

FINAL REPORT

Compound Specific Isotope Analysis of Mineral-Mediated Abiotic Reduction of Nitro Compounds

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14. ABSTRACT

Methods are needed to verify that abiotic attenuation of energetic compounds, such as trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), in groundwater is occurring, and it must also be possible to verify that strategies to enhance abiotic processes are having the desired effects. The overall objective of the project is to quantify the isotope fractionation factors of nitro compounds, including new components in insensitive munitions, during their abiotic reactions with iron bearing minerals. The central hypothesis is that abiotic attenuation processes will lead to specific fractionation of carbon (C) and nitrogen (N) contained with the pollutants. Specific objectives were to measure isotope enrichment factors during the abiotic reduction of nitroaromatic and nitramine explosives and assess how solution conditions and multiple reduction cycles influenced these values.

Testing of the overall objective was met by accomplishing the following overall tasks 1) Mineral Synthesis and Characterization, 2) Kinetic Studies with Synthetic Minerals, 3) Compound Specific Isotope Analysis (CSIA) method development, 4) Kinetic Studies with Natural Materials, and 5) Testing of Regeneration/Enhancement of Reactivity. CSIA was performed on samples collected from batch and column reactors in tasks 2, 4, and 5.

Reaction conditions (mineral identity, pH, presence of natural organic matter) influence reaction kinetics, but the isotope fractionation of N and C is unaffected by reaction conditions. Additionally, the fractionation measured with natural materials is similar to that with synthetic materials, and regeneration of reactivity with dithionite also leads to consistent isotope fractionation. Experiments in column reactors and samples from a field site reveal that transport processes make the interpretation of isotope fractionation more difficult, but ancillary data (including the presence of reaction products) allow assessment as to whether reduction of nitroaromatic and nitramine explosives is occurring.

This project has developed the tools necessary to assess if abiotic reduction is occurring in groundwater in situations where natural attenuation or an active remediation technology is being applied. While ancillary data is helpful in making the assessment, CSIA measurements are able to provide information as to 1) whether degradation is occurring, 2) the process responsible for the degradation, and 3) the extent of degradation versus dilution/non-degradative processes leading to concentration decreases. The methodologies will allow more robust information to be collected for evaluation of remediation success by responsible parties, practitioners, and stakeholders.

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Nitro explosives, groundwater, reduction, natural attenuation, remediation, compound specific isotope analysis

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4 LIST OF ACRONYMS

Acronym	Definitions
ACS	American Chemical Society
AKIE	Apparent kinetic isotope effect
2-ANAN	2-amino-2-nitroanisole
4-ANAN	4-amino-2-nitroanisole
ATO	3-Amino-1,2,4-triazol-5-one
C	carbon
c and c_0	concentration and initial concentration
CSIA	compound specific isotope analysis
D	Dispersion coefficient
Da	Dalton (molecular weight)
DAAN	2,4-diaminonitroanisole
DAD	Diode array detector
DNAN	2,4-dinitroanisole
DNP	Dinitrophenol
2,4-DNPH	2,4-dinitrophenylhydrazine
2,4-DNT	2,4-dinitrotoluene
DNX	1,3-dinitroso-5-nitro-1,3,5-triazacyclohexane
E	Element E
E_H	Electrochemical potential
EPA	Environmental Protection Agency
ESHA	Elliot Soil humic acid
F	extent of parent compound transformation ($1-c/c_0$)
Fe	iron
FeS	Iron sulfide, mackinawite
GC	gas chromatography
H	Hydrogen
HCHO	Formaldehyde
HMTA	hexamethylenetetramine
HMX	1,3,5,7-Tetranitro-1,3,5,7-tetrazocane
HPLC	high-pressure liquid chromatography
IAAAP	Iowa Army Ammunition Plant
ICP-AES	Inductively coupled plasma atomic emission spectroscopy
IHSS	International Humic Substances Society
IRMS	isotope ratio mass spectrometry
ISCR	In situ chemical reduction
KIE	kinetic isotope effect
k_{obs}	Pseudo-first order rate constant
k_R	rate constant of consumption of the reductant
L	Column length
LC	Liquid chromatography
m/z	Mass to charge ratio
MAE	Mean absolute error

MEDINA	methylenedinitramine
MNX	hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine
MOPS	3-(N-morphilino)propanesulfonic acid
MQL	Method quantification limit
MWCO	Molecular weight cutoff
n	Number of reactive atoms of a particular element
N	nitrogen
N ₂ O	nitrous oxide
NAC	nitroaromatic compound
NH ₄ ⁺	Ammonium
NOM	natural organic matter
NTO	3-nitro-1,2,4-triazol-5-one
PDF	Powder diffraction file
RDX	hexahydro-1,3,5-trinitro-1,3,5-triazine
S	Sulfur
SEM	Scanning electron microscopy
SPE	Solid phase extraction
SPME	Solid phase microextraction
t	Time
TAFB	Tinker Air Force Base
TCAAP	Twin Cities Army Ammunition Plant
TEM	Transmission electron microscopy
TNT	2,4,6-trinitrotoluene
TNX	hexahydro-1,3,5-trinitroso-1,3,5-triazine
V	Volume
V _R	Reactor volume
XRD	X-ray diffraction
$\delta^{13}\text{C}$	C isotope enrichment factor
$\delta^{15}\text{N}$	N-isotope signature
$\delta^{\text{hE}}, \delta^{\text{hE}_0}$	Isotope signature if element E with isotopomer atomic mass h. Subscript 0 indicates initial value.
ϵ_{C}	C-isotope signature
ϵ_{N}	N isotope enrichment factor
ϵ_{N}^*	Global (overall) N isotope enrichment factor
$\Lambda^{\text{N/C}}$	Correlation slope between N and C isotope fractionation
ρ_{b}	Bulk density
ρ_{p}	Particle density

5 ABSTRACT

Introduction and Objectives

Methods are needed to verify that abiotic attenuation of energetic compounds, such as trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), in groundwater is occurring, and it must also be possible to verify that strategies to enhance abiotic processes are having the desired effects. The overall objective of the project is to quantify the isotope fractionation factors of nitro compounds, including new components in insensitive munitions, during their abiotic reactions with iron bearing minerals. The central hypothesis is that abiotic attenuation processes will lead to specific fractionation of carbon (C) and nitrogen (N) contained with the pollutants. Specific objectives were to measure isotope enrichment factors during the abiotic reduction of nitroaromatic and nitramine explosives and assess how solution conditions and multiple reduction cycles influenced these values.

Technical Approach

Testing of the overall objective was met by accomplishing the following overall tasks 1) Mineral Synthesis and Characterization, 2) Kinetic Studies with Synthetic Minerals, 3) Compound Specific Isotope Analysis (CSIA) method development, 4) Kinetic Studies with Natural Materials, and 5) Testing of Regeneration/Enhancement of Reactivity. CSIA was performed on samples collected from batch and column reactors in tasks 2, 4, and 5.

Results

Reaction conditions (mineral identity, pH, presence of natural organic matter) influence reaction kinetics, but the isotope fractionation of N and C is unaffected by reaction conditions. Additionally, the fractionation measured with natural materials is similar to that with synthetic materials, and regeneration of reactivity with dithionite also leads to consistent isotope fractionation. Experiments in column reactors and samples from a field site reveal that transport processes make the interpretation of isotope fractionation more difficult, but ancillary data (including the presence of reaction products) allow assessment as to whether reduction of nitroaromatic and nitramine explosives is occurring.

Benefits

This project has developed the tools necessary to assess if abiotic reduction is occurring in groundwater in situations where natural attenuation or an active remediation technology is being applied. While ancillary data is helpful in making the assessment, CSIA measurements are able to provide information as to 1) whether degradation is occurring, 2) the process responsible for the degradation, and 3) the extent of degradation versus dilution/non-degradative processes leading to concentration decreases. The methodologies will allow more robust information to be collected for evaluation of remediation success by responsible parties, practitioners, and stakeholders.

6 EXECUTIVE SUMMARY

Introduction

Contamination of soil, sediment, and groundwater with nitroaromatic compounds (NACs), nitramines, and their structurally related precursors and manufacturing byproducts is a widespread problem at military training installations, abandoned production facilities, and munition disposal sites. Common contaminants include the explosives trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). Also of interest are newer materials that are components of insensitive munitions, such as 2,4-dinitroanisole (DNAN) and 3-nitro-1,2,4-triazol-5-one (NTO). Reductive transformation in the subsurface is often mediated by abiotic reductants including reduced Fe and S species and reduced quinone moieties of natural organic matter (NOM), which result from microbial carbon metabolism under anoxic conditions. Another important abiotic reductant in the environment is ferrous iron (Fe(II)) present in organic complexes and associated with Fe minerals such as iron oxides, sulfides, and clay minerals. A major challenge for the assessment of the degradation of nitro munitions (residues) in the subsurface is the large number of potential abiotic reductants. Depending on the contaminant and the reductant involved, rates of reduction vary by orders of magnitude, which makes it difficult to use pollutant concentrations to derive the extent of natural abiotic attenuation of nitro explosives. Assessing the transformation of NACs and nitramine compounds in the environment is also complicated by the possibility of several simultaneous (and potentially competing) transformation and transport processes (e.g., sorption, volatilization, (bio)degradation). Compound specific isotope analysis (CSIA) is useful for assessing degradation processes of nitro-compounds in such complex environmental systems with potentially competing attenuation processes. CSIA of nitro-compound (bio)transformations is based on characteristic changes in stable isotope ratios (e.g., $^{15}\text{N}/^{14}\text{N}$, $^{13}\text{C}/^{12}\text{C}$) that are indicative of a specific reaction process. Thus, it is possible to use CSIA to determine the reaction mechanism occurring without the need to detect reaction products, which may be not be amenable to detection, further degraded, bind to organic matter, or sorb to the matrix. Before such protocols can be used, it is necessary to establish the isotopic enrichment factors for these processes and their robustness under a variety of potential environmental conditions.

Objectives

The fundamental research presented herein responded to the statement of need by 1) implementing a compound specific isotope analysis (CSIA) methodology of carbon (C) and nitrogen (N) that will allow determination of the role of abiotic degradation of energetic compounds and insensitive munitions, including nitroaromatic and nitramine compounds, in subsurface systems by iron bearing minerals, 2) assessing the robustness of the isotope fractionation with different minerals under various solution conditions, and 3) evaluating enhancement of reactivity and quantifying the effectiveness of the enhancement by CSIA.

The CSIA methodology is particularly useful in this context because the measurement of isotope fractionation of the contaminants along a potential biogeochemical gradient is much more indicative of transformation than concentration measurements alone. Additionally, CSIA distinguishes not only between fractionating (e.g., chemical reactions) and non-fractionating

(e.g., dilution, sorption) processes, but also reductive, oxidative, and hydrolytic processes. It is critical to evaluate the reaction processes under conditions similar to those encountered in the field (e.g., presence of NOM, multiple contaminant exposures) to obtain a robust data set from the CSIA analyses and to evaluate the potential engineered interventions to enhance reactivity. Testing of the overall objective was met by accomplishing the following tasks: 1) Mineral Synthesis and Characterization, 2) Kinetic Studies with Synthetic Minerals, 3) CSIA method development, 4) Kinetic Studies with Natural Materials, and 5) Testing of Regeneration/Enhancement of Reactivity.

Technical Approach

Kinetic studies were performed in batch reactors, with mineral identity, pH, and the presence of natural organic matter being key variables. Natural soils and sediments containing iron oxides were collected from the Twin Cities Army Ammunition Plant (TCAAP, Arden Hills, MN) and Tinker Air Force Base (TAFB, Oklahoma City, OK). Monitoring of munitions compounds (DNAN, 2,4-dinitrotoluene (DNT), RDX, and NTO) and the products formed during degradation were used to establish reaction kinetics and pathways. Column experiments were performed with the natural materials to simulate in situ chemical reduction (ISCR) and test reactivity through multiple reduction cycles. Methods for CSIA of DNAN, DNT, RDX, and NTO by isotope ratio mass spectrometry (IRMS) were developed. CSIA was performed on samples collected from batch and column experiments in tasks 2, 4, and 5. The data were used to determine isotopic enrichment factors. The enrichment factors were then used to estimate the extent of transformation of DNAN and RDX in column reactors and of RDX in groundwater samples collected from the Iowa Army Ammunition Plant (IAAAP, Middletown, IA).

Results and Discussion

Reduction kinetics were affected by mineral identity and solution pH. In general, increasing pH led to faster reaction, but for NTO reduction by mackinawite (iron sulfide), reaction slowed with increasing pH. In most cases, reaction pathways (i.e., nitro group reduction) were unaffected by mineral identity or pH. The reaction of RDX with mackinawite was found to be different than that of iron oxides, with the accumulation of nitroso intermediates. The presence of natural organic matter had minimal effects on kinetics or pathways in all experiments.

Gas chromatography-IRMS was suitable for CSIA of DNAN, DNT, and RDX for both N and C isotopes, but interferences limited the C data collection for RDX. Liquid chromatography-IRMS allowed for the measurement of C isotopes of NTO, but a method suitable for N isotopes was not feasible.

Despite effects on reaction kinetics, the identity of minerals and pH did not affect the isotope fractionation of the target compounds. Results for DNAN (Figure 1a) revealed large changes in N isotope ratios as a function of reaction extent, and minimal changes in C isotopes. This is caused by the N-O bond breaking that occurs during nitro reduction. This was consistent for different materials, and for those dosed with ferrous iron or treated via ISCR. There were clear effects of the reaction mechanism on isotope fractionation. As shown in Figure 1, abiotic DNAN reduction led to large changes in N isotope ratios and small changes to those for C. Base-catalyzed hydrolysis and enzymatic hydrolysis led to markedly different results, with large fractionation of C and minimal fractionation of N (Figure 1b).

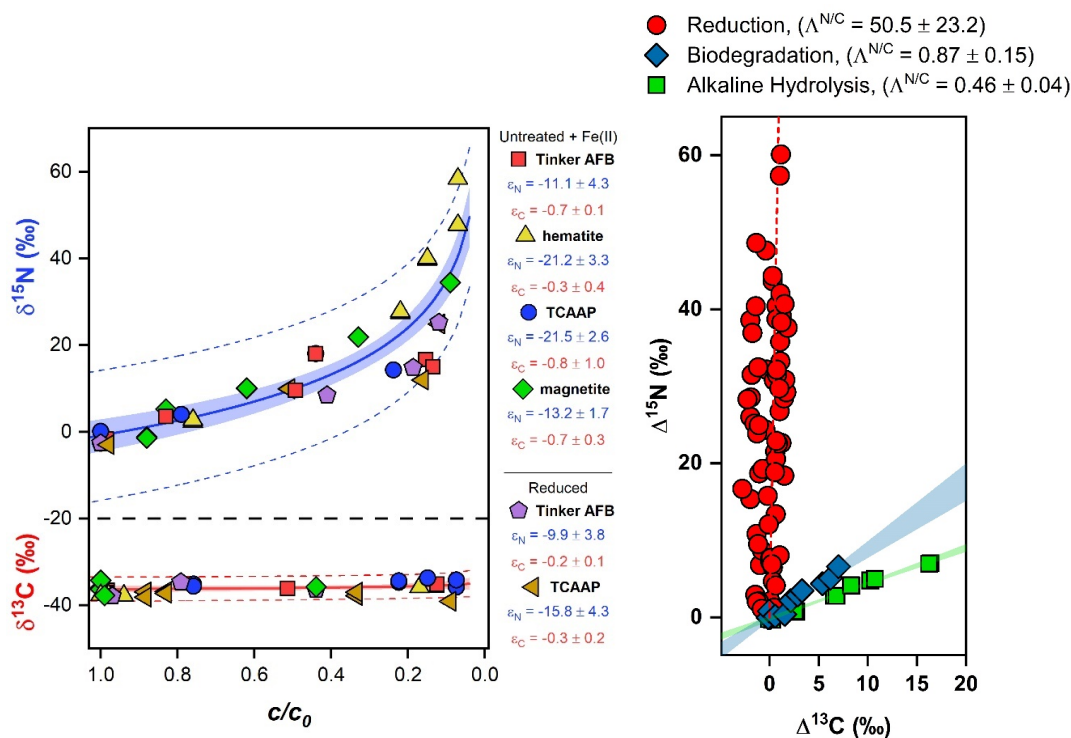


Figure 1. (Left) Nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) isotope signatures vs fraction of remaining substrate (c/c_0) during DNAN reduction by iron-bearing minerals. Solid lines represent fits from nonlinear regression with shaded portions indicating the 95% confidence intervals. Dashed lines designate 95% prediction intervals. N and C isotope enrichment factors (ϵ_N , ϵ_C) were determined from non-linear regression of the data points for each mineral type. Data are shown for untreated materials in the presence of Fe(II) and dithionite-treated materials. (Right) Two-dimensional isotope analysis for reductive and oxidative DNAN transformation pathways. Changes in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were monitored during abiotic reduction (circles), alkaline hydrolysis (squares), and biodegradation (diamonds). Any apparent inverse fractionation is an artifact of uncertainties in ^{13}C -AKIEs that are close to 1.

For RDX, reduction of the nitro group also led to enrichment of ^{15}N isotopes during reactions with iron oxide minerals (Figure 2a). Interferences prevented a full assessment of C isotopes, but results with green rust indicate fractionation of C is limited (Figure 2b). For mackinawite, the persistence of nitroso intermediates interfered with isotope measurements. Based on these results and literature data (Figure 2c), analysis of C and N isotopes for RDX should be able to distinguish between reductive, hydrolytic, and aerobic biodegradation processes.

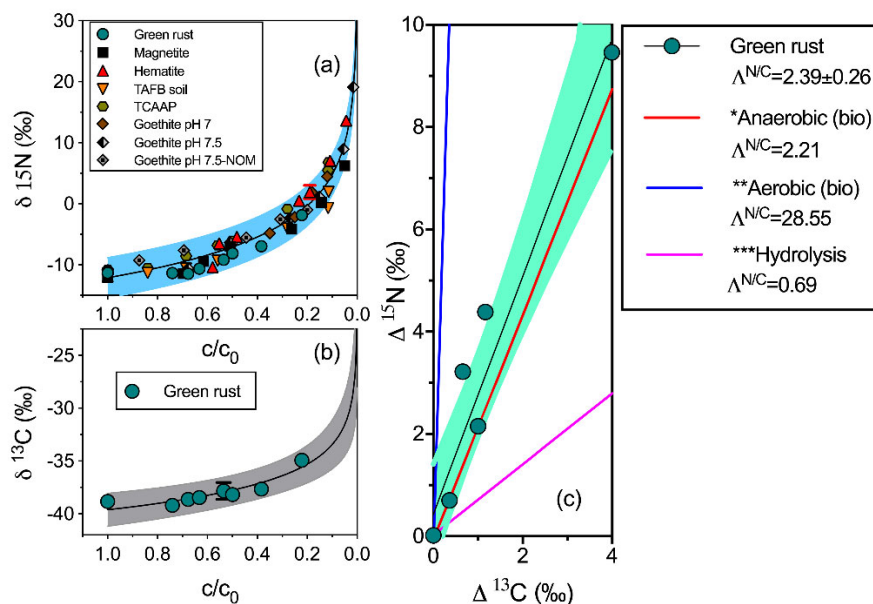


Figure 2. Nitrogen and carbon isotope fractionation during abiotic reduction of RDX at pH 7.5. (a) Nitrogen isotope ratios, $\delta^{15}\text{N}$, versus fraction of unreacted RDX (c/c_0) under various conditions. The goethite data represent experiments with Fe(II)/goethite at pH 7 and pH 7.5, and Fe(II)/goethite with ESHA at pH 7.5. The curve was fit using the data from each set of conditions to obtain a single ϵ_N value of $-7.4\pm0.2\text{‰}$ (individual values in Table 1). The error band shows the 95% confidence interval. (b) Carbon isotope signature, $\delta^{13}\text{C}$, versus fraction of unreacted RDX (c/c_0) during reduction by carbonate green rust. The error band shows the 95% prediction interval. (c) Two-dimensional N-C isotope analysis for RDX. For reduction mediated by carbonate green rust, $\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$ were calculated by subtracting the unreacted N and C isotope signatures from the measured values (e.g., $\Delta^{15}\text{N} = \delta^{15}\text{N} - \delta^{15}\text{N}_0$). The $\Lambda^{N/C}$ value was the slope of $\Delta^{15}\text{N}$ versus $\Delta^{13}\text{C}$. The error band shows the 95% confidence interval.

Column experiments using ISCR treatments gave consistent results over multiple cycles. The measured N isotope ratios were a function of the extent of transformation, with values returning to those of unreacted contaminant once reductive capacity was exhausted (Figure 3). Evaluation of the extent of DNAN reduction in the sediment columns from the $\Delta^{15}\text{N}$ of DNAN led to predicted values that differed from the actual extent of conversion (Figure 4). A linear regression of predicted versus measured quantities showed a correlation slope of 1.27 ± 0.18 ($r^2 = 0.96$). By not forcing the regression through zero, predicted values overestimated the extent of conversion at high c/c_0 and underestimated conversion at low c/c_0 . While the mean absolute error (MAE) is 0.091 ± 0.063 in c/c_0 , the inset in Figure 4 reveals that the relative error is greater at larger conversions. This illustrates that in our laboratory model system, the accuracy of estimates of the extent of degradation decreases with increasing turnover of the contaminant. That said, underprediction of the extent of conversion at low c/c_0 provides a margin of safety. Similar results were found for RDX (Figure 5).

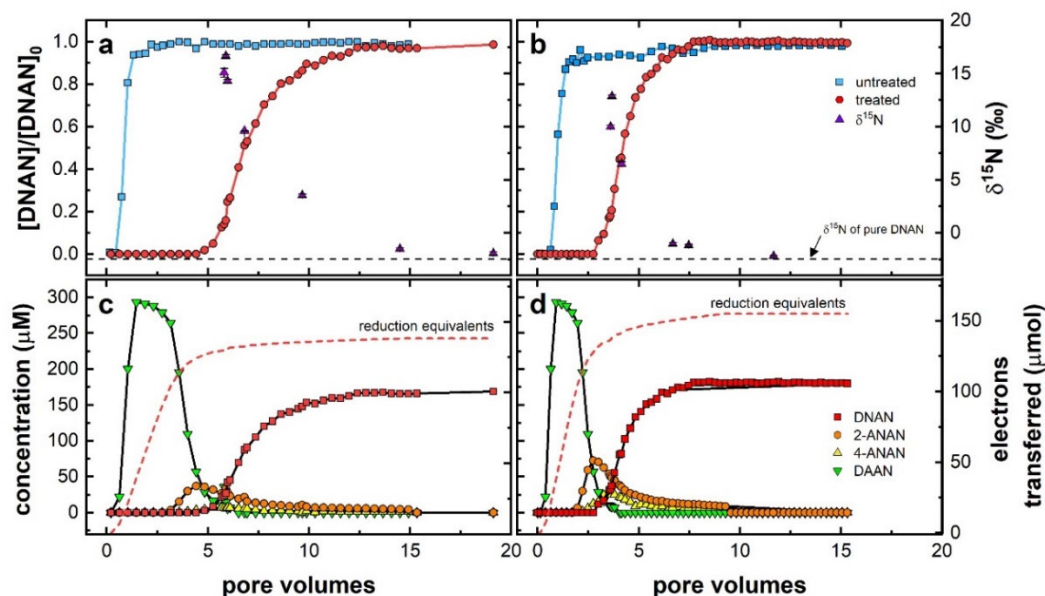


Figure 3. Breakthrough curves for 200 μM DNAN in (a) TCAAP and (b) Tinker AFB packed columns before (squares) and after (circles) receiving ISCR. Triangles denote measurements of $\delta^{15}\text{N}$ of DNAN in the effluent from treated columns. Error bars denote standard deviations from five sequential breakthrough experiments. Aqueous concentrations of DNAN (circles), 2-amino-4-nitroaniline (2-ANAN; squares), 4-amino-2-nitroanisole (4-ANAN; triangles), and 2,4-diaminoanisole (DAAN; diamonds) during DNAN exposure to (c) TCAAP and (d) Tinker AFB columns. Dashed lines represent the cumulative number of reduction equivalents transferred to DNAN during experiments.

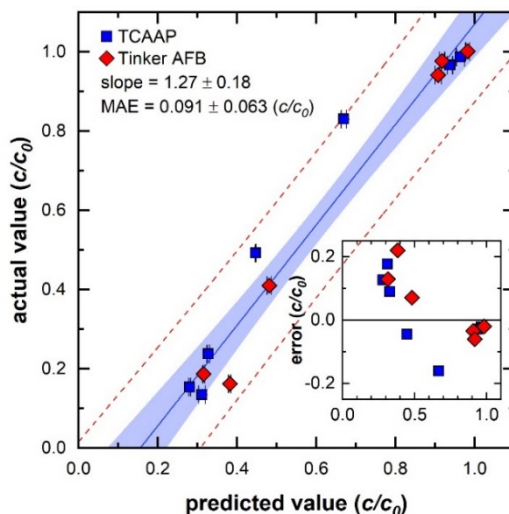


Figure 4 Predicted versus measured values of DNAN transformation by chemically reduced TCAAP and Tinker AFB materials in column reactors. Predictions were made according to eq 2 using an ϵ_{N}^* value of -14.9%. The solid line indicates the calculated fit by linear regression. Shaded portions indicate 95% confidence intervals of linear regressions and dashed lines represent the 95% prediction intervals. The inset shows the prediction error of each estimate.

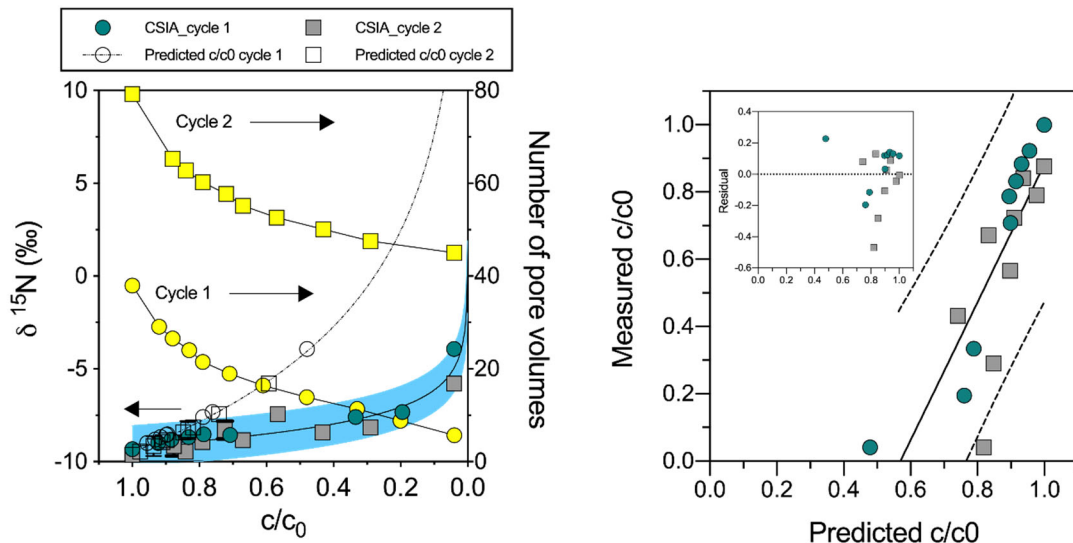


Figure 5. (a) Nitrogen isotope fractionation ($\delta^{15}\text{N}$, left y-axis) versus the extent of RDX reduction (c/c_0 , x-axis) in column reactors. The right y-axis indicates where isotope samples were taken on the column breakthrough curves during cycle 1 (yellow circle) and cycle 2 (yellow rectangle). The hollow squares and circles indicate predicted N-fractionation using $\varepsilon_{\text{N}} = -7.4$ ‰ from the batch reactors. (b) Measured c/c_0 versus predicted c/c_0 values of RDX in column effluent. The predicted c/c_0 values were obtained using $\varepsilon_{\text{N}} = -7.4$ ‰ from batch experiments. The solid line is a linear regression. The dashed lines are the 95% prediction intervals of linear regression. The inset is the residual plot.

CSIA of NTO revealed a secondary isotope effect for C during abiotic reduction by Fe(II)/goethite ($\varepsilon_{\text{C}} = 3.17 \pm 0.32$ ‰) that was caused by the minimal involvement of carbon during the abiotic reduction of $-\text{NO}_2$ moieties (Figure 6). For comparison, oxidative reactions of NTO with hydroxyl radicals were performed, and the bulk enrichment factor of $\varepsilon_{\text{C}} = 5.98 \pm 1.51$ ‰ was significantly higher than that for reduction. These results indicate that it is possible to use CSIA to distinguish reduction from reactions with hydroxyl radicals.

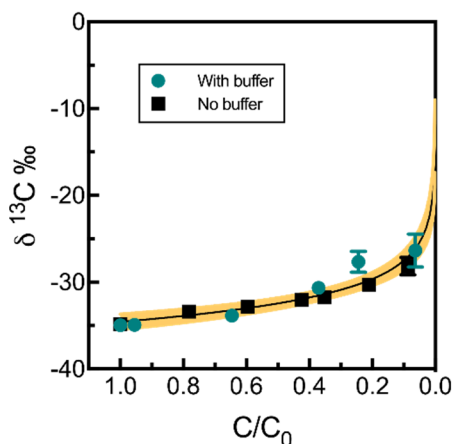


Figure 6. Carbon isotope fractionation during abiotic reduction of NTO by Fe(II)-goethite at pH 7, showing Carbon isotope signature, $\delta^{13}\text{C}$, versus fraction of unreacted NTO (c/c_0) under buffered and non-buffered conditions. All data were fit to obtain a single ε_{C} value. The error band shows the 95% confidence interval.

With the understanding of potential RDX reaction pathways and the associated isotope fractionation gained from laboratory experiments, CSIA was used with other lines of evidence including RDX concentrations and the presence of reaction products, to evaluate RDX transformation along a subsurface plume at the IAAAP. The G-20 sample ($\delta^{15}\text{N} = 0.66\text{‰}$) was assumed to represent the $\delta^{15}\text{N}$ value for unreacted RDX ($\delta^{15}\text{N}_0$) in this study because of the close proximity of the G-20 well to the source of the plume. The $\delta^{15}\text{N}_0$ of manufactured RDX typically spans a range of -17‰ to -4‰ depending on synthesis method and raw materials. As shown in Figure 7a, the overall $\Delta^{15}\text{N}$ was associated with a decreasing RDX concentration along the plume, suggesting that RDX concentration losses were due in part to N-O bond cleavage and not solely an artifact of non-degradative processes (i.e., sorption, dilution, volatilization, etc). Samples collected from L800-TT-MW09 ($\delta^{15}\text{N} = 6.4\text{‰}$) and 800-MW-25 ($\delta^{15}\text{N} = 6.5\text{‰}$) showed similar isotope ratios but distinct RDX concentrations ($2.8\text{ }\mu\text{M}$ and $0.8\text{ }\mu\text{M}$, respectively). Thus, the concentration of RDX in 800-MW-25 was assumed to be more affected by non-degradative processes compared to the other wells. Because of the consistent $\Delta^{15}\text{N}$ values, these data directly reflect the effects of mass transfer processes that cause apparent losses of contaminant concentration but do not elicit transformation.

The concentration of RDX in each well was predicted with the average isotope enrichment factor obtained from the earlier batch experiments ($\epsilon_{\text{N}} = -7.4\text{‰}$). It was assumed that RDX reduction along the groundwater transect was only caused by abiotic reduction. As with the column experiments, the predicted concentrations were consistently higher (less conversion; Figure 7a, blue bars) than the observed concentrations (Figure 7a, red bars), suggesting that non-fractionating processes affected the RDX concentration. Thus, while lower concentrations are at least partially due to reactive processes, total contaminant mass may not have decreased by a similar extent. The difference in predicted and measured conversions could be caused by the complexity of the hydrology and biogeochemistry in the subsurface. In heterogeneous aquifers with multiple flow paths, RDX (bio)degradation will not occur at an equal rate in each flow path. Groundwater extracted from a monitoring well contains contributions of residual RDX that has been isotopically fractionated (i.e., degraded) to different extents. Because our use of CSIA only considers the $\delta^{15}\text{N}$ of unreacted RDX, the measured values of $\Delta^{15}\text{N}$ in groundwater will more closely reflect the fractionation of unreacted RDX ($\Delta^{15}\text{N} = 0\text{‰}$) with increasing distance from the pollutant source (G-20 in this study). This complicates the interpretation of RDX degradation by CSIA because it suggests that RDX fractionation (i.e., RDX degradation) did not occur or was less than the actual amount and led to an underestimate of the extent of RDX reduction. Stakeholders must therefore exercise caution when using the results of CSIA as the only criterion to evaluate pollutant removal in sites with complex hydrogeology and geochemistry. Moreover, it supports the use of multiple lines of evidence to quantify pollutant (bio)degradation by monitored natural attenuation in the environment.

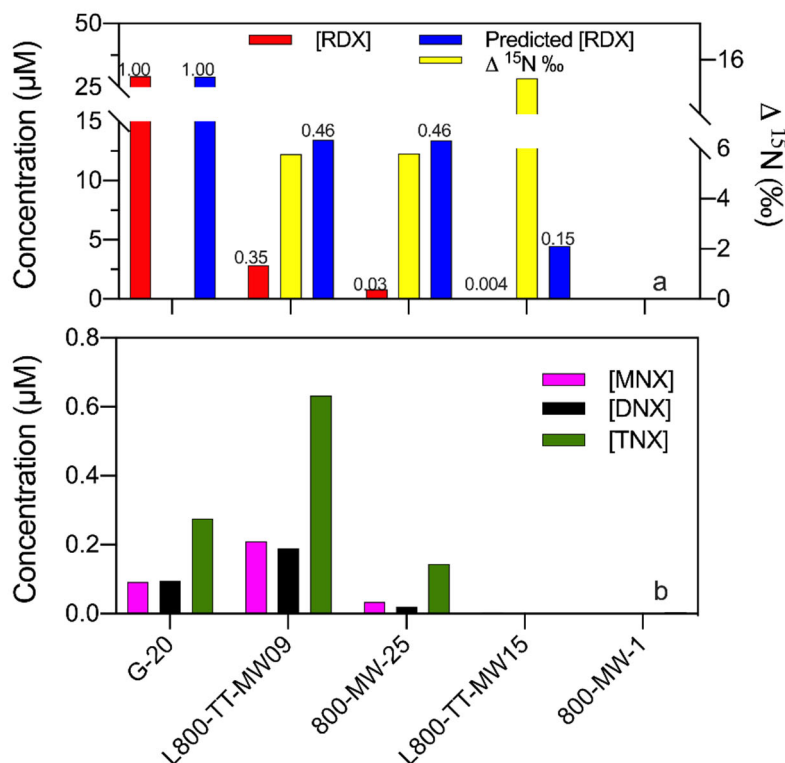


Figure 7. Evaluation of RDX transformation in monitoring wells along Line 800 at IAAAP. (a) The aqueous concentrations of RDX are plotted against the left Y-axis. The assumption was made that the concentration of RDX in G-20 represents the concentration and $\delta^{15}\text{N}_0$ of the contaminant source. The changes in N-isotope ratio ($\Delta^{15}\text{N} = \delta^{15}\text{N} - \delta^{15}\text{N}_0$) of RDX are plotted against the right Y-axis in yellow bars. The predicted RDX concentration in each well based on the lab-determined ^{15}N enrichment factor, $\epsilon_N = -7.4\text{‰}$ are plotted blue bars. The numbers on top of bars indicate the extents of transformation, measured (red bar) or predicted (blue bar), based on the assumption that G-20 has no RDX transformation (b) The aqueous concentrations of MNX, DNX, and TNX in the monitoring wells

Implications for Future Research and Benefits

This project has developed the tools necessary to assess if abiotic reduction is occurring in soil, sediment, and groundwater where natural attenuation or an active remediation technology is being applied. While ancillary data is required in making the assessment, CSIA provides information as to 1) whether contaminant degradation is occurring, 2) the process responsible for the degradation, and 3) the extent of degradative versus dilution and other non-degradative processes leading to concentration decreases. The methodologies will allow more robust information to be collected for evaluation of remediation success by responsible parties, practitioners, and stakeholders.

As remediation strategies are implemented, the adaptation of CSIA for pollutant monitoring in treated soil, sediment, and groundwater is critical for its successful integration into protocols for monitored natural attenuation and active remediation. To an extent, this has been accomplished, but only for the assessment of biodegradation processes. In this work, CSIA was shown to be a

robust technique to quantify abiotic reduction in various systems, but future work should include a survey of other *in situ* abiotic (e.g., calcium polysulfides, sodium persulfate) and biotic remediation strategies. Future studies should also address direct applications of CSIA to contaminated field sites using natural attenuation or ISCR as remediation methods. To accurately assess the efficiency of sediment reduction with external electron donors such as dithionite, these analyses will have to include critical phenomena which we were not able to evaluate in a laboratory model system. These include mass transport processes such as diffusive isotope fractionation, hydrodynamic dispersion, multiple contaminant sources, and the quality of stable isotope ratio measurements. Each of these phenomena can lead to either over- or underestimations of the extent of contaminant degradation. Understanding the local hydrogeology will therefore be necessary to accurately estimate the extent of contaminant (bio)degradation with CSIA. The observation, however, that isotope fractionation is unaffected by natural subsurface materials and ISCR can be leveraged to generate accurate estimates of contaminant removal strengthen our findings. Moreover, it provides strong support for the compulsory lines of evidence set by the US Environmental Protection Agency for evaluating the progress of natural attenuation.

7 OBJECTIVES

The statement of need ERSON-16-01 requested projects to develop methods to verify that abiotic attenuation of energetic compounds in groundwater is occurring and strategies to enhance such abiotic processes. The fundamental research presented herein responded to the statement of need by 1) implementing a compound specific isotope analysis (CSIA) methodology of carbon (C) and nitrogen (N) that will allow determination of the role of abiotic degradation of energetic compounds and insensitive munitions, including nitroaromatic and nitramine compounds, in groundwater systems by iron bearing minerals, 2) assessing the robustness of the isotope fraction with different minerals under various solution conditions, and 3) evaluating enhancement of reactivity and quantifying the effectiveness of the enhancement using CSIA.

The CSIA methodology is particularly useful in this context because the measurement of isotope fractionation of the contaminants along a potential biogeochemical gradient is much more indicative of transformation than concentration measurements alone. Additionally, CSIA is able to distinguish not only between fractionating (e.g., reactions) and non-fractionating (e.g., dilution, sorption) processes, but also reductive, oxidative, and hydrolytic processes. It is critical to evaluate the reaction processes under conditions similar to those encountered in the field (e.g., presence of natural organic matter (NOM), multiple contaminant exposures) to obtain a robust data set from the CSIA analyses and to evaluate the potential engineered interventions to enhance reactivity.

The specific research aims were to:

Establish reaction kinetics for various synthetic minerals

After synthesizing and characterizing various iron minerals (Task 1), the kinetics of reaction with the target contaminants were determined (Task 2). Such experiments are important for several reasons. First, using well characterized materials allows exploration of the effect of solution variables on mineral reactivity. Second, kinetic data are critical to interpretation of the CSIA data and calculation of isotope fractionation factors, and thus solution phase variables must be explored in detail. Third, these systems allow evaluation of multiple contaminant exposure cycles, which formed the basis of reactivity recovery/enhancement studies.

Identify isotope fractionation factors during abiotic degradation with synthetic minerals

While CSIA analysis of nitroaromatic compounds is well established, methods for analysis of nitramine compounds were developed. CSIA is used to analyze the evolution of N and C isotope ratios of the target contaminants during their reaction with the minerals (Task 3). Using different minerals and different solution conditions allows determination of the robustness of the fractionation factors for a given contaminant/mineral combination.

Establish reaction kinetics and fractionation factors with natural materials

The kinetic experiments and CSIA analyses were repeated with natural materials where abiotic attenuation is known or suspected to occur (Task 4). Natural materials contain multiple phases

which may sorb or react with the nitro compounds. While trace minerals present in the samples, potentially undetectable by bulk characterization methods, may be responsible for the observed degradation, the fractionation factor determined via CSIA allows the specific abiotic transformation process to be identified by comparison with experiments using synthetic materials. Samples from a contaminated site were used to evaluate the utility of CSIA.

Evaluate regeneration/enhancement of reactivity

Introduction of dithionite to the systems was used to regenerate reduction equivalents and assess potential changes in mineralogy (Task 5). Experiments were performed in both batch and column systems. These experiments provide the opportunity to not only evaluate the effectiveness of abiotic regeneration/enhancement of reactivity, but also to study the effects such manipulations have on the isotope fractionation of the contaminants. Such information is critical in determining if enhancements are having the desired effect in field systems.

8 BACKGROUND

8.1 Energetic compounds as contaminants

Contamination of soil, sediment, and groundwater with nitroaromatic compounds (NACs) and nitramines and their structurally related precursors and manufacturing byproducts are a widespread problem at military training installations, abandoned production facilities, and munition disposal sites.¹⁻³ Many of the frequently reported contaminants, such as the explosives 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), are of significant toxicological concern.⁴ Under anoxic conditions, the organic nitro contaminants are transformed reductively at the NO₂-moiety resulting in nitroso, hydroxylamino, and amino compounds (Figure 8-1). Some of the reduction products can be of even higher toxicological concern than the parent compounds. A quantification of the pathways and extent of contaminant reduction is therefore key for the assessment of contamination risks.

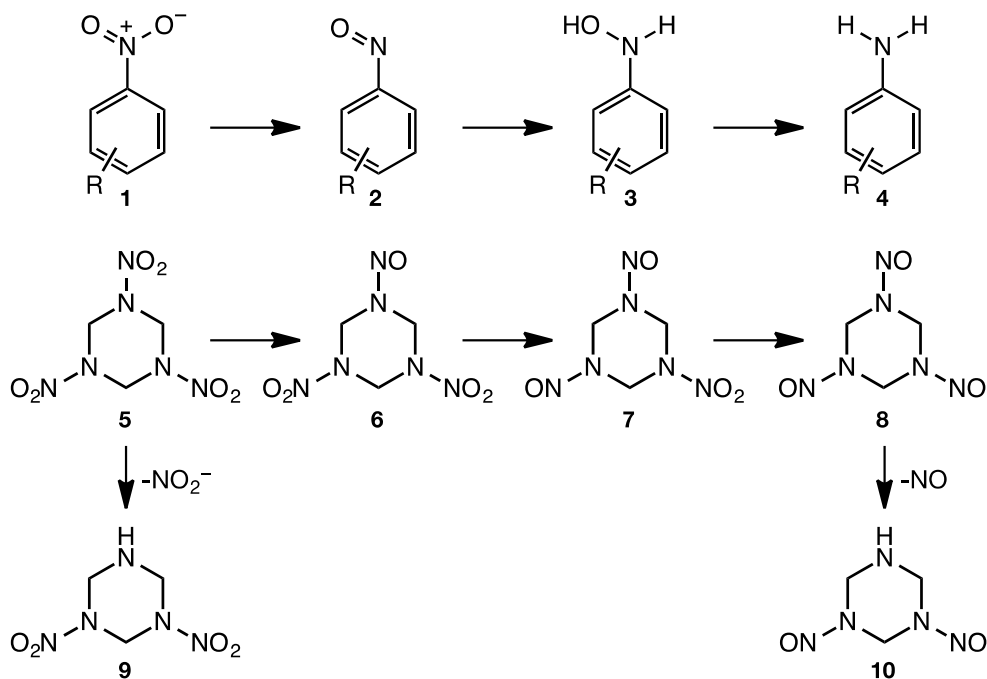


Figure 8-1. Abiotic reduction NACs (1) via nitroso (2) and hydroxylamino (3) intermediates to substituted anilines (4) and reductive transformation pathways of RDX (5) via nitroso intermediates (6) - (8) and denitrosations.¹

Reductive transformation in the subsurface is typically mediated by abiotic reductants including reduced iron (Fe) and sulfur (S) species as well as reduced quinone moieties of natural organic matter, which result from microbial carbon metabolism under anoxic conditions.⁵⁻⁸ The resulting Fe-bearing minerals and reduced NOM constitute a large portion of the (renewable) electron donating capacity in the subsurface.⁹ Many laboratory and field studies have shown that abiotic reductants, particularly iron oxide minerals, have considerable reactivity towards organic nitro compounds, including munitions.¹⁰⁻¹⁵ A comparison of the relative importance of abiotic versus

microbial reduction is difficult to establish under field conditions, but current evidence suggests that Fe-minerals are the predominant reductants of organic and inorganic pollutants.^{13,16–18}

A major challenge for the assessment of nitro munitions (residues) in the subsurface is the large number of potential abiotic reductants. Examples include hydroquinone moieties of reduced NOM, reduced S species, and Fe(II) species of organic complexes, Fe(II) associated with Fe minerals such as Fe oxides, sulfides, and clay minerals.^{5,19–22} Depending on the organic contaminant and the reductant involved, rates of reduction vary by orders of magnitude,²³ which makes it impossible to derive the extent of natural attenuation of nitro explosives by abiotic reductants from analyses of concentration dynamics. In addition, many nitro compounds sorb strongly to mineral soil and sediment constituents, where they may or may not be accessible for abiotic or biological reactions.¹

8.2 Compound specific isotope analysis (CSIA)

These issues are, in part, circumvented by applying CSIA to nitro contaminants. Stable isotope signatures in organic contaminants, which represent the ratio of heavy and light isotope of an element in a compound (e.g., for N isotopes in eq. 1), vary systematically as a consequence of (bio)chemical (degradation) reactions.^{24–27}

$$\delta^{15}\text{N} = \frac{^{15}\text{N}/^{14}\text{N}(\text{sample})+1}{^{15}\text{N}/^{14}\text{N}(\text{standard})+1} - 1 \quad (1)$$

where $\delta^{15}\text{N}$ is the N isotope signature, $^{15}\text{N}/^{14}\text{N}(\text{sample})$ is the $^{15}\text{N}/^{14}\text{N}$ -ratio measured in the organic compound, and $^{15}\text{N}/^{14}\text{N}(\text{standard})$ is the corresponding ratio of an internationally accepted standard material.

The enrichment factors (ϵ_E) and kinetic isotope effects (KIE_E) of element E (often C, N, or H) for many abiotic and microbial degradation routes of NACs have been quantified in the laboratory.^{6,27–33} Reduction of NACs leads to cleavage of N-O bonds of the NO_2 group. Rates of this reaction are sensitive to the N isotope at the reacting bond leading to changes of N isotope ratios in the remaining contaminant during the reaction (Figure 8-2a). C isotope ratios, in contrast, do not change significantly. This combination of large N and secondary C isotope fractionation produces typical trends for isotope fractionation analysis. As shown in Figure 8-2b, reductions produce distinctly different trends than oxidations. The unique combinations of C, N, and H isotope fractionation patterns also enable quantification of the extent of (bio)transformation.

As shown in eq. 2 for N isotope signatures ($\delta^{15}\text{N}$), shifts in contaminant isotope signatures are related to the extent of transformation, F , using ϵ_E values, which reflect the KIE of the transformation pathway (eq. 3).

$$F = 1 - c/c_0 = \left(\frac{\delta^{15}\text{N} + 1}{\delta_{x,t=0}^{15}\text{N} + 1} \right)^{1/\epsilon_N} \quad (2)$$

$$^{15}\text{N} - \text{KIE} = \frac{1}{1 + n \cdot \epsilon_{\text{N}}} \quad (3)$$

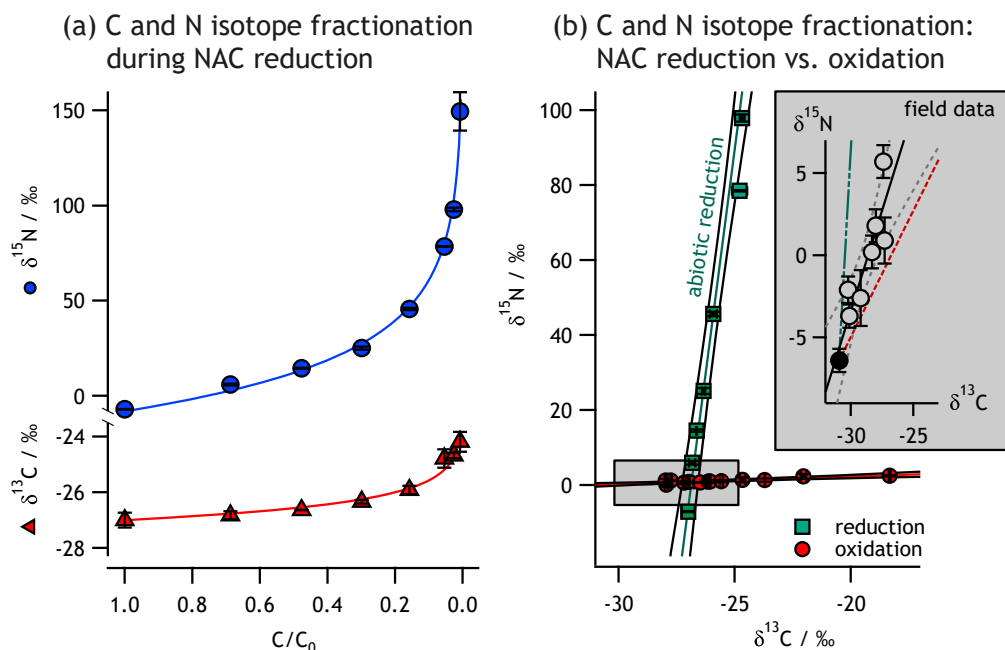


Figure 8-2 (a) Large nitrogen and moderate C isotope fractionation associated with the reduction of NACs, (b) Abiotic reduction generates patterns of C and N isotope fractionation that is distinctly different from (biotic) oxidations. Gray insert shows field data resulting from a combination of two isotope fractionating processes (modified from references^{28,34}).

where c/c_0 is the fraction of remaining substrate and $\delta_{x,t=0}^{15}\text{N}$ is the reference isotope signature for unreacted contaminant. ϵ_{N} is the N isotope enrichment factor, which relates the N isotope fractionation measured in the whole compound to the KIE at the reacting bond (^{15}N -KIE) according to eq. 3 based on the number of reactive atoms n .

