Groundwater Research Report
WR11R006

# The Effects of Particulate Organic Carbon Quantity and Quality on Denitrification of Groundwater Nitrate

**Robert Stelzer, Ph.D.**

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Project Number WR11R006

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# The Effects of Particulate Organic Carbon Quantity and Quality on Denitrification of Groundwater Nitrate **(WRI Project Number WR11R006)**

## FINAL REPORT

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## Project Period

July 1, 2011 to June 30, 2013

# TABLE OF CONTENTS

## LIST OF FIGURES

## PROJECT SUMMARY

**Title:** The effects of particulate organic carbon quantity and quality on denitrification of groundwater nitrate

**Project I.D.:** WR11R006

**Investigators:**
Principal Investigator: Robert Stelzer, Professor
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**Period of Contract:** July 1, 2011 to June 30, 2013

**Background/Need:** Groundwater nitrate concentrations are elevated and rising in many aquifers throughout the world, including those in Wisconsin. Elevated nitrate concentration in groundwater can lead to human health problems and can contribute to eutrophication in river networks and coastal ecosystems. Many comparative studies have shown that denitrification rates are positively related to organic carbon quantity in soils and sediments. However, there have been few manipulative studies in field settings that have addressed how organic carbon quantity and quality affects denitrification and nitrate retention in groundwater. The overall objective of the research project was to determine how particulate organic carbon (POC) quantity and quality influences groundwater nitrate removal and retention in stream sediments.

**Objectives:** The study was conducted in two phases. Phase I included an experimental manipulation of POC quantity and Phase II included an experimental manipulation of POC quality. The main objective of Phase I was to determine how the *quantity* of POC in sediments affected denitrification and nitrate retention in shallow groundwater. The main objective of Phase II was to determine how the *quality* of POC affected denitrification rate, nitrate retention, and dissolved organic carbon (DOC) production and quality in shallow ground water.

**Methods:** Both phases of the study were conducted in Emmons Cr., a third-order groundwater-fed stream in Portage County, WI. In Phase I POC quantity was manipulated and phase II included a manipulation of POC quality. Different amounts (Phase I) or types (Phase II) of POC were added to sediments in replicated mesocosms from which groundwater was sampled for assessment of nitrate retention, denitrification and dissolved organic carbon quantity and quality. In situ rates of denitrification were measured using membrane-inlet mass spectroscopy (MIMS) and denitrification potential was assessed using lab-based acetylene-block incubations. Particulate organic carbon quality was assessed by measuring C:N and lignin:N ratios and DOC quality was assessed using fluorescence excitation emission matrix (EEM) spectroscopy. Direction of groundwater flow was determined based on measurements of vertical hydraulic gradient and groundwater velocity, necessary for measuring nitrogen fluxes, was determined using NaCl injections. Pore water dissolved oxygen was measured using a dissolved oxygen microelectrode. ANOVA and PARAFAC statistical models were used to analyze the data.

**Results/Discussion:** POC addition affected nitrogen processing in groundwater and the rate of nitrogen processing was influenced by POC quality. In Phase I, POC addition drove oxic groundwater to severe hypoxia, lead to large increases in dissolved organic carbon (DOC) and strongly increased denitrification rates and nitrogen (nitrate and total dissolved nitrogen) retention relative to the Control. In situ denitrification accounted for 30 to 60 % of nitrate retention. In Phase II, POC quality had strong effects on nitrogen processing in shallow groundwater. Leaf treatments had much higher nitrate retention and denitrification rates than red maple wood and control treatments and red maple leaf