In recent years, many isotope enrichment factors for reduction reactions have been determined,^{28,29,31,33,35–37} but it is unclear to what extent they apply to those in the field. Previous work on biodegradation of NACs under oxic conditions suggest that it is possible to extrapolate such data from laboratory experiments and established analytical procedures (i.e., gas chromatography/isotope ratio mass spectrometry, GC/IRMS) for soil and water samples to allow analysis of field samples.^{27,37}

Assessing the transformation of NACs in the environment is complicated by the possibility of several simultaneous (and potentially competing) transformation and transport processes (e.g., sorption, volatilization, (bio)degradation). Likewise, NACs in the subsurface may be present in different phases^{38,39} and exhibit high soil and sediment sorption, rendering the interpretation of concentration dynamics difficult.^{40,41} CSIA has proven useful for assessing degradation processes of NACs in such complex environmental systems with potentially competing attenuation processes.^{26,28} CSIA of NAC (bio)transformations is based on characteristic changes in stable isotope ratios (e.g., $^{15}\text{N}/^{14}\text{N}$, $^{13}\text{C}/^{12}\text{C}$) that are indicative of a specific pathway.^{25,42} Thus,

it is possible to use CSIA to determine the reaction process occurring without the need to detect reaction products, which may not be amenable to detection, further degraded, bind to organic matter, or sorb to the matrix.^{43,44} These so-called isotope fractionation patterns arise from apparent kinetic isotope effects (AKIEs) controlled by changes in the bonding environment at the reactive position during the initial stages of NAC transformation.³⁴ Indeed, AKIEs have been well-characterized for the abiotic reduction of several NACs including chloro- and methyl-substituted nitrobenzenes, dinitrobenzene isomers, and 2,4,6-trinitrotoluene (¹⁵N-AKIE ~ 1.03–1.04, ¹³C-AKIE ~ 1.001).^{21,29–31} Abiotic reductions of NACs (Figure 8-1) result in large primary ¹⁵N-AKIEs, for which cleavage of the first N—O bond to form a nitroso intermediate is the rate-limiting step and thus dictates shifts in the isotope signature. In contrast, the corresponding ¹³C-AKIEs are typically small because C atoms are not directly involved in the rate-limiting step (i.e., secondary AKIEs).^{29–31}

Previous work on the abiotic reduction of NACs by ferrous iron (Fe(II)) associated with the surfaces of iron oxides and clay minerals suggests that other munitions compounds will display similar isotopic fractionation behavior.^{21,29–31} Those studies, however, focused on isomers of chloro- and methyl-substituted compounds and on a limited set of minerals (i.e., Fe(II) associated with goethite and structural Fe(II) in magnetite and ferruginous smectite). Showing that fractionation is independent of environmental matrix conditions is imperative for accurately quantifying the extent of pollutant degradation at contaminated field sites where the subsurface composition may be complex, changing, or unknown. Potential matrix conditions could include the presence of different minerals with different reactivity towards NACs, interferences of NOM through association with the mineral surface, and the transformation of Fe minerals after repeated exposure to NACs. Previous work has revealed that transformation rates of NACs may span orders of magnitude depending on the type of mineral involved owing to modulations in the electrochemical potential (E_H) of the oxide-associated Fe(II) system.^{7,23,45–47} The presence of NOM may also impact reaction rates through the formation of Fe(II)-NOM complexes.⁴⁸ NOM may also alter particle dispersion or change the availability of reactive sites.^{49–52} Repeated contaminant exposure may induce evolution of the mineral structure, potentially interfering with Fe(II) uptake or developing a new surface oxide.^{52,53} Because the magnitude of isotope fractionation during the reduction of NACs was proposed to be determined by changes of bonding in the aromatic NO₂ substituent(s) regardless of the rate of transformation (i.e., reduction of a nitro-group to form a nitroso-moiety), it is necessary to verify isotope fractionation associated with the reduction of new potential contaminants, such as insensitive munitions in the environment will be consistent with previously determined AKIE-values as well as independent of matrix conditions discussed above.^{21,28–30,35,54}

8.3 Insensitive munitions

Insensitive munitions have emerged as safer alternatives to traditional explosives such as TNT and RDX. The U.S. military has recently begun development of two insensitive munitions formulations (IMX-101 and IMX-104) containing 2,4-dinitroanisole and nitrotriazolone (DNAN and NTO, respectively; Figure 8-3).⁵⁵ These formulations exhibit a decreased sensitivity to shock and high temperatures relative to traditional munitions and are designed to minimize the risk of accidental detonations during handling and storage.^{56,57} Previous contamination by traditional munitions at military installations has caused concern for future contamination by insensitive munitions, motivating considerable effort towards understanding their subsurface fate and

transport properties. Limited data, however, is available concerning the fate and transport of these new energetic materials.^{1,3} Moreover, the toxicity and sediment-water partitioning behavior of DNAN and its transformation products have shown to be similar to that of TNT.^{38,55,58} NTO is highly soluble and thus mobile in the environment. A better understanding of the relevant natural attenuation processes for these compounds in subsurface environments is therefore needed.

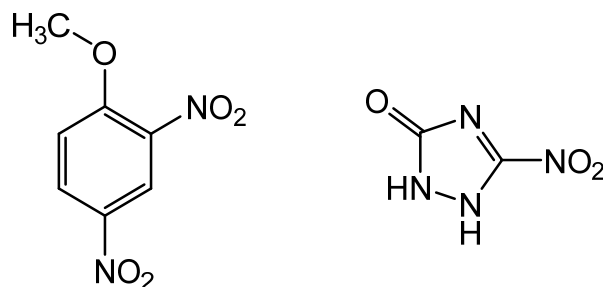


Figure 8-3. Structures of 2,4-dinitroanisole (DNAN; left) and nitrotriazolone (NTO; right).

The degradation of DNAN in the environment occurs through several abiotic transformation pathways including alkaline hydrolysis,^{59,60} photolysis,^{61,62} and also reduction in systems containing zero valent iron or other iron-bearing mineral species.^{63,64} Previous work has shown that ferrous iron associated with iron oxides, iron (oxy)hydroxides, and sulfide-bearing minerals mediates the abiotic transformation of NACs under anoxic conditions,^{23,45,65,66} reducing NACs to the corresponding substituted anilines (e.g., Figure 8-1).^{14,67} Such pathways have been observed for DNAN and can be attributed to mineral-bound Fe(II) species as well as electron-donating functional groups of natural organic matter.^{65,68} Substituted anilines may also form during biotic processes facilitated by Fe(III)-reducing microorganisms and other concomitant suites of aerobic and anaerobic microbiota.^{1,13,15,68–70} Complete reduction of DNAN in subsurface environments is often achieved through coupled abiotic-biotic transformation mechanisms. For example, Niedźwiecka et al.⁷¹ observed rapid DNAN reduction in microcosms containing Fe(III) following reduction of Fe(III) to Fe(II) by *Geobacter metallireducens*, compared to microcosms without ferric iron.

The abiotic transformation of NTO was found through advanced oxidation⁷² and reduction catalyzed by iron-bearing minerals such as green rust⁷³ or Fe/Cu bimetal particles.⁷⁴ The abiotic reduction of NTO under anoxic conditions has shown formation of aniline product, 3-amino-1,2,4-triazol-5-one (ATO; Figure 8-4). The biodegradation of NTO has also been observed in previous studies.^{75,76} Under aerobic conditions, mammalian cytochrome P450 enzymes transformed NTO to urazole and ATO, while under anaerobic conditions, NTO is primarily reduced to ATO.⁷⁷ Krzmarzick et al.⁷⁸ has observed that in anaerobic microcosms with soil inoculate using hydrogen as an electron donor, ATO is the final product that does not degrade unless exposed to aerobic biodegradation or oxidizing conditions.

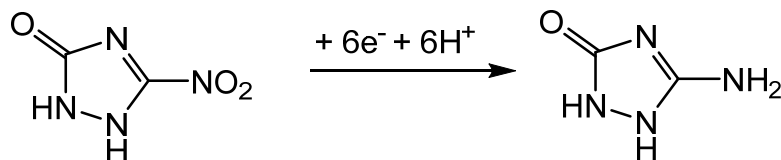


Figure 8-4. Reduction of NTO to ATO.

8.4 RDX

The synthetic cyclic N-nitrosamine munition compound RDX has been extensively used in military operations. RDX poses both chronic and acute toxicity threats to aquatic and terrestrial organisms and is considered a possible human carcinogen by the United States Environmental Protection Agency (EPA).^{79–81} The high solubility and low volatility of RDX⁸⁰ have led to widespread contamination in the subsurface,^{82–85} which mainly stems from manufacturing and weapons testing. Because of the leaching of RDX and the formation of nitroso intermediates under reducing conditions, these compounds have been widely detected in soil, sediment, and water at military training installations, abandoned production facilities, and munition disposal sites.^{86–88} Because the nitroso intermediates are more toxic to some terrestrial biota than RDX,^{89,90} understanding the extent of RDX reduction and the fate of the nitroso intermediates is also important.

For RDX, CSIA data have been obtained for biodegradation (aerobic and anaerobic) and hydrolysis.^{91–93} Anaerobic biodegradation of RDX undergoes two distinct pathways (Figure 8-5): (a) successive or simultaneous nitro group reduction to form nitroso intermediates (hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX), 1,3-dinitroso-5-nitro-1,3,5-triazacyclohexane (DNX), and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX))⁹² and (b) ring cleavage of RDX (or MNX) to form methylenedinitramine (MEDINA) and a bis(hydroxymethyl)nitramine intermediate followed by further degradation to nitrous oxide (N₂O), ammonium (NH₄⁺), and formaldehyde (HCHO).^{14,92,94} Detailed steps of nitro group reduction to form the nitroso intermediates are shown in Figure 8-5. During anaerobic biodegradation, isotope enrichment factors for ¹⁵N (ε_N) of -5‰ to -9.9‰ were observed for nitro group reduction and an ε_N of -12‰ was observed for ring cleavage.^{91,92} Aerobic biodegradation of RDX involves denitration originating from N atoms in the ring structure as the initial step (Figure 8-5). The enrichment of ¹⁵N (ε_N = -2.4‰) is larger than that of ¹³C (ε_C = -0.8‰) implying that C atoms are not involved in the initial step.^{91,92} For alkaline hydrolysis, the rate-limiting step of RDX reduction is rapid elimination of NO₂ followed by the breaking of a C-H bond and the formation of a C=N bond⁹⁵ (Figure 8-5) in which significant enrichment of ¹³C and ¹⁵N (ε_C = -7.8‰ and ε_N = -5.3‰, respectively) were observed.⁹³ Thus, sufficiently large isotope enrichment may enable a straightforward and conclusive application of CSIA to determine whether RDX degradation via a certain pathway is occurring.

In anoxic groundwater, RDX undergoes abiotic reduction mediated by Fe(II) associated with or structurally contained in iron minerals, such as iron oxides, iron sulfides, and clay minerals,^{14,73,80} or Fe(II) produced via in situ redox manipulation.⁹⁶ While nitro group reduction to form nitroso intermediates likely occurs in the anoxic subsurface,⁸⁰ there is a lack of N and C isotope fractionation data available for this degradation pathway. Because reduction leads to both

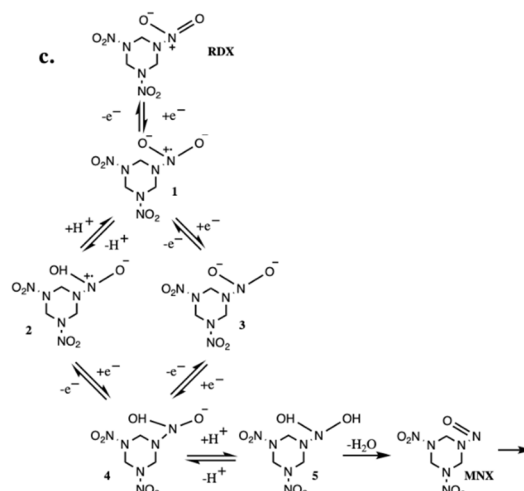
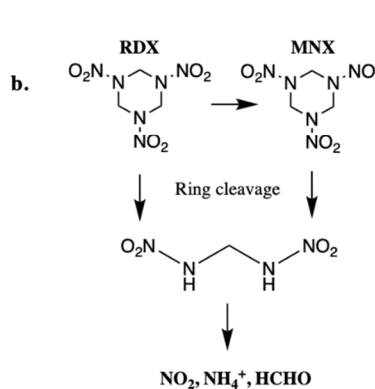
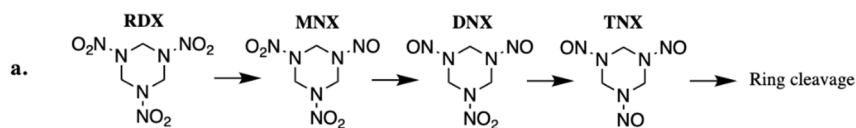
nitro group reduction and ring cleavage (the latter ultimately forming N_2O , NH_4^+ , and HCHO ; Figure 8-5),^{14,80} there are multiple possible reaction processes that could lead to isotope enrichment, depending on which one is rate limiting. Furthermore, previous research has not obtained isotope fractionation data for abiotic RDX abiotic reduction under controlled laboratory conditions and compared these values with samples acquired from column studies or from contaminated sites, for which isotope fractionation may be affected by other processes.

8.5 Summary

Overall, information about the isotope fractionating reactions of other nitro explosives is limited and has been measured under limited sets of abiotic conditions.^{92,97-99} *We hypothesize that C and N isotope enrichment factors for abiotic processes will be distinct from those for biological processes and that natural materials will display the same isotope enrichment factors as synthetic minerals.* Thus, we expect that for a given reaction process (i.e., reduction of a nitro group) the identity of the mineral, the reductants, and solutions conditions **will not affect** the isotope fractionation. Thus, once enrichment factors have been determined and tested for their robustness, the values determined in laboratory systems will be able to be used at contaminated sites by measuring isotope fractionation along a gradient to determine if abiotic (or even biological) degradation is occurring.

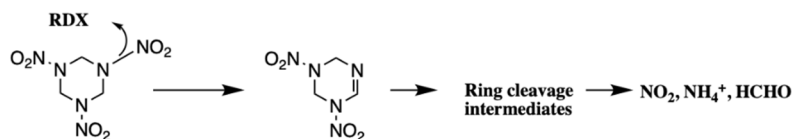
Anaerobic biodegradation

1



Aerobic biodegradation

2



Alkaline hydrolysis

3

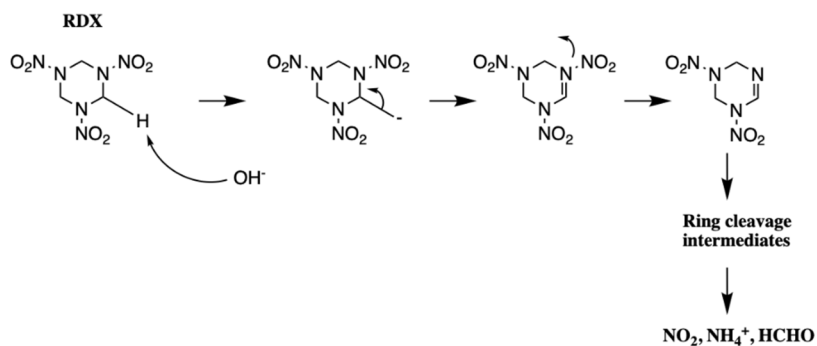


Figure 8-5. RDX degradation via anaerobic biodegradation (1), aerobic biodegradation (2), and alkaline hydrolysis (3) (derived from refs.^{19,92,100,101})

9 MATERIALS AND METHODS

9.1 Chemicals and Materials

All chemicals were ACS grade and used as received unless otherwise noted. All organic solvents were high pressure liquid chromatography (HPLC) grade. A full listing of chemicals and solvents is in Section 12.1.1. Mineral syntheses, except for goethite, were performed using deoxygenated ultrapure water (Milli-Q) inside an anaerobic chamber (95% N₂/5% H₂, Coy). Goethite was synthesized with ultrapure water under aerobic conditions. Dialysis bags used for mineral purification were purchased from Spectrum Laboratories Inc. (MWCO = 2000 Da). Elliot Soil humic acid (ESHA) was purchased from the International Humic Substances Society (IHSS, St. Paul, MN, Cat No. 4S102H).

9.2 Mineral synthesis and characterization methods

9.2.1 Iron (oxyhydr)oxides

Goethite was synthesized according to the methods of Anschutz and Penn.¹⁰² The produced goethite was stored in the laboratory atmosphere at pH 4 and 4 °C until further use. The mackinawite synthesis was adapted from Butler and Hayes.¹⁰³ Mackinawite (FeS) particles were washed by dividing the suspension into two dialysis bags and placing each bag in deoxygenated ultrapure water. The dialysate was exchanged three times per day for three days. The two portions were then combined and stored in the glovebag at pH 7 until further use. Magnetite nanoparticles were synthesized according to Schwertmann and Cornell.¹⁰⁴ The nanoparticles were dried at room temperature inside the anaerobic chamber and stored dry until use. Hematite was synthesized according to Schwertmann and Cornell.¹⁰⁴ The batch used in this study was obtained from Voelz et al.¹⁰⁵ The purity of the minerals were assessed by X-ray diffraction (XRD; see Section 9.2.4). Characterization of hematite was performed by Voelz et al.¹⁰⁵

9.2.2 Green rust

Carbonate green rust was synthesized by coprecipitation according to the methods of Larese-Casanova and Scherer.¹⁴ Briefly, solutions of 0.13 M FeCl₂•4H₂O and 0.025 M FeCl₃•6H₂O were prepared in deoxygenated, ultrapure water inside the anoxic chamber. The solutions were individually titrated to pH 7.0 with 1.0 M K₂CO₃ (ACS grade, Fisher), combined, adjusted to pH 8.4, and held under continuous stirring for 1 h. The resulting precipitate was collected by vacuum filtration on a glass fiber filter (Whatman 44, 70 mm), washed three times with ultrapure water, and immediately added to batch reactors (5 g/L). A small subset of the synthesized was preserved with glycerol and analyzed by XRD (see Section 10.1.2).

9.2.3 Chemically reduced smectites

The two nontronite clays (NAu-1 and NAu-2) were purchased from The Clay Minerals Society (Chantilly, VA, USA). The source of these materials is the Uley graphite mine in South Australia. NAu-1 is an Al-rich nontronite (90% nontronite, 4% kaolinite in bulk) and NAu-2 is an Al-poor subset (95% nontronite, 5% plagioclase in bulk). The chemical composition and

structure of each sample was previously determined by Keeling et al (see Table 9-1).¹⁰⁶ The nontronite clays were conditioned prior to use according to previously described methods.^{107,108} The purpose of conditioning was to simplify the water chemistry in clay suspensions to allow for easier quantification of the relevant species during experiments. Seventy-five grams of each nontronite were milled using a glass mortar and pestle and transferred to 0.5 L of 1 M NaClO₄. The suspension was then rotated on an end-over-end rotator for ~3 h and subsequently allowed to settle before discarding the supernatant. Nontronite materials were then resuspended in fresh 1 M NaClO₄ and the process was repeated for a total of three cycles. The resulting clay suspensions were then transferred to centrifuge bottles, suspended in deionized water, and shaken for 15 min. The bottles were then centrifuged at 600 g for 7 min to separate the ≤ 0.5 μm fraction from the bulk clay material. The supernatants were transferred to clean beaker and flocculated with 1 M NaClO₄. This process was repeated until a clear supernatant was obtained. The combined flocculated portion from the wash procedure was allowed to sit overnight before discarding the supernatant. The washed clays were acidified to pH 3.5–4 with HNO₃ to remove impurities and prevent hydrolysis and shaken for 30 min. The suspension was then centrifuged at 600 g for 5 min, the supernatant was removed and discarded, and the clay was resuspended in 1 M NaClO₄. The resuspension resulted in a circumneutral pH. The final clay suspension was stored in the dark at 4 °C prior to reduction.

Table 9-1. Chemical composition and structure of the two nontronites, NAu-1 and NAu-2.^a

Component	Source Clay Composition (%)	
	NAu-1	NAu-2
SiO ₂	53.33	56.99
Al ₂ O ₃	10.22	3.4
Fe ₂ O ₃	34.19	37.42
MgO	0.27	0.34
CaO	3.47	2.67
Na ₂ O	0.08	0.11
K ₂ O	0.03	0.02
Structure	$(M^{+})[Si_{7.00}Al_{1.00}][Al_{0.58}Fe_{3.38}Mg_{0.05}](M^{0.97+})[Si_{7.57}Al_{0.01}Fe_{0.42}][Al_{0.52}Fe_{3.32}Mg_{0.7}O_{20}(OH)_4]$	

^adata obtained from Keeling et al.¹⁰⁶

After conditioning, the nontronite suspensions were dithionite-reduced according to previous methods^{9,10} as adapted by Gorski et al.¹⁰⁸ Nontronite was reduced to increase the amount of available Fe(II) to serve as a reducing agent of NACs. The conditioned nontronite suspensions were deoxygenated with N₂ gas (~2 h) and transferred into the glovebag. Solutions of 1 M NaHCO₃ and sodium citrate were added to each suspension and the resulting mixture was heated to 70 °C and stirred using a magnetic stir plate. Sodium dithionite (3 g/g nontronite in suspension) was then added and the suspensions continued stirring overnight at 70 °C. Unwanted residual solutes and loosely-bound iron species were removed using dialysis. Dialysis tubing (MWCO = 12400 Da, MilliporeSigma) was rinsed with ultrapure water and filled with clay suspensions. Filled tubing was placed in a deoxygenated 0.1 M NaClO₄ solution and allowed to equilibrate for 8 h before being transferred to a fresh NaClO₄ solution. This was repeated for a total of four cycles. To assess the effects of reduction on reactivity, a subset of each nontronite

was subject to an identical process as described above except no dithionite was added. A mass loading experiment was performed to determine the nontronite mass concentration in each suspension. Total Fe was quantified by the ferrozine method¹¹⁰ (Section 12.1.2) following dissolution in 3 M HCl. Reduced materials were stored in the anaerobic chamber.

9.2.4 Characterization of synthetic minerals

All synthetic minerals were characterized by XRD to determine the crystalline phases present in each sample. Patterns were collected using a PANalytical X'Pert Pro XRD system equipped with a cobalt source (1.79 Å) and an X'Celerator detector. Diffraction patterns were collected over 10°–80° 2θ and compared to powder diffraction files (PDF) for goethite (No. 29-0713), magnetite (No. 19-0629), mackinawite (No. 15-0037) and carbonate green rust (No. 40-0172). Stoichiometry (Fe(II)/Fe(III) ratio) of the synthetic magnetite was quantified by acid digestion in 3 M HCl for 7 d and then measured using ferrozine. Details are in Section 12.1.2.

9.3 Synthesis of RDX

The synthesis of RDX was adapted from the methods of Just and Schnoor.¹¹¹ A two-necked, 100 mL round bottom flask equipped with a thermometer was suspended in a water bath set to 5 °C. Purum fuming nitric acid (20.59 g) was transferred to the 100 mL round bottom flask and cooled to 5 °C. Once the reaction vessel reached temperature, 1.2025 g of hexamethylenetetramine (HMTA) was added slowly to the round bottom flask over 90 min. During HMTA addition, the water bath was set to 1 °C and careful attention was given to maintain the reaction vessel between 5–10 °C. Once HMTA addition was complete, the water bath temperature was ramped from 1 °C to 16 °C in intervals of 3 °C with 5 min between intervals. The reaction mixture was allowed to react for 3 h once the temperature inside the flask reached 15 °C. The temperature inside the reaction vessel was maintained at 15–20 °C during this portion. Over the 3 h, the reaction mixture turned from a light yellow to a deep orange color with light bubbling. Following the 3 h reaction, the reaction mixture was poured into a pre-chilled, 100 mL beaker containing 30 mL of ultrapure water and a 10 mL ice cube made from ultrapure water to precipitate the synthesized RDX. The solution was then filtered through a Buchner funnel equipped with a glass fiber filter (Whatman 44, 70 mm) and washed with sodium carbonate (5% w/w) until the filtrate was neutral. The precipitate was then washed with approximately 250 mL ultrapure water and allowed to dry overnight. The following day, the RDX was transferred into 20 mL scintillation vials and dissolved in a 1:1 mixture of acetonitrile and methanol and stored at 4 °C until use. The synthetic RDX was characterized by HPLC and compared to an analytical standard. No detectable peaks other than RDX were present in the resulting chromatogram.

9.4 Batch experiments with synthetic minerals

All reactions were performed inside an anaerobic chamber unless otherwise noted. Batch experiments for DNAN, 2,4-dinitrotoluene, (2,4-DNT, used as a surrogate for TNT), and RDX were conducted according to previously established methods.^{52,53} Reactors were constructed by filling 35 mL glass serum bottles with the desired mineral phase suspended in 10 mM carbonate buffer (pH 7.0). Reactors were equilibrated for 21–24 h on an end-over-end rotator (40 rpm, Glas-Col) before the addition of a contaminant. If desired, amendments of ferrous iron (1 mM) were added to goethite, magnetite, and hematite suspensions from an aqueous FeCl₂ (175 mM)

stock solution before equilibration. To initiate reactions, a contaminant was spiked from a methanolic stock solution to an initial concentration of 200 μM . This concentration was selected to ensure sufficient parent compound remained for CSIA after 90-95% conversion. During reactions, aliquots were removed from reactors with a 1 mL syringe and immediately filtered through a 0.2 μm nylon syringe tip filter. The concentrations of parent compound and reduction products were evaluated by HPLC as described above. The pH and Fe(II) content were monitored throughout reactions and readjusted to 7.0 and 1 mM, respectively. Aqueous Fe(II) was quantified as described in Section 12.1.2. For experiments containing natural organic matter, ESHA was added to a specified concentration (as organic carbon) after the addition of aqueous Fe(II) but before the 21–24 h equilibration period. Repeated contaminant exposure reactions were performed by allowing reactions to proceed until no remaining contaminant was detected in the reactor at which point the pH and Fe(II) concentrations were returned to 7.0 and 1 mM, respectively, and reactors were equilibrated for ~ 20 h. Further contaminant spikes were performed following the methods described above.

For NTO, reduction experiments were conducted similarly, except goethite and hematite loadings were 0.5 g/L reactor and magnetite and FeS were 2.0 and 1.5 g/L, respectively. The reactions were performed at pH values ranging from 5.5. to 7 in bicarbonate buffer or MOPS buffer. Samples were taken as described above over a period of 1 to 4.5 hours. The effect of ESHA was tested for goethite. For the NTO oxidation experiments, 30% of H_2O_2 was spiked into reactors at a concentration of 50 mM, and the initial pH was adjusted to 3 with 5 N of H_2SO_4 . Because NTO reacts with Fenton reagent immediately, FeSO_4 was spiked at 1mM stepwise to control extent of reaction. Samples were taken after 5 minutes of mixing after each FeSO_4 spike. The samples were quenched with 1 M of NaOH (0.4 mL) for 30 minutes, filtered, neutralized with 20 drops of 5 N H_2SO_4 , and diluted as necessary before HPLC analysis.

9.5 Analytical methods and kinetics calculations

All measurements by HPLC were performed using an Agilent Technologies 1200 Series HPLC equipped with a photodiode array director (DAD). DNAN and its reaction products 2-amino-2-nitroanisole (2-ANAN), 4-amino-2-nitroanisole (4-ANAN), and 2,4-diaminonitroanisole (DAAN) were separated using an Inertsil ODS-3 column (4.6 $\times$ 250 mm, 5 μm particle size). The mobile phase (60 % acetonitrile/40 % ultrapure water) was operated at a flow rate of 1.00 mL/min. The injection volume was 20 μL . The detection wavelength was 230 nm.

The concentrations of RDX and three nitroso intermediates, MNX, DNX and TNX, were measured by HPLC with a ZORBAX Eclipse Plus C-18 column (4.6 mm $\times$ 250 mm, 5 μm). The mobile phase (40:60 acetonitrile: MilliQ water) flow rate was 1 mL/min and the injection volume was 10 μL . The detection wavelength was 230 nm for all compounds. Analytical standards of RDX and TNX were used to produce calibration curves for quantification. The concentrations of MNX and DNX were estimated by using a quantitative estimation method for a no-analytical standard scenario.¹¹² The TNX standard was used to validate that the nitroso intermediates had similar response factor as RDX at a detection wavelength of 230 nm.

Analysis of HCHO was adapted from Soman et al.¹¹³ Aqueous samples were derivatized by 2,4-dinitrophenylhydrazine (2,4-DNPH) under acidic conditions. A mixture of 10 mL diluted sample (1:5 dilution), 2 mL of 5.05 M 2,4-DNPH solution in acetonitrile, and 200 μL 5N phosphoric

acid were reacted for 30 min. The derivatized HCHO-DNPH was measured by HPLC with a Supelcosil LC-18-DB column (150×4.6 mm, Sigma Aldrich) and quantified using a calibration curve developed from the HCHO-2,4-DNPH standard. The mobile phase was water–acetonitrile (55:45, v/v) with a flow rate of 0.7 mL/min. A 10 µL injection volume and detection wavelength of 360 nm were used.

Ammonium (NH_4^+) was derivatized by ortho-phthalaldehyde (OPA) and Na_2SO_3 in tetraborate buffer to form a fluorophore, OPA- NH_4^+ -sulfite. The fluorophore was measured within 4 hours after derivatization using a spectrofluorometer (Aqualog, Horiba). The excitation wavelength was set to 360 nm. The sample signal was read from the emission spectrum at 422 nm. NH_4^+ standards were prepared from 0.4 µM to 10 µM and processed in the same manner as the samples.

The concentrations of NTO and its reaction product ATO were determined by HPLC with a Hypercarb porous graphitic column (4.6 mm × 100 mm, 3 µm). The mobile phase was 85% of MilliQ water with 2 mM of ammonium acetate and 15% of methanol with 2 mM of ammonium acetate. The flow rate of mobile phase was 0.3 mL/min. The detection of NTO was at 15 min at 320 nm, and ATO was at 7.3 min at 216 nm. ATO standards were prepared in the iron-reduction matrix, i.e. a mixture of NaHCO_3 , dissolved FeCl_2 , and iron minerals. ATO standards in different aqueous matrices resulted in different response factors on the HPLC, and thus to obtain mass balances, the standard matrix had to match that of the experimental systems (Section 12.1.4). The minerals were filtered out before the standards were measured on HPLC. ATO standards were immediately measured after preparation in the reaction matrix to prevent potential loss.

Pseudo-first order rate constants were determined by linear regression of $\ln(c/c_0)$ vs. time (t), where c and c_0 are the current and initial pollutant concentrations, respectively. All reported errors are 95% confidence limits propagated from the uncertainties associated with the regressed slopes for replicate experiments.

9.6 Natural Materials

9.6.1 Collection of Natural Materials.

Aquifer material from the Twin Cities Army Ammunition Plant (TCAAP) was extracted by sonic drilling at the south-central region of the site. Sediment was collected from four depth profiles between 41–52 m below ground surface into 5-gallon buckets and transported back to the laboratory. The materials were purged with N_2 gas, dried in the anaerobic chamber, and stored within the chamber until further use. Material was sieved to a uniform particle size of 350–425 µm before use. The magnetic portion of TCAAP material was separated using a neodymium magnet according to previously described methods.¹¹⁴ Material was collected from the Tinker Air Force Base (TAFB) into 1-gallon plastic bags for transportation back to the laboratory. The material was dried at 100 °C, sieved to 350–425 µm, and stored on the benchtop in amber glass vials until use.

9.6.2 Characterization of natural materials.

Natural materials were characterized by XRD as described above. Scanning electron microscopy (SEM) images were collected using a JEOL 6500 field emission gun microscope at 5 – 10 kV with elemental mapping collected by energy-dispersive X-ray spectroscopy using a Thermo Scientific Noran system. Samples were uncoated and prepared on carbon tape. For synthetic magnetite, transmission electron microscopy (TEM) was performed on a FEI Tecnai T12 microscope with a LaB₆ electron source (120 kV) and Gatan charge-coupled device camera. Samples were prepared by suspending a small amount (< 1 mg) of dried sample in 1 mL ultrapure water, sonicating 15 s, diluting 10 µL of that suspension to 1 mL, and air drying a drop of dilute suspension on a 200 mesh holey carbon coated copper grid (SPI Supplies). Fe percent (w/w) of the materials was quantified by inductively coupled plasma-atomic emission spectrometry (ICP-AES). The Fe(II)/Fe(III) ratio of the magnetic TCAAP extract was determined as described in Section 12.1.2.

9.6.3 Batch reactors with added Fe(II)

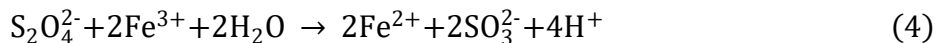
Protocols were identical to those with synthetic materials (Section 9.4) except the amount of material added was higher to match the mass of iron minerals to those for the synthetic minerals.

9.6.4 Batch experiments with reduced clays

Protocols were identical to those with synthetic materials (Section 9.4) except no aqueous Fe(II) was added. All of the available Fe(II) was generated during the reduction of the clay materials (see Section 9.2.3).

9.6.5 In situ chemical reduction experiments

In-situ chemical reduction (ISCR) experiments were carried out inside an anaerobic chamber and all aqueous solutions were prepared in deoxygenated ultrapure water. For each experiment, the dithionite dosage was selected to target a 10% reduction of the total Fe present in the TCAAP or TAFB material. Because one mole of dithionite can reduce two moles of Fe³⁺ (eq. 4), sodium dithionite was added to a ratio of 1 mol S₂O₄²⁻:4 mol Fe in all reactors. Anoxic dithionite stock solutions were prepared fresh before each experiment to avoid the loss of dithionite by aqueous disproportionation.¹¹⁵ All reductions were buffered with a solution of K₂CO₃ to neutralize the 4 moles of H⁺ released for every mole of dithionite consumed during iron reduction (eq. 4).



The Fe(II) content in aquatic materials after ISCR was quantified by acid dissolution followed by ferrozine method.

For ISCR experiments in batch reactors, materials were suspended in a solution of K₂CO₃/S₂O₄²⁻ (2:1 mol/mol) and placed on the benchtop rotator for 48 h. The exact concentrations of K₂CO₃ and dithionite varied depending on the total Fe content of the material being used. The resulting suspension was then separated by centrifugation and the supernatant was discarded. Reduced materials were washed three times with carbonate buffer (10 mM, pH 7) to remove excess

dithionite and any reduction products (e.g., sulfate, sulfite, thiosulfate).¹¹⁶ The reduced materials were then suspended in carbonate buffer and immediately used for batch experiments as described in Section 9.4, but aqueous Fe(II) was not added.

To prepare column reactors, borosilicate glass columns (Kimble FLEX-COLUMNS®; 2.5 cm I.D., 10 cm length) were dry packed with either TCAAP or Tinker AFB material to uniform bulk ($\rho_b = 1.66 \pm 0.04 \text{ g/cm}^3$) and particle ($\rho_p = 2.68 \pm 0.05 \text{ g/cm}^3$) densities. A flow adapter (Kimble) affixed with fluorinated ethylene propylene (FEP) tubing was attached to the column inlet to prevent migration of materials within each column. Column outlets were secured with a polypropylene endcap. All feed solutions were amended with 10 mM NaCl to prevent dissolution and sediment leaching by providing a uniform ionic strength. Columns were saturated with 10 mM NaCl to determine the pore volume and porosity; the mean porosity of all columns was 0.44 ± 0.05 . Sodium bromide (100 mM) was used as a step input conservative tracer to characterize column flow and estimate the dispersion coefficient (details in Section 12.1.3). Bromide concentrations were measured with a conductivity probe (Oakton).

The natural materials were conditioned prior to the introduction of dithionite by passing carbonate buffer (10 mM, pH 7) upwards through the column at 0.5 mL/min for approximately 5 pore volumes. A freshly prepared aqueous solution containing 1.25 mM sodium dithionite and 2.50 mM K_2CO_3 was then fed upwards through the column at 0.25 mL/min for ~18–24 h. The exact run time was selected based on the total Fe content of the material. The low flow rate and long run times were used to allow enough contact time for the reaction to occur without delivering excess dithionite. Columns were then flushed with 10 pore volumes of carbonate buffer (10 mM, pH 7) to remove unreacted dithionite and any reaction products and immediately used for contaminant reduction experiments. Aqueous Fe(II) was not introduced to column reactors receiving ISCR treatment.

Columns were equilibrated before contaminant reduction experiments by upward flow (0.5 mL/min) with 10 mM, pH 7 carbonate buffer including 10 mM NaCl for ~5 volumes. Reactions were initiated by adding the desired contaminant (200 μM) to the feed solution and collecting effluent samples with an automated fraction collector (Bio-Rad Laboratories Inc). All samples were acidified to pH <4 with HCl (Sigma, trace metals; ~0.1 mL) to prevent the oxidation of any structural or dissolved Fe(II). Experiments were terminated once the effluent concentration was equal to that of the feed solution. Concentrations of the parent compound and reaction products were determined by HPLC (see Section 9.5). Columns were flushed after reduction reactions with carbonate buffer for several pore volumes to remove residual contaminant and any reaction products. The column materials were then reduced again by sodium dithionite according to the method described above before further experiments.

9.7 Compound Specific Isotope Analysis

The GC-IRMS and LC-IRMS methods developed are described in Section 10.3.2.

9.7.1 Sample Collection from Reactors

Samples for CSIA were collected from batch reactors by conducting reactions in eight replicate reactors under the same conditions as those described above for the synthetic mineral and natural

material experiments. At each sampling point, the entire contents of a reactor were removed and immediately filtered through a 0.2 μm nylon syringe tip filter. A small portion ($\sim 1\text{ mL}$) was set aside for analysis by HPLC to determine the concentrations of the parent and reaction intermediates/products as described in Section 9.5, and the remaining extract was stabilized to $\text{pH} < 4$ with trace metals HCl and stored at $4\text{ }^{\circ}\text{C}$ until CSIA. Because of the low concentrations and interferences from reaction products, RDX was concentrated prior to CSIA using a previously established solid phase extraction (SPE) method.¹⁰¹ Briefly, SPE tubes (Supelclean™ ENVI™ -Chrom P, MilliporeSigma) were conditioned with 3 mL ethyl acetate followed by 3 mL methanol and 5 mL ultrapure water. Samples were passed through SPE cartridges by applying vacuum to achieve a sample flow rate of $\leq 10\text{ mL/min}$. SPE cartridges were then dried to dryness under vacuum flow and eluted with ethyl acetate.

NTO samples for CSIA were collected using the step injection method similar as mentioned in Section 9.4, to control NTO reaction extent. Replicate reactors were prepared as described above using goethite at $\text{pH } 7$, except that no FeCl_2 was spiked initially. The initial NTO concentration in each reactor was approximately $800\text{ }\mu\text{M}$. Different amount of FeCl_2 was spiked to each serum bottle to initiate reaction with NTO that leads to different extents of reaction. The entire liquid of each reactor was filtered using the same syringe tip filter as described above. A small portion ($\sim 1\text{ mL}$) was set aside for HPLC analysis of reactants and products. No further sample preparation was conducted before isotope analysis. NTO oxidation samples were also analyzed for CSIA as comparison. Sample collection was similar to reduction samples, except that reaction matrix and sample preparation were following protocols described in Section 9.4.

Samples for CSIA from column reactors were collected from the column outlet into borosilicate glass culture tubes ($16 \times 1000\text{ mm}$, 10 mL) and immediately filtered through a $0.2\text{ }\mu\text{m}$ syringe tip filter into a separate, clean culture tube. A portion ($\sim 1\text{ mL}$) of each sample was analyzed by HPLC and the remainder was preserved for CSIA as described for batch reactions. To ensure enough sample volume for CSIA ($\geq 15\text{ mL}$), each sample was collected over a 30 min period; thus, the concentration represents a composite of each 30 min sampling period. The reaction time of each sample was the middle time point of each sampling period (i.e., 15 min into each period).

9.7.2 Extraction of groundwater samples and preparation for CSIA

Seven groundwater samples were collected from monitoring wells along a subsurface plume at the Line 800 site of the Iowa Army Ammunition Plant (IAAAP, Middletown, IA). Each sample contained approximately 4 L of groundwater. Details of the well locations and sampling method are described in Section 10.4.5. Concentrations of RDX and the nitroso intermediates from separate samples collected at the same time were provided by Jacobs Engineering Group. SPE in preparation for isotope analysis was performed as described above, except the sample from each well was split into three $\sim 1.3\text{ L}$ aliquots and each was passed through a separate SPE cartridge. After elution, the three extracts were combined and processed as described below.

9.8 Data processing

For experiments in which the amount of reductant was not in excess, a theoretical approach was used to invoke a second order kinetic model that considers the concentration of both the pollutant (N) and reductant (R).¹¹⁷ Using this approach, the rate constant of consumption of the reductant

(k_R) was assumed to be equivalent to the rate constant of pollutant reduction (k_{obs}) divided by the concentration of the reductant (eq. 5).

$$k_R = \frac{k_{obs}}{[R]} \quad (5)$$

A system of ordinary differential equations was then used to model the reaction rate of pollutant (eq. 6) and reductant (eq. 7).

$$\frac{d[N]}{dt} = -k_R[R][N] \quad (6)$$

$$\frac{d[R]}{dt} = -k_R[R][N] \quad (7)$$

A solution to eqs. 6 and 7 for a set of initial conditions of the pollutant and reductant was proposed by simultaneously fitting the values of k_R and R_0 to measured pollutant concentrations (eq. 8).¹¹⁷

$$N(t) = \frac{N_0(N_0 - R_0)}{N_0 - R_0 \exp(-(N_0 - R_0)k_R t)} \quad (8)$$

where, N_0 and R_0 are the initial concentrations of pollutant and reductant, respectively. N_0 was 200 μ M in all experiments. Measurements of N and t were collected from triplicate reactions for each type of mineral suspension. The fitting of eq. 8 was performed with Origin 2019 and one combined value of k_R and R_0 were determined from each set of triplicate reactors. The fitted values of R_0 were compared to the estimated initial Fe(II) concentration that would be expected for a certain dosage of dithionite during in situ chemical reduction. The theoretical and calculated values were within reasonable error.

Reaction pathways for RDX were assessed based on a carbon mass basis, because the nitrogen mass balance requires the trapping and analysis of gaseous products.¹⁴ Concentrations of the carbon-containing analytes were converted to the concentration of carbon within that analyte, denoted as “C-analyte” (e.g., C-RDX = 3×[RDX]). The amount of C in the initial RDX represents the total C in the reactor. A hypothetical C-containing product (C-Unidentified) was introduced to the model to compensate for any loss of total C as reduction of RDX proceeded. The changes of RDX, intermediates, and product concentrations over time were expressed as sets of differential equations, with different sets of equations used to test whether ring cleavage occurred for RDX or the nitroso compounds (Section 12.1.8) and evaluated by the lowest value of the normalized residual. The assumption was made that the reaction kinetics of all compounds followed pseudo-first order expressions, with reductant concentration being constant. The sets of differential equations were solved in MATLAB (MathWorks, Version 9.5) using the initial concentration of C-RDX (i.e., [C-RDX]₀) and fixing the overall pseudo-first order reaction rate constants of RDX to those determined from ln(c/c₀) vs. t plots for the experiments were sacrificed as a function of time. The kinetic models were fit to experimental data via a least squares regression (code provided Section 12.1.8).

Isotope ratios were referenced against standard laboratory gases and DNAN of known isotopic composition ($\delta^{13}\text{C}_0 = -37.3 \pm 0.1$ and $\delta^{15}\text{N}_0 = -2.4 \pm 0.1$) as determined by an elemental analyzer using standard bracketing procedures.¹¹⁸

Carbon and nitrogen isotope enrichment factors (ϵ_{C} and ϵ_{N}) were derived according to methods described by Pati et al.¹¹⁸ Linear regression analyses of the isotope signature data were carried out using the log-linearized form of eq. 9:

$$\frac{\delta^{\text{h}}\text{E}+1}{\delta^{\text{h}}\text{E}_0+1} = \left(\frac{c}{c_0}\right)^{\epsilon_{\text{E}}} \quad (9)$$

where $\delta^{\text{h}}\text{E}_0$ is the C or N isotope signature of unreacted DNAN and c/c_0 is the fraction of remaining substrate.¹¹⁹ Apparent kinetic isotope effects (^{13}C -AKIE and ^{15}N -AKIE) were then derived from the calculated ϵ_{E} values using eq. 3 and the values above.^{26,120} The n values used in eq. 3 for DNAN (^{15}N - $n = 2$, ^{13}C - $n = 1$), RDX (^{15}N - $n = 6$, ^{13}C - $n = 1$), and NTO (^{13}C - $n = 1$) were determined from their chemical structures. Combined AKIEs were calculated for all sets of conditions for each mineral phase (i.e., mineral alone, presence of NOM, repeated contaminant exposures) according to the method of Scott et al.¹²¹ using the Pitman estimator.

To estimate the extent of pollutant transformation (F), all of the measurements of $\delta^{15}\text{N}$ from batch reactions with each mineral were combined and applied to eq. 9. This resulted in one global ϵ_{N} value (ϵ_{N}^*) for each pollutant. The extent of pollutant reduction was calculated with eq. 10 from the deviation of measured $\delta^{15}\text{N}$ values from $\delta^{15}\text{N}_0$ using the combined ϵ_{N}^* value. Note that eq. 10 is the result of a reorganization of eq. 2 to account for ϵ_{N}^* . We evaluated the accuracy of our estimates by performing a linear regression of the predicted vs measured values of c/c_0 and calculating the mean absolute error (MAE) of the predictions (eq. 11).

$$F = 1 - \frac{c}{c_0} = \left(\frac{\delta^{15}\text{N} + 1}{\delta^{15}\text{N}_0 + 1} \right)^{1/\epsilon_{\text{N}}^*} \quad (10)$$

$$\text{MAE} = \frac{\sum_{i=1}^n \left| (c/c_{0, \text{measured}})_i - (c/c_{0, \text{predicted}})_i \right|}{n} \quad (11)$$

10 RESULTS AND DISCUSSION

10.1 Task 1 – Mineral Synthesis and Characterization

10.1.1 Rationale

Reduction rates of nitro compounds are influenced by several factors, including mineral identity, pH, buffer type, and the presence of natural organic matter. By beginning with studies using synthetic, well characterized (in terms of phase, size, and shape) minerals, we were able to logically build an understanding of the magnitude of effects of various factors on kinetics and isotope fractionation. Fe-bearing minerals are ubiquitous in subsurface environments; thus, we aimed to represent several of the most commonly occurring minerals during reduction experiments. This included minerals of varying crystallinity, morphology, stability, and composition. The studies include iron (oxyhydr)oxides (goethite, magnetite, hematite), iron sulfides (mackinawite), phyllosilicate clays (nontronite), and other transient, less stable species (green rust).

10.1.2 Materials characterization

The desired minerals were synthesized successfully. The collected XRD patterns shown in Figure 10-1 match the reference patterns and do not show evidence of secondary mineral phases.

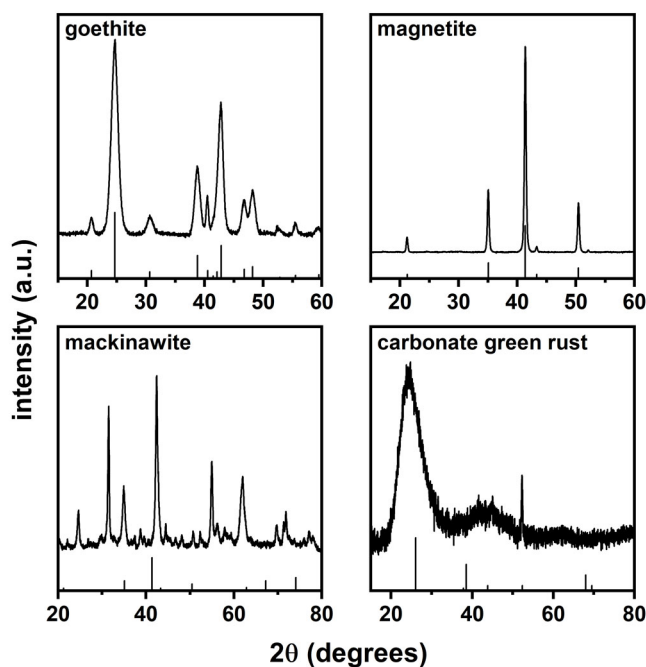


Figure 10-1. XRD patterns of synthetic minerals. Vertical lines indicate the associated powder diffraction file for each mineral phase.

Magnetite synthesis yielded pure magnetite nanoparticles with no secondary phases detected by XRD. Fe(II)/Fe(III) stoichiometry was determined to be 0.50 (Fe(II)_{1.00}Fe(III)_{2.00}O₄) by acid digestion, hydroxylamine reduction, and ferrozine colorimetry. A second magnetite synthesis was performed to check the reproducibility of this result and yielded magnetite with a stoichiometry of 0.49, although these magnetite nanoparticles were not further used in this study.

The nontronite clays were not analyzed by XRD because of their rapid oxidation and phase transformation under aerobic conditions. The purchased clays (NAu-1 and NAu-2) were conditioned with NaClO₄^{107,122} and chemically reduced by sodium dithionite^{20,109,122} before use (see Section 9.2.3) and used in batch experiments without further characterization.

10.2 Task 2 – Kinetic Studies

10.2.1 Rationale

While laboratory reaction kinetics may or may not be representative of those in the field (particularly given site to site variation in mineralogy and redox conditions), reaction kinetics and the fraction of substrate remaining are critical components in determining isotope fraction factors from CSIA data. Additionally, understanding how these factors (both rates and isotopic fractionation) change with solution conditions and over multiple contaminant spikes are critical to evaluating reaction enhancements.

10.2.2 DNAN Results

The reduction kinetics of DNAN were affected by the mineral identity and, in mackinawite suspensions, repeated contaminant exposures (Figure 10-2). Because amendments of aqueous Fe(II) were not supplied to mackinawite suspensions, the decreased reactivity in mackinawite experiments was likely an artifact of the depletion of reducing equivalents in mackinawite during the reduction reactions. Decreased pseudo-first-order rate constants (k_{obs}) were not observed during multispike experiments with goethite and magnetite wherein aqueous Fe(II) was maintained at 1 mM (Table 10-1). Similarly, no significant variation in rate constants were observed following the addition of ESHA. These results are consistent with previous work indicating negligible changes of NAC reduction rates in Fe(II)/mineral systems during multiple contaminant exposures.⁵² A k_{obs} value was also calculated for DNAN reduction by Fe(II)/hematite ($1.45 \pm 0.37 \text{ h}^{-1}$); hematite is another Fe(III)-oxide mineral known to contribute to pollutant reduction in the subsurface. The reactivity of hematite was tested only in single-spike reactors. The reactivity of each mineral suspension for DNAN reduction in increasing order was magnetite < mackinawite < hematite < goethite in k_{obs} values.

Measurements of the DNAN reduction products, 2-ANAN and DAAN, were also made during batch reactions (Figure 10-3). The overall mass balances indicate that approximately 80% of the DNAN in the initial contaminant spike was recovered. The incomplete mass balance could be due to formation of coupling products between hydroxylamine and nitroso intermediates.^{123,124}

Table 10-1. Pseudo-first-order rate constants, k_{obs} , for DNAN reduction.^{a,b} Rate constants were calculated in systems containing the mineral alone (Spike 1), the addition of NOM (ESHA), and during the fifth sequential contaminant exposures (Spike 5).

System	k_{obs} (h^{-1})			
	Mackinawite	Goethite	Magnetite	Hematite
Spike 1	1.15 ± 0.12	2.55 ± 0.14	0.79 ± 0.05	1.45 ± 0.37
ESHA	1.16 ± 0.09	2.49 ± 0.18	0.82 ± 0.04	ND
Spike 5	0.19 ± 0.04	2.52 ± 0.11	0.81 ± 0.07	ND

^aRate constants obtained from linear regression of natural log of concentration versus time data.

^bUncertainties represent standard deviations of triplicate reactors.

ND = Not determined

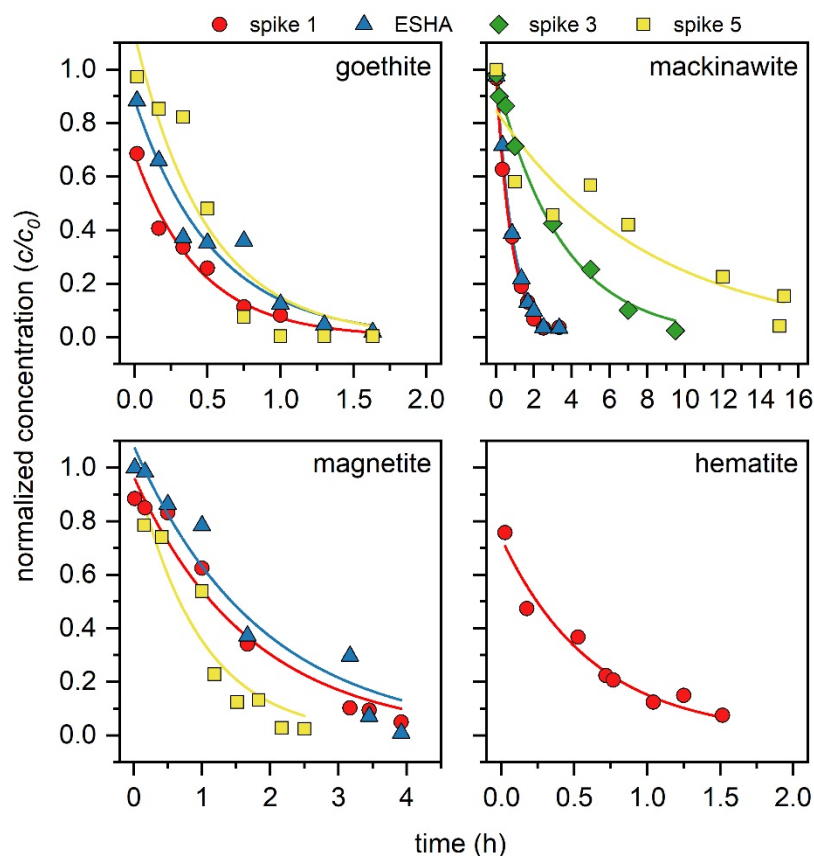


Figure 10-2. Reduction of DNAN in suspensions of Fe(II) with goethite, magnetite, mackinawite, and hematite. Kinetic profiles were obtained for one (circles), three (diamonds, mackinawite only), and five (squares) sequential contaminant spikes. The addition of ESHA (triangles) was also evaluated. Solid lines represent fits from a nonlinear regression analysis assuming pseudo-first order kinetics.

Moreover, an isomer of 2-ANAN, 4-amino-2-nitroanisole (4-ANAN), can also form but was not measured in this set of experiments. The limited accumulation of DAAN is likely due to lower reactivity of 2-ANAN compared to DNAN, because Fe(II) was maintained at 1 mM throughout the experiments with goethite and magnetite. The greater accumulation of 2-ANAN with mackinawite (Figure 10-3a) may be due to evolving mineralogy. Because the focus of this investigation was on DNAN, monitoring the reaction to complete conversion to DAAN was not necessary.

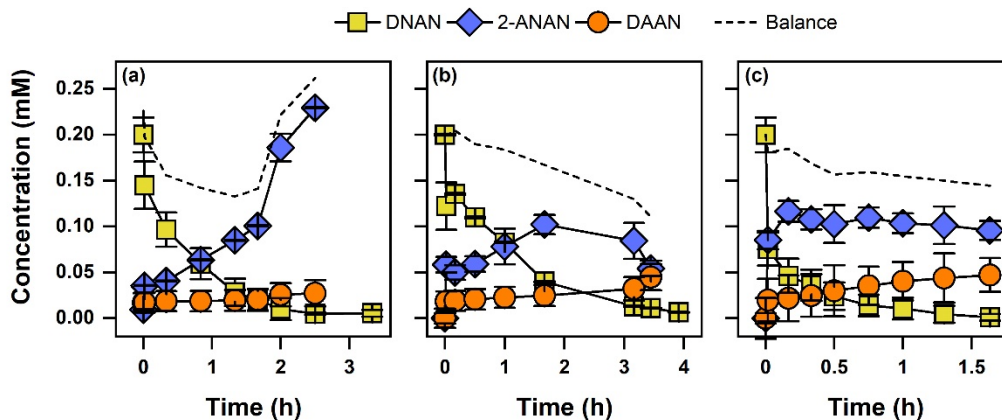


Figure 10-3. Aqueous concentrations of DNAN, 2-ANAN, and DAAN during DNAN kinetics experiments in suspensions of (a) mackinawite (1.5 g/L), (b) magnetite (2 g/L), and (c) goethite (1.0 g/L). Reactions were performed at pH 7 with 1 mM Fe(II) and are for the first spike of DNAN. Error bars represent standard deviations of triplicate reactors. Dotted lines represent mass balances (moles) of the three compounds.

10.2.3 2,4-DNT

The reduction of 2,4-DNT by Fe(II)/mineral suspensions was also evaluated. 2,4-DNT is an NAC structurally similar to DNAN and is thus expected to behave similarly. In addition, 2,4-DNT was a surrogate for the commonly used explosive, TNT, which was not used because of safety and availability restrictions. Because of the consistency observed during DNAN experiments, the reduction of 2,4-DNT was only assessed in single spike reactors containing Fe(II)/mineral suspensions (Figure 10-4). These reactions were performed under conditions identical to those with DNAN. In general, values of k_{obs} followed the same increasing trend of magnetite ($0.42 \pm 0.03 \text{ h}^{-1}$) < mackinawite ($0.65 \pm 0.07 \text{ h}^{-1}$) < hematite ($3.0 \pm 0.6 \text{ h}^{-1}$) < goethite ($5.3 \pm 0.2 \text{ h}^{-1}$) as observed for DNAN. These data suggest that the addition of NOM or multiple contaminant exposures will have the same effects on k_{obs} as observed above for DNAN.

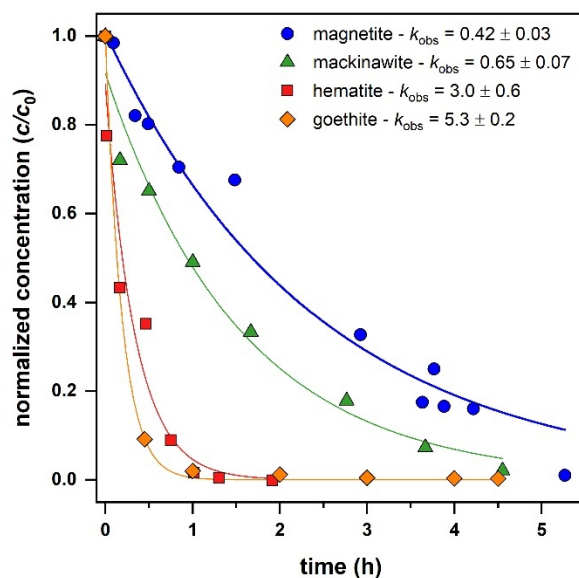


Figure 10-4. Reduction of 2,4-DNT in suspensions of 1 mM Fe(II) with magnetite (circles), mackinawite (triangles), hematite (squares), and goethite (diamonds). Kinetic profiles were obtained only during single contaminant exposures. Solid lines represent fits from a nonlinear regression analysis assuming pseudo-first order kinetics.

10.2.4 RDX

10.2.4.1 Kinetics and Pathways of RDX reduction

The pseudo-first order rate constants for RDX reduction by iron minerals are listed in Table 10-2. RDX was reduced in all of the tested systems as depicted in Figure 10-5a for Fe(II)/goethite at pH 7 and Figure 12-22 in Section 12.1.8 for the other systems. Consistent with past results,^{80,125,126} reaction rates increased with increasing pH of the solution for all minerals tested. For example, rate constants with Fe(II)/goethite were 0.12 ± 0.01 , 0.45 ± 0.10 , and $1.02 \pm 0.26 \text{ h}^{-1}$ at pH 6.5, 7, and 7.5, respectively (Table 10-2 and Table 10-10). This is consistent with increased adsorption of Fe(II) on the iron (oxy)hydroxides (the adsorption of Fe(II) by goethite was 0.15 ± 0.07 , 0.28 ± 0.08 , $0.39 \pm 0.07 \text{ mmol Fe (II)/g goethite}$ at pH 6.5, 7, and 7.5) and more favorable reduction potentials for Fe(II)/mineral systems with increasing pH.¹²⁵ The reactivity of FeS could be due to both reduced iron and sulfide contributing to the electron transfer.¹²⁷ Rate constants for TNX are shown in Table 10-3.

Table 10-2. Pseudo-first order rate constants (k_{obs}) of RDX reduction for multiple pH values. Errors represent 95% confidence propagated from the errors associated with regressed slopes of duplicate measurements. Single tests were conducted for green rust, TAFB soil, and TCAAP sediment at pH 6.5 and 7.

Mineral	Total mineral loading ^a (g L ⁻¹)	Fe loading (g L ⁻¹) ^b	Fe(II) _{aq} (mM)	pH	k_{obs} (h ⁻¹)
Mackinawite (FeS)	0.45	0.29	0	6.5	0.81 ± 0.34
				7	1.02 ± 0.21
				7.5	1.44 ± 0.20
Carbonate Green rust	5	2.64	0	6.5	0.05
				7	0.08
				7.5	0.18 ± 0.04
Fe(II)/goethite	0.5	0.26	1	6.5	0.12 ± 0.01
				7	0.45 ± 0.10
				7.5	1.02 ± 0.26
Fe(II)/hematite	0.5	0.35	1	6.5	0.15 ± 0.02
				7	0.35 ± 0.05
				7.5	0.58 ± 0.09
Fe(II)/magnetite	2	1.45	1	6.5	0.03 ± 0.002
				7	0.15 ± 0.01
				7.5	0.59 ± 0.15

^aMineral loading = mass of mineral/volume of the reaction solution.

^bFe loading = mineral loading × Fe content of the mineral (%). The iron content of synthetic minerals were determined by stoichiometry.

^cFe(II)_{aq} was supplied to maintain 1mM in the solution

Table 10-3. Pseudo-first order rate constants of TNX degradation by Fe(II)/goethite, Fe(II)/magnetite, and green rust under varying pH conditions

Mineral	pH	k_{obs} (h ⁻¹)
Fe(II)/goethite	6.5	0
	7	0.06
	7.5	10.98
Fe(II)/magnetite	7.5	34.68
Green rust	7.5	16.66

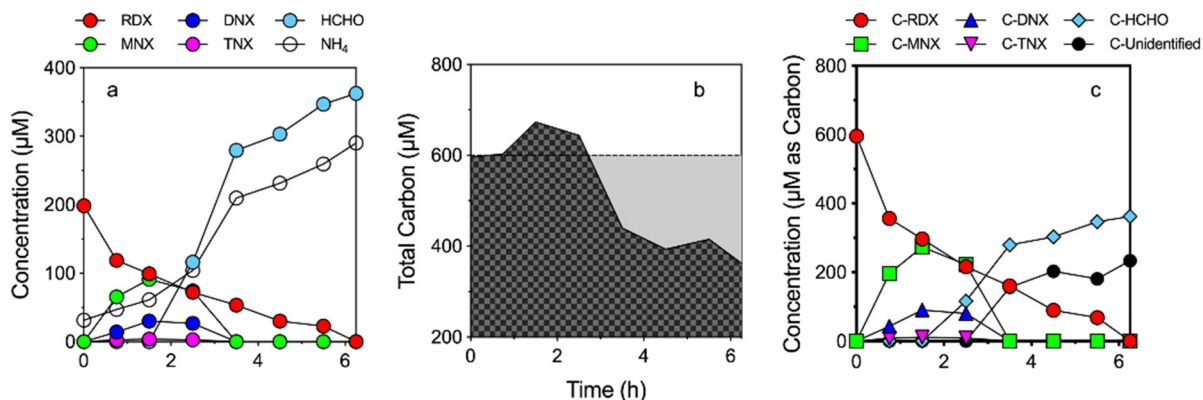


Figure 10-5. Concentration versus time data for RDX, intermediates, and end products during abiotic RDX reduction in the Fe(II)/goethite reactors at pH 7. (a) Measured concentrations of RDX, nitroso intermediates and final degradation products, (b) Total carbon mass balance, (c) Concentration of RDX, nitroso intermediates, and products plotted in μM as carbon (denoted as C-compound). A hypothetical carbon-containing product (C-Unidentified; black solid circle) was introduced to compensate for the incomplete carbon mass balance and was assumed to be a single reaction product in kinetic fitting.

Different minerals may react with RDX through different and/or multiple pathways (e.g., concurrent $-\text{NO}_2$ reduction and ring cleavage; see Figure 10-6) and cause different isotope fractionation. Thus, an assessment of reaction pathways is needed. The mass balance for each experimental system was converted to a carbon-basis as illustrated in Figure 10-5b for Fe(II)/goethite at pH 7. C-Unidentified was considered as a single, final ring cleavage product because carbon deficits began during late stages of the reaction (Figure 10-5c) and C-Unidentified and HCHO appear in parallel. MEDINA is unlikely responsible for the carbon deficit because it undergoes instantaneous degradation.¹⁴ C-Unidentified could be one or more carbon-containing products, but from the prospective pathways (Figure 10-6), this portion of the mass is an end-product.

Based on the concentration of carbon versus time data (Figure 10-5 and Figure 12-22), potential reaction pathways were tested via kinetic modeling (different branching scenarios are described in Section 12.1.8), with the assumption that the nitroso intermediates were sequentially produced ($\text{RDX} \rightarrow \text{MNX} \rightarrow \text{DNX} \rightarrow \text{TNX}$). The differences among the potential reaction pathways were the species from which C-HCHO and C-Unidentified were formed. The formation of these final products was considered to occur from RDX, one of the nitroso compounds, or a combination thereof. The experimental data and the rate constants given by the model with the lowest normalized residuals for the tested pH/mineral combinations are summarized in Section 12.1.8. The fitting results suggest that there are two possible reaction pathways, with the iron oxides reacting via the pathway shown in Figure 10-6a and mackinawite via that shown in Figure 10-6b. The goodness of fit for other proposed pathways, which had substantially higher residuals, is shown in Table 12-6 thru Table 12-8. Further discussion of the pathways is provided in Section 12.1.8, but the results establish that for the iron oxides, the rate limiting step is reaction of RDX to MNX, which allows interpretation of the RDX CSIA results.

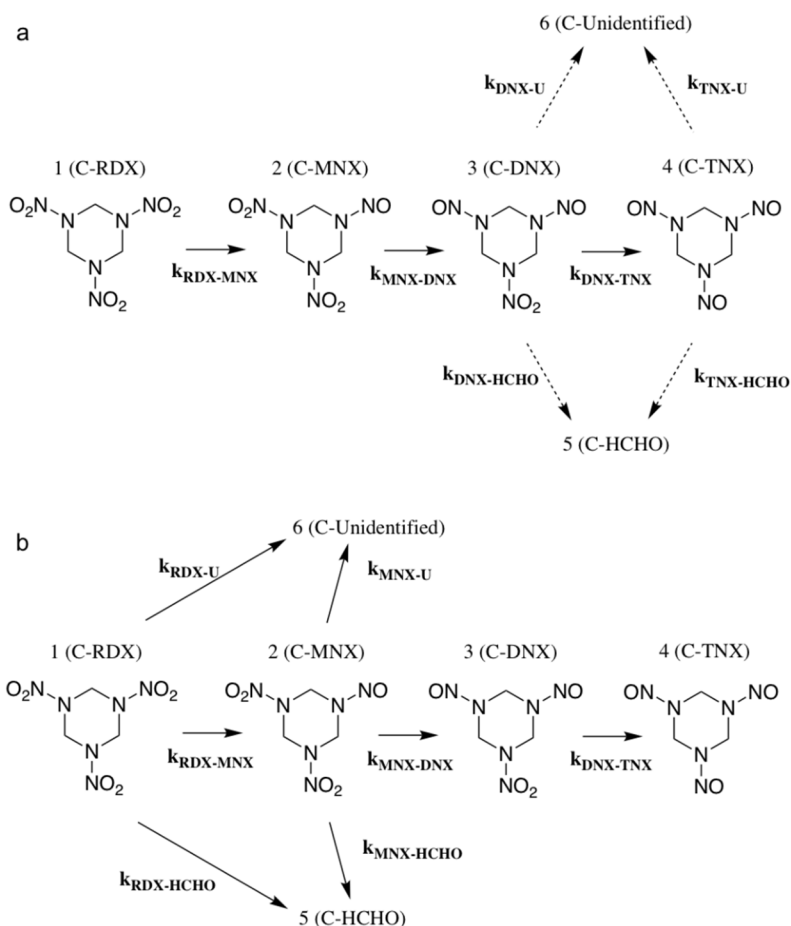


Figure 10-6. Possible abiotic reduction pathways of RDX in terms of the total carbon within the reduction system. a. reduction of RDX (C-RDX, 1) is initiated by the formation of MNX ($k_{\text{RDX-MNX}}$). The major ring cleavage events to produce C-HCHO (3) and C-Unidentified (4) occur via $k_{\text{TNX-HCHO}}$ and $k_{\text{TNX-U}}$, or $k_{\text{DNX-HCHO}}$ and $k_{\text{DNX-U}}$. b. reduction of RDX (C-RDX, 1) is initiated by the formation of MNX ($k_{\text{RDX-MNX}}$) in parallel with ring cleavage to form C-HCHO ($k_{\text{RDX-HCHO}}$) and C-Unidentified ($k_{\text{RDX-U}}$). Ring cleavage to produce C-HCHO (3) and C-Unidentified (4) originated from C-MNX via $k_{\text{MNX-HCHO}}$ and $k_{\text{MNX-U}}$ is also possible (see Section 12.1.8).

10.2.4.2 Repeated exposure of RDX

Sequential additions of RDX to batch reactions offer unique insight into environmental natural attenuation processes of munitions, to reveal evolving reactivity of these reactions after continuous exposure to contaminant. The first injection of RDX was spiked, allowing complete degradation before the second injection of RDX. In Figure 10-7, RDX degradation and nitroso intermediates' lifespan were compared for iron sulfides and goethite over three (FeS) and five (Fe(II)/goethite) RDX spikes, respectively, with the latter also including a maintained 1 mM aqueous Fe(II) concentration. No significant changes in RDX reduction rate were observed with each sequential spike, except for the fifth spike of RDX in goethite reactors. In this spike, however, careful monitoring of pH revealed that the buffer was failing and attempts to maintain the pH using HCl and NaOH were not successful. After three spikes of RDX in FeS reactors, TNX showed a stable concentration, supporting the inability of FeS to reduce TNX. The

lifespans of MNX and DNX were also extended after five spikes of RDX in goethite reactors. Thus, long term exposure to RDX of reductive system might lead to failure of buffering capability in the reactors and decreasing reactivity towards reduction intermediates.

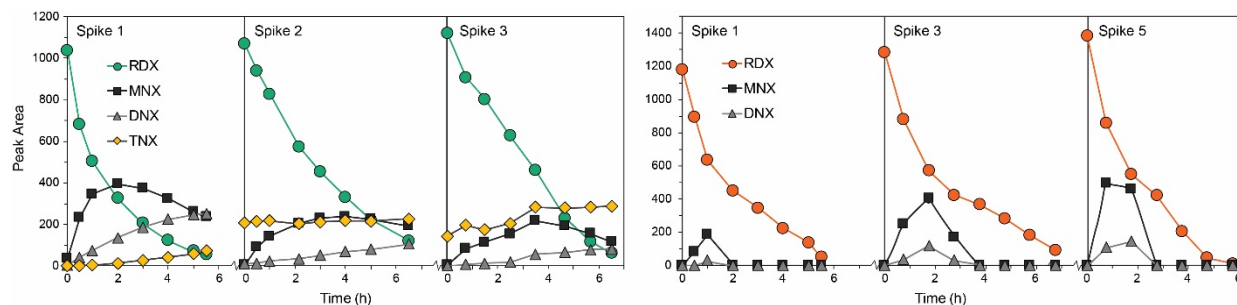


Figure 10-7. Peak area of RDX and nitroso derivatives MNX, DNX, and TNX over reaction time and each sequential spike with either iron sulfides (green, left) or goethite (orange, right) in 10 mM bicarbonate pH 7.

10.2.5 NTO

10.2.5.1 *Impact of buffer type and pH on NTO reduction by minerals*

The impact of the buffer solution was examined for the Fe(II)/goethite system for multiple pH values. Figure 10-8 displays the reduction of NTO in NaHCO₃ or MOPS buffer. NTO lifespan is shorter in MOPS than that in NaHCO₃. Similarly, RDX reduction is faster in MOPS buffer than in NaHCO₃ buffer, possibly due to better suspension of mineral particles in MOPS buffer, which provides more accessible adsorption sites for Fe(II) (Section 10.4.3.2). NaHCO₃ is more representative of typical groundwater conditions and thus was used during all NTO reduction experiments.

The reduction of NTO by iron (hydro)oxides and iron sulfide is strongly affected by the pH of the reaction medium. We evaluated the reduction kinetics of NTO at pH 5.5, 6, 6.5, 7, and 7.5 (Figure 10-9). In general, pH had a positive impact on the rate of NTO reduction for Fe(II)/goethite, Fe(II)/hematite, and Fe(II)/magnetite-Fe (Figure 10-9a-c). These variations in reaction rate due to pH and mineral type resemble those previously observed during RDX reduction (Table 10-2). The reduction of NTO by goethite-Fe(II) and hematite-Fe(II) at pH 7 and 6.5 were so rapid that it was not possible to determine the rate constant under these conditions. Hence, given the controllable rate of NTO reduction at pH 6, experiments were conducted at this pH to collect the reduction rate constants for iron (hydro)oxides. Interestingly, the impact of pH on NTO reduction by FeS showed a different trend from the other minerals. For FeS, NTO reduction was slower at pH 6.5 and 7 than that at pH 5 and pH 6 (Figure 10-9d).

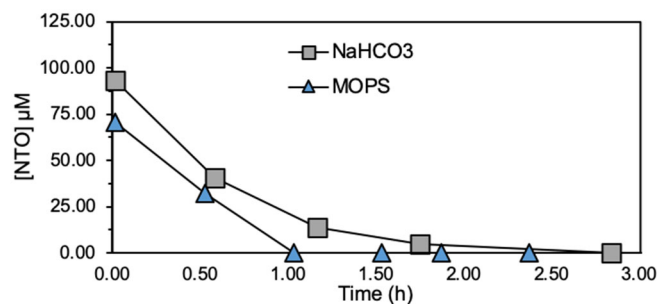


Figure 10-8. Decreasing of NTO concentration in MOPS and NaHCO₃ buffer each at 10 mM and pH 7.

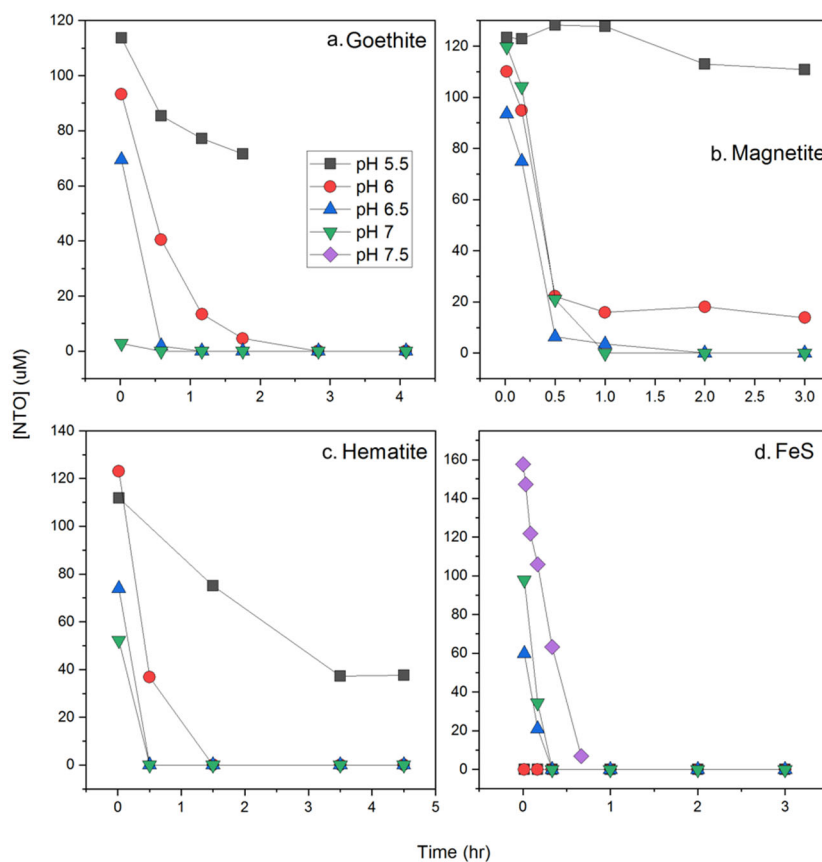


Figure 10-9. NTO reduction by (a) goethite, (b) magnetite, (c) hematite, and (d) FeS at pH ranging from 5.5 to 7.5. Experiments were conducted in 10 mM NaHCO₃ buffer. FeCl₂ was supplied to a, b, and c to sustain a concentration of 0.5 mM Fe(II). The concentration of minerals was consistent with that in RDX experiments.

10.2.5.2 *Impact of mineral phase, NOM, and repeated exposure of NTO on reduction rate constant*

Given the impact of pH on NTO reduction rate, rate constants were measured at pH 6 for iron (oxy)hydroxides and at pH 7.5 for FeS. Table 10-4 summarizes the measured rate constants. NTO reduction rates vary substantially, depending upon the mineral identity. Based on results for DNAN and RDX, it was hypothesized that NOM would not affect NTO degradation. As shown in Table 10-4, the presence of ESHA increased NTO reduction 5 and 10 mg C/L for the Fe(II)-goethite system. It is possible that NOM facilitates reduction of NTO. In the presence of only ESHA or ESHA/Fe(II) without goethite, no reduction for NTO was observed (Table 10-4).

Table 10-4. Pseudo-first order rate constants (k_{obs}) of NTO reduction under the impact of mineral phase and NOM. Errors represent standard deviations from triplicate reactors. Single tests were conducted for FeS (Mackinawite), goethite with the presence of NOM, and mineral-absent tests.

Mineral	NOM (mg C L ⁻¹ , ESHA)	Fe(II) (mmol L ⁻¹)	pH	k_{obs} (h ⁻¹)
FeS	0	0	7.5	4.62
Fe(II)/goethite	0	1	6	1.75 ± 0.21
Fe(II)/goethite	5	1	6	2.79
Fe(II)/goethite	10	1	6	3.85
Fe(II)/magnetite	0	1	6	0.51 ± 0.31
Fe(II)/hematite	0	1	6	0.95 ± 0.54
NA	10	0	6	0
NA	10	1	6	0

NA = No minerals were added

The reactivity of Fe(II)/goethite, Fe(II)/magnetite, and FeS in pH 6 NaHCO₃ buffer was tested with repeated exposure of NTO. Figure 10-10 shows comparisons of NTO degradation rates for each spike with three synthetic minerals. For goethite, an increase in reduction rate constant was observed after the second spike of NTO. NTO reduction changed to zero order after the third spike. There was no obvious change of reduction rate in the magnetite reactor. For the FeS reactor, reactivity slowed with the second and third spike. The data indicate potential evolving reactivity for minerals over multiple exposures to NTO.

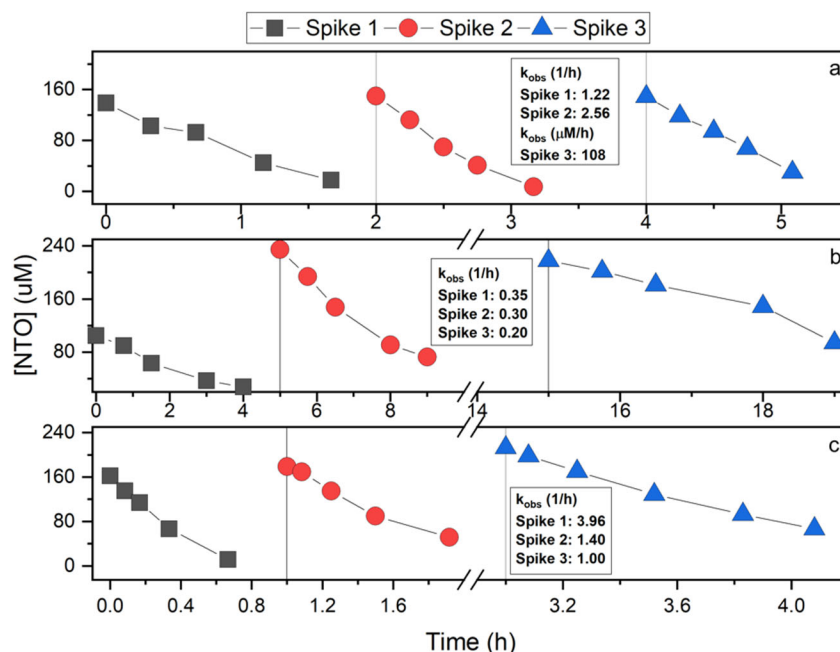


Figure 10-10. Reactivity of goethite/Fe(II) (a), magnetite/Fe(II) (b), and FeS (c) over NTO, under repeated exposure of NTO. The goethite and magnetite reactors were prepared in 10 mM NaHCO_3 buffer at pH 6, and the FeS reactor was prepared in 10 mM NaHCO_3 at pH 7.5.

10.2.5.3 Formation of ATO

For abiotic reduction of NTO by iron minerals, ATO is produced, and based on past work, should be stable. As noted in Section 9.5, accurate measurement of ATO required the analytical standards to be made in the experimental matrix (details in Section 12.1.4). ATO formation was quantified in goethite and magnetite-containing reactors at pH 6.

Figure 10-11 shows the degradation of NTO, formation of ATO, and the mass balance of the two compounds. The mass balance was ~75%. It does appear that ATO concentration slowly decreases after NTO is depleted, and using ATO as the starting material also leads to slow loss over time (see Figure 10-12). It is unclear if this is slow reaction of ATO or an analytical issue caused by evolving composition of the reaction matrix such that ATO response on the instrument varies compared to standards made in the initial composition of the matrix.

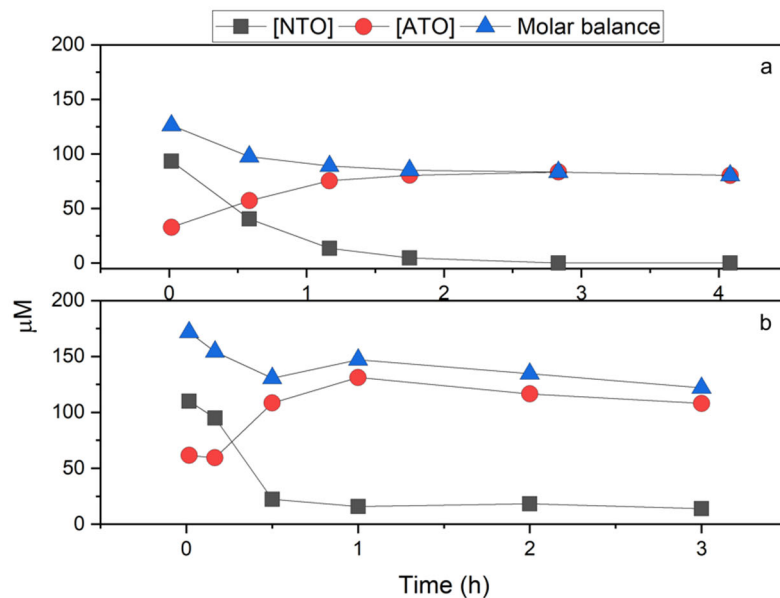


Figure 10-11. The concentration change and molar balance of NTO and ATO in a) goethite/Fe(II), b) magnetite/Fe(II) reactors in pH 6, 10 mM NaHCO_3 buffer.

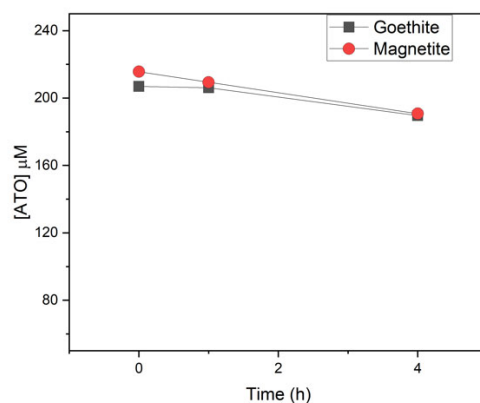


Figure 10-12. The concentration of ATO in reaction matrix over time. ATO was added to goethite or magnetite reaction matrix at a concentration of approximately 200 μM .

10.3 Task 3 – Compound Specific Isotope Analysis

10.3.1 Rationale

The accurate measurement of $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ ratios in organic nitro compounds is key for identification of the mechanism and pathways of transformation and for assessing the extent of degradation in the environment. We have established analytical procedures for CSIA of many NACs in laboratory experiments and field samples from highly contaminated soil. Further

refinement is required for aqueous samples at $\mu\text{g/L}$ concentrations and in the development of new procedures for CSIA of nitramines. The methods CSIA on the experiments conducted with synthetic mineral as well as with natural materials.

10.3.2 Method Development

10.3.2.1 NACs

C- ($\delta^{13}\text{C}$) and N- ($\delta^{15}\text{N}$) isotope ratios for DNAN and 2,4-DNT were determined by gas chromatography isotope ratio mass spectrometry (GC-IRMS) using a Trace GC (Thermo Electron Corp.) coupled to an IRMS (DeltaPLUS XL, Delta V, Thermo Electron Corp.) via a combustion interface (GC Combustion III, Thermo) equipped with a Ni/Pt reactor. The GC contained an Rtx-5MS capillary column (0.32 mm ID, 1 μm film thickness, 30 m length). The injector and GC oven were operated differently depending on the analyte and extraction method (see below). During $\delta^{15}\text{N}$ analysis a liquid nitrogen trap was used to trap CO_2 produced from combustion. The custom-made oxidation reactor was operated as described previously.¹²⁸ Analytes were measured against standard laboratory gases that were introduced at the beginning of each run (CO_2 and N_2 for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analyses, respectively). Subsets of six samples were bracketed with standards in duplicate or triplicate to allow correction for any drift in signal observed during each run. Method quantification limits (MQLs) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures were determined according to the Moving Mean procedure.^{129,130}

NACs were extracted from samples by solid phase micro extraction (SPME) prior to isotope analysis. A method was modified from established SPME procedures¹³¹ using the PAL SPME Arrow (DVB/PDMS sorption phase, 120 μm phase thickness, 1.1 mm diameter) instead of a normal SPME fiber, such that improved sensitivity could be obtained with a larger sorption phase.¹³² Automated SPME was carried out using a PAL autosampler equipped with a PAL SPME Arrow Tool and a Heatex Stirrer. All samples were initially diluted with 10 mM phosphate buffer (pH = 7, prepared with ultrapure water) to obtain concentrations within a range of linear response, and sodium chloride (200 g/L) was added to maximize extraction efficiency. Following equilibration at 50 °C for 10 min, the SPME Arrow was immersed in the sample for 70 minutes at 50 °C (600 RPM stir rate), and the analyte was thermally desorbed from the arrow in an injector equipped with a deactivated liner (270 °C, 6 minutes splitless time). The GC oven was operated with the following temperature program: 50 °C for 1 minute, ramp 10 °C/min to 250 °C, hold for 5 minutes.

C and N isotope analysis of DNAN by GC-IRMS with the SPME arrow showed good precision in measured signatures. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ MQLs were 20 $\mu\text{g/L}$ and 200 $\mu\text{g/L}$, respectively (Figure 10-13a-b), which are nearly an order of magnitude lower than previously reported MQLs obtained by conventional SPME of structurally similar nitroaromatic compounds.¹³¹ Moreover, the SPME Arrow GC-IRMS method was especially effective for 2,4-DNT. MQLs for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were 4.5 $\mu\text{g/L}$ and 10 $\mu\text{g/L}$, respectively (Figure 10-13c-d), which are more than two orders of magnitude lower than previously reported DNT MQLs using conventional SPME (i.e., $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ MQLs of 600 $\mu\text{g/L}$ and 9000 $\mu\text{g/L}$, respectively, using a conventional polyacrylate SPME fiber).

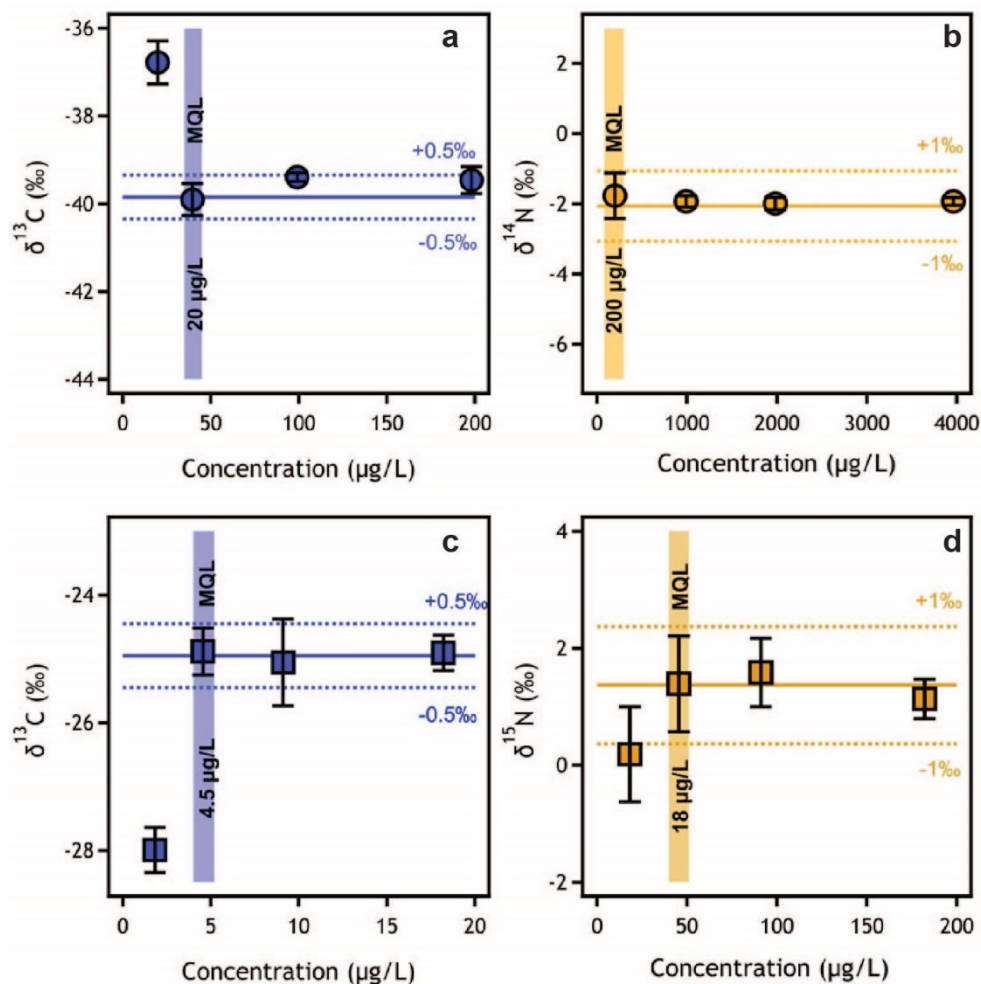


Figure 10-13. Determination of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ method quantification limits (MQLs) for DNAN and 2,4-DNT isotope analysis by SPME Arrow-GC-IRMS. (a) $\delta^{13}\text{C}$ and (b) $\delta^{15}\text{N}$ in DNAN and (c) $\delta^{13}\text{C}$ and (d) $\delta^{15}\text{N}$ in 2,4-DNT. The solid lines represent the iterative mean value determined according to the moving mean procedure,¹³⁰ and the dotted lines represent acceptable uncertainty ranges for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of $\pm 0.5\text{‰}$ and $\pm 1\text{‰}$, respectively).

10.3.2.2 RDX

An RDX standard solution (4.5 mM in 50:50 methanol:acetonitrile, AccuStandard) was used for all RDX method development experiments. A method for GC-IRMS analysis of RDX was adapted from previously published methods^{91,93} and optimized for our instrumentation in N mode. Briefly, 2 µL of ethyl acetate solution containing was injected into a CIS-4 PTV injector (Gerstel) for which an optimal injection temperature of 180 °C was identified to balance the needs of maximum sensitivity and minimal decomposition (Figure 10-14). Following a splitless time of 1 min (splitless flow 50 mL/min) the injection temperature was raised to 300 °C for cleaning for the remainder of the run. Chromatography was carried out using an Rtx-5MS column (30 m × 0.32 mm, 1 µm film thickness, Restek) with the following oven program adapted from Gelman et al.⁹³: hold at 50 °C for 1 min, ramp 15 °C/min to 180 °C, ramp 45 °C/min to 250 °C, hold 15 min (the final hold time was necessary to allow re-oxidation of the

combustion reactor). This resulted in an RDX retention time of 770 s (Figure 10-15). To determine method detection limits (MQLs) for the optimized method in N and C mode, solutions of RDX diluted with ethyl acetate at concentrations ranging from 0.1 mM to 4.5 mM were analyzed in triplicate (Figure 10-16). According to the Running Mean method¹³⁰, the MQLs for RDX were 0.5 nmol RDX per injection for both C and N (or 0.25 mM for a 2 μ L injection). For N mode the measured isotope ratio was stable for $m/z=28$ peak amplitudes up to 3500 mV (Figure 10-16a). However, in C mode the isotope signatures began to shift to more positive values for the $m/z=44$ peak amplitudes above 1000 mV (Figure 10-16b). This was potentially due to an increasing background with increasing RDX concentration in C mode. We therefore determined that for RDX analysis in C mode, only data for which peak amplitudes between 300-1000 mV should be used to produce a reliably consistent signature.

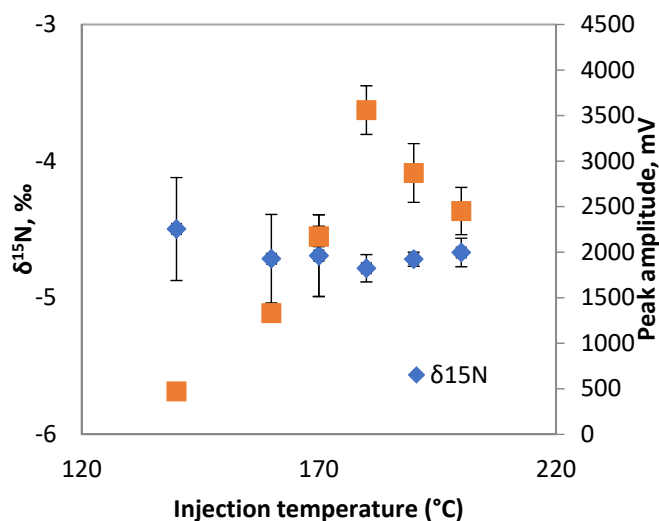


Figure 10-14. Identification of optimal injection temperature for RDX isotope ratio measurements. At low temperatures RDX is not fully flushed from the injector, and at high temperatures (above 180 °C) RDX is thermally degraded within the injector.

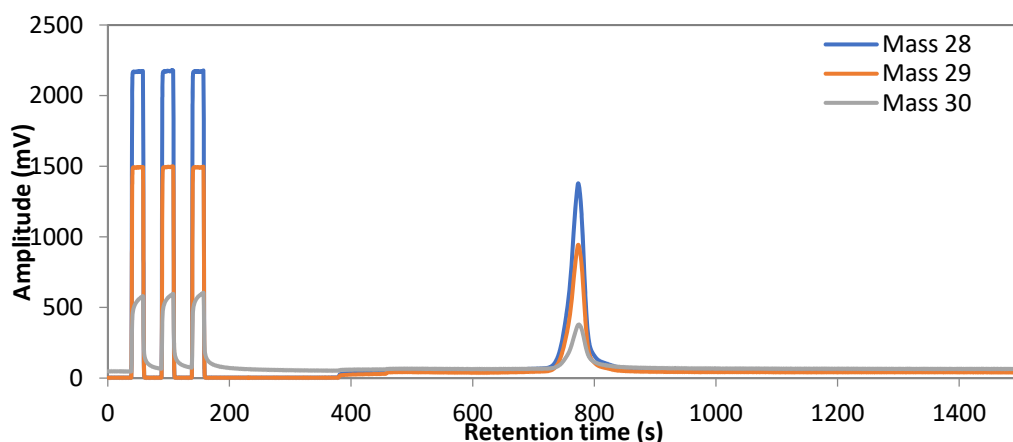


Figure 10-15. Typical RDX chromatogram for the GC-IRMS method in N mode.

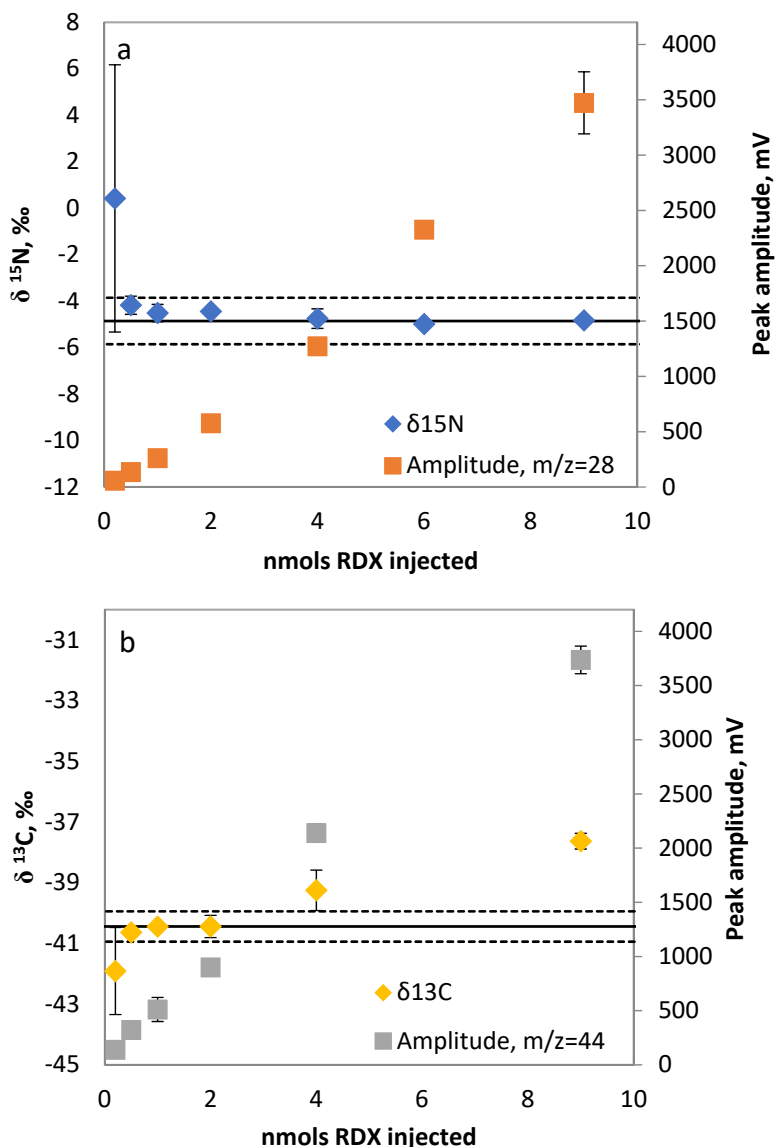


Figure 10-16. Determination of method quantification limits (MQLs) for analysis of N (a) and C (b) isotope signatures ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$). The solid lines represent the running mean¹³⁰, and the dotted lines represent acceptable error boundaries of $\pm 1\text{‰}$ (for $\delta^{15}\text{N}$) and $\pm 0.5\text{‰}$ (for $\delta^{13}\text{C}$). The MQL was 0.5 nmol RDX injected in both C mode and N mode.

10.3.2.3 NTO

Carbon isotope ratio measurements of NTO were determined by liquid chromatography isotope ratio mass spectrometry (LC-IRMS) using a Dionex UltiMate 3000 System coupled to an IRMS (DeltaPLUS XL, Delta V, Thermo Electron Corp.) with wet chemical oxidation interface. Due to its polarity and low volatility, NTO is not amenable to gas chromatography for the determination of N isotope ratios by GC/IRMS. Given that NTO is not available as pure substance C isotope ratios by flow injection analysis (Figure 10-17) were used to estimate its $\delta^{13}\text{C}$ ($-44.4 \pm 0.2\text{‰}$).

LC/IRMS measurements were carried out with an Ascentis RP-Amide column (15cm x 4.6mm, 3 μ m, Supelco) at flow rates of 1 mL/min at room temperature with dilute H₂PO₄ (25 mM, pH 2.0) as eluent. ¹³C/¹²C ratios of NTO were determined over a nominal NTO mass range of 19 to 190 nmol C with injection volumes of up to 100 μ L.

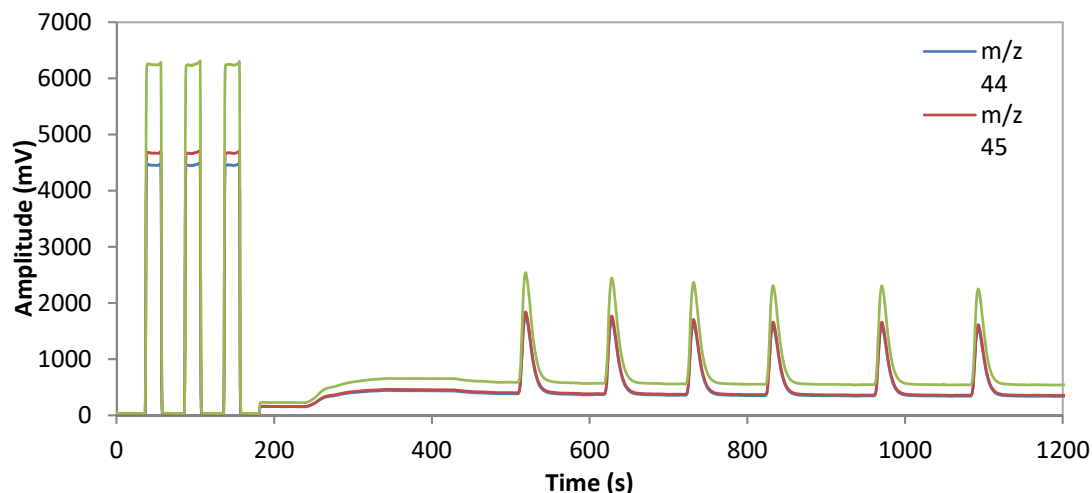


Figure 10-17. Chromatogram for $\delta^{13}\text{C}$ analysis of NTO by LC-IRMS via flow injection analysis. The resulting $\delta^{13}\text{C}$ determined was -44.4 ± 0.2 ‰.

10.3.2.4 HMX

Significant HMX decomposition occurred during analysis by the same GC-IRMS method developed for RDX, as indicated by the presence of two peaks in the chromatogram (Figure 10-18). When the injector and column temperatures were lowered to 160 °C the peak shape improved (though a shoulder was still present), at the cost of substantially decreased peak amplitude (i.e., peaks of less than 100 mV for injection of 54 nmols N).

While initial tests with LC-IRMS for HMX indicated that measurement of C isotopes could be possible, the avenue was not pursued further because i) data from RDX indicated that measurement of N isotopes was critically important and ii) sufficient HMX was not available for a robust set of experiments.

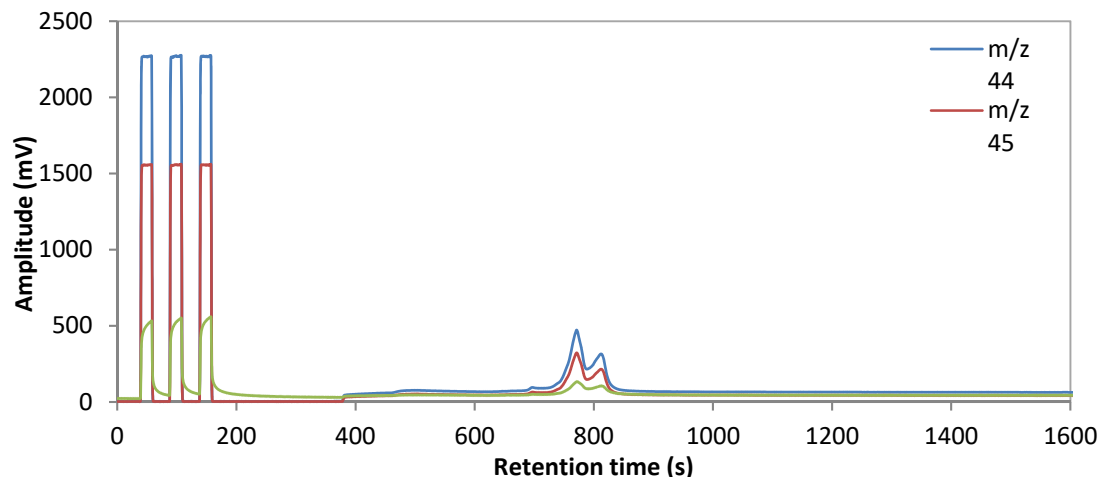


Figure 10-18. Chromatogram showing the thermal decomposition of HMX during ^{15}N GC-IRMS analysis by the method developed for RDX.

10.3.3 CSIA During DNAN Reduction

Abiotic reduction of DNAN introduced strong enrichment of ^{15}N (up to 60‰; Figure 10-19) in the remaining DNAN with only slight variations in magnitude in response to the type of mineral used, presence of natural organic matter or repeated contaminant exposures (Table 10-5).

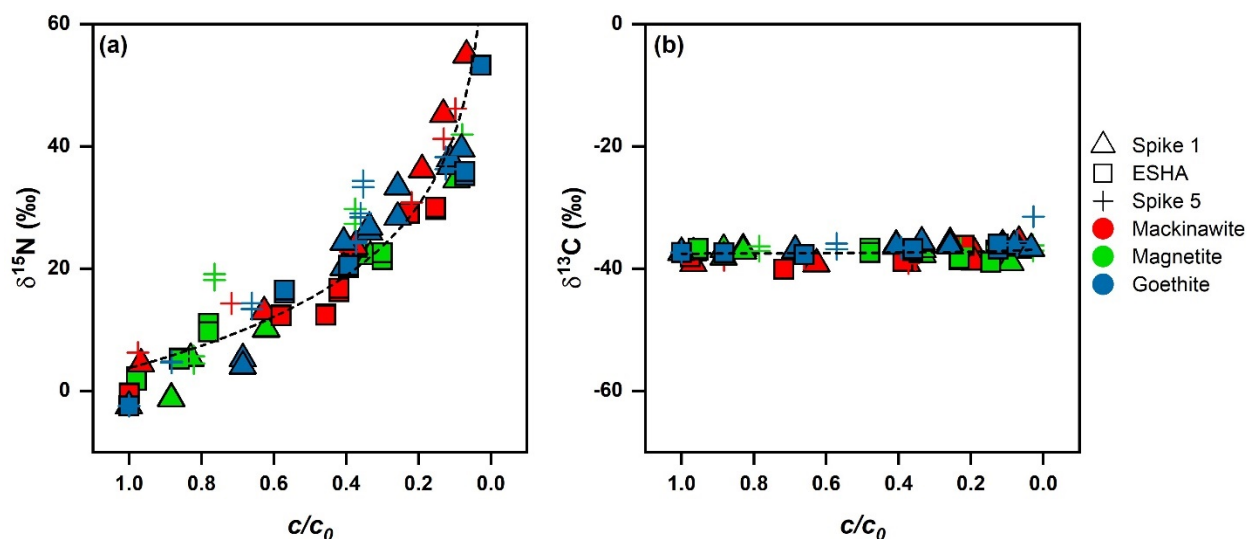


Figure 10-19. Nitrogen and carbon isotope fractionation of DNAN during abiotic reduction. (a) $\delta^{15}\text{N}$ and (b) $\delta^{13}\text{C}$ vs. fraction of remaining substrate (c/c_0) with dotted lines provided to guide the eye along the data. Detailed N and C enrichment values are provided in Section 12.1.6 (see Figure 12-12 for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ vs. c/c_0 data separated by reaction condition).

Moreover, the associated enrichment of ^{13}C during DNAN reduction was small (≤ 2.4 ‰), thus suggesting reductive transformation as the primary reaction mechanism. ^{15}N -isotope enrichment factors, ϵ_N , ranged from -9.0 ± 2.0 ‰ to -21.5 ± 1.0 ‰ in response to ^{15}N -AKIEs of 1.021 ± 0.002 to 1.045 ± 0.001 . These results are typical for nitro-group reduction in NACs by mineral-bound

Fe(II) species (^{15}N -AKIE = 1.030–1.045).^{21,29–31} Because no C atoms are involved during nitro-group reduction, the observed C isotope fractionation was small and resulted in secondary ^{13}C -AKIEs near unity.

Table 10-5. N and C bulk isotope enrichment factors (ϵ_{N} , ϵ_{C})^{a,b,c} and apparent kinetic isotope effects (^{15}N -AKIE, ^{13}C -AKIE)^{b,c} during the reduction of DNAN in different mineral systems.

System	ϵ_{N} (‰)	ϵ_{C} (‰)	^{15}N -AKIE (-)	^{13}C -AKIE (-)
Mackinawite				
Spike 1	-19 ± 1	-0.8 ± 0.6	1.039 ± 0.001	1.0008 ± 0.0008
ESHA	-16 ± 1	-0.7 ± 1.4	1.034 ± 0.001	1.0007 ± 0.0014
Spike 5	-16 ± 2	-1.5 ± 1.2	1.034 ± 0.002	1.0015 ± 0.0012
Average	-19 ± 2	-0.3 ± 0.6	1.039 ± 0.004	1.0003 ± 0.0006
Goethite				
Spike 1	-17 ± 3	-0.1 ± 0.3	1.035 ± 0.003	1.0000 ± 0.0003
ESHA	-16 ± 5	-0.6 ± 0.4	1.034 ± 0.005	1.0006 ± 0.0004
Spike 5	-11 ± 1	-1.2 ± 0.9	1.022 ± 0.001	1.0012 ± 0.0009
Average	-16 ± 3	-0.5 ± 0.3	1.033 ± 0.007	1.0005 ± 0.0003
Magnetite				
Spike 1	-15 ± 3	-0.7 ± 0.6	1.031 ± 0.003	1.0007 ± 0.0006
ESHA	-17 ± 3	-1.3 ± 0.2	1.036 ± 0.003	1.0013 ± 0.0005
Spike 5	-9 ± 2	-0.1 ± 0.2	1.018 ± 0.002	1.0000 ± 0.0002
Average	-17 ± 3	-0.3 ± 0.5	1.035 ± 0.006	1.0000 ± 0.0005
Hematite				
Spike 1	-21 ± 1	-0.3 ± 0.4	1.044 ± 0.007	1.0000 ± 0.0049
Alkaline Hydrolysis ^d	-2.7 ± 0.4	-6.0 ± 0.5	1.0027 ± 0.0004	1.0445 ± 0.0028
Enzymatic hydrolysis by O-demethylase ^d	-3.2 ± 0.1	-3.7 ± 0.1	1.0032 ± 0.0003	1.0269 ± 0.0053

^aValues derived from nonlinear regression analysis of eq. 9.

^bUncertainties represent 95% confidence intervals.

^cAveraged values according to the methods of Scott et al.¹²¹ using the Pitman estimator.

^dData from Ulrich et al.¹³³

^eObtained from previous work and references therein.^{27,29–31,42}

Slight decreases in fractionation were observed during repeated contaminant exposure in Fe(II)/goethite and Fe(II)/magnetite systems (Table 10-5). This suggests that morphological changes (e.g., phase evolution and growth) occurring on the mineral structure during repeated surface oxidation and Fe(II) exposure may have limited the accessibility of reactive Fe(II) and thus slightly masked the isotope fractionation (Figure 12-13). These changes were within experimental error of the initial spike experiment (ϵ_{N} = -17 ± 3‰ to -11 ± 1‰ and -15 ± 3‰ to -9 ± 2‰ between the single and multiple spike experiments for goethite and magnetite, respectively).

The collective C and N isotope fractionation data from all DNAN reduction experiments is plotted in Figure 10-19. The values of $\delta^{15}\text{N}$ follow the general trend of ϵ_{N} values between -19 ± 1‰ to -9 ± 2‰ from individual DNAN reduction experiments. The scatter of $\delta^{15}\text{N}$ values illustrates that experimental and analytical uncertainties are larger in experiments with ESHA and after repeated

spikes of DNAN. We conclude that those uncertainties are primarily responsible for the observed variations of ϵ_N values from the individual experiments as well as for the large confidence intervals (typically $<0.5\%$).¹¹⁸ Based on this interpretation, we derived average ^{15}N -AKIE for DNAN reduction by each mineral, that is 1.039 ± 0.004 , 1.033 ± 0.007 , and 1.035 ± 0.005 for mackinawite, goethite, and magnetite, respectively (Table 10-5) which are again identical within uncertainty.

An analysis of eqs. 2 and 9 illustrates that a variation of ϵ_N for DNAN by $\pm 2.8\%$, that is variations of ^{15}N -AKIE of ± 0.006 as observed in the average uncertainty of ^{15}N -AKIEs in this study, will introduce uncertainty in estimating the extent of transformation (i.e., $100 \times (1-c/c_0)$). This uncertainty will be higher when the observed N isotope fractionation is small (e.g., 15% for $\Delta^{15}\text{N}$ of 5‰) and level off as the N isotope fractionation increases (e.g., $<7.5\%$ at $\Delta^{15}\text{N}$ of 25‰²⁸ see Figure 10-20). Given the large magnitude of ^{15}N -AKIEs observed here, the extent of DNAN reduction has to exceed only approximately 12% to generate N isotope fractionation beyond the typical total uncertainties of N isotope ratio measurements of $\pm 1\%$ ²⁵ based on an average ϵ_N value of -16% . The N isotope fractionation data for DNAN reduction reveal two important findings. First, abiotic reduction of DNAN will give rise to similar isotope enrichment factors across a variety of reaction conditions (i.e., regardless of the presence of NOM and previous contaminant exposures). Moreover, it is notable that ^{15}N -AKIE and ϵ_N values calculated in this study closely agree with those observed for the reduction of other model NACs by Fe-bearing mineral phases.^{21,29-31} This comparison confirms our hypothesis that previous N isotope fractionation data for the abiotic reduction of NACs by Fe(II) bearing minerals can be

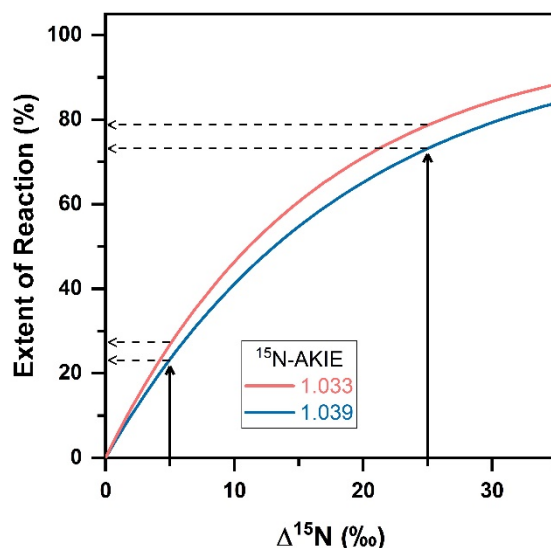


Figure 10-20. Changes in N isotope ratios ($\Delta^{15}\text{N} = \delta^{15}\text{N} - \delta^{15}\text{N}_0$) vs the extent of DNAN reaction ($100 \times (1-c/c_0)$). Equations 2 and 9 were used to illustrate variations in the extent of reaction given uncertainties in calculated AKIEs. Isotope fractionation profiles were generated using ϵ_N values of -16.0% (red) and -18.8% (blue), values typical for abiotic reductions of NACs but also within the range of isotope effects derived for the reduction of other NACs by other minerals. These ϵ_N values represent the extrema observed in this work. Dotted lines represent uncertainties in calculating the extent of reaction from uncertainties in ^{15}N -AKIEs.

extrapolated to new contaminants such as DNAN. This observation also indicates that N isotope fractionation is a robust indicator for monitoring the extent of reduction of DNAN and other NACs in complex environmental matrices, even in the absence of knowledge relating to reaction kinetics and product formation. Indeed, this technique has previously been employed to provide quantitative estimates of the extent of organic contaminant transformations at contaminated field sites.¹³⁴ Our work, therefore, serves to qualify the prospective use of CSIA to assess the abiotic reduction of novel insensitive munitions in the environment.^{28,97}

The combined C and N isotope analysis for DNAN reduction derived here is compared to the data for alkaline hydrolysis and aerobic biodegradation from Section 12.1.5 in Figure 10-21 and confirms that the different transformation pathways can also be discerned by CSIA. These data support the claim that N fractionation of DNAN during abiotic reduction is distinct from the

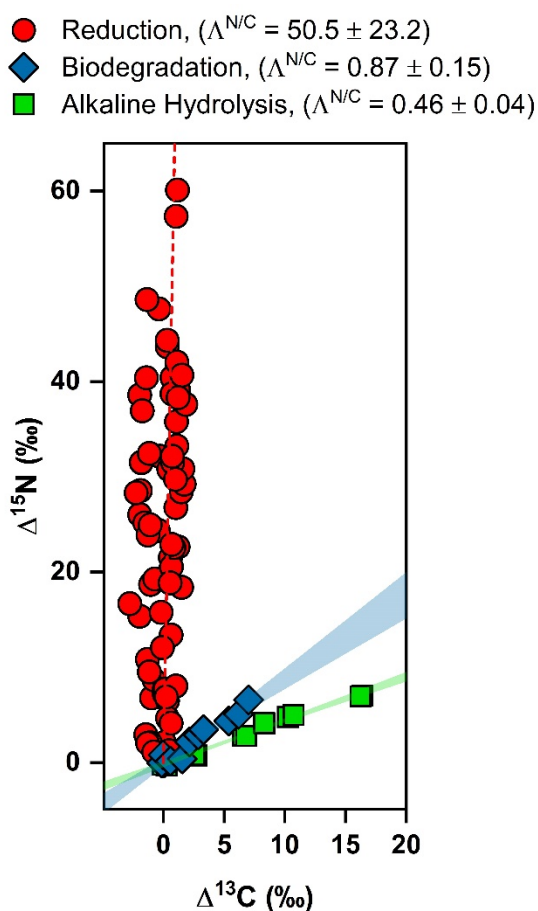


Figure 10-21. Two-dimensional isotope analysis for reductive and oxidative DNAN transformation pathways. Changes in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were monitored during abiotic reduction (circles), alkaline hydrolysis (squares), and biodegradation (diamonds). Alkaline hydrolysis and biodegradation data are from Section 12.1.5 Reduction data separated by mineral type are provided in Figure 12-14. The dotted line along the reduction data is provided simply to guide the eye. Moreover, any apparent inverse fractionation is an artifact of uncertainties in ^{13}C -AKIEs that are close to 1.

isotopic fractionation observed in alkaline hydrolysis (nucleophilic aromatic substitution) and enzymatic hydrolysis (nucleophilic aliphatic substitution). Due to the vertical nature of the $\Lambda^{N/C}$ for the abiotic reduction data, a standard linear regression failed. Thus, the slope was found by plotting all of the $\Delta^{13}\text{C}$ vs $\Delta^{15}\text{N}$ data, finding the slope, and then taking the inverse of this value. The $\Lambda^{N/C}$ calculated via this method for abiotic reduction was 50.5 ± 23.2 (shown as the dotted line in Figure 5) in contrast to 0.46 ± 0.04 and 0.87 ± 0.18 for alkaline hydrolysis and biodegradation, respectively.¹³³ These results therefore suggest that an identification of those DNAN reaction pathways and their respective contributions in the environment would be possible.¹³⁵ Abiotic and biotic transformations of NACs typically elicit variable degree of ^{15}N , ^{13}C , and ^2H enrichment,^{31,42,136} so that the observable isotope fractionation of processes occurring simultaneously can, in principle, be described by linear combinations of the enrichment factors pertinent to the individual, contributing processes.^{35,135} Such dual or triple isotope analysis²⁸ can also circumvent masking interferences, which so far, have been reported primarily for oxidative NAC degradation processes.^{26,32,136–139}

10.3.4 2,4-DNT

The N and C isotope ratios were measured during the reduction of 2,4-DNT. Because of the consistency observed of isotope fractionation during the abiotic reduction of other NACs, only a subset of the DNAN experiments were replicated with 2,4-DNT. No experiments were performed using natural organic matter or multiple contaminant spikes. Reaction rate constants were calculated for mackinawite, goethite, magnetite, and hematite; however, the isotope fractionation behavior was only assessed during reactions with magnetite and hematite (Table 10-6). These results combined with past results with NACs indicate that large N and small C isotope fraction is a consistent trait for reduction of NACs.

Table 10-6. N and C bulk isotope enrichment factors (ϵ_N , ϵ_C)^{a,b,c} and apparent kinetic isotope effects (^{15}N -AKIE, ^{13}C -AKIE)^{b,c} during the reduction of 2,4-DNT in different mineral systems.

System	ϵ_N (‰)	ϵ_C (‰)	^{15}N -AKIE (-)	^{13}C -AKIE (-)
Magnetite	-7.3 ± 3.1	-1.9 ± 1.2	1.015 ± 0.006	1.0002 ± 0.0002
Hematite	-10.3 ± 2.2	-2.6 ± 1.3	1.021 ± 0.004	1.0003 ± 0.0003

^aValues derived from log-linear regression analysis of eq. 9.

^bUncertainties represent 95% confidence intervals.

10.3.5 RDX

Nitrogen and carbon fractionation during RDX degradation was assessed for each mineral phase (Figure 10-22). Initial analyses showed that the nitroso intermediates were also retained by SPE and co-eluted with RDX on the GC column, thus introducing interferences to the instrumental signal. To circumvent these issues, only samples with >60% of RDX molar percentage, i.e. experiments performed at pH 7.5 for the iron oxides, green rust, and selected samples from Fe(II)/goethite experiments at pH 7 were analyzed by CSIA. All FeS samples were excluded due to the substantial accumulation of nitroso compounds, and we were not able to gather additional insights into the pathway shown in Figure 10-6b via CSIA.

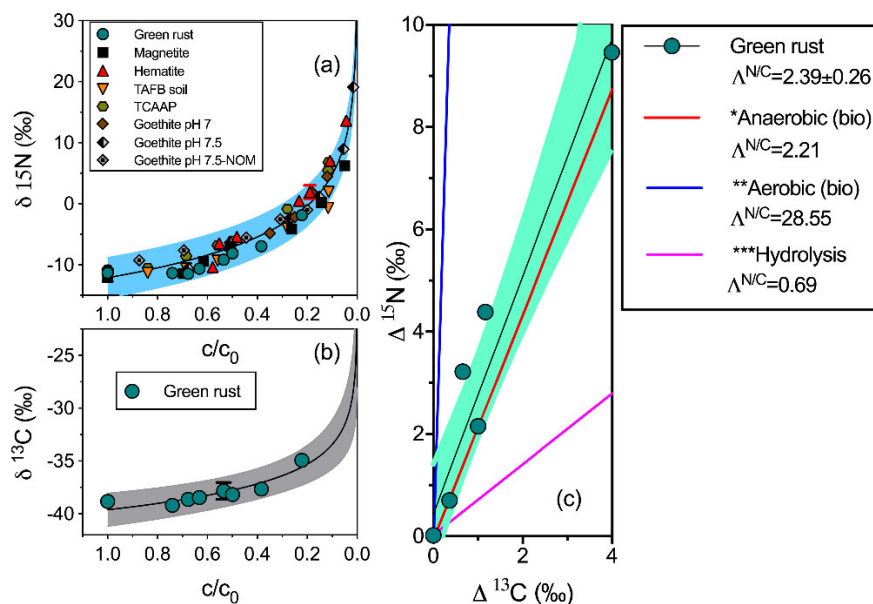


Figure 10-22. Nitrogen and carbon isotope fractionation during abiotic reduction of RDX at pH 7.5. (a) Nitrogen isotope ratios, $\delta^{15}\text{N}$, versus fraction of unreacted RDX (c/c_0) under various conditions. The goethite data represent experiments with Fe(II)/goethite at pH 7 and pH 7.5, and Fe(II)/goethite with ESHA at pH 7.5. The curve was fit using the data from each set of conditions to obtain a single ϵ_N value of $-7.4 \pm 0.2\text{‰}$ (individual values in Table 10-7). The error band shows the 95% confidence interval. (b) Carbon isotope signature, $\delta^{13}\text{C}$, versus fraction of unreacted RDX (c/c_0) during reduction by carbonate green rust. The error band shows the 95% prediction interval. (c) Two-dimensional N-C isotope analysis for RDX. For reduction mediated by carbonate green rust, $\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$ were calculated by subtracting the unreacted N and C isotope signatures from the measured values (e.g., $\Delta^{15}\text{N} = \delta^{15}\text{N} - \delta^{15}\text{N}_0$). The $\Lambda^{N/C}$ value was the slope of $\Delta^{15}\text{N}$ versus $\Delta^{13}\text{C}$. The error band shows the 95% confidence interval. *adapted from the composited isotope data from anaerobic degradation of RDX by multiple anaerobic strains via nitro reduction. Fuller et al.⁹¹ **adapted from the composited isotope data from aerobic degradation of RDX by multiple aerobic strains via N-denitrification. Fuller et al.⁹¹ ***adapted from the isotope data from RDX alkaline hydrolysis via N-denitrification and ring cleavage. Gelman et al.⁹³

The isotope ratios of N and C in the unreacted RDX were approximately -10‰ and -40‰ , respectively. A significant ^{15}N enrichment of $\sim 30\text{‰}$ in the remaining unreacted RDX as reaction proceeds is attributed to nitro group reduction (Figure 10-22a).^{91,92} The bulk enrichment factors for each set of conditions, ϵ_N , was found from Eqn. 1 and are given in Table 10-7. Bulk ^{15}N enrichment factors ranged from $-6.3 \pm 0.3\text{‰}$ to $-8.2 \pm 0.2\text{‰}$ across the tested synthetic and natural minerals. The ^{15}N -AKIE values derived from ϵ_N values ranged from 1.039 ± 0.002 for magnetite to 1.051 ± 0.001 for hematite. The isotope fractionation of nitrogen was assessed by combining using all of the collected data as shown in Figure 10-22a, because the rate-limiting step for the iron (hydr)oxide minerals was determined to be the initial nitro reduction. The combined ϵ_N and ^{15}N -AKIE values were $-7.4 \pm 0.2\text{‰}$ and 1.046 ± 0.002 , respectively. The values of ^{15}N -AKIE under different conditions suggest a primary kinetic isotope effect and are

consistent with previous research on anaerobic biodegradation of RDX (Table 10-7).^{91,92,140} Due to interferences during C isotope analysis (suspected to be from leaching of material from the SPE cartridge), ¹³C enrichment data was only obtained for the green rust samples. The ¹³C enrichment of ~12‰ (Figure 10-22b) introduced bulk ϵ_C and ¹³C-AKIE values of $-2.8 \pm 0.5\%$ and 1.003 ± 0.001 , respectively (Table 10-7). The ¹³C-AKIE is similar to that for anaerobic biodegradation (1.004, Table 10-7). The two-dimensional isotope analysis for green rust samples was characterized by a $\Lambda^{N/C}$ value of 2.39 ± 0.26 via linear regression (Figure 10-22c) and 2.98 ± 0.20 via the York method. By adapting data from Fuller et al.,⁹¹ a $\Lambda^{N/C}$ value of 2.22 (Figure 10-22c) was obtained for anaerobic RDX biodegradation. This two-dimensional isotope correlation falls within the error band of RDX abiotic reduction (Figure 10-22). Aerobic degradation of RDX, which is initiated by the initial enzymatic attack on an N-N bond, gives rise to large enrichment of ¹⁵N and less enrichment of ¹³C (Table 10-7) with an estimated $\Lambda^{N/C}$ of 28.55 (Figure 10-22c, adapted from Fuller et al.⁹¹). The ¹⁵N enrichment for aerobic biodegradation ($\epsilon_N = -2.3 \pm 0.5$) is smaller than that for abiotic reduction and anaerobic biodegradation. During alkaline hydrolysis of RDX, significant enrichment of the ¹³C and ¹⁵N (-7.8% and -5.3% , respectively) was observed,⁹³ however, the estimated $\Lambda^{N/C}$ was the lowest among all reaction pathways (Figure 10-22c).

Table 10-7. N and C bulk isotope enrichment factors ϵ_N and ϵ_C during abiotic reduction, biodegradation, and hydrolysis of RDX

Mineral	ϵ_N^a (‰)	¹⁵ N-AKIE (-)	ϵ_C^a (‰)	¹³ C-AKIE (-)
Green rust	-6.9 ± 0.8	1.043 ± 0.005	-2.8 ± 0.5	1.003 ± 0.001
Hematite	-8.1 ± 0.2	1.051 ± 0.001	ND	ND
Magnetite	-6.3 ± 0.3	1.039 ± 0.002	ND	ND
Goethite, pH 7	-7.7 ± 0.4	1.049 ± 0.001	ND	ND
Goethite, pH 7.5	-7.3 ± 0.3	1.046 ± 0.002	ND	ND
Goethite, pH 7.5-NOM	-6.3 ± 0.6	1.039 ± 0.003	ND	ND
TAFB soil	-7.9 ± 0.4	1.049 ± 0.002	ND	ND
TCAAP sediment	-8.2 ± 0.2	1.050 ± 0.001	ND	ND
Anaerobic biodegradation ^b	-9.9 ± 0.7	1.063 ± 0.005	-4.7 ± 1.1	1.005 ± 0.001
Anaerobic biodegradation ^c	-5.0 ± 0.3	1.031 ± 0.002	NA	NA
Aerobic biodegradation ^d	-2.3 ± 0.5	1.006 ± 0.003	-0.8 ± 0.5	1.001 ± 0.001
Hydrolysis ^e	-5.3	1.033	-7.80	1.008

^abulk isotope enrichment factor.

^bcomposited isotope data from RDX anaerobic degradation by multiple anaerobic strains via nitro reduction.⁹¹

^cRDX anaerobic degradation by non-specific sediment strains via nitro reduction.⁹²

^dcomposited isotope data from RDX aerobic degradation by multiple anaerobic strains via N-denitration.⁹¹

^eisotope data from RDX hydrolysis via ring cleavage.⁹³

ND = no data. Samples were not subject to C analysis. NA=data not available

The primary ¹⁵N-AKIE arose from the reduction of the first nitro group to a nitroso group as the rate-limiting step (Figure 8-5). For the reaction at pH 7.5 that is proposed to proceed via Figure 10-6a, the modeled initial rate constant, $k_{RDX-MNX}$, is smaller than the subsequent MNX reduction (Table 12-5). This rate-limiting effect of one-nitro group reduction is in agreement with the primary ¹⁵N-AKIE. Even though it is hard to directly measure nitroso compounds due to minimal

to zero accumulation at pH 7.5 (Figure 10-5a), the primary ^{15}N -AKIE could still justify Figure 10-6a because of the absence of nitroso compounds. The goethite reaction at pH 7 showed comparable ^{15}N enrichment and ^{15}N -AKIE with all systems tested at pH 7.5 (Figure 10-22a). The small (i.e., secondary) ^{13}C enrichment of green rust samples indicates that direct ring cleavage at an N-C bond^{94,141} is not involved in the isotope fractionating step. This fact further supports that RDX reduction by the iron oxide minerals is controlled by the first nitro group reduction. Although variations in reaction conditions, i.e. pH, mineral identity, and presence of ESHA, led to differences in rate constants, the primary N isotope effects were consistent throughout the experiments. Thus, CSIA represents a potential tool for monitoring natural attenuation of RDX. From the comparison of N isotope enrichment factors given in Table 10-7 and Figure 10-23, it is clear that while aerobic biodegradation and hydrolysis can be distinguished from reductive processes, anaerobic biodegradation and abiotic reduction will lead to similar isotope fractionation.

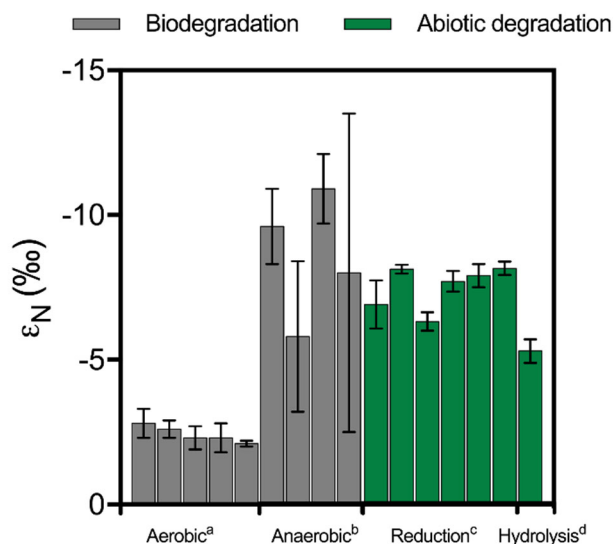


Figure 10-23. Nitrogen enrichment factors during RDX collected from this study and adapted from previous research under varying experimental conditions. (a) Bernstein et al.⁹² and Fuller et al.⁹¹; (b) Fuller et al.⁹¹; (c) this study; (d) Gelman et al.⁹³ Error bars represent the 95% confidence intervals.

10.3.6 NTO

It is possible that other C-containing constituents, particularly the bicarbonate buffer, could interfere with measurement of NTO via LC-IRMS. To assess any effect influence of C-containing compounds during LC-IRMS analysis, NTO was reduced by Fe(II)-goethite in NaHCO_3 buffered or non-buffered systems at pH 7. NTO reduction in buffered solution at pH 7 was instantaneous. The extent of NTO degradation was therefore controlled by stepwise injection of Fe(II) into the mixture. In non-buffered system, NTO reduction followed zero order kinetics with the rate constant of $156.3 \pm 32.6 \text{ M s}^{-1}$. For both conditions, we observed similar C isotope fractionation, which could be analyzed together as shown in Figure 10-24. Thus, the

$^{13}\text{C}/^{12}\text{C}$ ratio measurements were not affected by the NaHCO_3 buffer, and C isotope fractionation was independent of reaction kinetics.

CSIA of NTO reveals a secondary isotope effect during abiotic reduction by Fe(II) /goethite, with an enrichment factor of $3.17 \pm 0.32\text{‰}$ associated with the reduction of $-\text{NO}_2$ moieties and a less involvement of carbon during the process (Table 10-8). As a comparison, we examined the carbon isotope effect during oxidation of NTO. Previous research¹⁴² has shown that NTO degradation by $\bullet\text{OH}$ radicals occurred via attack at the C-N bond. Hence, a larger C enrichment during oxidation of NTO should be observed. The generation of $\bullet\text{OH}$ radicals was performed via Fenton reaction (FeSO_4 and H_2O_2 at pH 3.) Because NTO reacted rapidly with $\bullet\text{OH}$ under the tested conditions, we injected FeSO_4 step-wise to obtain samples with different extents of reaction. There were nine spikes of FeSO_4 to obtain approximately 90% degradation of NTO (Figure 10-25). We quenched the samples using NaOH to prevent unexpected NTO degradation before IRMS measurement. The bulk ϵ_{C} value of $5.98 \pm 1.51\text{‰}$ for NTO oxidation was significantly higher than that for reduction (Table 10-8), suggesting a more prominent involvement of C in the rate limiting step. These results indicate it is possible to distinguish abiotic reduction from reaction with hydroxyl radical for NTO using C isotopes. Given the relatively small difference in ϵ_{C} values, robust data and sufficient replication will be needed to apply this tool in practice. Additionally, measurement of ϵ_{C} for other potential degradation processes (e.g., aerobic and anaerobic biodegradation) are needed to have a robust data set to identify which process is relevant for NTO in natural and engineered treatment schemes.

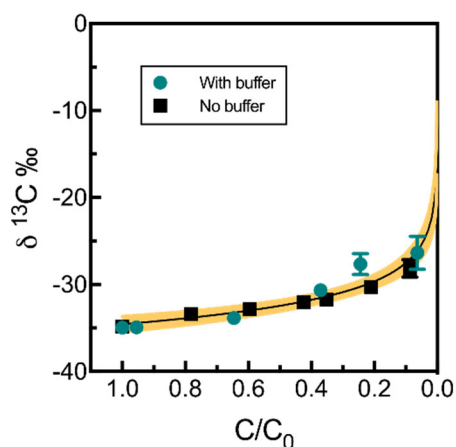


Figure 10-24. Carbon isotope fractionation during abiotic reduction of NTO by Fe(II) -goethite at pH 7, showing Carbon isotope signature, $\delta^{13}\text{C}$, versus fraction of unreacted NTO (c/c_0) under buffered and non-buffered conditions. All data were fit to obtain a single ϵ_{C} value. The error band shows the 95% confidence interval.

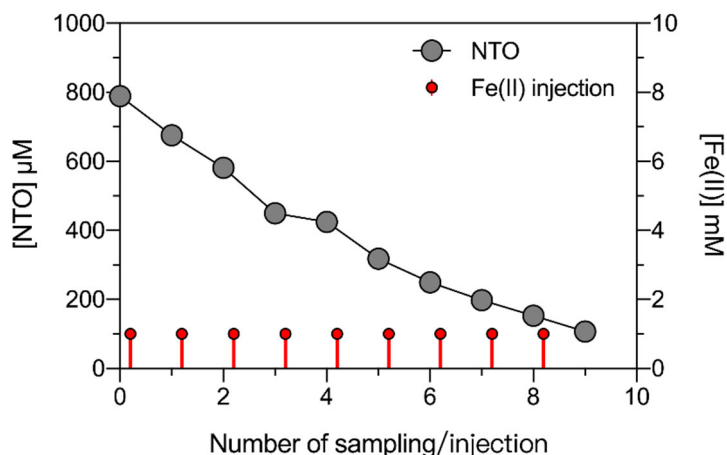


Figure 10-25. The degradation of NTO via Fenton reaction. The gray circles indicate the concentration decreasing of NTO. The red bars indicate Fe(II) spikes that drive the oxidation.

Table 10-8. C bulk isotope enrichment factors (ϵ_C) and ^{13}C -AKIEs during abiotic reduction of NTO by Fe(II)/goethite and oxidation through the Fenton reaction.

	NaHCO ₃ buffer	ϵ_C^a (‰)	^{13}C -AKIE (-)
Reduction by mineral			
Fe(II)/Goethite	Y	-3.55 ± 0.59	1.0036 ± 0.0006
Fe(II)/Goethite	N	-2.78 ± 0.26	1.0027 ± 0.0003
	Combined ^a	-3.17 ± 0.32	1.0032 ± 0.0003
Fenton reaction	N	-5.98 ± 1.51	1.0060 ± 0.0015

^aFe(II)/goethite data were combined to include measurements from reactions with and without NaHCO₃ buffer.

10.4 Task 4 – Natural Materials

10.4.1 Rationale

While synthetic materials with added natural organic matter allow careful study of kinetics and isotope fractionation under well-controlled conditions, they will not represent all the complexities of a natural sample. Thus, the capacity of natural materials to lead to abiotic transformation of the target munitions compounds must also be tested.

10.4.2 Material characterization

Aquifer material collected from the south-central area of TCAAP was primarily a mixture of sand and silt, with quartz the only detectable mineral by XRD (Figure 10-26). A simple magnetic extraction on dried material revealed a significant amount of magnetite in the aquifer matrix. Although the extract was observably darker in color, extraction did not yield pure magnetite and both quartz and clay minerals were still detectable by XRD (Figure 10-26). Because of quartz and other mineral impurities in the extract, percent magnetite in the whole aquifer material was instead estimated using ICP-AES analysis, which measured $3.02 \pm 0.16\%$ total Fe (w/w).

Although this value does not directly correspond to magnetite, it is much closer to the reported ~ 0.3% magnetite determined through magnetic susceptibility by Ferrey et al.¹⁴³ instead of the bulk iron content of pure magnetite (~72% Fe(w/w)). The TCAAP extract was 6.9% iron, with an Fe(II)/Fe(III) ratio of 0.1. This supports the XRD results that the magnetic extract is not comprised of iron oxides alone and indicates that the magnetite stoichiometry is below the ideal 0.5 value.

XRD of the TAFB material revealed the dominant iron oxide was hematite, and quartz was the bulk mineral phase. Detailed physical and chemical properties of natural materials are provided in Table 10-9 and Figure 10-26. The collected patterns were compared to the previously listed PDFs for magnetite, hematite, and quartz (SiO₂, PDF #46-1045). A pattern was also collected from the TCAAP extract to show the occurrence of magnetite as the primary reactive iron phase. Because of the low abundance of hematite relative to quartz in the Tinker AFB sediment, a zoomed in portion of the Tinker AFB diffraction pattern is provided in Figure 10-26.

Table 10-9. Chemical properties of natural materials and the synthetic analogs* used in this study. Mineral phases were determined by X-ray diffraction (XRD).

Material	Reactive Iron Phase ^a	Bulk Mineral Phase ^a	Fe (% w/w) ^b	Fe(II)/Fe(III) ^c	Organic Content (%)	Inorganic Content (%)	CaCO ₃ (%)
TCAAP	Magnetite (Fe ₃ O ₄)	Quartz (SiO ₂)	3.03	<LOD	0.46	97.21	2.36
TCAAP extract	Magnetite (Fe ₃ O ₄)	Quartz (SiO ₂)	6.9	0.1	ND	ND	ND
Magnetite* ^d	Magnetite (Fe ₃ O ₄)	NA	72 ^e	0.5	ND	ND	ND
Tinker AFB	Hematite (Fe ₂ O ₃)	Quartz (SiO ₂)	2.04	<LOD	0.88	97.83	1.29
Hematite* ^f	Hematite (Fe ₂ O ₃)	NA	70 ^e	<LOD	ND	ND	ND

^aDetermined by XRD.

^bCalculated by ICP-AES.

^cDetermined using the ferrozine method¹¹⁰ following dissolution in 5 M HCl.

^dData obtained from Strehlau et al.¹¹⁴

^eEstimated from structural formulas of pure minerals.

^fObtained from Voelz et al.¹⁰⁵

LOD = limit of detection. ND = not determined. NA = no additional mineral phases detected

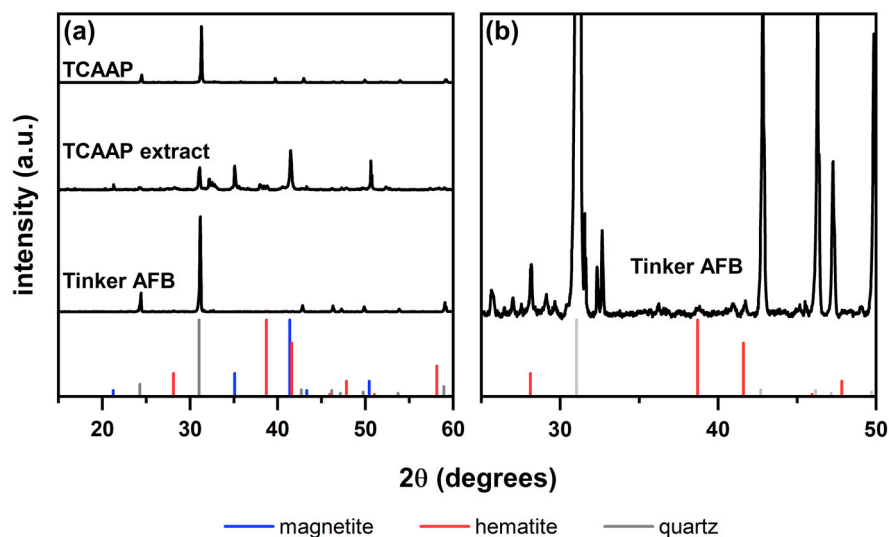


Figure 10-26. XRD patterns of natural materials.

10.4.3 Kinetic Studies of Natural Materials

10.4.3.1 $DNAN + Fe(II)$

Batch reactions were performed identical to those for DNAN reduction by the synthetic minerals (Section 9.4). The amount of TCAAP (143 g/L) or Tinker AFB (1.5 g/L) materials added to batch reactors was selected such that DNAN transformation occurred in a similar time period to the synthetic minerals (Section 10.2.2).

DNAN reduction occurred in all reactors receiving amendments of aqueous Fe(II) (Figure 10-27, open symbols). DNAN reduction did not occur in reactors containing only mineral suspensions of aqueous Fe(II) alone (see Figure 12-11). When aqueous Fe(II) was maintained at 1 mM throughout the batch experiments, all of the added DNAN was reduced. DNAN reduction was considerably slower in reactors containing synthetic magnetite, TCAAP sediment, and the TCAAP extract than in reactors containing TAFB soil or synthetic magnetite. Moreover, the reactivity of natural materials was lower than for synthetic minerals because the natural materials contain a greater amount of low-reactivity surface sites (e.g., quartz). There was also a decreased reactivity of TCAAP extract as compared to synthetic magnetite. This was likely caused by the lower Fe stoichiometry of the TCAAP extract ($x = 0.1$) than synthetic magnetite ($x = 0.5$) and the fact that it is difficult to remove all of the impurities and low-reactive components of natural materials. These data, however, still suggest that pollutant reduction will occur in ferruginous soils and sediments in the presence of dissolved iron.

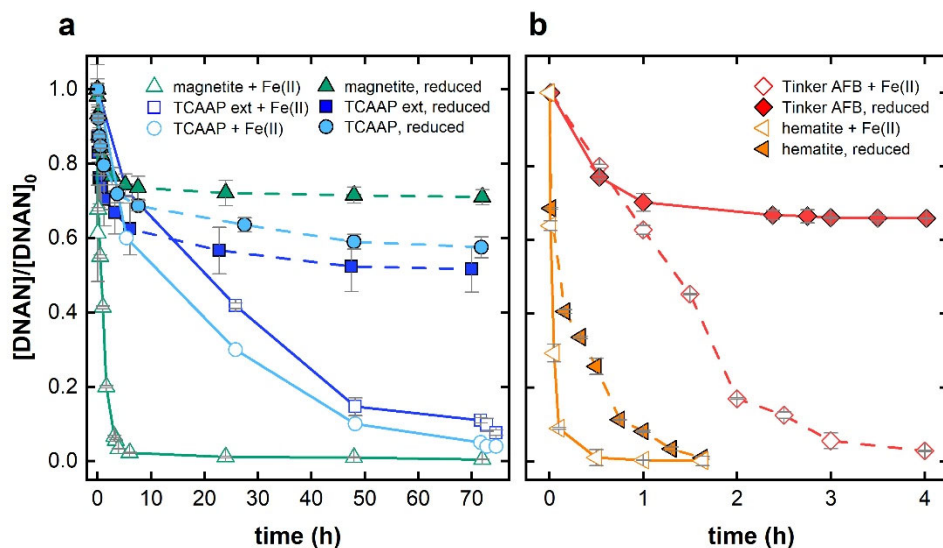


Figure 10-27. Concentration of DNAN during abiotic reduction by synthetic and natural materials. Reactors contained (a) synthetic magnetite, TCAAP sediment, TCAAP extract, (b) Tinker AFB soil, and synthetic hematite. Reactions were performed with either untreated materials in the presence of 1 mM aqueous Fe(II) (open symbols) or with dithionite-reduced materials without additional Fe(II) (closed symbols; see Section 10.5). All error bars represent standard deviations of triplicate reactors.

10.4.3.2 RDX

Comparisons between synthetic mineral and natural aquifer material revealed the effects that nonreactive minerals have on mineral-mediated natural attenuation processes (Table 10-2 and Table 10-10). Nonreactive minerals such as quartz in natural aquifer materials significantly decreased RDX reaction rates, likely through loss of accessible reactive surface sites either through heteroaggregation and subsequent blocking of reactive surface sites on the magnetite particles or through competitive Fe(II) adsorption to less reactive surfaces. During these experiments, TCAAP aquifer material reactions required approximately three times more aqueous Fe(II) addition to maintain a 1 mM Fe(II) concentration at each sample point as compared to TCAAP magnetic extract, indicating that competitive Fe(II) adsorption was occurring. Furthermore, extracting the magnetic portion of TCAAP aquifer material led to faster RDX degradation rates in comparison to whole aquifer material (Table 10-10). Similar results were seen with the TAFB material. Rate constants were slower than those with pure hematite. Increasing the pH from 6.5 (no reactivity) to 7.5 increased the observed pseudo-first order rate constant (Table 10-10).

Given recent controversy regarding the role of magnetite in reduction of pollutants,¹⁴⁴ detailed comparisons of the reaction of synthetic magnetite, TCAAP extract, and the bulk TCAAP material, both with and without added Fe(II), were performed to provide guidance on appropriate

conditions to assess natural material reactivity. Several factors contributed to varying observed rate constants for RDX reduction in the presence of magnetite. All tested conditions and corresponding pseudo-first order rate constants are outlined in Table 10-10. Variables that resulted in significant differences in rate constants included the presence of aqueous Fe(II), buffer identity, magnetite source, and presence of nonreactive minerals, providing evidence that experimental parameters are critical considerations when using microcosm experiments as predictors for natural environments.

No degradation of RDX was observed in bicarbonate buffer alone or with aqueous Fe(II). The greatest variable across all experimental conditions with minerals was whether aqueous Fe(II) was present. No reaction was detected over 21 d in reactors with mineral phases that did not contain aqueous Fe(II). This indicates no biodegradation of RDX. Magnetite has structural Fe(II), suggesting that the direct transfer of electrons from mineral to oxidized groundwater contaminants is possible. While it is expected that aqueous Fe(II) would increase contaminant reduction rate through adsorption and electron delocalization within the mineral, a review of current literature found variability in the extent of direct electron transfer from magnetite to contaminant in the absence of aqueous Fe(II).^{145,146} Researchers have noted quantifiable $-\text{NO}_2$ reduction rates for nitroaromatic compounds and RDX over time-scales of minutes or days by magnetite alone, with magnetite stoichiometry controlling reaction rate.^{147–149} Others did not detect $-\text{NO}_2$ reduction by magnetite under similar conditions, even after weeks of sampling,^{150,151} and the results in Table 10-10 support this latter finding.

One study detected loss of RDX in magnetite suspensions but noted that sorption on the mineral may be influencing RDX concentration.¹⁴⁸ The synthetic magnetite used in our study had a stoichiometry of 0.50, suggesting that a sub-stoichiometric Fe(II)/Fe(III) ratio was not controlling reaction rate. Possible variations of the minerals, including how they were synthesized, their surface and chemistry, and residual aqueous Fe(II) may explain the widespread differences in the literature regarding observed synthetic magnetite reactivity. Thus, interpretations of magnetite reactivity based on literature data must include careful consideration of material preparation and experimental conditions. These discrepancies warrant future review of the physical and chemical properties of magnetite that drive its reactivity toward oxidized molecules in the presence and absence of aqueous Fe(II).

Buffer identity also affected RDX reduction rates by adsorbed Fe(II) on synthetic magnetite, with pseudo-first order rate constants nearly one order of magnitude faster in 50 mM MOPS buffer than in 10 mM bicarbonate buffer at similar pH. An experiment with 10 mM MOPS was also performed to normalize ionic strength to that of bicarbonate buffered reactions. These results revealed similar pseudo-first order rate constants to reactions containing 50 mM MOPS, thus supporting our observation that significant rate constant increases are due to buffer identity, and not ionic strength. Aqueous Fe(II) concentration after equilibration with synthetic magnetite in each buffer was comparable, with aqueous Fe(II) concentration decreasing from 1 mM to 0.76 ± 0.01 mM in bicarbonate buffer and 0.71 ± 0.01 mM in MOPS, so the effect is not related to amount of Fe(II) associated with the mineral surface. While aggregate size was not monitored

Table 10-10. Reaction conditions and pseudo-first order rate constants (k_{obs}) for RDX reduction by natural minerals and synthetic magnetite. Reactions are in 10 mM NaHCO₃ buffer at pH 7, unless otherwise specified.

Material	Collection depth (m)	Total mineral mass (g)	Fe loading (g/L) ^a	[Fe(II)] (mM)	k_{obs} (h ⁻¹) ^b
TCAAP	44	5.00 ± 0.05	0.89	0	ND
TCAAP	44	5.00 ± 0.05	0.89	1	1.8 (± 0.4) × 10 ⁻³
TCAAP	48	5.00 ± 0.05	0.89	1	5.8 (± 1.8) × 10 ⁻⁴
TCAAP	51	5.00 ± 0.05	0.73	1	1.3 (± 0.1) × 10 ⁻³
TCAAP	54	5.00 ± 0.05	0.80	1	1.3 (± 0.2) × 10 ⁻³
TCAAP extract	44	0.170 ± 0.005	0.34	0	ND
TCAAP extract	44	0.170 ± 0.005	0.34	1	4.5 (± 0.3) × 10 ⁻³
TAFB	NA	1.00±0.05	0.19	0	ND
TAFB	NA	1.00±0.05	0.19	1	6.0 (±1.0) × 10 ⁻²
Effect of pH					
	pH	Total mineral mass (g/L)	Fe loading (g/L)	[Fe(II)] (mM)	k_{obs} (h ⁻¹)
TCAAP	6.5	171	5.18	1	~0
	7	171	5.18	1	0.001
	7.5	171	5.18	1	0.06 ± 0.01
TAFB	6.5	28.6	0.69	1	~0
	7	28.6	0.69	1	0.05
	7.5	28.6	0.69	1	0.06 ± 0.01
Effect of buffer					
	Buffer (pH 7)	Total mineral mass (g)	Fe loading (g/L)	[Fe(II)] (mM)	k_{obs} (h ⁻¹) ^a
Synthetic magnetite	bicarbonate	0.030 ± 0.005	0.62	0	ND
Synthetic magnetite	bicarbonate	0.030 ± 0.005	0.62	1	8.9 (± 1.2) × 10 ⁻²
Synthetic magnetite	50 mM MOPS	0.030 ± 0.005	0.62	0	ND
Synthetic magnetite	50 mM MOPS	0.030 ± 0.005	0.62	1	5.4 (± 0.9) × 10 ⁻¹
Synthetic magnetite	10 mM MOPS	0.030 ± 0.005	0.62	1	3.3 (± 0.5) × 10 ⁻¹
Controls					
None		0	0	0	ND
None		0	0	1	ND

^a Based on the iron content of the solid material

^b Errors are 95% confidence intervals

ND = RDX loss was not detected within 21 days of sampling. NA = Not applicable

here, qualitative observations of magnetite particles noted faster settling in bicarbonate buffer (settled in under 24 h) than in MOPS buffer (still suspended after 3 d). These results with magnetite support that aggregate size, which is influenced by buffer identity and associated with the amount of accessible reactive surface area,¹⁵² affects observed reaction rates of mineral-mediated redox reactions. Buffer identity has significant effects on observed reaction rates for mineral-mediated redox reactions by adsorbed Fe(II) on goethite (α -FeOOH),⁵⁰ which has been attributed to larger aggregate sizes in bicarbonate buffer than in MOPS buffer more so than amount of adsorbed Fe(II). MOPS buffer has been shown to alter redox reactions at the goethite surface¹⁵³ and the corrosion process of zero-valent iron.¹⁵⁴ It has also been shown that Good's buffers affect both the kinetics and reaction pathways of carbon tetrachloride in the presence of magnetite.¹⁵⁵ Thus, buffers should not be considered passive, and bicarbonate or other environmentally relevant buffers may better predict reaction rates in environmental systems.

Comparisons between synthetic magnetite and magnetite in natural aquifer material revealed not only the importance of mineral size and morphology but also the effects that nonreactive minerals have on mineral-mediated natural attenuation processes. Although surface area was not quantified for natural TCAAP magnetite, general particle sizes (50 – 100 μ m for natural magnetite and 0.05 – 0.1 μ m for synthetic magnetite) from SEM and TEM micrographs (Figure 10-28) provided evidence of higher specific surface area for synthetic magnetite, making surface area the most likely explanation for the differences in rate constants between synthetic and natural magnetite. This is supported by the fact that normalization of the rate constants by the amount of iron present in the minerals provides the same overall trends in rate constant. Furthermore, extracting the magnetic portion of TCAAP aquifer material led to faster RDX degradation rates in comparison to whole aquifer material. The loadings (5.00 g and 0.17 g) were chosen such that the total amount of Fe in the two sets of experiments were approximately equal (assuming that the magnetic extract contains all of the iron present), and in actuality, less Fe was present in the extract given that other minerals were also collected along with the magnetic material. In natural TCAAP sediment, the observed reactions rates of RDX were significantly decreased. Potential reasons are loss of accessible reactive surface sites either through heteroaggregation and subsequent blocking of reactive surface sites on the magnetite particles or through competitive Fe(II) adsorption to less reactive surfaces.¹⁵⁶ As noted above, Fe(II) adsorption to, and reaction on, less reactive surfaces was likely occurring. Given that the aqueous concentration was maintained at 1 mM Fe(II), we suspect aggregation and blocking of sites limits reaction on the magnetite.

Mineral characterization data, including XRD patterns and elemental mapping in SEM micrographs (Figure 10-28), of the reacted minerals supported competitive Fe(II) adsorption through detection of oxidized iron oxides, such as goethite, in the TCAAP extract following several anaerobic washes and removal from the anaerobic glove bag. The XRD pattern for reacted TCAAP extract with Fe(II) revealed goethite production, which was not observed in the pattern of reacted synthetic magnetite with Fe(II). Both the TCAAP aquifer material and the extract with Fe(II) present also had a notable orange color after washing (inset images in Figure 10-28a). In the SEM element maps, Fe and O were detected on all grains in reacted TCAAP extract + Fe(II) where only select grains contained exclusively Fe and O in the unreacted material, providing evidence of a layer of iron oxide on all grains including clays and quartz. Thus, it is likely that, at the very least, adsorption of Fe(II) on other minerals led to reaction

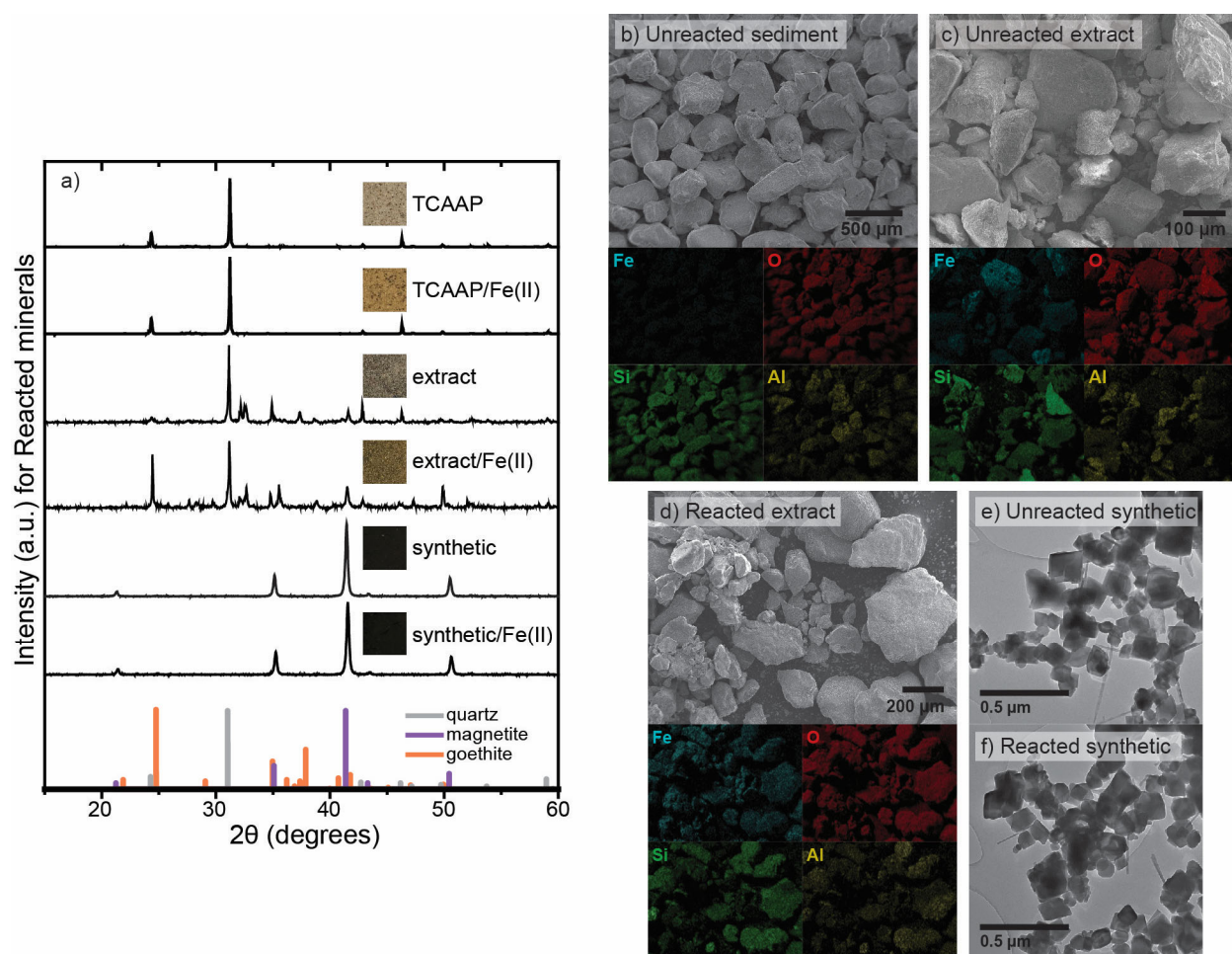


Figure 10-28. a) XRD patterns of minerals collected and washed anaerobically following reaction with 0.2 μM RDX in 10 mM bicarbonate buffer at pH 7. Inset images are photo representations of each sample. b-d) Representative SEM micrographs and corresponding elemental mapping by EDS for unreacted TCAAP aquifer material, unreacted TCAAP magnetic extract, and TCAAP magnetic extract with Fe(II) reacted with RDX in the presence of aqueous Fe(II). Micrographs only pertain to material collected at a depth of 44 m. e-f) Representative TEM micrographs for synthetic magnetite particles either unreacted or reacted with RDX in the presence of aqueous Fe(II). For reactions, buffer identity was 10 mM bicarbonate buffer at pH 7.

occurring on other surfaces, contributing to the slower RDX reduction rate observed with TCAAP sediment, and reaction did not exclusively occur on the magnetite. Because of the large abundance of these other materials, Fe(II) is adsorbed to these less reactive surfaces, and the reaction with Fe(II) occurring on quartz or clays (leading to the formation of goethite) is slower than the reaction on magnetite, as shown by the normalized rate constants in Table 10-10. Good's buffers are also known to lead to higher reaction rates than carbonate buffer with goethite,¹⁵² and thus the effect of the buffer effect on rate could be due to effects on both magnetite and goethite surfaces.^{153,155} It is interesting that reactions on these other surfaces also appear to occur in the TCAAP extract, where a larger fraction of magnetite is present initially.

Further *in situ* imaging would be necessary to quantify any impact of heteroaggregation on limited reaction of the magnetite particles.

Buffer identity not only affected observed rate constants for RDX degradation but also the quantity of intermediates detected. Three nitroso derivatives of RDX were detected during abiotic RDX reduction and peak areas for RDX, MNX, DNX, and TNX are provided in Figure 10-29.

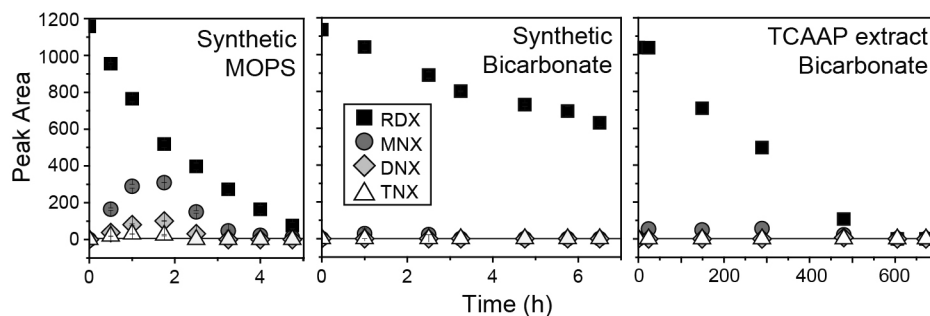


Figure 10-29. Peak area of RDX and nitroso derivatives MNX, DNX, and TNX over reaction time with different magnetite sources (either synthetic or TCAAP extract) and different buffers (either 50 mM MOPS pH 7 or 10 mM bicarbonate pH 7).

The accumulation of all three nitroso derivatives was significantly greater in reactions containing MOPS buffer as compared to similar reactions containing bicarbonate buffer. The products in MOPS buffer are similar to those observed previously with MOPS in the presence of minerals,^{150,157} and additional products observed in other studies include formaldehyde, NH_4^+ , and N_2O .^{19,150} The accumulation of nitroso compounds could be due to 1) slower reaction of the intermediates in the presence of MOPS buffer, 2) competition for reactive sites between RDX and the intermediates, or 3) an alteration in the reaction pathway caused by the different buffers. To evaluate these possibilities, TNX was introduced to and quantified for reactors containing synthetic magnetite and aqueous Fe(II) in either MOPS or bicarbonate buffer. No degradation of TNX in MOPS or bicarbonate buffer alone was observed. In the experiments containing synthetic magnetite, Fe(II), and bicarbonate buffer, TNX would degrade slowly for 4+ hours, whereas in MOPS buffer, there was a sudden disappearance (Figure 10-30). Pre-exposure to RDX shortened, but did not eliminate, this induction period for both buffers, indicating that alteration of the magnetite surface is required for TNX reaction. While these data point to additional complexities in the reaction pathways, it does not appear that slower reaction rates of intermediates in the MOPS buffer is a viable explanation for the differences seen in Figure 10-29.

Results from varying the initial concentration of RDX are shown in Figure 10-31. According to Langmuir-Hinshelwood-Hougen-Watson (LHHW) kinetics,^{158,159} a linear relationship between reaction rate and initial concentration is indicative of adsorption to the surface being the rate limiting step, and a plateau in reaction rate is reached when reaction is the rate limiting step. The data demonstrate that adsorption is rate limiting in MOPS buffer (Figure 10-31a) and reaction is rate limiting in carbonate buffer (Figure 10-31b). The aggregation of particles is greater in the bicarbonate buffer meaning fewer reactive sites are available^{50,160} which could be partially responsible for the lower overall rates of reaction in the bicarbonate buffered reactors. The site

availability does not drive whether a reaction is adsorption or reaction limited in LHHW kinetics, but we cannot rule out that the plateau in rate versus concentration has not been reached in the MOPS buffered system due to a weaker binding of RDX to the surface. The concentration range tested, however, is already beyond that expected in most contaminated groundwater systems. Thus, we conclude there is likely a difference in rate limiting step between the two buffers.

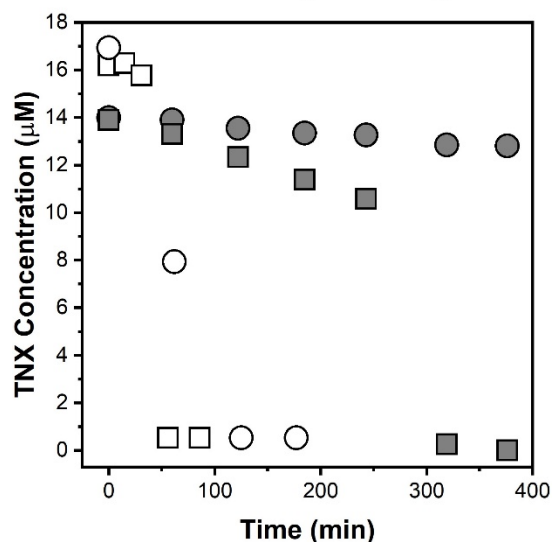


Figure 10-30. Reaction of TNX with Fe(II)/synthetic magnetite bicarbonate buffer (circles) and in MOPS buffer (squares). Closed symbols indicate experiments in which batch reactions were performed following the standard procedure described above. Open symbols indicate experiments where reactors were pre-exposed to RDX and allowed to react to completion before initiating experiments with TNX.

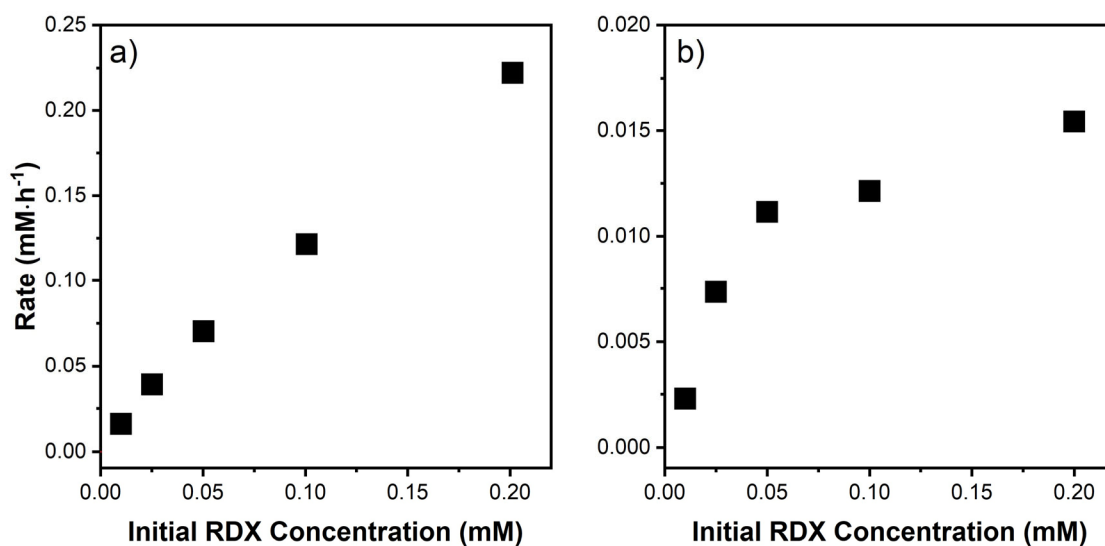


Figure 10-31. Initial reaction rate ($k_{obs}C_0$) as a function of initial RDX concentration in either a) 50 mM MOPS or b) 10 mM bicarbonate at pH 7. Note the difference in scales for the y-axes.

If the rate is adsorption limited, reactive surface sites for both RDX and intermediates would be available. On the other hand, intermediates should accumulate in the reaction limited system, especially if intermediates desorb before reacting further and if the intermediates are less reactive than the parent compound. From the results above with TNX, it is unclear whether TNX reacts more slowly than RDX, but TNX reactivity is obviously not straightforward.

Given that the rate limiting step for RDX degradation varies depending on buffer identity, the most likely explanation for the different reaction intermediate profiles is that the buffer identity affects the reaction pathway of RDX, with intermediate products other than MNX, DNX, and TNX forming in bicarbonate buffer. This suggests that Good's buffers affect reaction pathways of not only chlorinated solvents,^{153,155} but also nitramines. In the TCAAP material and the extract, the pathways of reaction may be altered by the buffer on both the original magnetite present and the goethite formed. Evaluating results from previous studies including various mineral surfaces performed in Good's buffers^{150,157} (MNX, DNX, TNX produced in high yield), those in carbonate buffer or simulated groundwater^{13,161} (low yield of MNX, DNX, TNX), and taking into account that ring cleavage products dominated in systems with Good's buffers and complexed iron,¹² it appears that the combination of Good's buffers with mineral surfaces gives both kinetics and product distributions that may not match those likely to be found in the field.

These results have important implications for the assessment of the reactivity of RDX with iron oxide minerals. The data presented here further highlight the significance of solution variables and mineral characteristics when relying on microcosm experiments for predicting natural attenuation of groundwater contaminants. Given that aqueous Fe(II) was necessary for RDX degradation in the presence of magnetite for our experiments, and that there is much variability in literature about the reaction between oxidized organic molecules and magnetite in the absence of aqueous Fe(II), it is not yet clear whether the presence of aqueous Fe(II) is necessary for reaction of oxidized groundwater contaminants in sediments containing magnetite. In addition, the use of synthetic magnetite, while ideal for controlling mineralogy and decreasing experiment times, will not always correspond to results using natural magnetite-containing sediments. Even with the consideration of varying size and morphology, nonreactive minerals such as clays and quartz will affect contaminant degradation rates indirectly through competition for dissolved Fe(II). Finally, comparisons of RDX degradation rates and reaction pathway under similar conditions, with the exception of buffer identity, revealed that buffer choice and concentration may also affect assessment of both reaction kinetics and pathways. Here, bicarbonate buffer at low concentration, which is representative of typical groundwater chemistry, slowed reaction rates, changed the rate limiting step, and led to variable detection and accumulation of certain intermediates compared to MOPS buffer, which would have large impacts on predicting RDX degradation pathways in natural systems. In combination, these results indicate that while idealized and controllable laboratory conditions are useful in assessing reactions that may occur, conditions as close to those expected in field systems are necessary to evaluate the reaction rates and pathways of RDX in reduced groundwater systems.

10.4.3.3 NTO

Natural aquifer minerals including TCAAP sediment and TAFB soil were added to NTO reactors at similar concentrations as above. Batch reactions were performed as described in Section 9.6.3. Similarly as in RDX experiments, NTO was reduced more slowly by TCAAP sediment ($0.49 \pm$

0.11 h⁻¹) and TAFB soil (0.08 ± 0.01 h⁻¹) than the synthetic analogs (see Section 10.2.5) due to the presence of non-reactive phases.

10.4.4 CSIA of Natural Materials

10.4.4.1 DNAN

The N and C AKIEs and the associated bulk isotope enrichment factors (Table 10-11) for DNAN reduction by natural materials with added aqueous Fe(II) were similar to those from experiments with synthetic minerals (Table 10-5 and Figure 10-19). The results indicate strong enrichment of ¹⁵N with minimal C fractionation. These results are typical for nitro-group reduction on NACs by Fe(II) species associated with minerals (i.e., large ¹⁵N-AKIE = 1.030–1.045 and ¹³C-AKIE close to unity).^{21,29–31} There was some variability of isotope effects between the natural materials and the synthetic analogs (~10–12‰). Because a different number of samples with different extent of reactant conversion were collected from each experiment, we posit that this introduced uncertainties associated with the experimental and data evaluation procedures (e.g., regression analysis).¹¹⁸ This variability was also observed during DNAN reduction by synthetic iron (oxyhydroxides) (see Section 10.3.3). Those results revealed ε_N values between -9 ± 2‰ and -19 ± 1‰ corresponding to ¹⁵N-AKIEs of 1.018 ± 0.002 and 1.039 ± 0.001, respectively, as well as the small ε_C and ¹³C-AKIE values that are consistent with previous studies.¹⁶²

Table 10-11. Bulk N and C isotope enrichment factors (ε_E) and AKIEs in batch reaction for DNAN reduction by natural materials. All reactors were dosed with aqueous Fe(II) (1 mM). Errors represent 95% confidence intervals. Data for the synthetic analogs of Tinker AFB (hematite) and TCAAP (magnetite) materials are provided for reference.

Material	ε _N (‰)	¹⁵ N-AKIE (-)	ε _C (‰)	¹³ C-AKIE (-)
Tinker AFB	-11.1 ± 4.3	1.023 ± 0.009	-0.7 ± 0.1	1.0007 ± 0.0002
Hematite	-21.2 ± 3.3	1.044 ± 0.007	-0.3 ± 0.4	1.0003 ± 0.0008
TCAAP	-21.5 ± 2.6	1.045 ± 0.005	-0.8 ± 1.0	1.0008 ± 0.0020
Magnetite	-13.2 ± 1.7	1.027 ± 0.003	-0.7 ± 0.3	1.0007 ± 0.0006

10.4.4.2 RDX

Although variations in iron minerals/natural material identity led to difference in rate constants (Table 10-2 and Table 10-10), the primary N isotope effect was consistent throughout the experiments (Table 10-6 and Table 10-12). The fact further supports that RDX reduction by iron mineral is controlled by the first nitro group reduction regardless the presence of non-reactive minerals. The C isotope effect was not available, because of interferences.

Table 10-12. N bulk isotope enrichment factor ϵ_N during abiotic reduction of RDX by natural aquifer materials

Mineral	ϵ_N (‰)	^{15}N -AKIE (-)
Fe(II)/TAFB	-7.91 ± 0.40	1.0495 ± 0.0024
Fe(II)/TCAAP	-8.16 ± 0.23	1.0505 ± 0.0014

10.4.5 Case Study – Iowa Army Ammunition Plant

10.4.5.1 Overview.

Compound specific isotope analysis (CSIA) was used to evaluate the extent of RDX transformation along a subsurface plume at the Iowa Army Ammunition Plant (IAAAP; Middletown, IA). The N isotope signature ($\delta^{15}\text{N}$) in four groundwater monitoring wells was measured and ^{15}N -enrichment was detected in each sample. These data suggest that RDX transformation is occurring and contributing to decreases in RDX concentration along the hydrological gradient.

10.4.5.2 Description of Study Site.

The Iowa Army Ammunition Plant (previously Iowa Ordnance Plant) consists of 77 km² in Des Moines County in southeast Iowa (inset of Figure 10-32). The site served as a loading, assembly, and packing plant of military munitions from 1947–1975. The IAAAP was added to the United States National Priority List as a Superfund site in 1990. Samples for this study were taken from the Line 800 Pinkwater Lagoon region (L800) near the center of the IAAAP and situated adjacent to Brush Creek. The lagoon was installed to serve as a settling pond for the treatment of explosives contaminated wastewater prior to discharge into Brush Creek. Detections of several energetic compounds, including RDX, from L800 have been previously recorded with maximum RDX concentrations of 2900 mg/kg and 42 µg/L in soil and surface water, respectively. The explosives-contaminated soil has since been removed and relocated to an onsite landfill. All monitoring wells sampled are overburden wells in which the overburden layer is approximately 6–18 m thick and consists primarily of low-permeability clay-rich till. The underlying bedrock (~20–24 mbgs) is composed of limestone with intermittent shale and silt deposits. Groundwater at L800 exhibits radial flow away from the lagoon (Figure 10-32) towards the water table (~2 mbgs) and precipitation-driven recharge of the lagoon serves as the primary source of hydraulic head.

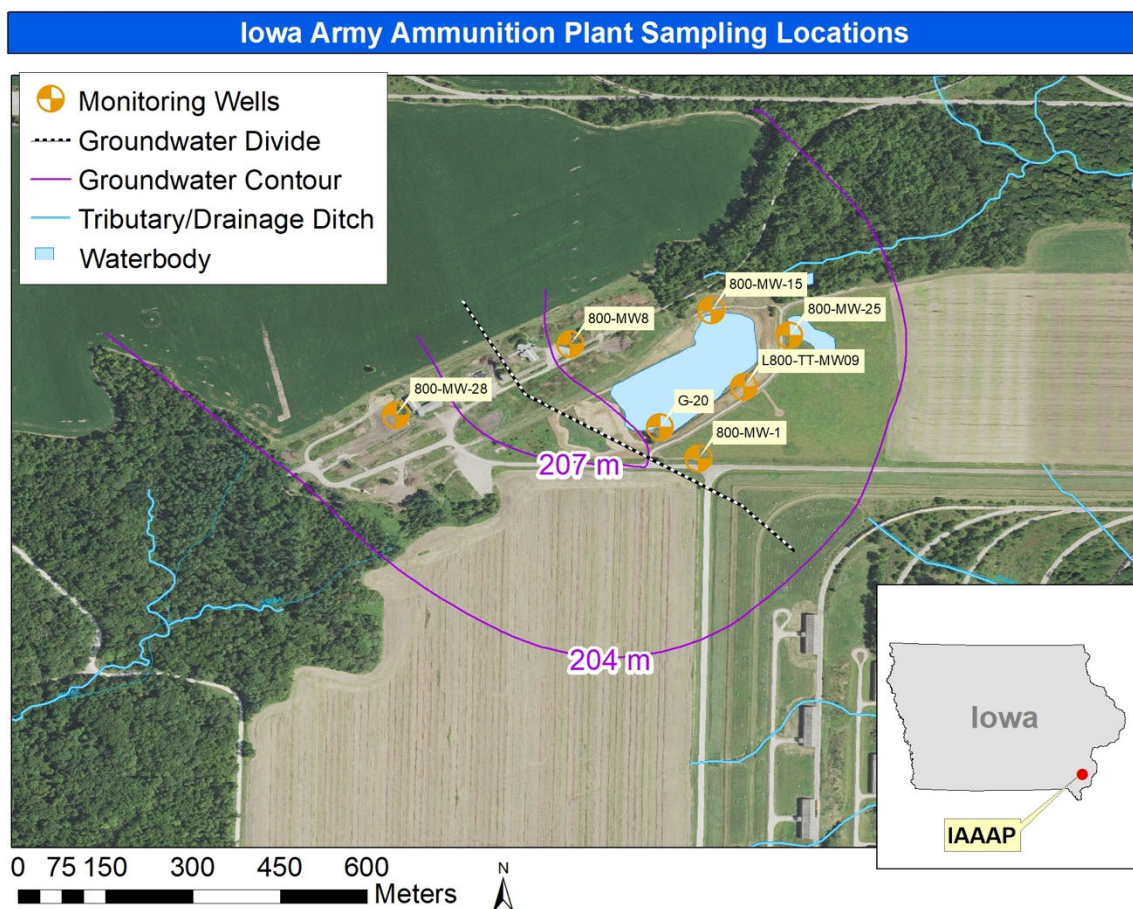


Figure 10-32. Local map of IAAAP monitoring wells sampled for the determination of N enrichment during RDX transformation. The read labels are the $\delta^{15}\text{N}$ value of RDX in each well.

10.4.5.3 Sample Collection and Processing.

Seven groundwater samples were extracted from monitoring wells in the Line 800 area. Samples were collected into clean 1-gallon glass jugs, filtered, and shipped on ice to the laboratory. Sample collection and shipment was coordinated by Jacobs Engineering. Filtered samples were enriched for RDX content by SPE¹⁰¹ and the $^{15}\text{N}/^{14}\text{N}$ content of each sample was determined by GC-IRMS. Details of the SPE and CSIA methods are provided in Section 9.7.2.

10.4.5.4 Results and Discussion.

The list of the groundwater monitoring wells surveyed for this study is provided in Table 10-13. Concentrations measurement were made at the time of sampling for RDX and three of the characteristic abiotic reduction intermediates (MNX, DNX, TNX). Measurements of $\delta^{15}\text{N}$ could only be performed on samples with enough RDX present to yield accurate detection by the GC-IRMS following SPE extraction; thus, three wells were not analyzed (800-MW-1, 800-MW-8, 800-MW-28).

Table 10-13. List of groundwater monitoring wells assessed in our study including aqueous concentrations

Well	Sampling date	Concentration ($\mu\text{g/L}$)			
		RDX	MNX	DNX	TNX
G-20	08-28-2018	6500	19	18	48
L800-TT-MW09	08-28-2018	630	43	36	110
800-MW-25	08-18-2018	180	6.9	3.9	25
L800-TT-MW15	08-28-2018	23	0.69	0.11	0.21
800-MW-1	08-18-2018	18	0.33	0.083	0.78
800-MW-8	08-19-2018	1.7	0.15	0.1	0.2
800-MW-28	08-27-2018	0.1	0.82	0.17	0.2

With the understanding of potential RDX reaction pathways and the associated isotope fractionation gained from laboratory experiments, CSIA was used with other lines of evidence, including RDX concentrations and the presence of reaction products, to evaluate RDX transformation along a subsurface plume at the IAAAP. The G-20 sample ($\delta^{15}\text{N} = 0.66\text{‰}$) was assumed to represent the $\delta^{15}\text{N}_0$ value for RDX in this study because of the close proximity of the G-20 well to the source of the plume.^{163,164} The $\delta^{15}\text{N}_0$ of manufactured RDX typically spans a range of -17‰ to -4‰ depending on synthesis method and raw materials.¹⁶⁵ As shown in Figure 10-33a, the overall $\Delta^{15}\text{N}$ was associated with a decreasing RDX concentration along the plume, suggesting that RDX concentration losses were due in part to N-containing bond cleavage and not solely an artifact of non-degradative processes (i.e., sorption, dilution, volatilization, etc). Samples collected from L800-TT-MW09 ($\delta^{15}\text{N} = 6.4\text{‰}$) and 800-MW-25 ($\delta^{15}\text{N} = 6.5\text{‰}$) showed similar isotope ratios but distinct RDX concentrations ($2.8 \mu\text{M}$ and $0.8 \mu\text{M}$, respectively). Thus, the concentration of RDX in 800-MW-25 was assumed to be more affected by non-degradative processes compared to the other wells. Because of the consistent $\Delta^{15}\text{N}$ values, these data directly reflect the effects of mass transfer processes that cause apparent losses of contaminant concentration but do not elicit transformation.

We predicted the concentration of RDX in each well with the average isotope enrichment factor obtained from the batch experiments ($\epsilon_{\text{N}} = -7.4\text{‰}$) and the assumption that RDX reduction along the groundwater transect was only caused by abiotic reduction. As with the column experiments (see section 10.5.2.6), the predicted concentrations are consistently higher (less conversion) (Figure 10-33a, blue bars) than the observed concentrations of (Figure 10-33a, red bars), suggesting that other non-fractionating processes are affecting RDX concentration. Thus, while lower concentrations are at least partially due to reactive processes, total contaminant mass may not have decreased by a similar extent. The difference in predicted/measured conversions could be caused by the complexity of the hydrology and biogeochemistry in the subsurface. In heterogenous aquifers with multiple flow paths, RDX (bio)degradation will not occur at an equal rate in each flow path. Groundwater extracted from a monitoring well will thus contain contributions of residual RDX that has been isotopically fractionated (i.e., degraded) to different

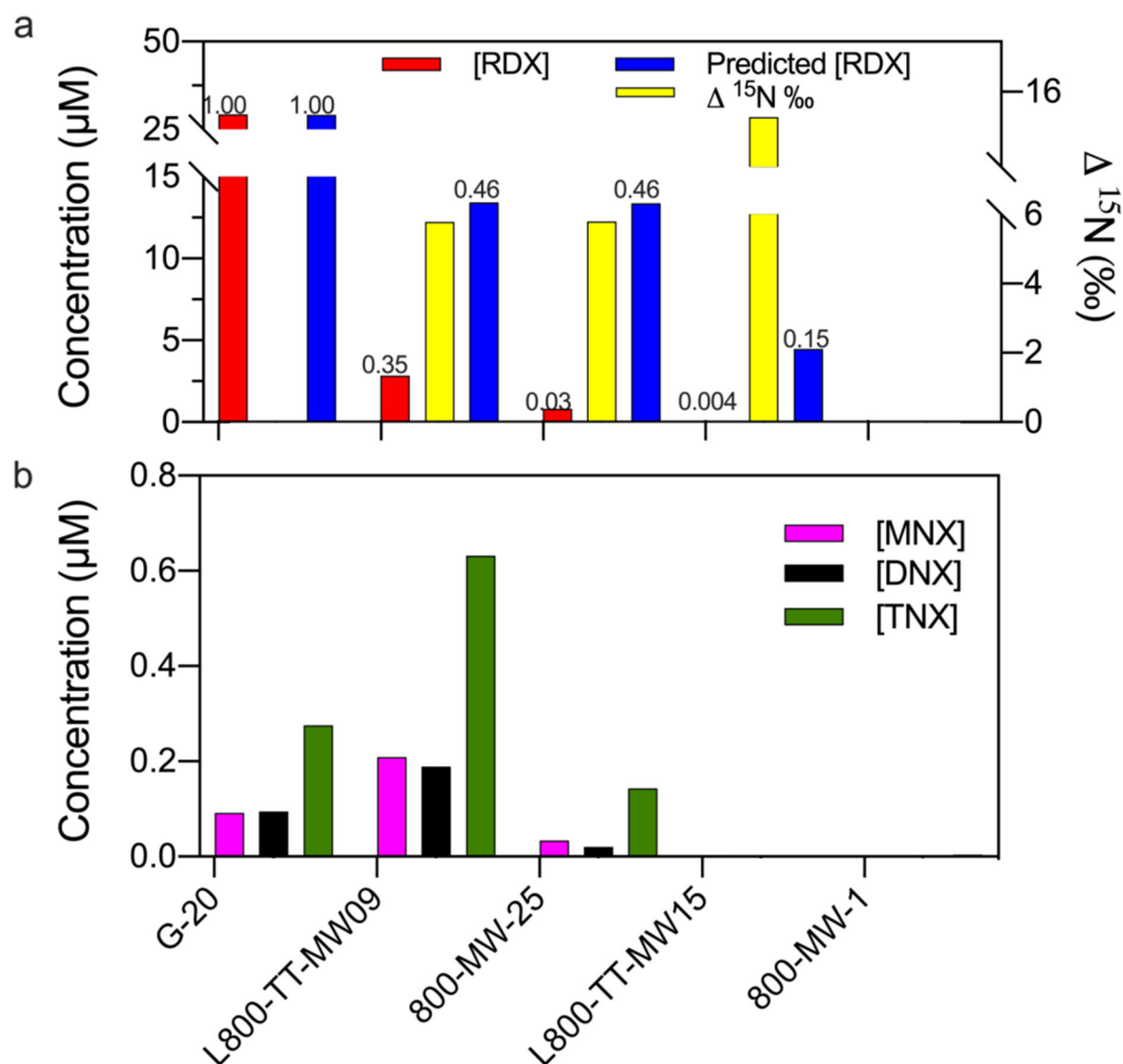


Figure 10-33. Evaluation of RDX transformation in monitoring wells along Line 800 at IAAAP. (a) The aqueous concentrations of RDX are plotted against the left Y-axis. The assumption was made that the concentration of RDX in G-20 represents the concentration and $\delta^{15}\text{N}_0$ of the contaminant source. The changes in N-isotope ratio ($\Delta^{15}\text{N} = \delta^{15}\text{N} - \delta^{15}\text{N}_0$) of RDX are plotted against the right Y-axis in yellow bars. The predicted RDX concentration in each well based on the lab-determined ^{15}N enrichment factor, $\epsilon_N = -7.4\text{‰}$ are plotted blue bars. The numbers on top of bars indicate the extents of transformation, measured (red bar) or predicted (blue bar), based on the assumption that G-20 has no RDX transformation (b) The aqueous concentrations of MNX, DNX, and TNX in the monitoring wells

extents. Because our use of CSIA only considers the $\delta^{15}\text{N}$ of unreacted RDX, the measured values of ^{15}N in groundwater will more closely reflect the fractionation of unreacted RDX with increasing distance from the pollutant source (G-20 in this study). This complicates the

interpretation of RDX degradation by CSIA because it suggests that RDX fractionation (i.e., RDX degradation) did not occur or was less than the actual amount and leads to an underestimate of the extent of RDX reduction.^{163,166} Stakeholders must therefore exercise caution when using the results of CSIA as the only criterion to evaluate pollutant removal in sites with complex hydrogeology and geochemistry. Moreover, it supports the use of multiple lines of evidence to quantify pollutant (bio)degradation by monitored natural attenuation in the environment.¹³⁴

The simultaneous identification of reaction products is an important line of evidence to produce more accurate estimates. For example, the combination of nitroso intermediates in monitoring wells (Figure 10-33b), the decreasing concentration of RDX, and the enrichment of ¹⁵N support the occurrence of nitro group reduction at IAAAP.^{14,91} Even though CSIA is useful in demonstrating that reactions are occurring, the formation of reaction intermediates is important in identifying the responsible reaction processes when fractionation profiles are confounded by other degradative and non-degradative factors. Nonetheless, the results demonstrated the potential of CSIA in combination with product analysis to validate abiotic reduction as a dominant pathway for RDX degradation, with further efforts needed in improving analytical methods to mitigate interference from nitroso intermediates and thorough characterization of geochemistry and hydrology at field sites for field application of CSIA.

10.5 Task 5 – Regeneration/Enhancement of Reactivity

10.5.1 Rationale

In natural systems, ferrous iron is replenished by iron reducing bacteria. It is possible that munitions compounds will consume the reactive capacity present in a contaminated groundwater system. It may also be desired to enhance reactivity by manipulation of the system to effect treatment. One approach to enhance reactivity is through the application of an in-situ chemical reduction strategy wherein a chemical reductant is supplied to a subsurface system to promote the generation of reaction-accessible reduction equivalents. Sodium dithionite was selected as the chemical reductant in this work because of its previous success remediating contaminated soils and sediments. Reduction experiments were performed in batch reactors using each synthetic and natural material and in packed column reactors using the natural materials.

10.5.2 Results and Discussion

10.5.2.1 Fe content after ISCR

The measured Fe(II) percent contents in the natural materials after ISCR were mostly much lower than the anticipated theoretical percentage (Table 10-14), except that the theoretical and measured values were identical at 3% reduction of TCAAP. The lower value of measured Fe(II) content was likely due to the incomplete consumption of ISCR reagent. Additionally, the extent of iron (oxy)hydroxide dissolution using HCl is likely incomplete¹⁶⁷ which could also contribute to the imbalance of Fe(II) content in the measured values.

Table 10-14. Fe(II) content in natural aquifer materials after dithionite reduction.

Material	Mineral mass (g)	Total Fe in raw material (μmol)	Theoretical Fe(II)/Fe _{tot.} after dithionite (%)	Fe(II) after dithionite (μmol)	Measured Fe(II)/Fe _{tot.} after dithionite (%)
			3	78.4	3.1
TCAAP	6	2571	15	161.0	6.3
			20	210.7	8.2
TAFB	1	535.71	12	15.8	2.9

10.5.2.2 DNAN reduction with ISCR treated materials in batch reactors

To evaluate the extent of contaminant reduction by the reduced materials, electron balances were calculated for each set of reactions. The quantity of electrons transferred to DNAN from each reduced material was calculated with the assumption that 6 moles of electrons are required to reduce one nitro-moiety to the corresponding amine. Thus, 6 and 12 electrons are required for each mole of 2- or 4-ANAN and DAAN formed during DNAN reduction, respectively (eq. 12).

$$\text{total electrons transferred} = (6c_{2\text{-ANAN}} + 6c_{4\text{-ANAN}} + 12c_{\text{DAAN}}) * V_R \quad (12)$$

where V_R is the reactor volume (35 mL) and the analyte concentrations (c) are those at the endpoint of batch experiments. Similarly, the cumulative number of electrons transferred at any point during column experiments were calculated by integrating the curves of c/c_0 vs. pore volumes according to eq. 13.

$$\text{total electrons transferred} = \int_0^V (6c_{2\text{-ANAN}} + 6c_{4\text{-ANAN}} + 12c_{\text{DAAN}}) dV \quad (13)$$

where c is the analyte concentration measured at the column outlet and V is the volume of DNAN solution introduced to the column.

In reactors receiving ISCR, only a portion of the DNAN was removed (Figure 10-27, closed symbols), likely because of a limited supply of available electron equivalents (i.e., mineral-associated Fe(II)) generated during ISCR. To understand the extent of DNAN reduction in systems receiving ISCR, an electron balance was computed for each set of reactions (Figure 10-34, dashed line). Accounting for the number of electrons transferred to DNAN as a function of the mineral-associated Fe(II) (i.e., mol e^- /mol Fe(II)) allowed for an evaluation of the efficiency of ISCR to generate reactive Fe(II). As observed during DNAN reduction in reactors of untreated minerals with aqueous Fe(II) (Figure 10-27, open symbols), the transformation products detected during the experiments were 2-ANAN, 4-ANAN, and DAAN.

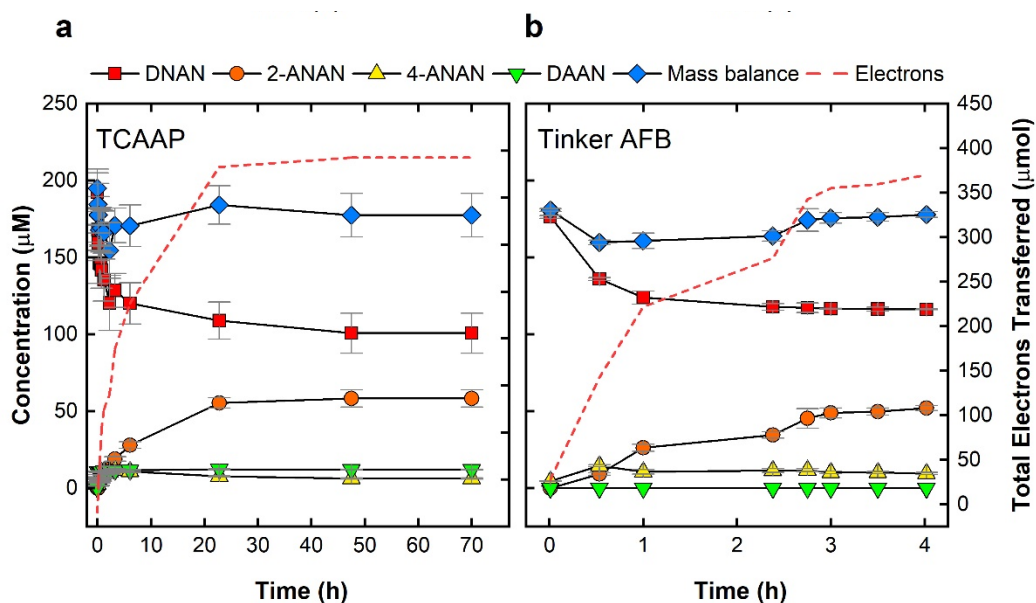


Figure 10-34. Concentrations of DNAN, 2-ANAN, 4-ANAN, DAAN, and the cumulative number of electrons transferred during DNAN reduction by reduced (a) TCAAP and (b) Tinker AFB materials. All error bars represent standard deviations of triplicate reactors. Note the difference in time scales.

Dithionite-reduced magnetite, TCAAP extract, and hematite promoted DNAN reduction with extents of electron transfer ranging from 0.359–0.603 mol e^- /mol Fe(II) (Table 10-15). These results suggest that not all available iron (Fe_T) was reduced to reaction-accessible Fe(II)¹⁶⁷ during ISCR. The washing of materials following dithionite treatments may have caused the release of Fe(II) that was not retained on mineral surfaces. It is also possible that potentially reducible oxide-Fe(III) was inaccessible to dithionite.^{104,168–170}

The lower standard reduction potential (E_H^0) of hematite/Fe(II) (+0.793 V) versus magnetite/Fe(II) (+1.053 V)¹⁷¹ may further explain the difference in electron transfer between the two systems. The reactivity of Fe-bearing minerals for pollutant reduction increases with lower E_H values as evidenced by hematite experiments. Moreover, magnetite stoichiometry ($x = Fe(II)/Fe(III)$) directly correlates with its intrinsic E_H values²¹ and reactivity towards NAC reduction.¹⁴⁶ The magnetite used in this study ranged from partially oxidized ($x = 0.1$, TCAAP extract) to fully reduced, stoichiometric ($x = 0.5$, synthetic magnetite) materials. Low stoichiometry magnetite is a better oxidant and was thus more easily reduced by dithionite than the high stoichiometry magnetite (Table 10-15). These results emphasize the potential benefits of ISCR to magnetite-bearing soils and sediments in which partially oxidized magnetite is common and may be amenable to ISCR leading to more stoichiometric and thus reactive magnetite.^{172,173}

Table 10-15. Total iron (Fe_T) and Fe(II) content of materials after dithionite treatments, and the cumulative number electrons transferred during DNAN reduction experiments.

System	Fe_T (μmol)	Fe(II) (μmol) ^c	Total e^- transferred (μmol) ^{d,e}	mol e^- /mol Fe(II) ^e
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Batch				
TCAAP	3430 ^a	170	393 ± 1	2.31 ± 0.01
TCAAP extract	865 ^a	432	232 ± 1	0.536 ± 0.003
Magnetite	903 ^b	451	162 ± 0.3	0.359 ± 0.001
Tinker AFB	2180 ^a	79.1	370 ± 0.4	4.68 ± 0.01
Hematite	439 ^b	219	132 ± 1	0.603 ± 0.006
Column				
TCAAP	25800 ^a	1280	183 ± 30	0.143 ± 0.022
Tinker AFB	17000 ^a	616	109 ± 10	0.176 ± 0.020

^aDetermined by ICP-OES.

^bCalculated from structural formulas of pure minerals.

^cCalculated by acid dissolution and quantification by the ferrozine method.¹¹⁰

^dCalculated using eqs. 12-13.

^eUncertainties represent standard deviations of triplicate reactors.

The number of reduction equivalents transferred to DNAN from dithionite-reduced TCAAP (2.31 ± 0.01 mol e^- /mol Fe(II)) and Tinker AFB (4.68 ± 0.01 mol e^- /mol Fe(II)) materials exceeded the initial amount of Fe(II) present after dithionite treatments (Table 10-15). Despite targeted dithionite dosages to reduce one-tenth of the Fe_T , the calculated reduction efficiencies (i.e., Fe(II)/ Fe_T from data in Table 10-15) of TCAAP and Tinker AFB materials during ISCR were only 4.95% and 3.62%, respectively. The low reduction efficiencies suggest that other reducible moieties were present in the natural materials which were reduced by dithionite and subsequently provided reducing equivalents for DNAN reduction.^{174,175} For example, the reversible transfer of electrons in the environment is often mediated by natural organic matter and humic substances, specifically those containing quinone moieties,^{176–179} and the TCAAP and Tinker materials contained 0.46% (w/w) and 0.88% (w/w) organic matter, respectively. The electron-carrying capacity of these species plays a key role in both the reduction of substituted nitrobenzenes¹⁸⁰ and ferric iron.¹⁸¹ Another possibility is that acid digestion of the materials to determine Fe(II) was incomplete. This is especially likely in materials with high Si content, such as phyllosilicates, because the Fe(III) is easily reduced by dithionite but Fe(II) extraction requires a rigorous treatment with H_2SO_4 and HF.^{167,182,183} This fraction of Fe(II) is a known strong reductant of NACs.^{184,185} The prominence of SiO_2 (Figure 12-15) and low Fe_T content of TCAAP and Tinker AFB materials suggests that a large share of Fe_T was associated with silicates.

DNAN reduction by the dithionite-reduced nontronite clays (Figure 10-35) followed a similar pattern to the other dithionite-treated minerals (see Figure 10-27). During the experiments with clays, the initial amount of DNAN (200 μM) was reduced to approximately 20 μM and 75 μM for NAu-1 and NAu-2, respectively. Both clays have a similar amount of structural iron (34% as Fe_2O_3 in NAu-1 and 37.4% as Fe_2O_3 in NAu-2; Table), however, NAu-1 contains a higher amount of aluminum (10.2% as Al_2O_3) than NAu-2 (3.4%; Table). The difference in the amount of DNAN conversion between the two systems is therefore best explained by the greater presence of Al in NAu-1. Previous research has shown that Al-containing moieties on mineral surfaces also form Fe(II)-surface complexes that promote pollutant reduction.^{186–189}

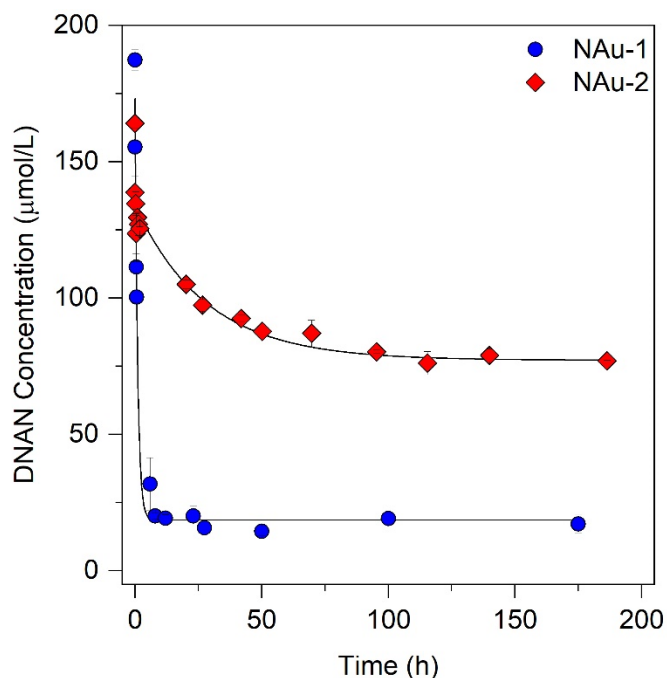


Figure 10-35. Reduction of 200 μM DNAN by two dithionite-reduced nontronite clays: NAu-1 (circles) and NAu-2 (diamonds).

DNAN reduction in batch reactors introduced strong ^{15}N enrichment in the remaining contaminant with variations in magnitude comparable to those observed in earlier studies (Figure 3). ^{15}N isotope enrichment factors, ϵ_{N} , ranged from $-11.1 \pm 4.3\text{‰}$ to $-21.5 \pm 2.6\text{‰}$ corresponding to ^{15}N -AKIEs of 1.023 ± 0.009 to 1.045 ± 0.005 (eq. 3). Because no bonds to C atoms are involved during the reactions leading to nitro-group reduction, C isotope fractionation was small and resulted in secondary ^{13}C -AKIE values (1.0005 ± 0.0002). These results are typical for nitro-group reduction on NACs by Fe(II) species associated with minerals (i.e., large ^{15}N -AKIE = 1.030–1.045 and ^{13}C -AKIE close to unity).^{21,29–31} The variability of isotope effects is related to uncertainties associated with experimental and data evaluation procedures such as a different number of samples with variable extent of reactant conversion included in the regression analysis.¹¹⁸ We postulate these effects as the likely sources of variation in ϵ_{N} values reported for experiments with reduced sediments (Figure 10-36). In fact, our previous report of DNAN reduction by iron (oxyhydr)oxides revealed ϵ_{N} values between $-9 \pm 2\text{‰}$ and $-19 \pm 1\text{‰}$ corresponding to ^{15}N -AKIEs of 1.018 ± 0.002 and 1.039 ± 0.001 , respectively, as well as the small ϵ_{C} and ^{13}C -AKIE values that are consistent with previous studies.¹⁶² The kinetics of electron and proton transfers preceding the isotope sensitive step of aromatic nitro-group reduction could also lead to variation of ^{15}N -AKIE through partially masking of the isotopically sensitive bond cleavage reaction(s)^{21,29,47} and smaller ^{15}N -AKIEs were found with increasing rates of NAC reduction.²⁹ However, no such trends were observed in our studies with DNAN (Table 12-3) and further investigation of this phenomenon is beyond the scope of this work.

The agreement in combined ^{15}N - and ^{13}C -AKIEs between this and other reports of abiotic NAC reduction^{29–31} suggests that DNAN reduction followed the same reaction mechanism (i.e., nitro-group reduction) in the presence of Fe(II)-amended synthetic and natural materials and with the naturally collected materials after ISCR. Dual-element isotope analysis ($\delta^{15}\text{N}$ vs $\delta^{13}\text{C}$) was used

to support this interpretation by illustrating the distinction of our results from other known DNAN transformation pathways (see Figure 12-5).¹³³ For example, the correlation slope ($\Lambda^{N/C}$) calculated from this study was 43.8 ± 28.6 (38.9 ± 4.3 calculated based on Ojeda et al.¹⁹⁰) which is in clear contrast with independent evidence from biodegradation ($\Lambda^{N/C} = 0.87 \pm 0.15$) and alkaline hydrolysis ($\Lambda^{N/C} = 0.46 \pm 0.04$) experiments (Section 12.1.6.4). This is consistent with the $\Lambda^{N/C}$ of 50.5 ± 23.2 during Fe(II)-mediated DNAN reduction by synthetic iron (oxyhydr)oxides.

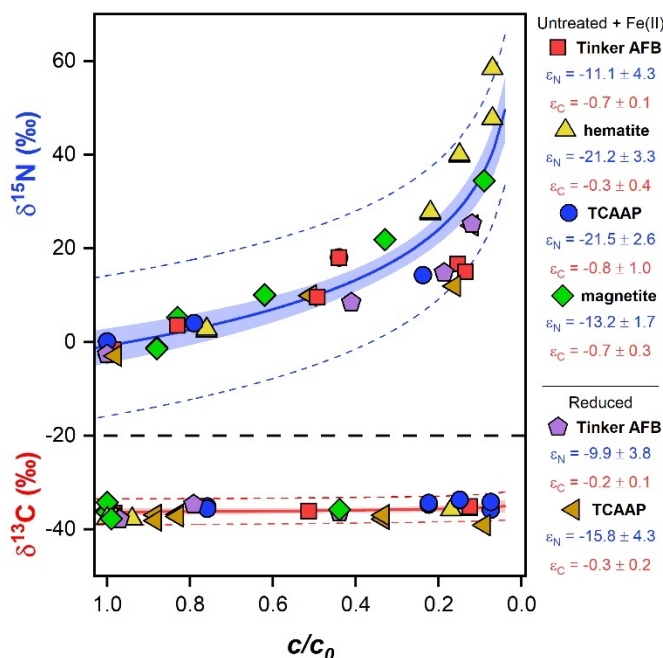


Figure 10-36. Nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) isotope signatures vs fraction of remaining substrate (c/c_0) during DNAN reduction by iron-bearing minerals. Solid lines represent fits from nonlinear regression with shaded portions indicating the 95% confidence intervals. Dashed lines designate 95% prediction intervals. N and C isotope enrichment factors (ϵ_N , ϵ_C) were determined from non-linear regression of the data points for each mineral type. Data are shown for untreated materials in the presence of Fe(II) and dithionite-treated materials.

10.5.2.3 Limited reductant DNAN batch experiments

DNAN was partially reduced by synthetic and natural materials that received in situ chemical reduction when no additional Fe(II) was added (Figure 10-37). For each set of experiments, the dosage of dithionite was selected to theoretically reduce half of the total Fe originally present in each mineral. Details of the fitting procedure are provided in Section 9.8. In general, the second-order rate constants for DNAN reduction in each mineral suspension were between 0.004–0.012 $\mu\text{M}/\text{h}$ except for hematite ($0.006 \pm 0.002 \mu\text{M}/\text{h}$). Approximately one half of the initial DNAN (200 μM) was reduced by each mineral (vertical arrows in Figure 10-37); higher extents of reduction occurred with NAu-1 (90%) and carbonate green rust (GR_{CO_3} , 88%) and less DNAN was reduced by hematite (16%). For these reactions, the amount of DNAN reduced is equivalent to the initial reductant concentration (R_0) described in eq. 8.

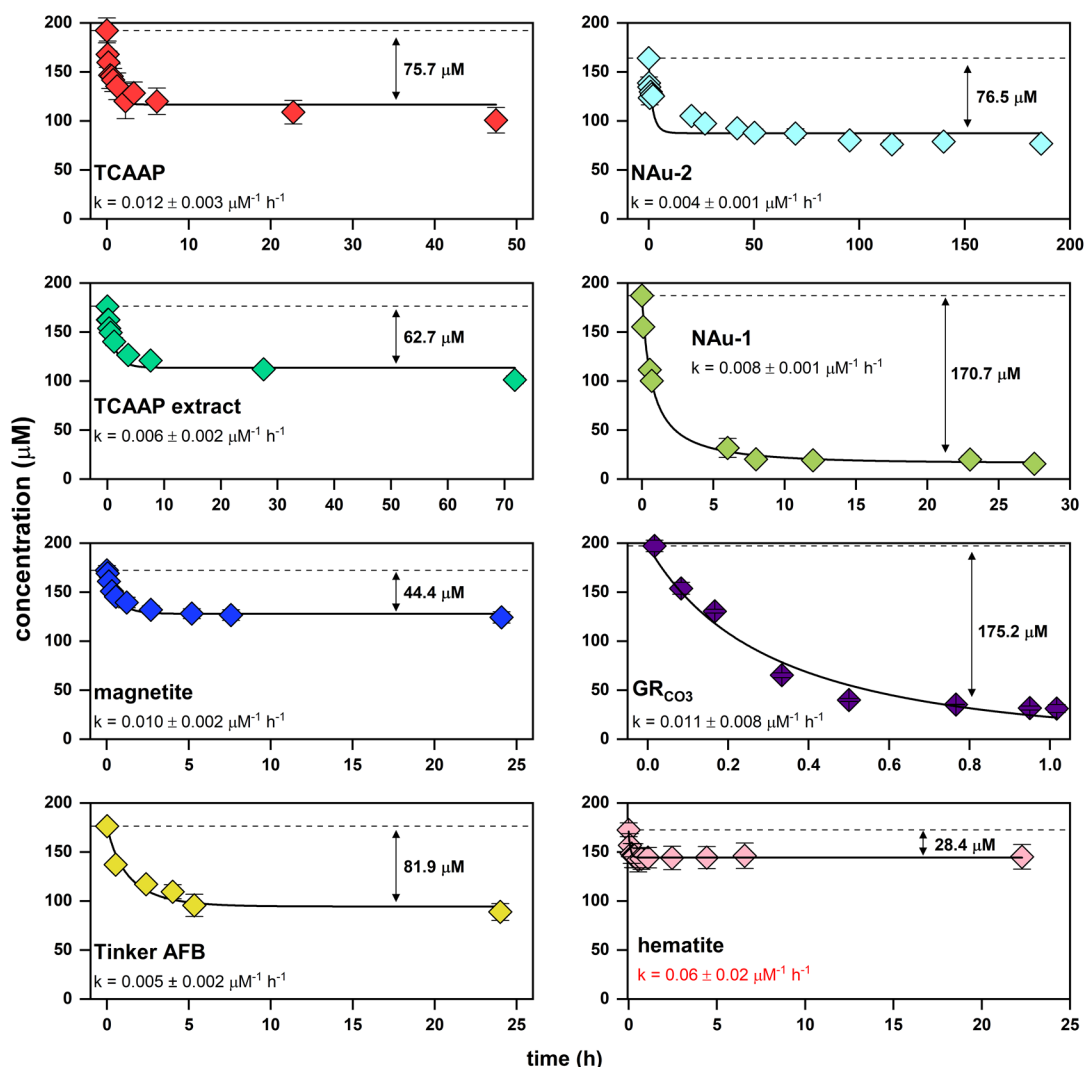


Figure 10-37. DNAN reduction by dithionite-reduced synthetic and natural materials during batch reactions without supplemental Fe(II). Values of k represent the calculated second-order rate constants. Dotted lines indicate the initial concentration of DNAN in each reactor. The amount of DNAN reduction is shown by the vertical arrows.

10.5.2.4 DNAN reduction in ISCR treated columns

To probe for the availability of reduction equivalents in flow-through systems, DNAN (200 μM) was introduced to column reactors containing dithionite-reduced TCAAP or Tinker AFB materials for five cycles of Fe(III) reduction by dithionite followed by DNAN exposure (Figure 10-38a-b). As shown in Figure 10-38c-d, ISCR in column reactors generated reaction-accessible electron equivalents in natural materials and promoted the reductive transformation of DNAN with a similar product distribution to the batch experiments (Figure 10-36c-d, Figure 12-17). It should be noted that DAAN concentrations exceeded the input DNAN concentration (up to 150%) during the early stages of experiments. To assess this phenomenon, the cumulative amount of DAAN measured in the column effluent was compared to the total amount of DNAN introduced. We found that the amount of DAAN in the peaks did not exceed the total amount of DNAN introduced to the columns. This indicated that DAAN was either associated with or

retained on mineral surfaces and was then displaced by another solute, namely DNAN or 2/4-ANAN. The initial rapid production of DAAN leading to high concentration may have driven this interaction, and as aqueous concentrations dropped, other components in

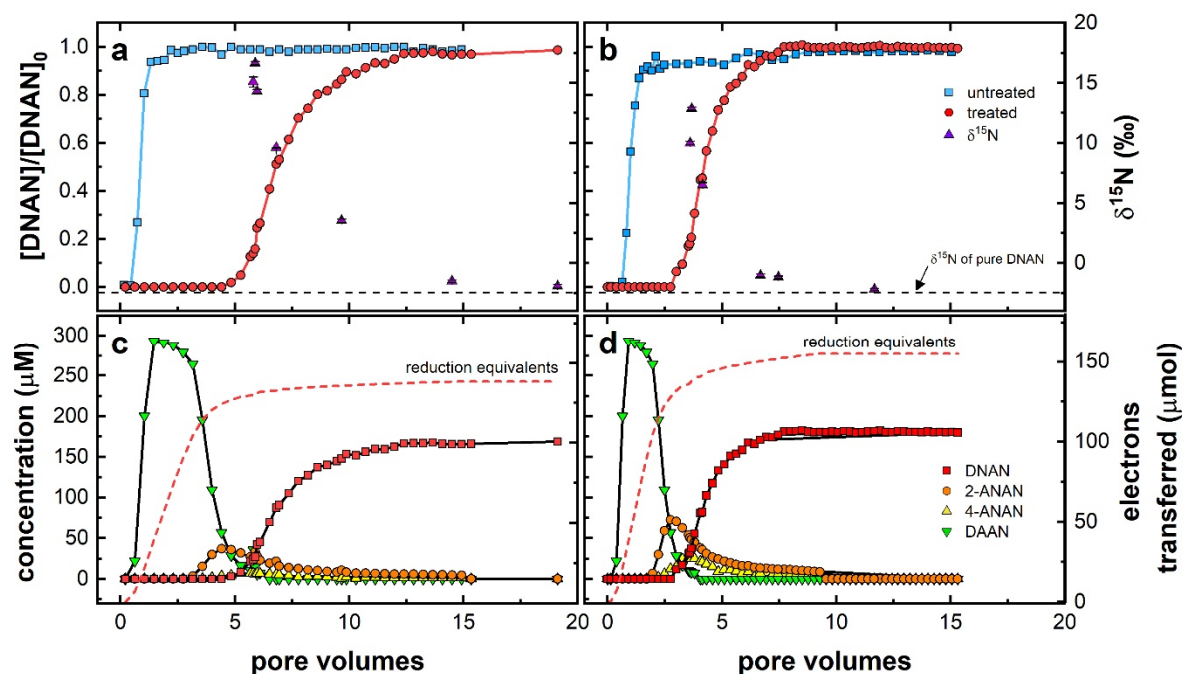


Figure 10-38. Breakthrough curves for 200 μM DNAN in (a) TCAAP and (b) Tinker AFB packed columns before (squares) and after (circles) receiving ISCR. Triangles denote measurements of $\delta^{15}\text{N}$ of DNAN in the effluent from treated columns. Error bars denote standard deviations from five sequential breakthrough experiments. Aqueous concentrations of DNAN (circles), 2-ANAN (squares), 4-ANAN (triangles), and DAAN (diamonds) during DNAN exposure to (c) TCAAP and (d) Tinker AFB columns. Dashed lines represent the cumulative number of reduction equivalents transferred to DNAN during experiments. The data provided in panels c and d are a representation of one of the reduction-reaction cycles shown in panels a and b, respectively. Individual cycles are shown in Figure 12-17.

the solution displaced DAAN from the surface. Such competition and effluent concentrations exceeding influents have been observed for ion sorption on activated carbon.¹⁹¹ Another possible explanation is that a reaction intermediate (e.g., a hydroxylamine) is more strongly retained by the solid phase, as previously observed during the reduction of cyanonitrobenzene,¹⁹² and the excess DAAN observed occurs once this intermediate has been reduced and released.

Despite ISCR targets that were equivalent to batch experiments (i.e., 10% reduction of Fe_T), the extent of electron transfer to DNAN from the reduced materials was markedly lower in columns than in batch experiments (0.143 ± 0.022 and 0.176 ± 0.020 mol e^- /mol $\text{Fe}(\text{II})$ for TCAAP and Tinker AFB columns, respectively; Table 10-15). This suggests that either (i) reactive $\text{Fe}(\text{II})$ was removed from the column, (ii) the transport of dithionite or DNAN to mineral surfaces was

restricted by conditions within the column, or (iii) the extent of mineral reduction was affected by the rate of dithionite decomposition.

Flow-through systems introduce the potential for newly generated Fe(II) or other reducible species to be removed from the column before association with mineral surfaces or pollutant reduction occurs. Monitoring aqueous Fe(II) in column effluents, however, negligible losses of Fe(II) from the column were observed during and after exposure to dithionite (Figure 12-18). Only 1.6 and 5.3 μmol Fe(II) in TCAAP columns and 2.6 and 4.0 μmol Fe(II) in Tinker AFB columns were removed during the first and fifth exposures to dithionite, respectively, corresponding to 0.06–0.20% (w/w) of Fe_T. This suggests that Fe(II) generated during ISCR was mostly retained within the column either because it was not released from the mineral surfaces or it was effectively adsorbed by or bound within the materials within the column. The presence of silicates may also indicate a portion of Fe(II) that is not readily removed from natural soils and sediments. The consistency of breakthrough curves during the five cycles of ISCR and DNAN exposure (Figures S3) indicates that the removal of reduction equivalents by column flow likely did not affect the potential for contaminant reduction.

It is also possible that the transport of reducing equivalents or pollutants to mineral surfaces is restricted in confined systems composed of heterogeneous media.¹⁹³ Moreover, the iron content of natural sediments can be distributed throughout the bulk mineral as opposed to being concentrated at singly-coordinated oxygen atoms on mineral surfaces, leaving Fe(III) inaccessible to dithionite. To account for these potential limitations, the columns in this study were packed to a uniform porosity (0.44 ± 0.05), bulk density ($1.66 \pm 0.04 \text{ g cm}^{-3}$), and particle density ($2.68 \pm 0.05 \text{ g cm}^{-3}$) to resemble typical subsurface conditions in sandy soils similar to the TCAAP and Tinker AFB (Table 12-2).¹⁶⁸ The dispersion coefficients in TCAAP ($2.37 \pm 0.22 \text{ cm}^2 \text{ s}^{-1}$) and Tinker AFB ($2.53 \pm 12 \text{ cm}^2 \text{ s}^{-1}$) columns were also determined and indicated advection-dominated regimes with negligible effects from flow retardation.¹⁹⁴ Column porosities and dispersion coefficients were remeasured following all experiments and were not appreciably different from the initial values (Table 12-2), indicating that ISCR did not affect the physical properties of columns or restrict material transport.

Lastly, the decomposition of dithionite can unevenly distribute iron reduction by ISCR in columns. In batch reactors, the entire supply of dithionite was simultaneously exposed to all of the solid material in a well-mixed heterogeneous suspension, whereas, in columns only the materials located immediately following the inlet were exposed to unreacted dithionite. Because dithionite undergoes rapid disproportionation in aqueous media, the combination of Fe(III) reduction and dithionite decomposition limits mineral reduction at locations away from the point of application.^{116,195} These differences explain the difference between the batch and column experiments and provide an explanation for the lower extent of DNAN reduction in the column.

It is possible to quantify DNAN transformation from ^{15}N enrichment in the residual contaminant. As shown in Figure 10-38a-b, $\delta^{15}\text{N}$ values of DNAN at the breakthrough front (~ 5 pore volumes) were isotopically enriched in ^{15}N corresponding to an increase of $\delta^{15}\text{N}$ by 15–20‰. Measured values subsequently decreased and approached the value for unreacted DNAN ($-2.4 \pm 0.1\text{‰}$) as the effluent concentrations approached the input level. This trend of $\delta^{15}\text{N}$ is consistent with the elution of an increasing share of DNAN that has not been reduced by surface-associated

Fe(II). While the data provide evidence that ISCR promoted DNAN reduction, it should be noted that $\delta^{15}\text{N}$ values were not fully restored to the input $\delta^{15}\text{N}$ value ($-1.9 \pm 0.1\text{‰}$ and $-2.2 \pm 0.1\text{‰}$ in columns with TCAAP and Tinker AFB sediment, respectively) suggesting the retention and slow release of enough residual ^{15}N -enriched DNAN from the mineral surfaces to influence the $\delta^{15}\text{N}$ after the reaction capacity was exhausted or residual, low level transformation activity.^{196–198}

Based on the above reasoning for the variability of N isotope fractionation associated with NAC reduction, we aggregated all of our current data¹⁶² for isotope fractionation during Fe(II)-catalyzed DNAN reduction in batch experiments. The combined N isotope ratio measurements resulted in an averaged ϵ_{N}^* value of $-14.9 \pm 1.3\text{‰}$, which is based on one of the most comprehensive data sets for the stable N isotope fractionation related to the transformation of a single nitroaromatic contaminant (Figure 12-20a). This value was used to establish a quantitative relationship of c/c_0 vs $\Delta^{15}\text{N}$ (Figure 12-20b) to estimate the amount of DNAN degradation from $\delta^{15}\text{N}$ measurements made during column experiments (Figure 10-38a-b). Values of ϵ_{N} calculated from TCAAP ($-8.6 \pm 1.8\text{‰}$) and Tinker AFB ($-7.2 \pm 0.8\text{‰}$) column experiments were lower than batch experiments which could indicate longitudinal mixing with unreacted DNAN in the feed solution. This interpretation agrees with systematic evaluations of the applicability of eq. 2 to assess contaminant transformation in groundwater plumes,¹⁹⁹ where physical heterogeneity, geometry of the contaminant plume, and the extent of degradation can lead to an underestimation of isotope enrichment factors.

Application of the higher average ϵ_{N}^* value of $-14.9 \pm 1.3\text{‰}$ from batch experiments for the evaluation of the extent of DNAN reduction in the sediment columns from the $\Delta^{15}\text{N}$ of DNAN leads to predicted values that differ from the actual extent of conversion (Figure 10-39). A linear regression of predicted versus measured quantities showed a correlation slope of 1.27 ± 0.18 ($r^2 = 0.96$). By not forcing the regression through zero, predicted values overestimate the extent of conversion at high c/c_0 and underestimate conversion at low c/c_0 . While the mean absolute error (MAE) is 0.091 ± 0.063 in c/c_0 , the inset in Figure 10-39 reveals that the relative error is greater at larger conversions and illustrates that in our laboratory model system, the accuracy of the assessment of the extent of degradation decreases with increasing turnover of the contaminant. That said, underprediction of the extent of conversion at low c/c_0 provides a margin of safety.

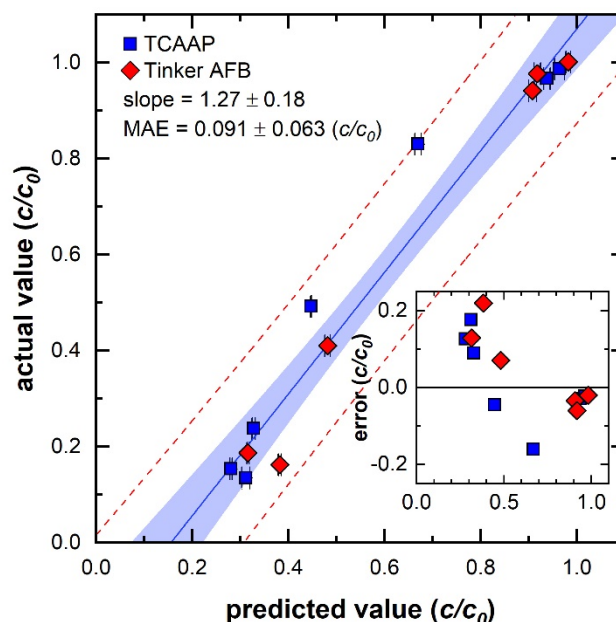


Figure 10-39. Predicted versus measured values of DNAN transformation by chemically reduced TCAAP and Tinker AFB materials in column reactors. Predictions were made according to eq 2 using an ϵ_N^* value of -14.9‰. The solid line indicates the calculated fit by linear regression. Shaded portions indicate 95% confidence intervals of linear regressions and dashed lines represent the 95% prediction intervals. The inset shows the prediction error of each estimate.

While a number of additional factors pertinent to the hydrology of the contaminated subsurface will contribute to the successful application of CSIA,²⁰⁰ Figure 10-39 illustrates that under the assumption that the same reaction is occurring, there is a basis for using the N isotope fractionation of DNAN measured during laboratory experiments as a proxy of transformation in the field. To confirm the specificity of our model to N isotope fractionation associated with DNAN reduction, we also measured the accompanying $\delta^{13}\text{C}$ values of DNAN to the $\delta^{15}\text{N}$ data shown in Figure 10-38a-b. The observed minimal C isotope fractionation of DNAN at the breakthrough front ($\Delta^{13}\text{C} < \sim 3\text{‰}$; data in Figure 12-21) is in agreement with the postulated reduction reaction. Because this C isotope fractionation was not observable after 6-7 pore volumes where N isotope fractionation continued, we also consider it unlikely that another reaction such as concurrent (bio)degradation processes contributed to our observations where C isotope fractionation would be more substantial based on the reported ϵ_C of -3‰ vs. -0.3‰ shown in Figure 10-36.¹³³ The distribution of $\delta^{13}\text{C}$ against $\delta^{15}\text{N}$ from column experiments also reflected the batch systems (Figure 10-36 and Figure 12-19) and supports that, in both cases, abiotic reduction was the primary reactive pathway. While we cannot exclude small contributions of sorption processes to the observed DNAN isotope fractionation in dithionite treated columns, data with untreated sediment (Figure 12-16) suggest that this process is of minor importance.

10.5.2.5 RDX reduction by ISCR treated material in batch reactors

TCAAP sediment was treated with sodium dithionite to theoretically achieve 3%, 15%, and 20% of the iron reduction in the material. The actual Fe(II) content in the reduced sediment is shown in Table 10-14. Figure 10-40 shows the degradation of RDX over time by reduced TCAAP sediments. The values of measured Fe(II) content ($\text{Fe(II)}_{\text{total}}$) are given in Figure 10-40. The

degradation of RDX followed the second-order kinetics, because the reductant, i.e. reactive Fe(II) in the ISCR treated sediment, was limited. Because the actual amount of reductant was unknown, a second-order kinetic model assumed an equivalent reductant value, R_{eq} (eq. 8, which was calculated by fitting the kinetic model to the experimental data. The total Fe(II) in the sediment was not exhausted, because the R_{eq} values were much smaller than $Fe(II)_{total}$. Even though Fe(II) originating from the reduction of structural Fe(III) is reactive towards reducible munition pollutants, the availability and reduction capacity of the reactive Fe(II) species could be a function of natural material properties.²⁰¹ The rate constant of 3% reduced TCAAP ($k=0.01634 \mu M^{-1} h^{-1}$) is much higher than the 6% and 8% reduced TCAAP. This suggested the disparity in the availability of reactive Fe(II) in the materials, i.e. a limited pool of Fe(II) in 3% reduced TCAAP was highly reactive and available. The nitroso intermediates accumulated in the reactor (Figure 10-41). The mass balance is incomplete (Figure 10-41, solid line) indicating the formation of other products from RDX or the nitroso compounds.

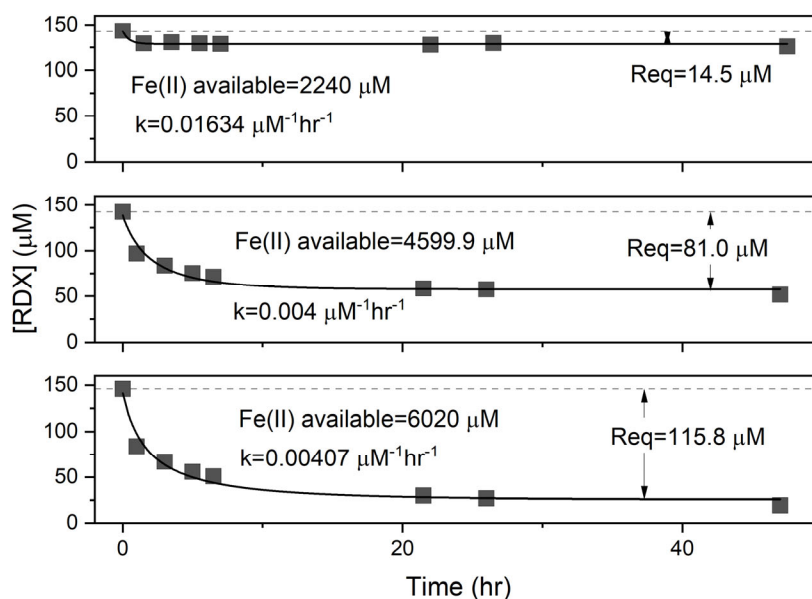


Figure 10-40. RDX degradation over time by dithionite treated TCAAP sediment. The reaction followed second-order kinetics. In theory, the iron mineral in TCAAP soil was reduced to 3% (top), 6.3% (middle), and 8.2% (bottom). Experiments were conducted in 10 mM $NaHCO_3$ buffer at pH 7.

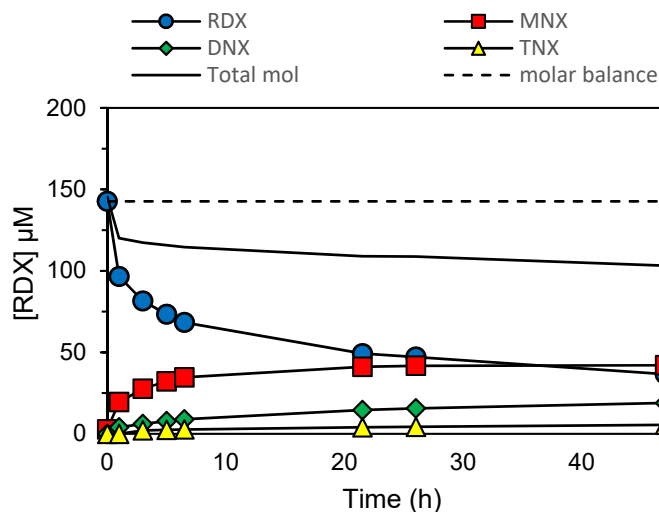


Figure 10-41. Concentration of RDX, TNX, DNX, and MNX during reduction by dithionite-treated TCAAP (target 6.3%) in a batch reactor. The solid line is the observed mass balance, and the dashed line is the initial concentration of RDX.

10.5.2.6 Results for RDX in an ISCR column

We continued ISCR experiments in a borosilicate glass column (Kimble FLEX-COLUMNS®, I.D. 2.5 cm). The column was packed with 4.8 cm of TCAAP sediment (porosity=33.5%). A 100 mM NaBr solution was continuously fed as a tracer (Figure 10-42). The column was then conditioned with 10 mM NaHCO₃ buffer for several pore volumes to remove the residual NaBr. An RDX reservoir was prepared in 10 mM NaHCO₃ buffer with 200 μM of RDX. The RDX solution was fed in the TCAAP column at 0.5 mL/min, and there was immediate RDX breakthrough similar as the tracer, indicating that there was no RDX degradation or adsorption (Figure 10-42). The TCAAP sediment was then reduced by feeding sodium dithionite and K₂CO₃ mixed solution at 0.25 mL/min for 18 hours, followed with feeding RDX solution to interact with reduced TCAAP for 36 pore volumes. This dithionite treatment-RDX reduction was repeated for three cycles, with column flushing using NaHCO₃ buffer in between cycles. After treated with dithionite, iron minerals in TCAAP sediment reduced RDX (Figure 10-42). The RDX breakthrough pattern was consistent for the first two cycles. We obtained samples indicated as yellow data points in Figure 10-42, for further isotope measurement. We were able to conduct CSIA because the low accumulation of nitroso intermediates in the effluent (Figure 10-43). RDX breakthrough during the third cycle indicated that there was more reductive capacity after the third treatment of TCAAP sediment, but this was not explored further.

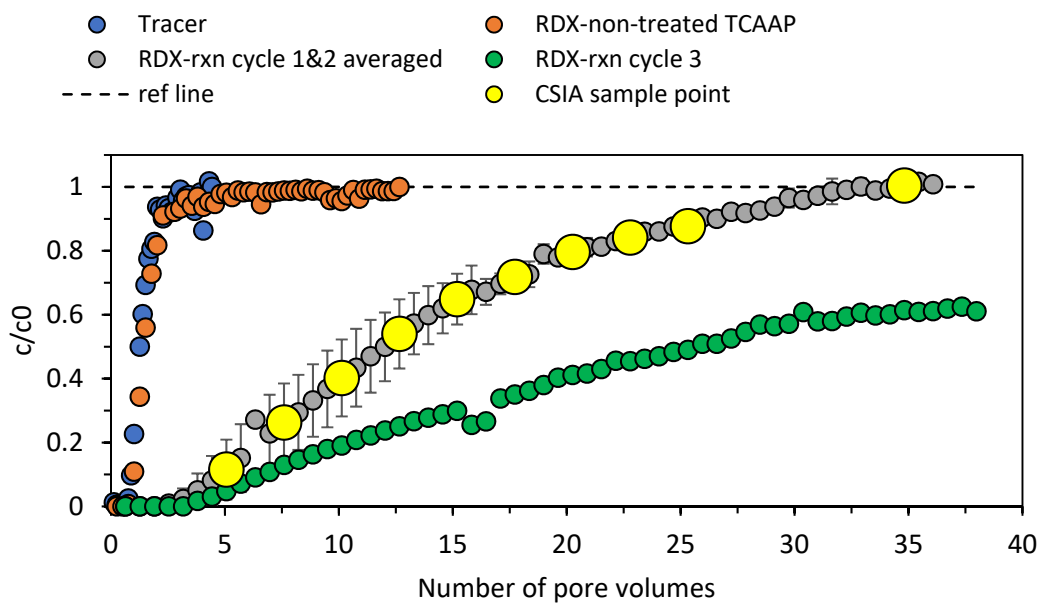


Figure 10-42. Breakthrough of NaBr tracer and RDX in TCAAP sediment column. RDX interacted with the non-treated TCAAP sediment, as well as the dithionite-treated sediment. Dithionite treatment was repeated three times.

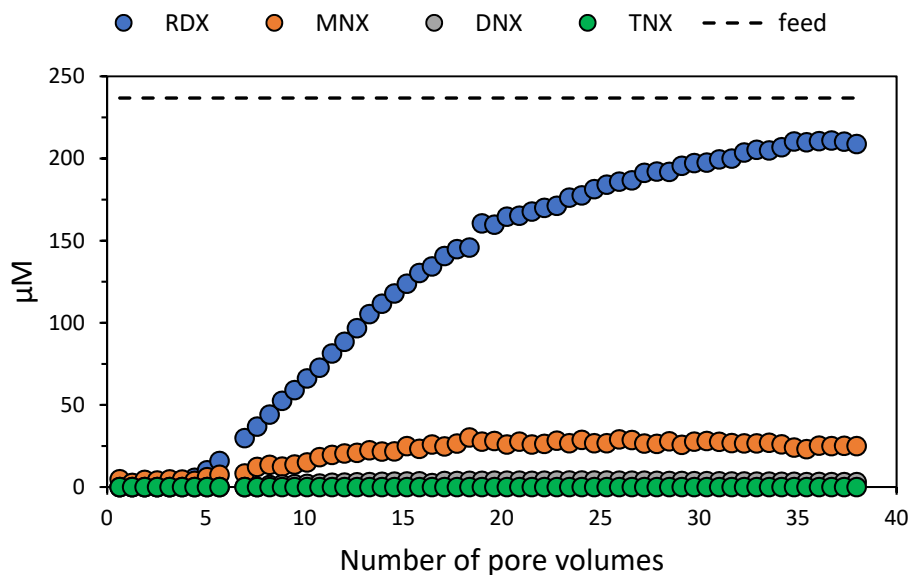


Figure 10-43. RDX, MNX, DNX, and TNX concentrations in the dithionite-treated TCAAP column effluent.

In Figure 10-44, the $\delta^{15}\text{N}$ values and extent of RDX conversion as a function of number of pore volumes is shown. Because of sample size requirements for GC-IRMS analysis, each sample contained approximately two pore volumes of effluent solution (17 mL), and thus the RDX concentration represents an average of this period. The $\delta^{15}\text{N}$ of RDX as a function of conversion (c/c_0) is similar for the two cycles (Figure 10-44a). Compared with batch experiments, the extent of N fractionation was lower during the column experiments (Figure 10-44a, change of isotope ratio $\Delta^{15}\text{N} = \delta^{15}\text{N} - \delta^{15}\text{N}_0 \sim 10\text{‰}$). Using the measured N enrichment factor $\epsilon_{\text{N}} = -7.4\text{‰}$ from the batch reactors, the predicted $\delta^{15}\text{N}$ as a function of c/c_0 reveals that a greater extent of fractionation was expected than what was observed in the column reactor.

A comparison of the predicted and measured extents of RDX transformation (Figure 10-44b) resulted in a linear correlation (slope = 2.05 ± 0.76 , $r^2 = 0.67$) that did not pass through the origin. The offset of the x-intercept (0.57 as c/c_0) suggests that using the results from batch experiments to predict conversion in column reactors will cause overestimates of the extent of RDX transformation. This may be caused by processes that decrease the RDX concentration but are not reflected in the isotope fractionation data (e.g., dispersion, variable flow patterns, inconsistent mixing). Despite these uncertainties, the predicted values underestimate the actual conversion and the isotope fractionation data will produce a conservative estimate of the extent of RDX transformation.

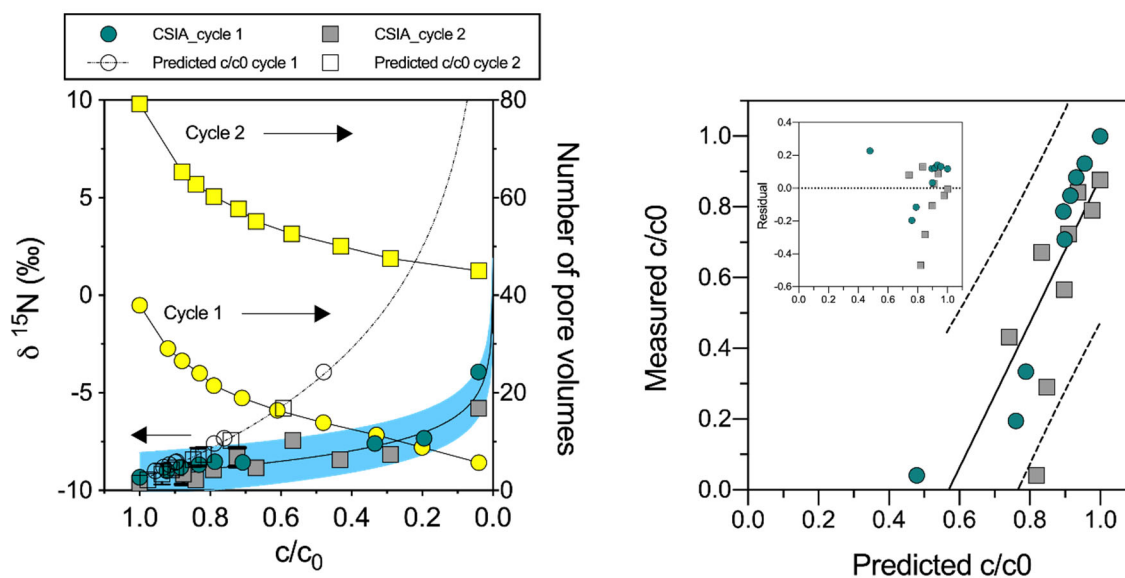


Figure 10-44. (a) Nitrogen isotope fractionation ($\delta^{15}\text{N}$, left y-axis) versus the extent of RDX reduction (c/c_0 , x-axis) in column reactors. The right y-axis indicates where isotope samples were taken on the column breakthrough curves during cycle 1 (yellow circle) and cycle 2 (yellow rectangle). The hollow squares and circles indicate predicted N-fractionation using $\epsilon_{\text{N}} = -7.4\text{‰}$ from the batch reactors. (b) Measured c/c_0 versus predicted c/c_0 values of RDX in column effluent. The predicted c/c_0 values were obtained using $\epsilon_{\text{N}} = -7.4\text{‰}$ from batch experiments. The solid line is a linear regression. The dashed lines are the 95% prediction intervals of linear regression. The inset is the residual plot.

10.5.2.7 NTO batch experiments

NTO was reduced by the dithionite-reduced TCAAP sediment at pH 6. The degradation of NTO also followed the second-order kinetics. The reductant equivalent values for reducing NTO, R_{eq} , were much higher than those for reducing RDX, and more NTO was reduced than RDX when providing similar amount of structural Fe(II) (Figure 10-45).

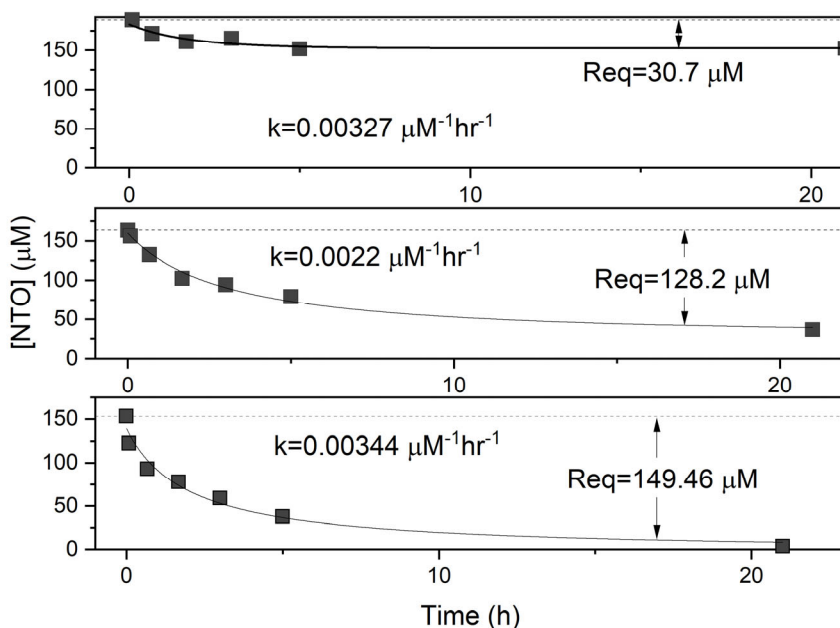


Figure 10-45. NTO degradation over time by dithionite-treated TCAAP sediment. The reaction followed second-order kinetics. The iron mineral in TCAAP soil was reduced to 3% (top), 6.3% (middle), and 8.2% (bottom). NTO was reduced in 10 mM NaHCO_3 buffer at pH 6

ATO produced during the reduction of NTO by dithionite-reduced TCAAP sediment did not show depletion within the experiment timeframe (Figure 10-46). It was assumed that NTO to ATO was the predominant pathway (Figure 10-45). The quantity of electrons transferred to NTO was calculated with the assumption that 6 moles of electrons are required to form one mole of ATO from NTO during NTO reduction (eq. 4). Dithionite-reduced TCAAP sediment reduced NTO with extents of electron transfer ranging from 0.04–0.11 mol e /mol Fe(II). This result again indicated that Fe(II) generated during dithionite treatments did not provide enough electron equivalents to completely reduce all of the added NTO.

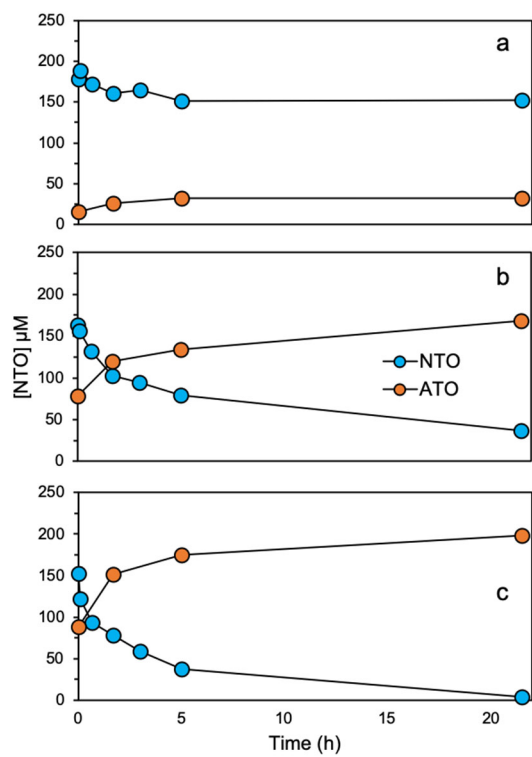


Figure 10-46. The reduction of NTO to form ATO by 3% (a), 6.3% (b), and 8.2% (c) reduced TCAAP sediment. The reaction was in 10 mM NaHCO_3 buffer at pH 6

11 CONCLUSIONS

Our study investigated laboratory conditions and future work should address direct applications of CSIA to contaminated field sites receiving ISCR. In addition, to assess the efficiency of sediment reduction with external electron donors such as dithionite, these analyses will have to include critical phenomena which we were not able to evaluate in a laboratory model system. Several studies have shown that mass transport related processes including diffusive isotope fractionation, hydrodynamic dispersion, multiple contaminant sources, and the quality of stable isotope ratio measurements can lead to both over- and underestimations of the extent of contaminant degradation.^{200,202–204} For ISCR, the understanding of the local hydrogeology will be necessary to accurately estimate the extent of contaminant (bio)degradation with CSIA.

As remediation strategies are implemented, the adaptation of CSIA for pollutant monitoring in treated soil, sediment, and groundwater is critical for its successful integration into protocols for monitored natural attenuation and active remediation. To an extent, this has been accomplished for the assessment of biodegradation of nitroaromatic explosives in soil.²⁸ In this work, we reported CSIA as a robust technique to quantify abiotic DNAN reduction in systems receiving repeated dithionite treatments, but future work should include a survey of other *in situ* abiotic remediation strategies (e.g., calcium polysulfides,²⁰⁵ sodium persulfate²⁰⁶). The observation that isotope fractionation is unaffected by natural subsurface materials and ISCR can be leveraged to generate accurate estimates of contaminant removal strengthen our findings. Moreover, it provides strong support for the compulsory lines of evidence set by the US Environmental Protection Agency for evaluating the progress of natural attenuation.¹³⁴ Considering DNAN as a surrogate for other NACs allows for this approach to be applied to a wide range of environmental pollutants.

12 APPENDICES

12.1 Additional and Supporting Data

12.1.1 Chemicals

2,4-Dinitroanisole (DNAN, 98%) was purchased from Alfa Aesar. The DNAN transformation products 2-amino-4-nitroaniline (2-ANAN, 98%), 4-amino-2-nitroaniline (4-ANAN, 97%), and 2,4-diaminoanisole (DAAN, $\geq 98\%$) were purchased from Fisher. Analytical standards for RDX and one of its transformation products, TNX, were purchased from AccuStandard. HMX was also purchased from AccuStandard. 3-nitro-4,5-dihydro-1H-1,2,4-triazol-5-one (NTO, 95%) was purchased from Enamine. The NTO transformation product 5-Amino-2,4-dihydro-[1,2,4]triazol-3-one (ATO, 95%) was purchased from Princeton BioMolecular Research. Analytical standards for NTO and ATO were purchased from AccuStandard.

$\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ ($\geq 98\%$), methanol (ACS grade), NaHCO_3 (ACS grade), $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (ACS grade), KNO_3 ($\geq 99\%$), and HCl (trace metals grade) were purchased from Sigma. $\text{FeCl}_2 \cdot 6\text{H}_2\text{O}$ (ACS grade), HCl (37%, ACS grade), NaOH (ACS grade), NaOH (50% in H_2O), KOH (ACS grade), H_2SO_4 (ACS grade), acetonitrile (HPLC grade), ferrozine (B-(2-pyridyl)-5,6-bis(4-sulfophenyl)-1,2,4-triazine disodium salt hydrate, $>98\%$), and NaCl (ACS grade) were purchased from Fisher. Elliot Soil Humic Acid (ESHA, Cat. No. 4S102H) was purchased from the International Humic Substances Society (IHSS) in St. Paul, MN. Elemental composition was performed by the IHSS and characterized the ESHA sample as 7.62% (w/w) water, 0.44% (w/w) ash, and 59.51% (w/w) C. Solutions containing ESHA were prepared to 5 mg/L as organic carbon.

N_2 gas ($>99.9\%$, Matheson) was used for deoxygenating ultrapure water (MilliQ, 18.2 $\text{M}\Omega$ cm, Millipore). Sodium dithionite ($\text{Na}_2\text{S}_2\text{O}_3$, 97% technical grade, MilliporeSigma) and potassium carbonate (K_2CO_3 , ACS grade, Fischer Scientific) were used for reducing minerals. Other chemicals used include sodium bromide (NaBr, ACS grade, Sigma Aldrich), sodium dihydrogen phosphate (NaH_2PO_4 , ACS grade, Sigma Aldrich), phosphoric acid (H_3PO_4 , ACS grade, Fluka), 2,4-dinitrophenylhydrazine (2,4-DNPH, HPLC grade, Sigma Aldrich), formaldehyde-2,4-DNPH (HCHO -2,4-DNPH, analytical standard grade, Sigma Aldrich), ortho-phthalaldehyde (OPA, HPLC grade, Sigma Aldrich), sodium tetraborate ($\text{Na}_2\text{B}_4\text{O}_7$, reagent grade, Sigma Aldrich), sodium sulfite (Na_2SO_3 , reagent grade, Sigma Aldrich), ammonium chloride (NH_4Cl , ACS grade, Macron), HPLC grade methanol, and ethyl acetate (Sigma Aldrich).

All glassware, cuvettes, stir bars, and Nalgene bottles were soaked in 0.1 M oxalic acid (pH 3.5) for at least 2 days and rinsed with ultrapure water prior to use to remove any residual iron.

12.1.2 Ferrozine Method for Fe(II) Quantitation

The Fe(II) content was determined by the method of Viollier et al.¹¹⁰ Aliquots from the batch reactors were filtered with a 0.2 μm PTFE syringe filter. To measure the Fe(II) content, 0.10 mL of reaction filtrate was transferred to a clean, dry polystyrene cuvette with 2.70 mL deoxygenated, ultrapure water and 0.20 mL of a 5 g/L aqueous ferrozine solution. Each sample was capped, mixed by inversion, and the absorbance was measured at 562 nm using a Shimadzu UV-1601PC UV-Vis spectrophotometer. A five-point calibration curve was constructed by producing serial dilutions from 0.0–0.005 mM Fe(II) in ultrapure water. A ferrozine/ultrapure

water solution was used as the blank. The Fe(III) content of a sample can also be quantified by the ferrozine assay by using hydroxylamine hydrochloride to reduce the Fe(III) to Fe(II). The difference between the measurements with and without hydroxylamine give the Fe(III) value. Solid samples were digested in 3 M HCl prior to analysis.

12.1.3 Fitting Details for Dispersion Coefficients

Dispersion coefficients (D) in packed columns were determined from eq. 14 adapted from Kreft and Zuber.²⁰⁷

$$\frac{c}{c_0} = \frac{1}{2} \operatorname{erfc}\left(\frac{L - uz}{\sqrt{4Dz}}\right) + \frac{1}{2} \exp\left(\frac{uL}{D}\right) \operatorname{erfc}\left(\frac{L+uz}{\sqrt{4Dz}}\right) \quad (14)$$

where c/c_0 is the normalized DNAN concentration at the column outlet, u is the mean flow velocity in the column, L is the column height, and z is the vertical displacement in the column. Because our measurements were made at the column outlet, L was equal to z in all cases. Fitting was performed using the Levenberg-Marquardt algorithm in Origin Version 2019b (OriginLab Corporation).

12.1.4 ATO calibration data

For analysis of ATO via HPLC a Hypercarb column (Thermo Fisher, 100 mm, 3 μ m, 4.6 ID) was used. The mobile phase was 85% ultrapure water (2 mM ammonium acetate) +15% methanol (2 mM ammonium acetate) with a flow rate of 0.3 mL/min. The retention time of ATO was $\sim$ 7.3 min, and ATO was detected at 216 nm with DAD or UV detector. The response of the ATO measured, however, was dependent upon the matrix in which standards were prepared (Figure 12-1). Thus, ATO standards were prepared in the reaction matrix, i.e. a mixture of NaHCO_3 , dissolved FeCl_2 , and iron minerals. The minerals were filtered out before the standards were measured on HPLC. Figure 12-2 shows the enhancement of response factors for ATO in goethite/Fe(II) and magnetite/Fe(II) systems. The response factor increased $\sim$ 30 fold compared with the standards in MilliQ water.

The reaction matrix, HPLC buffer, or HPLC column could be responsible for the enhancement of ATO signal. Standards of ATO in MilliQ water, the reaction matrix or HPLC buffer (NH_4 buffer) only, or a combination of the matrix and HPLC buffer were prepared and filtered after 30 minutes. The absorbance was measured on a standalone UV-Vis spectrophotometer. Figure 12-3a shows that for 5 μ M of ATO, which is the closest with the ATO concentration that is injected into HPLC sample loop, the presence of reaction matrix+HPLC buffer increased the signal about 17 fold comparing with ATO in MilliQ water. To test the effect of HPLC column, we used an off-line LC pump to flow ATO in the reaction matrix+HPLC buffer through the Hypercarb column. The ratio of the constituents in the influent were similar to that in real HPLC operation. We took samples in the reservoir before the pump and after the column. The samples were analyzed on UV-Vis. There was no obvious increase in the signal after passing the HPLC column (Figure 12-3b). Hence, the matrix in which ATO standards are prepared is the critical factor in correctly quantifying the concentration.

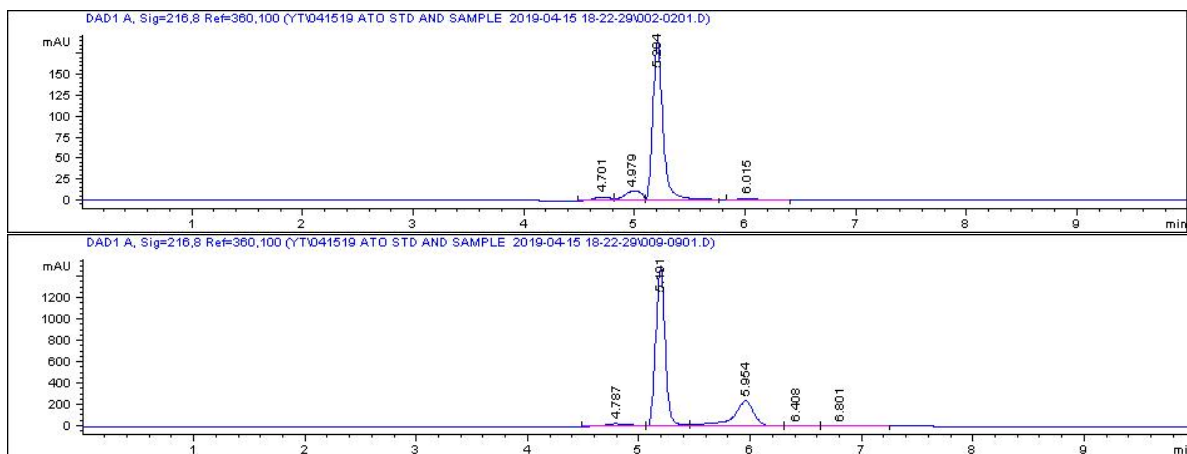


Figure 12-1. Signals of ATO in HPLC-DAD. ATO retention time is ~5.20 min. The top figure is 20 μ M of ATO standard in MilliQ water, and the bottom figure is 20 μ M of ATO filtered from the matrix for reduction experiments. Note the difference in the y-axis scales

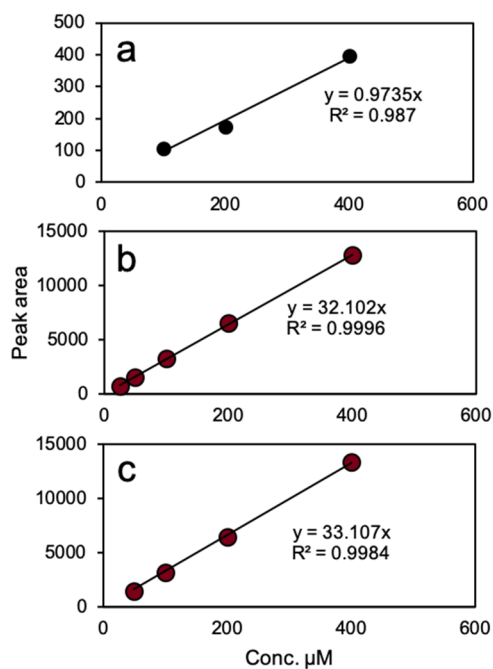


Figure 12-2. ATO calibration curves for ATO standards in different solution matrices: a) ATO in MilliQ water, b) ATO in goethite/Fe(II) reaction matrix, c) ATO in magnetite/Fe(II) reaction matrix.

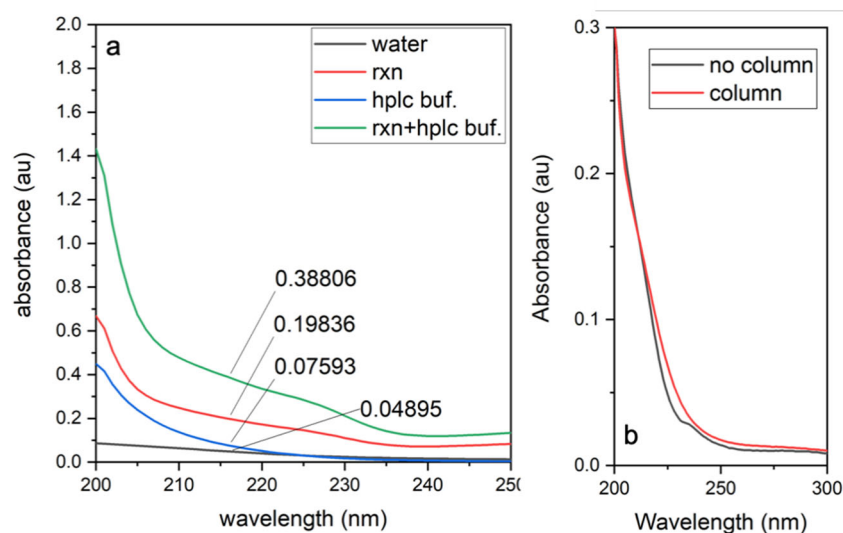


Figure 12-3. The impact of background matrix and HPLC column on the signal of ATO measured via a UV-Vis spectrometer. a) UV-Vis spectra of ATO in different background matrices: MilliQ water, reaction matrix (rxn), HPLC buffer (hplc buf.), and reaction matrix+HPLC buffer (rxn+hplc buf.). Annotations were added for signals at 216 nm where ATO was detected on HPLC-DAD. b) UV-Vis spectra of ATO in rxn+hplc buf and in the buffer after being pumped through the Hypercarb HPLC column.

12.1.5 Alkaline and Enzymatic Hydrolysis of DNAN

12.1.5.1 Alkaline and Enzymatic Hydrolysis Experiments

The procedure for the alkaline hydrolysis reactions was adapted from a method previously published by Salter-Blanc et al.⁶⁰ Briefly, reactions were initiated upon introduction of 200 μ L of a DNAN stock solution (50.5 mM in acetonitrile) into amber glass vials containing 20 mL of 50 mM phosphate buffer at pH 12, and were carried out at room temperature. Reactions were quenched at appropriate intervals by adjusting the pH of the reaction mixture to 7.0 with 2 mL of dilute sulfuric acid. Samples of quenched reactions were analyzed by high performance liquid chromatography (HPLC) with UV-vis detection to quantify DNAN and dinitrophenol (DNP). DNAN degradation by intact cells of *Nocardioide*s sp. strain JS1661 cells was conducted in 1/4 strength Stanier's minimal medium²⁰⁸ (pH 7.0, 25 $^{\circ}$ C) and DNAN degradation by the partially purified enzyme preparation was conducted in HEPES buffer (0.02 M, pH 7.5), both of which initially contained 300 μ M DNAN. Samples were removed from the incubation mixture at appropriate intervals, acidified to stop the reaction, filtered to 0.2 μ m, and stored at 5 $^{\circ}$ C until analyzed by HPLC. Procedures for the growth of *Nocardioide*s sp. strain JS1661 cells and the partial purification of DNAN *O*-demethylase were adapted from Karthikeyan and Spain.²⁰⁹

12.1.5.2 Alkaline Hydrolysis

Transformation of DNAN by alkaline hydrolysis at pH 12 followed pseudo-first order kinetics and concomitantly produced DNP as the only reaction product (Figure 12-4). This interpretation

is supported by an accurate description of the concentration dynamics with the stoichiometric transformation of DNAN to DNP with a single bimolecular hydrolysis rate constant, k_{OH^-} , of $(2.5 \pm 0.2) \cdot 10^{-4} \text{ M}^{-1}\text{s}^{-1}$. Even though this number is determined only at pH 12, it is in good agreement with data from Salter-Blanc et al.⁶⁰ determined for DNAN disappearance over a wider range of pH values $((7.1 \pm 0.5) \cdot 10^{-4} \text{ M}^{-1}\text{s}^{-1})$. DNP was identified through comparison of UV-vis spectra and by its exact mass through analysis by LC-HRMS. DNAN and DNP concentrations accounted for 98% of the mass balance after 14 days and we did not find any evidence for a stable Meisenheimer intermediate postulated by others^{59,60,210}.

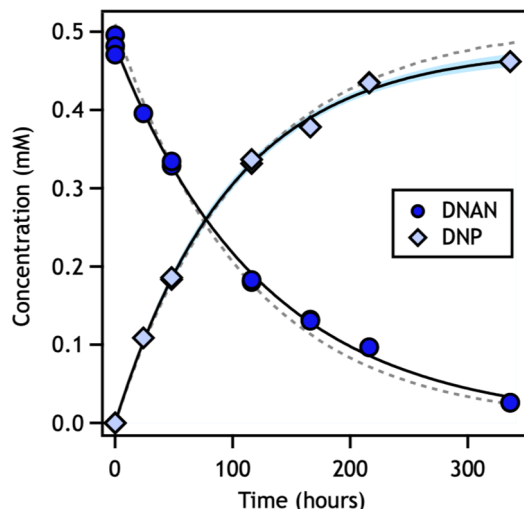


Figure 12-4. Kinetics for alkaline hydrolysis of DNAN at pH 12 and formation of DNP. Solid lines show the pseudo-first order kinetic description of concentration dynamics.

Alkaline hydrolysis of DNAN was associated with substantial C and N isotope fractionation (Figure 12-5) corresponding to isotope enrichment factors, ϵ_{C} and ϵ_{N} , of $-6.0 \pm 0.5\text{‰}$ and $-2.7 \pm 0.4\text{‰}$, respectively (Table 12-1). Based on evidence from previous works^{60,211,212} and the stoichiometric formation of DNP from the alkaline DNAN hydrolysis observed here, we derived apparent ^{13}C and ^{15}N kinetic isotope effects (AKIEs) of 1.0445 ± 0.0028 and 1.0027 ± 0.0004 , respectively, assuming transformation of DNAN through nucleophilic aromatic substitution (Scheme 1a). Although there is no precedent for ^{13}C and ^{15}N isotope effects of such reactions, it is plausible that the substantial bonding changes at the aromatic C atoms cause large ^{13}C AKIEs, whereas the ^{15}N AKIE reflects a secondary effect of N atoms not directly involved in the nucleophilic attack. A comparison with the ¹¹⁹ similarly large ^{13}C -AKIE of 1.047 ± 0.002 for the alkaline hydrolysis of atrazine at pH 12 supports this interpretation.^{212,213} The C isotope signature of DNP increases substantially with reaction progress. The ϵ_{C} of $-8.9 \pm 1.4\text{‰}$ calculated with

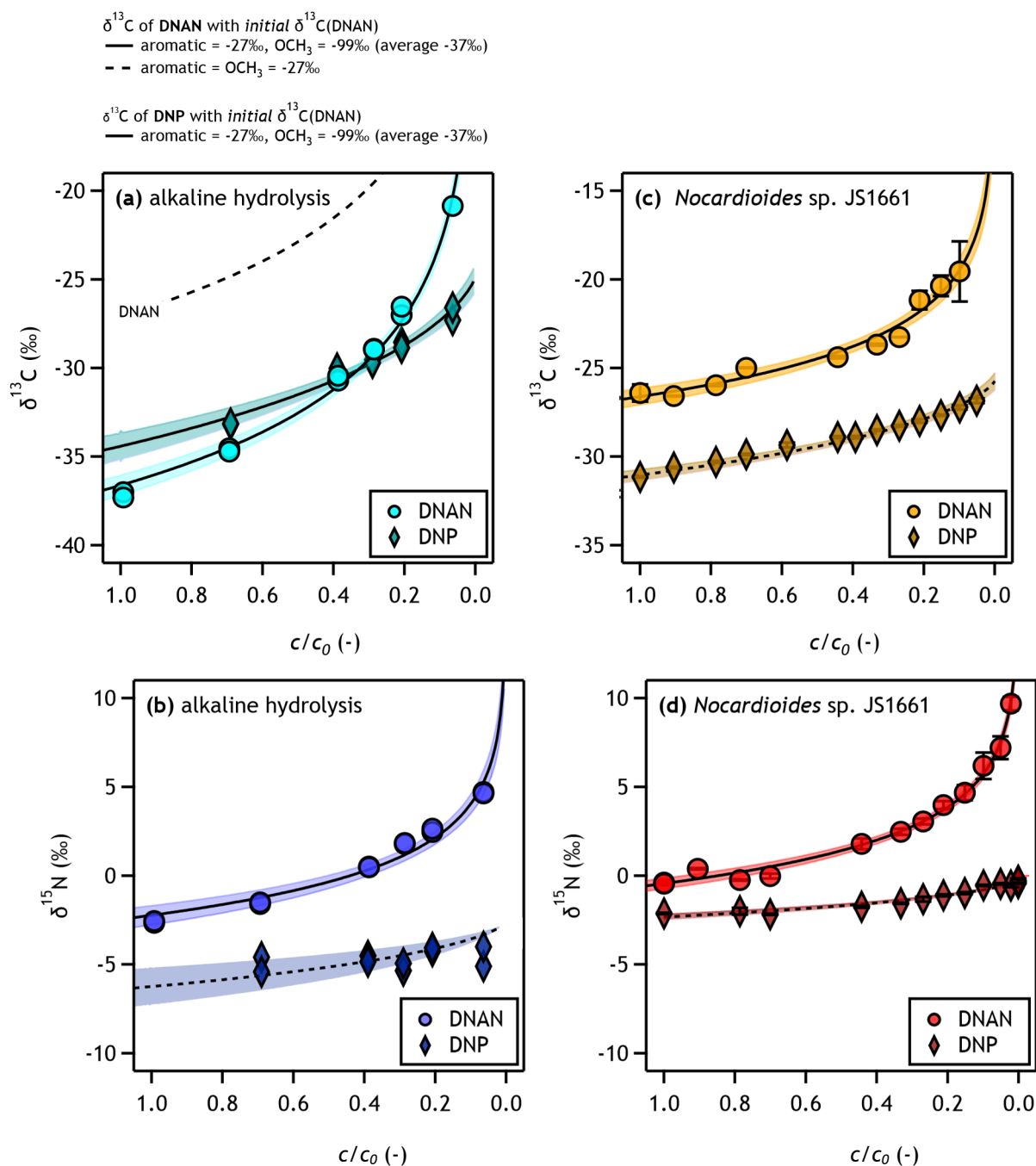


Figure 12-5. C and N isotope fractionation associated with the transformation of DNAN to DNP by alkaline hydrolysis at pH 12 (panels a and b) and during biodegradation by *Nocardioideis* sp. JS1661 (panels c and d). Panels (a) and (b) show $\delta^{13}\text{C}$ of DNAN and DNP vs. fraction of remaining DNAN, c/c_0 ; panels (c) and (d) show the corresponding $\delta^{15}\text{N}$ trends. Lines represent fits of C isotope fractionation (eqs. 1 and 2) for DNAN and DNP with different assumptions for intramolecular $\delta^{13}\text{C}$ distribution among aromatic and aliphatic C atoms in panel (a) (see text for details). The solid and dotted lines are nonlinear fits to the substrate and product isotope fractionation with eqs. 1 and 2, respectively (data in Table 12-1). The shaded areas indicate the 95% confidence intervals.

Table 12-1. Carbon and nitrogen isotope enrichment factors (ϵ_C , ϵ_N), apparent ^{13}C and ^{15}N kinetic isotope effects (^{13}C -AKIE, ^{15}N -AKIE), and correlation of C and N isotope fractionation ($\Lambda^{\text{N/C}}$) associated with the alkaline and enzymatic hydrolysis of DNAN.^a

System	ϵ_C	ϵ_N	^{13}C -AKIE	^{15}N -AKIE	$\Lambda^{\text{N/C}}$
Alkaline hydrolysis	-6.0 ± 0.5	-2.7 ± 0.4	1.044 ± 0.003	1.027 ± 0.0004	0.46 ± 0.04
<i>Nocardioide</i> sp JS1661					
Whole cell experiments	-2.8 ± 0.1	-2.5 ± 0.1	1.020 ± 0.003	1.0025 ± 0.0005	0.87 ± 0.15
Partially purified enzyme	-3.7 ± 0.1	3.2 ± 0.1	1.027 ± 0.005	1.032 ± 0.0003	1.06 ± 0.25

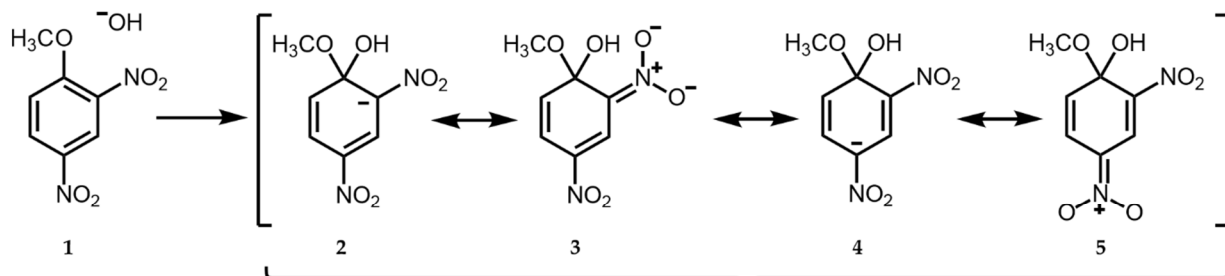
^a Combination of replicate experiments according to methods of Scott et al.¹²¹

assumptions for calculations ^{13}C -AKIE: $n^c = 7$, $x = z = 1$; ^{15}N -AKIE: $n = x = z = 1$ according to the assumption of a secondary isotope effect

^b Slope of a linear regression analysis of $\delta^{15}\text{N}$ vs. $\delta^{13}\text{C}$, uncertainties denote 95% confidence intervals.

$\delta^{13}\text{C}$ of DNP exceeds the one derived from data for DNAN because C isotope fractionation in DNP is not diluted by the presence of the non-reactive C in the methoxy group ($-\text{OCH}_3$). This observation is consistent with a reaction mechanism in which only aromatic C atoms are affected by nucleophilic attack (Figure 12-6). However, contrary to expectations from the large normal ^{13}C -AKIE, which implies a preferential transformation of ^{12}C isotopologues of DNAN, DNP is not enriched in ^{12}C compared to DNAN. In fact, even after 30% of DNAN conversion (i.e., $c/c_0 = 0.7$, Figure 12-5) the $\delta^{13}\text{C}$ of DNP is still larger than that of DNAN, meaning that DNP contains more ^{13}C than DNAN. This positive shift of $\delta^{13}\text{C}$ value of DNP reflects an uneven intramolecular $^{13}\text{C}/^{12}\text{C}$ distribution between aromatic and aliphatic C atoms in DNAN. The non-reactive aromatic C atoms of DNAN exhibit a $\delta^{13}\text{C}$ of between -25‰ to -27‰ . A mass balance calculation reveals that the C atom of the OCH_3 group was isotopically very light (-99‰ to -110‰). While the magnitude of C isotope fractionation due to alkaline hydrolysis of DNAN to DNP quantified with eqs. 1 and 2 is independent of the intramolecular C isotope distribution in DNAN, the position of the isotope fractionation trajectories on the y-axis in Figure 12-5a may shift considerably. As a comparison, Figure 12-5a also illustrates the behavior expected for isotopically homogenous DNAN. If the $\delta^{13}\text{C}$ of the OCH_3 group would correspond to that of the aromatic C atoms (e.g., -27‰), the DNAN fractionation curve would be shifted upwards in Figure 12-5a (dashed line). At low stages of conversion, such as $c/c_0 > 0.9$, the offset of the DNAN and DNP C isotope fractionation curve would be identical to the C value for alkaline hydrolysis of -6.0‰ . The N isotope enrichment factor for the alkaline hydrolysis determined from DNAN and DNP agree within uncertainty ($-2.7 \pm 0.4\text{‰}$ and $-3.6 \pm 1.0\text{‰}$, Table 12-1). Contrary the above observations, the N isotope fractionation not affected by intramolecular isotope distribution because DNAN and DNP contain the equal number of N atoms in the non-reactive NO_2 substituents. However, the $\delta^{15}\text{N}$ for DNP at 95% substrate conversion was still slightly lower than that for the unreacted substrate ($-4.6 \pm 1.0\text{‰}$ versus $-2.6 \pm 0.2\text{‰}$, respectively), which was likely due to a combination of analytical error and the reaction not quite reaching completion.

(a) Alkaline hydrolysis



(b) Enzymatic hydrolysis

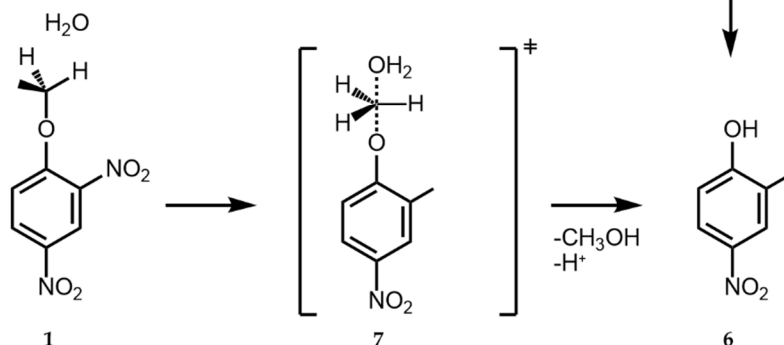


Figure 12-6. Initial steps for the transformation of 2,4-dinitroanisole (1) by (a) alkaline hydrolysis through nucleophilic aromatic substitution to 2,4-dinitrophenol (6). Compounds 2 to 5 are resonance structures of the Meisenheimer complex intermediates.²¹¹ (b) Hypothesized enzymatic hydrolysis of DNAN by *Nocardioides* sp. JS1661 and enzyme assays containing DNAN *O*-demethylase. Compound 7 shows one of several possible transition states for a hydrolytic *O*-demethylation at the aliphatic C atom of the methoxy group.

12.1.5.3 Enzymatic Hydrolysis

Transformation of DNAN by *Nocardioides* sp. JS1661 led to the stoichiometric formation of DNP after 80 minutes of reaction time (Figure 12-7a), in agreement with previous work.²¹⁴ Compared to alkaline hydrolysis, the disappearance of DNAN was associated with smaller C isotope fractionation and similar N isotope fractionation (Figure 12-5c and d), with ϵ_C and ϵ_N values of $-2.8 \pm 0.1\text{‰}$ and $-2.5 \pm 0.1\text{‰}$, respectively. Experiments with partially purified *O*-demethylase (Figure 12-7b) revealed DNAN C and N isotope fractionation that was approximately 30% stronger than for whole cell experiments (Figure 12-8). ϵ_C and ϵ_N were $-3.7 \pm 0.1\text{‰}$ and $-3.2 \pm 0.1\text{‰}$, respectively (Table 12-1). The correlations between C and N isotope fractionation, which are indicative of the reaction mechanisms, resulted in identical slopes, $\Lambda^{N/C}$ within experimental uncertainty (0.87 ± 0.15 vs. 1.06 ± 0.25 , respectively, Table 12-1 and Figure 12-9). Moreover, $\Lambda^{N/C}$ also corresponded well with the ratio $\Lambda^{N/C}$ (0.86 ± 0.04 and 0.89 ± 0.05 , respectively).

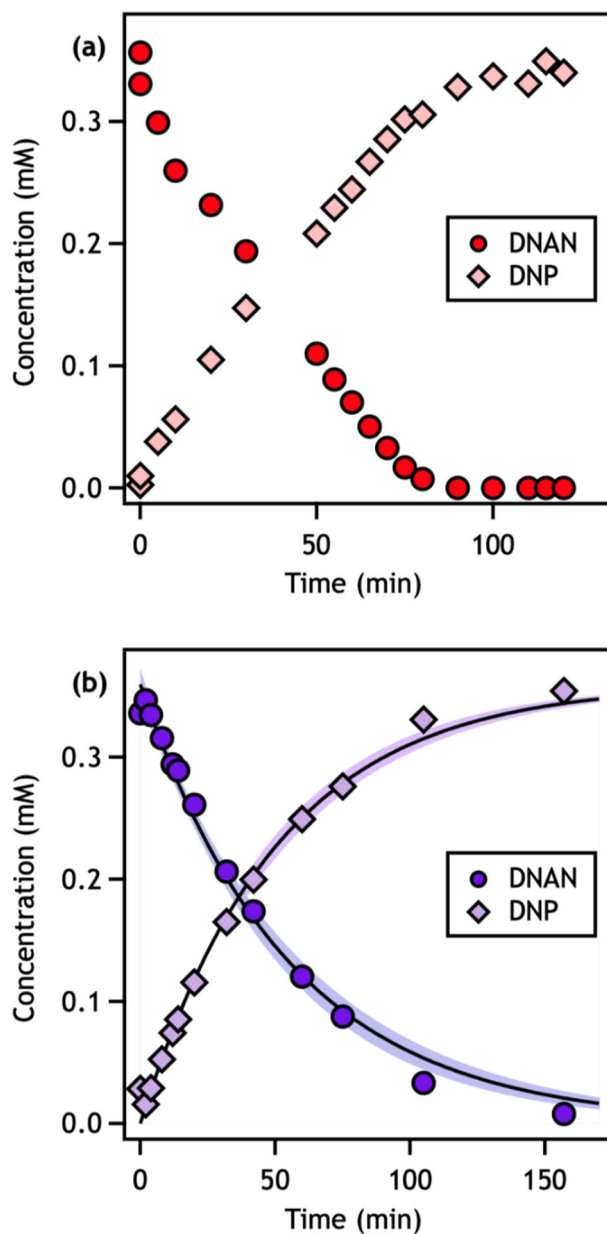


Figure 12-7. Kinetics for DNAN transformation to DNP by (a) *Nocardioide s p . JS1661* whole cells and (b) partially purified DNAN O-demethylase. Fitted lines correspond to Solid lines show the pseudo-first order kinetic description of concentration dynamics with $k_{\text{obs}}(\text{DNAN}) = 0.018 \pm 0.002 \text{ min}^{-1}$ and $k_{\text{obs}}(\text{DNP}) = 0.020 \pm 0.002 \text{ min}^{-1}$. Parameter uncertainties and colored areas in panel (b) stand for 95% confidence intervals of the fit.

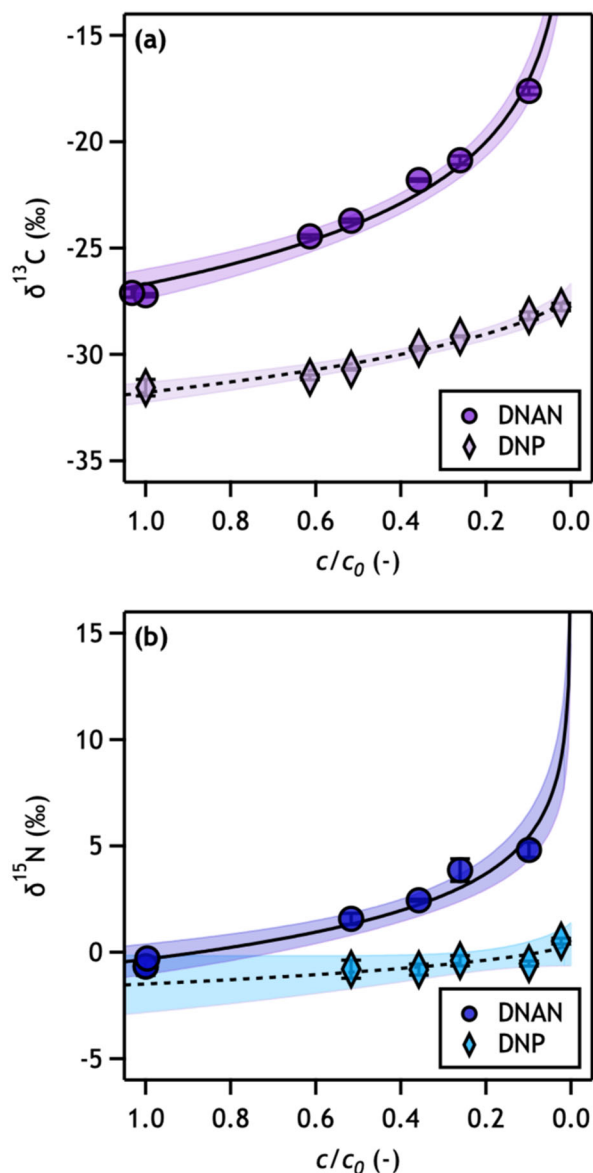


Figure 12-8. (a) C isotope fractionation of DNAN and DNP during enzyme-catalyzed hydrolysis by the partially purified DNAN O-demethylase and (b) the corresponding N isotope fractionation. Solid and dotted lines indicate the substrate and product isotope fractionation. The shaded areas indicate the 95% confidence interval of the fit.

Therefore, the differences between the experiments with whole cells and partially purified enzyme were presumably caused by the masking of isotope fractionation through mass transfer limitations associated with substrate uptake.²⁰⁴ Based on the assumption made for alkaline hydrolysis regarding reactive positions, we obtained ^{13}C and ^{15}N -AKIEs of 1.0269 ± 0.0053 and 1.0032 ± 0.0003 , respectively for enzyme-catalyzed DNAN hydrolysis (Table 12-1). Whereas the ^{15}N -AKIEs of alkaline and enzymatic DNAN are identical and consistent with reactions that do not involve the aromatic NO_2 groups, the ^{13}C -AKIEs differ substantially. These differences also result in distinctly different $\Lambda^{\text{N/C}}$ (Table 12-1) and imply that the hydrolysis of DNAN catalyzed

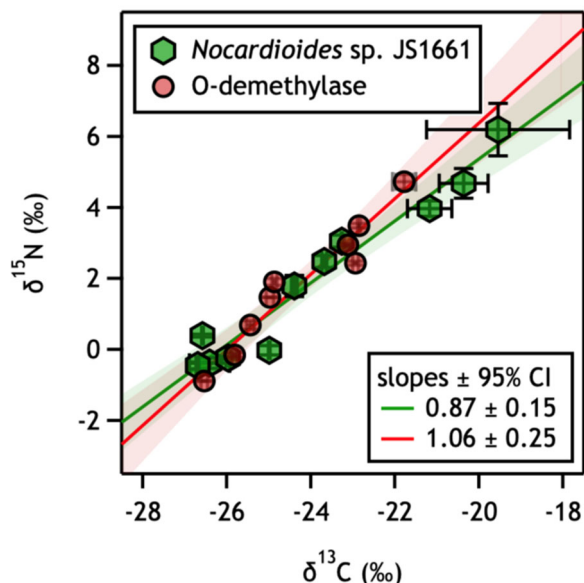


Figure 12-9. Correlation of C and N isotope fractionation of DNAN biodegradation by *Nocardioioides* sp. JS1661 vs. enzyme-catalyzed hydrolysis by the partially purified DNAN O-demethylase. The slopes denote $\Lambda^{N/C}$ values $\pm$ 95% confidence intervals (shaded areas) .

by the *O*-demethylase occurs by a mechanism that is different from that for alkaline hydrolysis. The isotope effects reported here for DNAN hydrolysis by *O*-demethylase are consistent with a nucleophilic substitution reaction at the aliphatic C of the OCH_3 group (Figure 12-6, **1** $\rightarrow$ **6**). This interpretation is supported by the known ^{13}C AKIEs for nucleophilic substitutions at aliphatic C atoms in C–O bonds, for example, an ^{13}C -AKIE of 1.025 for hydrolysis of methyl tert-butyl ether.⁴⁶ Note that bimolecular nucleophilic substitutions through formation of a tetrahedral intermediate such as **7** in Figure 12-6 also exhibit very moderate secondary H isotope fractionation consistent with our tentative evaluation of H isotope fractionation for the enzyme-catalyzed hydrolysis of DNAN. Secondary ^{15}N -AKIEs ≤ 1.005 agree with evidence from the attack of nucleophilic oxygen at several types of acyl groups including carboxylic esters and amides through formation of tetrahedral intermediates.^{215–217} An alternative mechanisms for enzymatic DNAN hydrolysis at the aliphatic C of the OCH_3 group is further supported by the C isotope fractionation observed in the reaction product DNP (Figure 12-5c). Whereas the magnitude of N isotope fractionation of DNP is identical to that determined in DNAN as well as to the N isotope fractionation of DNAN and DNP pertinent to alkaline hydrolysis, the $\delta^{13}\text{C}$ trends of DNP from enzymatic and alkaline hydrolysis shown in Figure 12-5a and Figure 12-5c, respectively, are substantially different. The enrichment of ^{13}C in DNP during its formation from enzymatic hydrolysis spans a smaller range of $\delta^{13}\text{C}$ values than for DNP formed during alkaline hydrolysis. The moderate C isotope fractionation in DNP is consistent with the above assumption that DNP formed enzymatically originates from a reaction that, in contrast to alkaline hydrolysis, did not involve aromatic C atoms.

Because alkaline and enzymatic hydrolysis of DNAN showed differences in C isotope fractionation but not in N isotope fractionation, the correlation of C and N isotope fractionation results in distinct trendlines shown in Figure 12-10 ($\Lambda^{N/C}$ of 0.46 ± 0.04 vs. 0.87 ± 0.15 for

alkaline and enzymatic hydrolysis, respectively). This result not only implies that the abiotic and biological hydrolysis of DNAN occur by different mechanisms, but also that the observed C and N fractionation patterns enable delineation of those processes by CSIA. These trends are also distinctly different from previously reported isotope fractionation for biological or abiotic reductions of structurally similar NACs exhibiting large $\Lambda^{N/C}$ (green trajectories in Figure 12-10).^{33,37,125,218,219} Therefore, the findings of this study are important for ongoing efforts to assess DNAN environmental reactivity because they can be used to distinguish among DNAN degradation processes in contaminated environments. Further, these results may also provide a basis for the application of CSIA to assess the effectiveness of engineered systems for DNAN removal, for example, by determining extent of reaction during treatment of contaminated soil in bioreactors.²⁰⁹ Finally, the finding that the $\delta^{13}C$ of DNAN may differ depending on the isotope signature of the methoxy C may be useful for a forensic tracking of DNAN contamination sources.

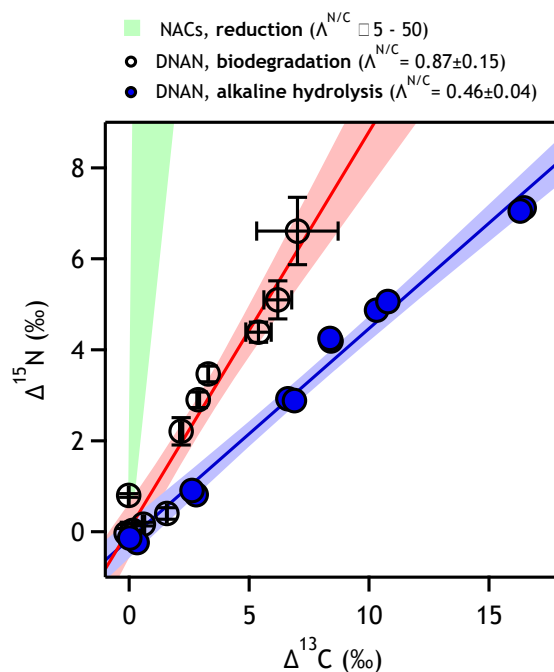


Figure 12-10. Correlation of C and N isotope fractionation for the alkaline and enzymatic hydrolysis of DNAN compared to expected trends for DNAN reduction (according to trends previously observed for the biotic and abiotic reduction of other NACs).^{33,37,218,219} Solid lines and shaded areas denote linear regressions and 95% confidence intervals, respectively, for alkaline and enzymatic hydrolysis. The expected range for DNAN reduction is shaded in green.

12.1.6 Additional Data for DNAN reduction by synthetic minerals

12.1.6.1 Control Experiments for DNAN Transformation Kinetics

A control experiment was performed to determine if DNAN transformation occurs in the absence of a mineral phase. Reactors were prepared in triplicate with bicarbonate buffer (10 mM, pH 7), Fe(II) (1 mM), and DNAN (0.2 mM). The results (Figure 12-11) revealed that no DNAN transformation occurred without an associated mineral phase after 21 days.

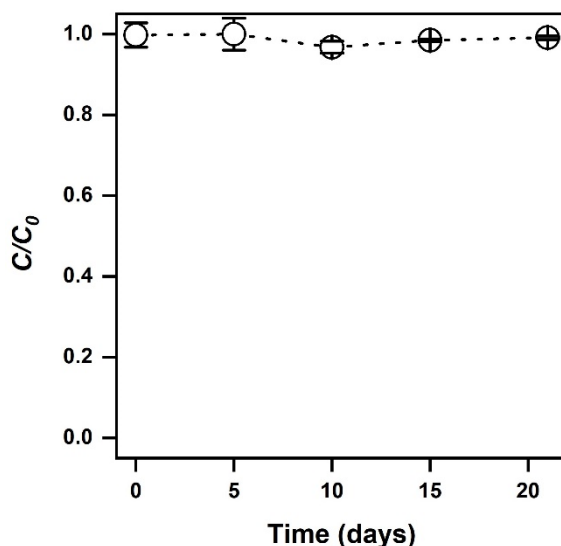


Figure 12-11. Control experiment for DNAN transformation without an added mineral. No DNAN transformation was observed in the control reactor with aqueous Fe(II) as compared to systems containing an Fe-bearing mineral. Reactors were prepared according to the methods described in the main report. Error bars represent standard deviations in triplicate reactors.

12.1.6.2 Detailed Carbon Fractionation Results

In the main text (Figure 10-19) all isotope fractionation results are plotted together, for simplicity, because minimal fractionation was observed across all sample sets. Here, values of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ vs. c/c_0 (Figure 12-12) are provided separately for each combination of reaction condition and mineral type.

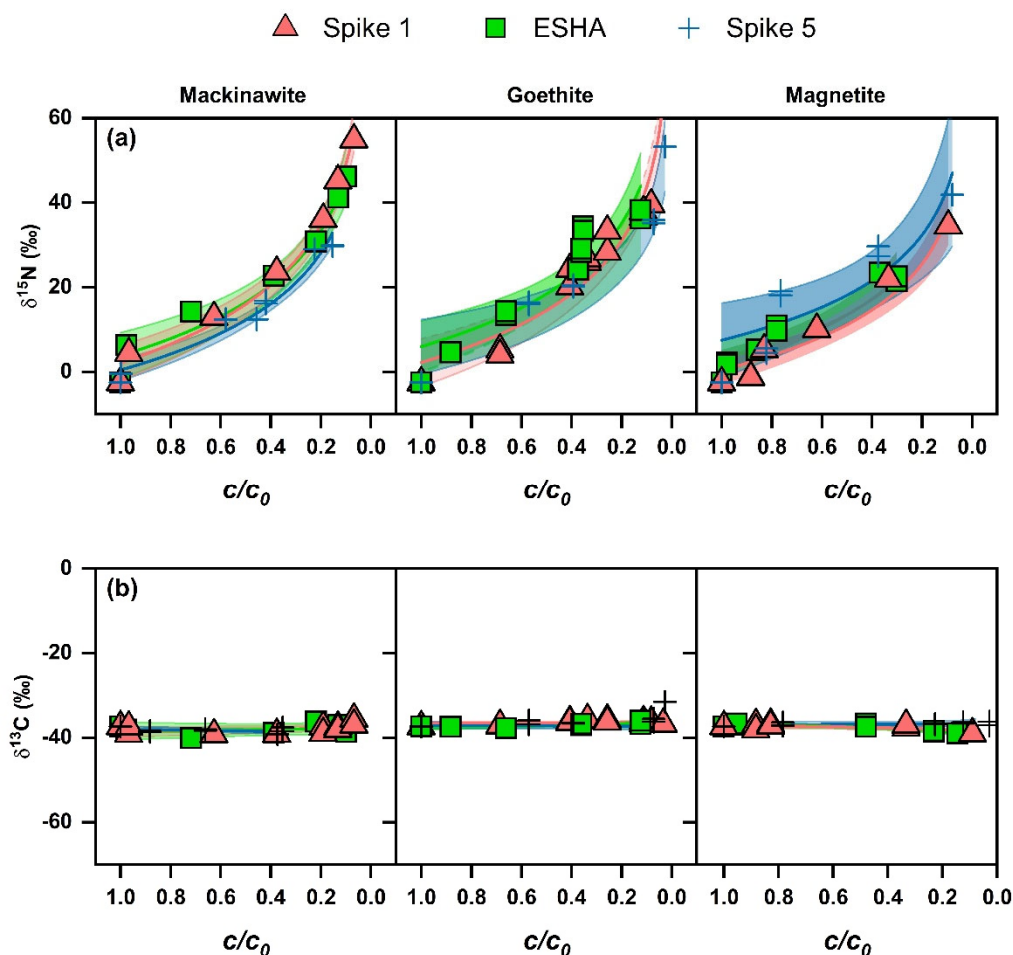


Figure 12-12. Complete (a) N and (b) C isotope fractionation results for abiotic reduction of DNAN calculated using nonlinear regression analyses of eq.1. All shaded regions represent 95% confidence intervals for each data set.

12.1.6.3 Decreased Enrichment Factors during Repeated Contaminant Exposure

A general trend of slightly reduced N fractionation was observed over 5 sequential DNAN spikes (Figure 12-13). Several factors may contribute to this result and we hypothesize the observed effects to be caused by the evolution of mineral surfaces. For example, Chun et al.⁵³ observed the oxidative growth of goethite nanoparticles during the reduction of 4-chloronitrobenzene in sequential-spike batch reactions containing Fe(II)/goethite, similar to those in the present study. Even though particle growth had occurred, the newly formed surfaces displayed marked increases in roughness leading to decreased mineral reactivity and negligible (even decreases on some facets) changes in surface area. Losses in reactivity were attributed to a lower Fe(II) uptake capacity of the roughened surfaces (approximately 16% decrease over five spikes). In addition, changes in mineral morphology may inhibit proton and electron transfer reactions that occur prior to the initial N—O bond cleavage. These transfer reactions typically elicit isotope effects that are minute in comparison to N—O bond cleavage but may be diminished over multiple contaminant

reductions as the mineral evolves resulting in decreased total isotope enrichment.²⁹ The trends in reductive pathways ($\epsilon_N \approx -19$ to -9 ‰ and $\epsilon_C \approx -0.1$ to -1.5 ‰), however, are still easily distinguished from oxidation ($\epsilon_N = -2.7 \pm 0.4$ ‰, $\epsilon_C = -6.0 \pm 0.5$ ‰ and $\epsilon_N = -3.2$ to -2.5 ‰, $\epsilon_C = -3.7$ to 2.8 ‰ for alkaline hydrolysis and biodegradation, respectively).¹³³

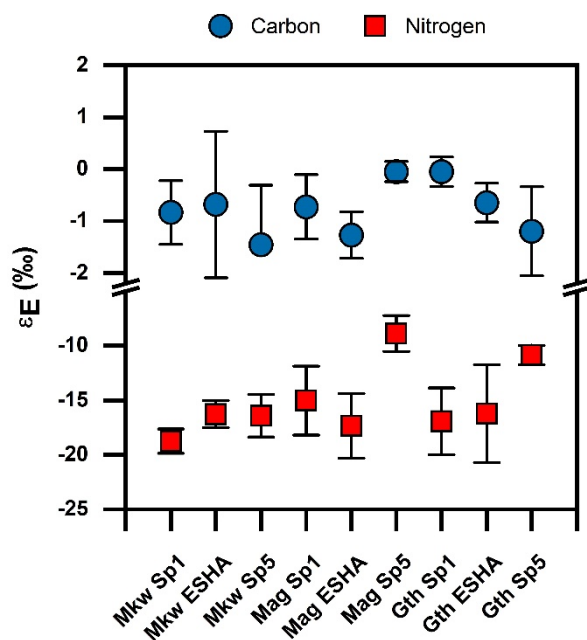


Figure 12-13. Carbon and nitrogen isotope enrichment factors (ϵ_E) in mackinawite (Mkw), magnetite (Mag), and goethite (Gth) suspensions derived using log-linear regression of eq. 1. Error bars represent the 95% confidence intervals.

12.1.6.4 Two-Dimensional Isotope Analysis

The results of two-dimensional CSIA with the reduction data separated by mineral type to indicate that isotope fractionation in each mineral suspension followed the same trend.

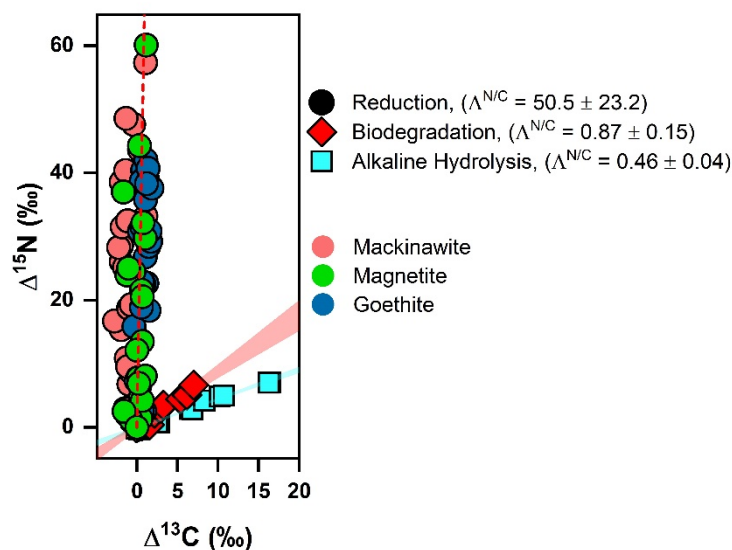


Figure 12-14. Two-dimensional isotope fractionation for reductive and oxidative DNAN transformation pathways. Isotope fractionation observed during abiotic reduction (circles) is shown with respect to the mineral type and compared to alkaline hydrolysis (squares), and biodegradation (diamonds). Alkaline hydrolysis and biodegradation data were reproduced with permission from Ulrich et al.¹³³

12.1.7 Additional Data for DNAN reaction with natural materials

12.1.7.1 Electron balance calculations

Batch experiments. The total amount of electrons transferred from Fe-bearing materials to DNAN in batch reactors was calculated according to eq 12.

$$\text{total electrons transferred} = (6c_{MENA} + 6c_{iMENA} + 12c_{DAAN}) * V_R \quad (12)$$

where V_R is the reactor volume (35 mL) and the analyte concentrations (c) are those at the endpoint of batch experiments.

Column experiments. In column experiments, the cumulative amount of electrons transferred were calculated by integrating the concentration measurements shown in Figure 10-38 according to eq 13.

$$\text{total electrons transferred} = \int_0^V (6c_{MENA} + 6c_{iMENA} + 12c_{DAAN})dV \quad (13)$$

where c is the measured analyte concentration at the column outlet and V is the volume of DNAN solution introduced to the column.

12.1.7.2 Column Characteristics

Table 12-2. Physical parameters of columns. Mean diffusion coefficients (D) determined from the nonreactive tracer were small and slightly greater for Tinker AFB ($2.53(12) \times 10^{-4} \text{ cm}^2 \text{ s}^{-1}$) than TCAAP ($2.37(22) \times 10^{-4} \text{ cm}^2 \text{ s}^{-1}$) columns. Similarly, large values of Pe (> 40) and uninhibited breakthrough of the nonreactive tracer (~ 1 PV) indicated convection-dominated regimes without preferential flow. All columns were 2.5 cm I.D. by 10 cm in length.

Type	Column no.	Mass (g)	ρ_p (g mL ⁻¹)	ρ_b (g mL ⁻¹)	PV (mL)	Before		After	
						ϕ	D (cm ² s ⁻¹)	ϕ	D (cm ² s ⁻¹)
TCAAP									
	1	47.7	2.64	1.59	11.9	0.40	2.69x10 ⁻⁴	0.41	2.69x10 ⁻⁴
	2	32.2	2.60	1.64	9.0	0.46	2.35x10 ⁻⁴	0.46	2.35x10 ⁻⁴
	3	40.2	2.76	1.64	10.0	0.41	2.11x10 ⁻⁴	0.43	2.11x10 ⁻⁴
Tinker AFB									
	1	27.5	2.68	1.70	11.5	0.37	2.48x10 ⁻⁴	0.37	2.48x10 ⁻⁴
	2	30.2	2.72	1.72	10.5	0.49	2.41x10 ⁻⁴	0.50	2.41x10 ⁻⁴
	3	33.1	2.70	1.68	10.7	0.48	2.70x10 ⁻⁴	0.49	2.70x10 ⁻⁴

12.1.7.3 Additional CSIA results

Bulk carbon and nitrogen isotope enrichment factors (ϵ_C and ϵ_N) and AKIEs were calculated from all experiments (Table 12-3). The values were obtained from experiments for DNAN reduction with untreated materials receiving a constant supply of 1 mM dissolved Fe(II). These represent the values used to calculate ϵ_N^* (see Section 10.5.2.4). The predictions made by applying the Rayleigh fractionation equation (eq. 2) to our results from column experiments are shown in Table 12-4. Details of the calculations are provided in the main text (Section 9.8).

Table 12-3. Bulk N and C isotope enrichment factors (ϵ_N) and AKIEs in batch reaction for DNAN reduction. All errors represent 95% confidence intervals. These values correspond to the data presented in Figure 3 in the main text.

Material	ϵ_N (‰)	^{15}N -AKIE (-)	ϵ_C (‰)	^{13}C -AKIE (-)
Tinker AFB				
Untreated + Fe(II)	-11.1 ± 4.3	1.023 ± 0.009	-0.7 ± 0.1	1.0007 ± 0.0002
Reduced	-9.9 ± 3.8	1.020 ± 0.008	-0.2 ± 0.1	1.0002 ± 0.0002
Hematite	-21.2 ± 3.3	1.044 ± 0.007	-0.3 ± 0.4	1.0003 ± 0.0008
TCAAP				
Untreated + Fe(II)	-21.5 ± 2.6	1.045 ± 0.005	-0.8 ± 1.0	1.0008 ± 0.0020
Reduced	-15.8 ± 4.3	1.033 ± 0.009	-0.3 ± 0.2	1.0003 ± 0.0004
Magnetite	-13.2 ± 1.7	1.027 ± 0.003	-0.7 ± 0.3	1.0007 ± 0.0006
Goethite ^a	-16.9 ± 3.0	1.035 ± 0.003	-0.1 ± 0.3	1.0000 ± 0.0003
Mackinawite ^a	-18.8 ± 1.4	1.039 ± 0.001	-0.8 ± 0.6	1.0008 ± 0.0008

^adata obtained from Berens et al.¹⁶²

Table 12-4. Estimated values of predicted vs measured DNAN transformation in sediment columns. All errors represent 95% confidence intervals.

Material	Measured (c/c_o)	$\Delta^{15}\text{N}$ (‰)	Predicted (c/c_o)	Error (c/c_o)
TCAAP	0.98	-1.94 ± 0.16	0.96 ± 0.010	0.02
	0.97	-1.52 ± 0.11	0.94 ± 0.007	0.03
	0.83	3.51 ± 0.15	0.67 ± 0.007	0.16
	0.50	9.59 ± 0.02	0.45 ± 0.001	0.04
	0.24	14.28 ± 0.16	0.33 ± 0.003	0.09
	0.15	16.64 ± 0.13	0.28 ± 0.002	0.13
	0.14	15.06 ± 0.43	0.31 ± 0.009	0.18
Tinker AFB	1.00	-2.22 ± 0.08	0.98 ± 0.005	0.02
	0.98	-1.20 ± 0.11	0.92 ± 0.007	0.06
	0.94	-1.04 ± 0.91	0.91 ± 0.008	0.03
	0.41	8.47 ± 0.18	0.48 ± 0.006	0.07
	0.19	14.82 ± 0.11	0.32 ± 0.002	0.13
	0.16	11.95 ± 0.12	0.38 ± 0.003	0.22

12.1.7.4 Additional figures.

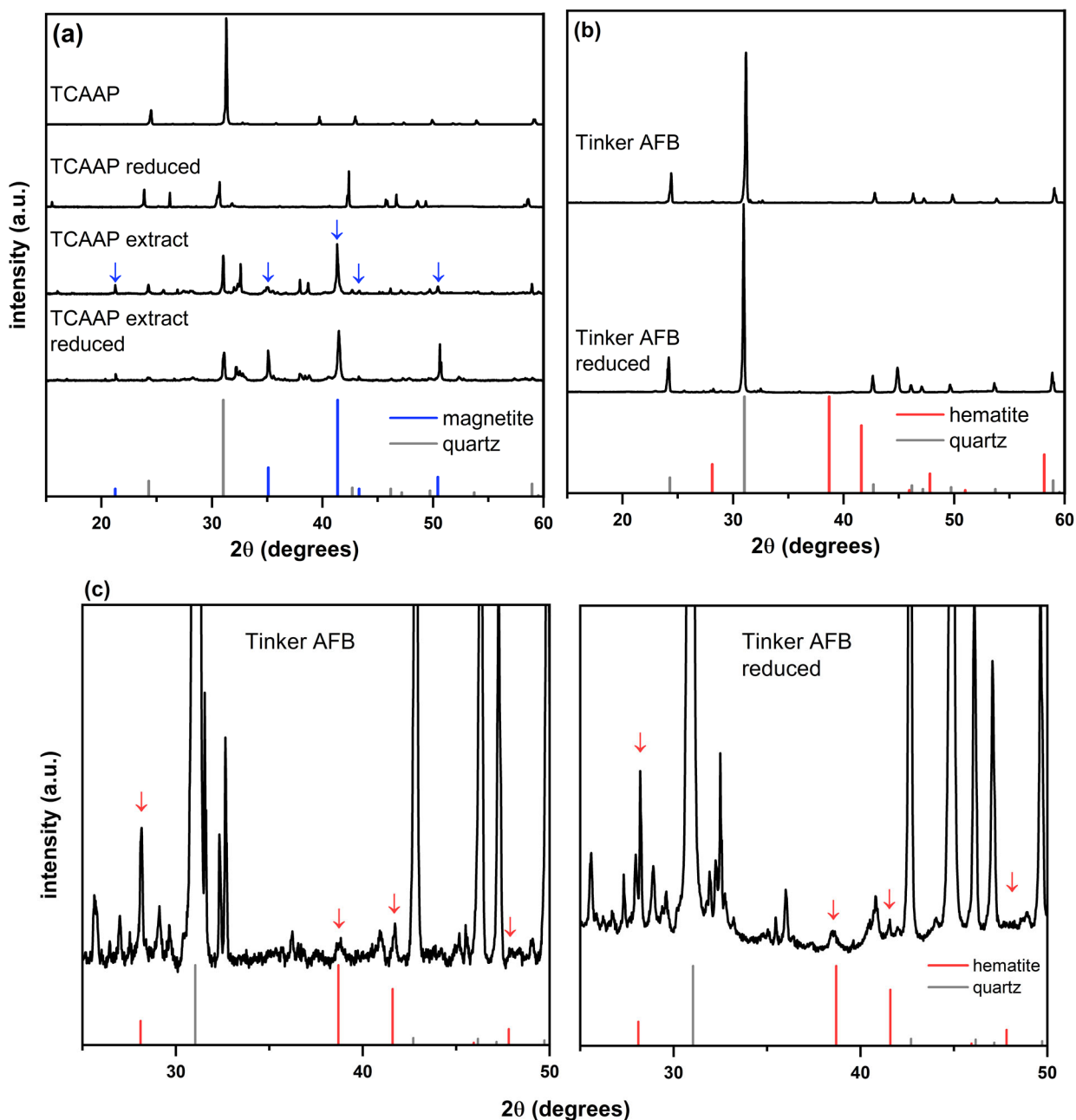


Figure 12-15. X-ray diffraction patterns of native and dithionite-reduced minerals used in this study. Both the bulk TCAAP material (a, top two patterns) and the magnetic extract (a, bottom two patterns) indicated that magnetite was the dominant iron-bearing mineral phase. (b) Hematite was the dominant iron phase in Tinker AFB sediment. Because of the strong quartz signal in Tinker AFB patterns, an enlarged subsection is provided in (c). These data reveal that negligible phase transformation occurred during ISCR and suggest that the primary effects of the treatment on the underlying mineralogy was the (re)generation of surface Fe(II).

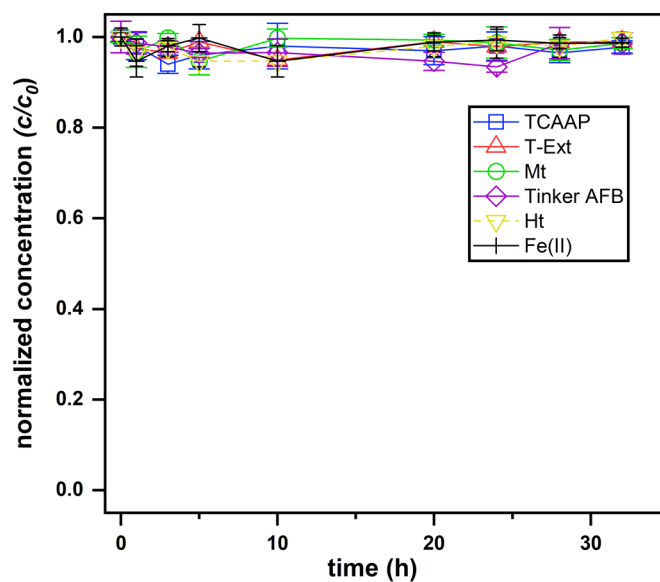


Figure 12-16. Control experiments for DNAN removal in suspensions of untreated minerals and dissolved Fe(II) without a mineral present. No reduction was observed over 32 h. Reactors remained on the rotator for three weeks with no detectable concentration loss (data not shown).

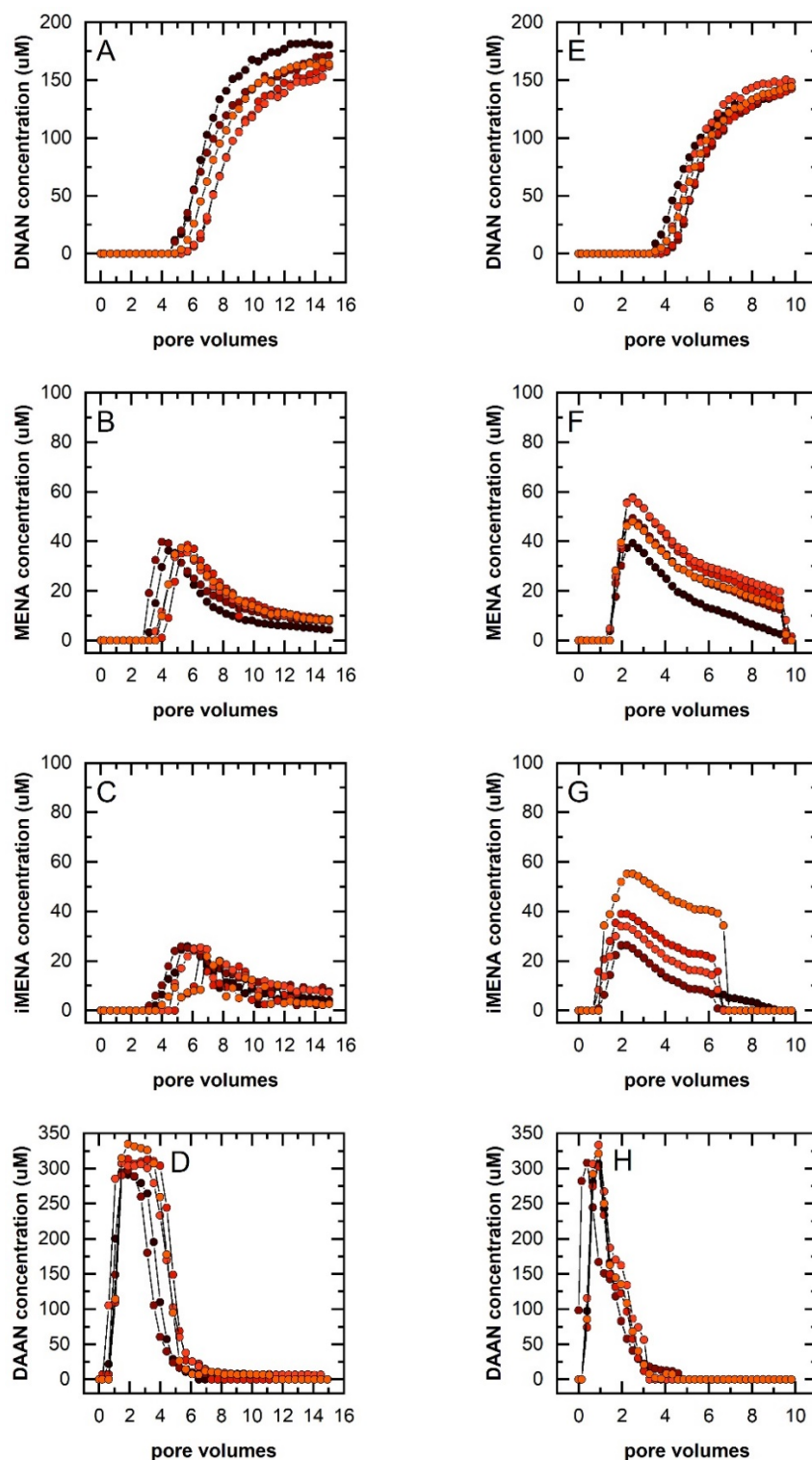


Figure 12-17. Breakthrough curves for 200 μM DNAN in (A-D) TCAAP and (E-H) Tinker AFB sediment columns following five sequential cycles of dithionite exposure and DNAN reduction. These are the data from Figure 2 (main text) expanded to show each individual cycle.

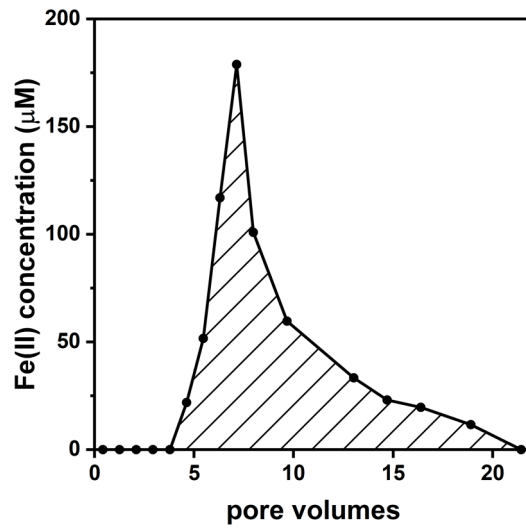


Figure 12-18. Representative concentration profile of Fe(II) detected in column effluents during dithionite treatment.

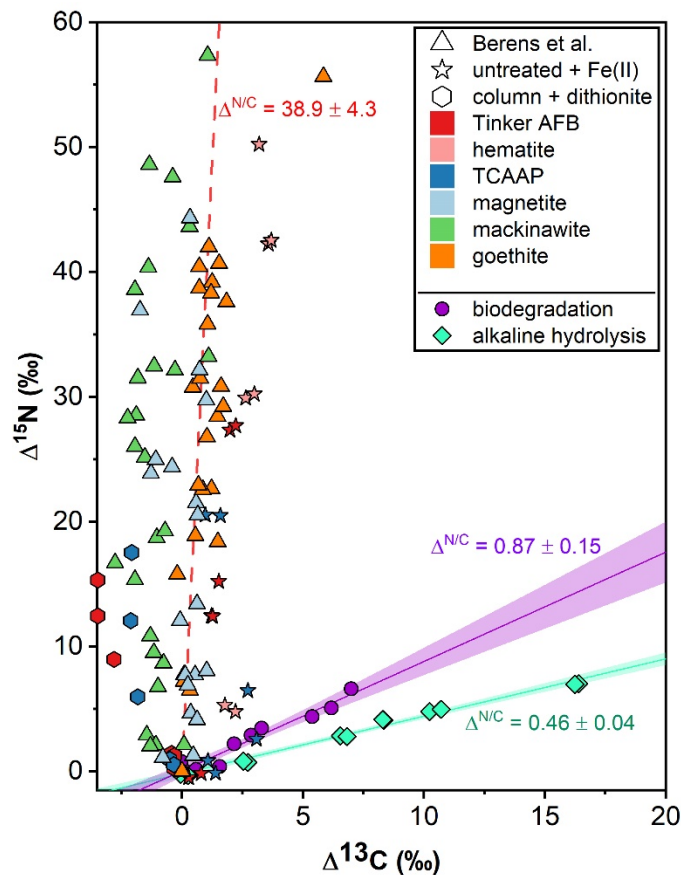


Figure 12-19. Dual-element (N vs C) isotope analysis to indicate multiple potential DNAN transformation pathways. Data from the present study for abiotic reduction by untreated minerals + Fe(II) (stars) and from columns receiving ISCR (hexagons) are shown. The observations from our previous work¹⁶² is also provided (triangles). The dotted line represents the fit from linear regression by the York method as described by Ojeda et al.¹⁹⁰ The isotope fractionation observed during biodegradation (circles) and alkaline hydrolysis (diamonds) are provided for reference. The latter data sets were reproduced with permission from Ulrich et al.¹³³ Shaded portions represent the 95% confidence intervals from nonlinear regression analysis.

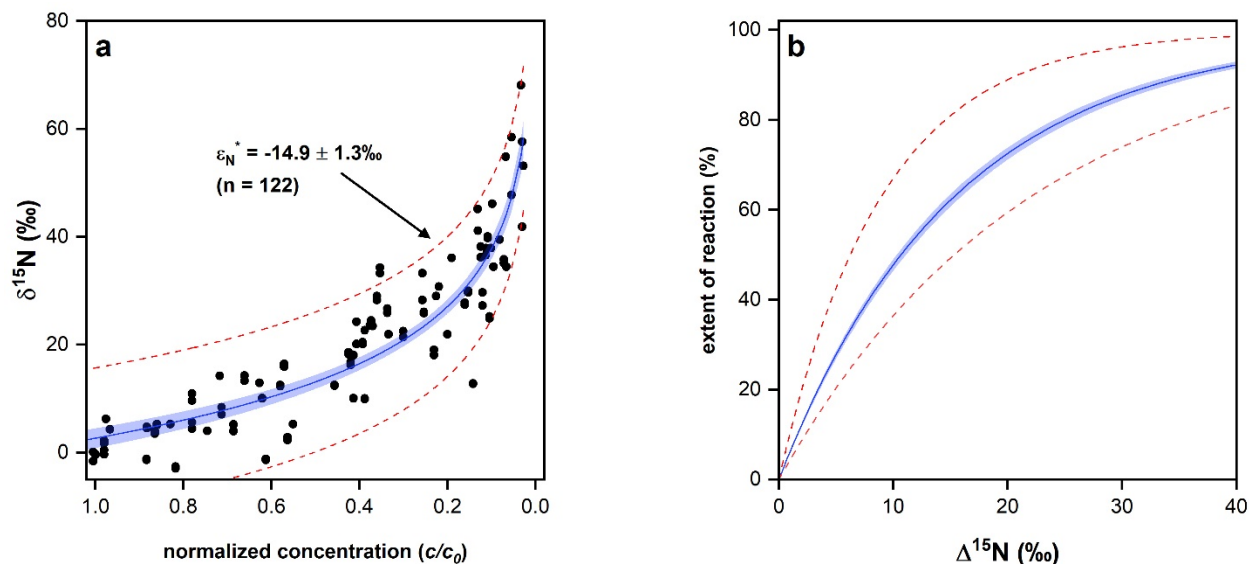


Figure 12-20. $\delta^{15}\text{N}$ vs c/c_0 values were combined from all batch experiments to evaluate consistencies in fractionation and calculate the associated ε_N^* value. Theoretical plot relating the extent of DNAN transformation to measured $\delta^{15}\text{N}$ values using eq 2.

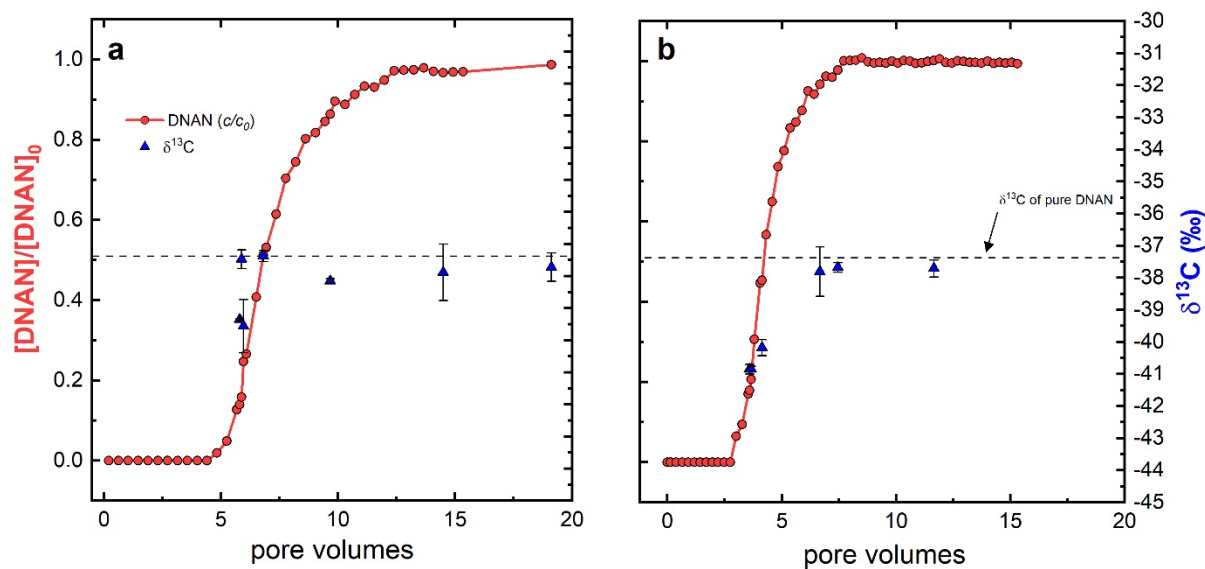


Figure 12-21. Extent of DNAN conversion ($[\text{DNAN}]/[\text{DNAN}]_0$, circles) and C isotope signatures ($\delta^{13}\text{C}$, triangles) measured at the breakthrough front during column experiments with dithionite-reduced (a) TCAAP and (b) Tinker AFB materials. Concentration data represent the average of five sequential breakthrough experiments. Error bars in $\delta^{13}\text{C}$ values indicate the standard deviations from triplicate measurements.

12.1.8 Additional RDX results

12.1.8.1 Kinetics equation sets for RDX abiotic reduction pathways

Kinetics equations 1-12 are for Figure 10-6a pathways. [C-M] = [M] × Number atoms of C in Compound M.

In case of ring cleavage at TNX:

$$\frac{d[C-RDX]}{dt} = -k_{RDX-MNX}[C - RDX] \quad 1$$

$$\frac{d[C-MNX]}{dt} = k_{RDX-MNX}[C - RDX] - k_{MNX-DNX}[C - MNX] \quad 2$$

$$\frac{d[C-DNX]}{dt} = k_{MNX-DNX}[C - MNX] - k_{DNX-TNX}[C - DNX] \quad 3$$

$$\frac{d[C-TNX]}{dt} = k_{DNX-TNX}[C - DNX] - k_{TNX-HCHO}[C - TNX] - k_{TNX-U}[C - TNX] \quad 4$$

$$\frac{d[C-HCHO]}{dt} = k_{TNX-HCHO}[C - TNX] \quad 5$$

$$\frac{d[C-Unidentified]}{dt} = k_{TNX-U}[C - TNX] \quad 6$$

Or in case of ring cleavage at DNX:

$$\frac{d[C-RDX]}{dt} = -k_{RDX-MNX}[C - RDX] \quad 7$$

$$\frac{d[C-MNX]}{dt} = k_{RDX-MNX}[C - RDX] - k_{MNX-DNX}[C - MNX] \quad 8$$

$$\frac{d[C-DNX]}{dt} = k_{MNX-DNX}[C - MNX] - k_{DNX-TNX}[C - DNX] - k_{DNX-HCHO}[C - DNX] - k_{DNX-U}[C - DNX] \quad 9$$

$$\frac{d[C-TNX]}{dt} = k_{DNX-TNX}[C - DNX] \quad 10$$

$$\frac{d[C-HCHO]}{dt} = k_{DNX-HCHO}[C - DNX] \quad 11$$

$$\frac{d[C-Unidentified]}{dt} = k_{DNX-U}[C - DNX] \quad 12$$

The kinetics equations 13-18 for Figure 10-6b are:

$$\frac{d[C-RDX]}{dt} = -(k_{RDX-MNX} + k_{RDX-HCHO} + k_{RDX-U})[C - RDX] \quad 13$$

$$\frac{d[C-MNX]}{dt} = k_{RDX-MNX}[C - RDX] - k_{MNX-DNX}[C - MNX] - k_{MNX-HCHO}[C - MNX] - k_{MNX-U}[C - MNX] \quad 14$$

$$\frac{d[C-DNX]}{dt} = k_{MNX-DNX}[C - MNX] - k_{DNX-TNX}[C - DNX] \quad 15$$

$$\frac{d[C-TNX]}{dt} = k_{DNX-TNX}[C - DNX] \quad 16$$

$$\frac{d[C-HCHO]}{dt} = k_{RDX-HCHO}[C - RDX] + k_{MNX-HCHO}[C - MNX] \quad 17$$

$$\frac{d[C-\text{Unidentified}]}{dt} = k_{RDX-U}[C - RDX] + k_{MNX-U}[C - MNX]$$

18

Numbers in square brackets represent RDX and its reduction products in the form of total carbon (as shown in Figure 10-6 in the main text) in μM as concentration. Boundary conditions to solve the equations are RDX degradation rate constant, k_1 , determined by experiments and RDX initial concentration in terms of total carbon, c_0 . MATLAB code is shown as below (taking green rust pH 7.5 as an example):

```
%function RDX_kinetics
t=[0.00;1.00;2.00;3.00;4.00;5.00];
c=[1126.49 6.50 2.80 0.00 0 0.00
    929.33 120.69 8.76 0.00 14.09 62.92
    654.46 22.65 0.00 0.00 120.74 337.94
    642.48 6.08 0.00 0.00 187.35 299.88
    506.77 2.62 0.00 0.00 225.16 401.23
    283.63 0.00 0.00 0.00 281.41 570.74];

k0=[0.25;1;1;1;1];
[k,resnorm,residual,exitflag,output,lambda,jacobian]=lsqcurvefit(@kinetics,k0,t,c);
conf=nlparci(k,residual,'jacobian',jacobian);
fprintf(1,'\tRate Constants:\n')
for k1 = 1:length(k)
    fprintf(1, '\t\tk(%d) = %8.5f\n', k1, k(k1))
end
fprintf('\t\tresnorm=%d\n', resnorm)
tv = linspace(min(t), max(t));
Cfit = kinetics(k, tv);
%rate equations
function dC=DifEq(t,c,k)
    dc=zeros(6,1);
    dc(1) = -0.25*c(1);
    dc(2) = 0.25*c(1) - k(2)*c(2);
    dc(3) = k(2)*c(2) - k(3)*c(3)-k(4)*c(3)-k(5)*c(3);
    dc(4) = k(3)*c(3);
    dc(5) = k(4)*c(3);
    dc(6) = k(5)*c(3);
    k(4)+k(5)==16.66;
    dC=dc;
end
%solve rate equations
function C=kinetics(k,t)
c0 = [1126.5; 0; 0.00; 0.00; 0; 0.00];
[T,Cv]=ode45(@DifEq(t,c,k),t,c0);
C=Cv;
end
```

12.1.8.2 *Fitting to kinetic model and determination of reaction pathways.*

Fe(II)/goethite, Fe(II)/magnetite, and green rust model fits (Figure 12-22a-e) caused immediate MNX formation and lagged formation of other intermediates and ring cleavage products. For goethite reactors, increasing the pH yielded higher reaction rate constants and a decreased accumulation of the intermediates (Table 12-5 and Figure 12-22a-c). The reaction pathways were best explained mathematically by Figure 10-6a in which HCHO and C-Unidentified were assumed to form from either DNX or TNX. The slowest step of the reaction is the reduction of the first nitro group in RDX, and reactions of the nitroso compounds are at least two, and in some cases, hundreds of times faster. This indicates a sequential reduction of RDX to the nitroso compounds, instead of direct ring cleavage from the parent compound, with minimal accumulation of nitroso intermediates. Because reduction of the $-\text{NO}_2$ group to $-\text{NO}$ occurs, this indicates that monitoring of N enrichment during RDX abiotic reduction is appropriate for evaluating this reaction pathway.

The reduction of RDX by FeS is best explained by equations built upon Figure 10-6b, where nitro group reduction and direct ring cleavage of RDX molecules are both important. The modeled curves and experimental data for FeS samples are shown in Figure 12-22f-h. The distribution of intermediates and products over time for FeS is clearly different than that for the iron oxides (i.e., faster RDX degradation and longer lifespan of nitroso intermediates were observed with FeS). Direct ring cleavage of RDX was previously reported for anaerobic biodegradation.^{91,94} The experimental and simulated data presented here imply that abiotic reduction mediated by FeS may also lead to direct ring cleavage and thus different N isotope fractionation. Further experiments should be carried out to assess the products formed during FeS mediated reduction to assess at which step ring cleavage occurs.

Some of the errors associated with the rate constants in Table 12-5 have magnitudes equal to or greater than the predicted values. The fitting exercise, however, was valuable because it provided both insight into which species could undergo ring cleavage and the relative importance of pathways that lead to HCHO and other carbon products as a function of mineral identity. Also, the persistence of nitroso compounds in the reactions with FeS and potential parallel degradation pathways for RDX revealed important differences in reactivity among minerals that, as shown below, have implications for the utility of CSIA.

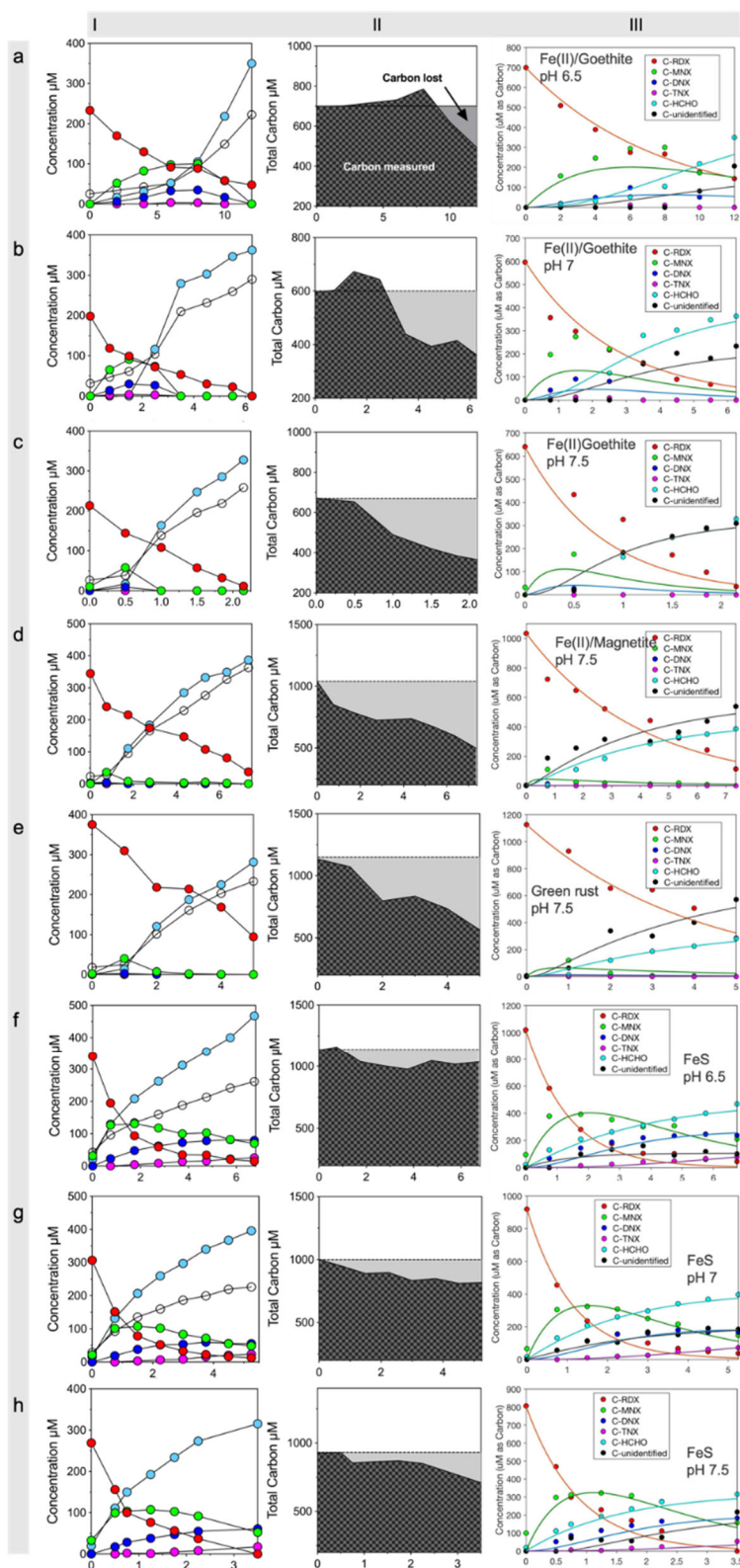


Figure 12-22. Concentration versus time for RDX and intermediates in all tested reductive systems. Column I: Measured concentrations of RDX, nitroso intermediates and final

degradation products in μM . Column II: Mass balance of total carbon. Column III: Experimental data (symbols) and model fits (lines) for RDX reduction on a carbon basis and product formation under various mineral and pH conditions. A hypothetical carbon-containing product (C-Unidentified; black solid circle) was introduced to compensate for the incomplete carbon mass balance and was assumed to be a single reaction product in kinetic fitting. Panel (a)-(c) Reductions mediated by surface-bound Fe(II) with goethite at pH 6.5, 7, and 7.5, respectively. RDX reduction at pH 7.5 by (d) Fe(II)/magnetite and (e) carbonate green rust. RDX reduction by FeS at pH (f) 6.5, (g) 7.0, and (h) 7.5. Rate constants for Column III are in Table 12-5.

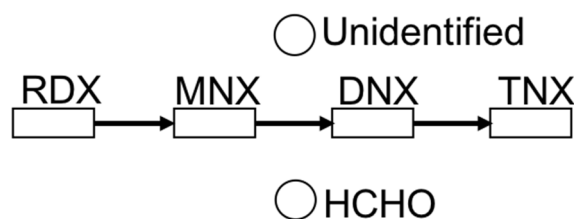
Table 12-5. Rate constant (k) values obtained from the fitting of RDX reduction and product formation to the kinetic equations that showed the lowest normalized residual errors. Errors associated with the rate constants are 95% confidence intervals.

Scheme 1a Case 1: Ring cleavage from DNX	^a k _{RDX-MNX} (h ⁻¹)	k _{MNX-DNX} (h ⁻¹)	k _{DNX-TNX} (h ⁻¹)	k _{DNX-HCHO} (h ⁻¹)	k _{DNX-U} (h ⁻¹)	residual (×10 ⁴ μM)		
Fe(II)/Goethite								
pH 6.5	0.13±0.03	0.21±0.06	0±0.07	0.48±0.33	0.19±0.17	8.59		
pH 7	0.38±0.16	0.98±0.41	0±0.13	1.78±1.84	0.96±1.03	10.67		
Scheme 1a Case 2: Ring cleavage from TNX	k _{RDX-MNX} (h ⁻¹)	k _{MNX-DNX} (h ⁻¹)	k _{DNX-TNX} (h ⁻¹)	^b k _{TNX-HCHO} (h ⁻¹)	^b k _{TNX-U} (h ⁻¹)	residual (×10 ⁴ μM)		
Fe(II)/Goethite pH 7.5	1.24±0.28	4.61±2.7	13.94±23.68	5.61±6.92	5.62±6.93	5.78		
Fe(II)/Magnetite pH 7.5	0.25±0.04	4.92±5.19	302±17962	506±68013	664±89118	7.47		
Green rust pH 7.5	0.25±0.06	3.51±2.52	19.26±78.03	5.36±17.18	10.50±33.45	5.43		
Scheme 1b Ring cleavage from RDX and MNX	k _{RDX-MNX} (h ⁻¹)	k _{MNX-DNX} (h ⁻¹)	k _{DNX-TNX} (h ⁻¹)	k _{RDX-HCHO} (h ⁻¹)	k _{RDX-U} (h ⁻¹)	k _{MNX-HCHO} (h ⁻¹)	k _{MNX-U} (h ⁻¹)	residual (×10 ⁴ μM)
FeS								
pH 6.5	0.54±0.09	0.17±0.03	0.08±0.05	0.12±0.08	0.07±0.07	0.13±0.07	0.01±0.06	6.32
pH 7	0.65±0.08	0.21±0.03	0.12±0.05	0.18±0.07	0.07±0.06	0.16±0.07	0.09±0.07	2.31
pH 7.5	0.99±0.18	0.26±0.08	0.10±0.16	0.26±0.14	0±0.23	0.14±0.16	0.26±0.17	4.41

^aAll k_{RDX-MNX} values were experimentally determined from separate sets of batch experiments. The fixed k_{RDX-MNX} values were used as an initial condition.

^bThe rate constants of TNX degradation were experimentally determined (see Section 10.2.4.1) and applied as a constraint of the kinetic model (k_{TNX} = k_{TNX-HCHO} + k_{TNX-U}). The values of k_{TNX-HCHO} and k_{TNX-U} in this table were derived from the model.

Other branching options for reaction pathways were proposed and tested. The legend for the pathways tested is depicted below. The rectangular blocks represent RDX and nitroso intermediates, and circles stand for ring cleavage products, i.e. HCHO and the unidentified compound. The branching scenarios to the HCHO and unidentified compound are depicted by arrows connecting RDX and/or a nitroso intermediate to these species.



Some branching options could be excluded from consideration according to the patterns of degradation and formation of intermediates and products. For instance, for FeS, branching of ring cleavage products from TNX is unlikely to occur, because of the increasing in TNX concentration during the reaction. Additionally, the formation of HCHO and unidentified compound shows no lag phase, suggesting an early-stage ring cleavage. Hence, four-branch pathways including two branches stemming from RDX were tested to compare goodness of fit for the FeS experimental data (Table 12-6).

Table 12-6. Goodness of fit in terms of model residual, for FeS at varying pHs

FeS		
pH 6.5	6.58×10^4	2.34×10^5
pH 7	2.31×10^4	9.97×10^4
pH 7.5	4.41×10^4	1.05×10^5

For green rust and iron (hydro)oxides at pH 7.5, the minimal TNX accumulation is likely due to fast formation and degradation of TNX (Table 10-3). The lag phase of ring cleavage product formation suggests that early stage ring cleavage unlikely occurs. Thus, two-branch pathways that stem from compounds other than TNX is unlikely to occur. Pathways including branches from TNX are compared for the best goodness of fit (Table 12-7).

Table 12-7. Goodness of fit in terms of model residual, for green rust, goethite and magnetite at pH 7.5

pH 7.5			
Fe(II)/goethite	1.15×10^6	1.14×10^6	5.78×10^4
Fe(II)/magnetite	2.52×10^6	2.50×10^6	7.74×10^4
Green rust	1.51×10^6	1.49×10^6	5.43×10^4

For goethite at pH 6.5 and 7, TNX had negligible degradation under these conditions, the minimal TNX accumulation is therefore likely due to negligible formation of TNX from DNX. The lag phase of ring cleavage product formation suggests that early stage ring cleavage unlikely occurs. Hence, pathways including branches from DNX are compared for the best goodness of fit (Table 12-8).

Table 12-8. Goodness of fit in terms of model residual, for goethite at pH 6.5 and 7. Note that the modeled $k_{\text{MNX-HCHO}}$ and $k_{\text{MNX-U}}$ for the four-branch pathway are all negative, suggesting that the branches stemming from MNX is unlikely to occur.

Fe(II)/goethite		
	8.74×10^4	
pH 6.5	$k_{\text{MNX-HCHO}} = -11.1$ $k_{\text{MNX-U}} = -10.5$	8.56×10^4
pH 7	10.25×10^4 $k_{\text{MNX-HCHO}} = -12.1$ $k_{\text{MNX-U}} = -10.4$	10.67×10^4

12.1.9 HMX synthesis and degradation experiment

HMX (1,3,5,7-Tetranitro-1,3,5,7-tetrazocane) synthesis was attempted several times but did not successfully yield pure HMX. Procedures are briefly summarized here. HMX synthesis requires a two-step process: 1) partial acetylation and nitration of HMTA to 1,5-diacetyloctahydro-3,7-dinitro-1,3,5,7-tetrazocine (DADN), and 2) complete nitration of DADN to HMX. In the first step, a 25 mL three-necked round bottom flask was equipped with a reflux condenser and magnetic stirrer in a circulating water bath. Water (0.8 g) was added to the flask and equilibrated to 5 – 10 °C before adding 1.6 g HMTA and 0.7 g ammonium acetate slowly over 10 min. With this solution still stirring at 5 – 10 °C, 3.5 g of acetic anhydride was added dropwise over 60 min. The solution was stirred an additional 30 min and transferred to an addition funnel perched above the water bath. The water temperature was raised to 18 – 20 °C. In a 250 mL beaker in the water bath, a mixture of 7.17 g of pure fuming nitric acid (> 99%) and 25.15 g of 96% fuming sulfuric acid (20% oleum) was vigorously stirred using a mechanical stirrer. Once equilibrated to temperature, the HMTA solution in the addition funnel was added very slowly to the beaker over 80 min, carefully maintaining the temperature below 20 °C. After stirring an additional 20 min, 230 g of ice made from ultrapure water was added. In the first attempt, no product (DADN) precipitated, despite adding an additional 250 mL of ice cold water. In other attempts, a small amount of white product precipitated (cloudy) but then dissolved or was otherwise lost upon filtration. Efforts to recollect it were unsuccessful. Slight adaptations of this procedure did not produce a solid that could be used in the second step. Thus, the limited experiments performed with HMX used analytical standard materials.

Results from early kinetic experiments of HMX with goethite and aqueous Fe(II) in bicarbonate buffer demonstrate that HMX is reduced via abiotic reduction under these conditions (Figure 12-23). Because a GC- and LC-IRMS methods were unable to be fully developed and the limitations on HMX material, additional studies were not pursued.

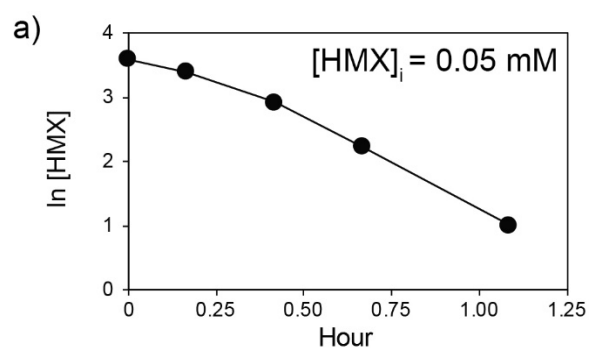


Figure 12-23. HMX degradation by adsorbed Fe(II) on goethite. Initial conditions were 10 mM bicarbonate buffer pH 7 with 0.5 g L^{-1} goethite and 1 mM aqueous Fe(II).

12.2 List of publications and abstracts

12.2.1 Publications

Strehlau, J. H.; Berens, M. J.; Arnold, W. A. Mineralogy and Buffer Identity Effects on RDX Kinetics and Intermediates during Reaction with Natural and Synthetic Magnetite. *Chemosphere* **2018**, *213*, 602–609.

Ulrich, B.A.; Palatucci, M; Bolotin, J; Spain, J.C.; Hofstetter, T. B. Different Mechanisms of Alkaline and Enzymatic Hydrolysis of the Insensitive Munition Component 2,4-Dinitroanisole Lead to Identical Products. *Environ. Sci. Technol.*, **2018**, *5*, 456-471.

Berens, M. J.; Ulrich, B. A.; Strehlau, J. H.; Hofstetter, T. B.; Arnold, W. A. Mineral Identity, Natural Organic Matter, and Repeated Contaminant Exposures Do Not Affect the Carbon and Nitrogen Isotope Fractionation of 2,4-Dinitroanisole during Abiotic Reduction. *Environ. Sci. Process. Impacts*, **2019**, *21*, 51–62.

Berens, M. J.; Hofstetter, T. B.; Bolotin, T; Arnold, W. A. Assessment of 2,4-Dinitroanisole Transformation Using Compound-Specific Isotope Analysis after In Situ Chemical Reduction of Iron Oxides. *Environ. Sci. Technol.*, **2020**, *54*, 5520-5531.

Berens, M. J.; Hofstetter, T. B.; Bolotin, T; Arnold, W. A. Assessment of 2,4-Dinitroanisole Transformation Using Compound-Specific Isotope Analysis after In Situ Chemical Reduction of Iron Oxides. **2020**. ChemRxiv doi: <https://chemrxiv.org/s/c7af599f0eaa4bced497>

12.2.2 Conference abstracts

12.2.2.1 Oral presentations

Berens, M. J., Bolotin, J., Hofstetter, T. B., Arnold, W. A. Assessment of 2,4-dinitroanisole transformation after in situ chemical reduction of iron oxides using CSIA. ACS, Philadelphia, PA, Spring 2020 (virtual conference).

Berens, M. J., Ulrich, B.A., Strehlau, J. H., Hofstetter, T. B., Arnold, W. A. Compound specific isotope analysis of nitroaromatic compounds during reaction with Fe-bearing minerals. ACS, Orlando, FL, March 31-April 4, 2019.

Hofstetter, T.B. Ulrich, B., Berens, M.J., Spain, J., Arnold, W.A. Compound-specific isotope analysis reveals the transformation processes of the insensitive munition component 2,4-dinitroanisole and its reaction products. Oral presentation, 258th ACS National Meeting & Exposition. Orlando, FL, March 31-April 4, 2019.

Tong, Y., Strehlau, J.H., Ulrich, B., Bolotin, J., Hofstetter, T.B., Arnold, W.A. Reduction of nitro explosives RDX and NTO by iron-bearing minerals: A study on the kinetics and using compound specific isotope analysis to assess mechanisms. Oral presentation, 258th ACS National Meeting & Exposition. Orlando, FL, March 31-April 4, 2019.

Tong, Y.; Strehlau, J. H.; Ulrich, B. A.; Hofstetter, T. B.; Arnold, W. A. Understanding the Mechanisms of Mineral-Mediated Abiotic Reduction of RDX with Compound Specific Isotope Analysis. Talk. Water Technology Accelerator (WaTA) Seminar Series at the University of Wisconsin-Milwaukee, October 18th, 2018

Berens, M. J., Ulrich, B. A., Strehlau, J. H., Hofstetter, T. B., Arnold, W. A. Mineral-mediated attenuation of nitroaromatic contaminants in groundwater systems. Society of Environmental Toxicology and Chemistry North America Meeting, Minneapolis, MN, 2017.

12.2.2.2 Poster presentations

Berens, M. J., Bolotin, J., Hofstetter, T. B., Arnold, W. A. Reduction of 2,4-dinitroanisole after in situ chemical reduction of iron oxides. SERDP and ESTP Symposium, Washington, DC, December 3, 2019.

Tong, Y., Berens, M.J., Strehlau, J.H., Hofstetter, T.B., Arnold, W.A. Using compound specific isotope analysis (CSIA) to assess abiotic reduction of energetic nitro compounds: A study on lab and field samples. Poster presentation, SERDP symposium. Washington D.C., December 3, 2019

Berens, M. J.; Strehlau, J. H.; Ulrich, B. A.; Hofstetter, T. B.; Arnold, W. A. Compound Specific Isotope Analysis of Nitroaromatic Compounds Upon Reaction with Fe-bearing Minerals Poster. Presented at the SERDP & ESTCP Symposium 2018, November 27th-29th 2018, Washington DC

Tong, Y.; Strehlau, J. H.; Berens, M. J.; Arnold, W. A. The Kinetics of RDX abiotic reduction by synthetic and natural iron-bearing minerals. Poster. Presented at the SERDP & ESTCP Symposium 2018, November 27th-29th 2018, Washington DC.

Tong, Y., Berens, M.J., Strehlau, J.H., Hofstetter, T.B., Arnold, W.A. Assessing RDX natural attenuation in groundwater using compound specific isotope analysis. Poster presentation, COE. Minneapolis, MN, November 7, 2019

Berens, M. J., Ulrich, B. A., Strehlau, J. H., Hofstetter, T. B., Arnold, W. A. Evaluating the effects of matrix conditions and transformation processes on the nitrogen and carbon isotope fractionation of 2,4-dinitroanisole. Minnesota Water Resources Conference, St. Paul, MN, October 17-18, 2018

Tong, Y.; Strehlau, J. H.; Berens, M. J.; Arnold, W. A. The Kinetics of RDX abiotic reduction by synthetic and natural iron-bearing minerals. Poster. Presented at the Minnesota Water Resources Conference, St. Paul, MN, October 17-18, 2018

Ulrich, B.A., Spain, J., Arnold, W.A., Hofstetter, T.B. Development of an Improved Microextraction Method to Enable Compound-Specific Isotope Analysis of Munitions-Contaminated Groundwater (Poster presentation) SERDP & ESTCP Symposium 2017, November 28th-30th 2017, Washington DC

Strehlau, J.H., Berens, M., Ulrich, B.A., Hofstetter, T.B., Arnold, W.A. Compound specific isotope analysis of the abiotic reduction of munitions in iron mineral suspensions (Poster presentation) SERDP & ESTCP Symposium 2017, November 28th-30th 2017, Washington DC

Berens, M., Strehlau, J., Ulrich, B., Hofstetter, T., Arnold, W. Mineral-Mediated Attenuation of Nitroaromatic Contaminants in Groundwater Systems. 38th Society of Environmental Toxicology and Chemistry North America Meeting, Minneapolis, Minnesota, November, 2017

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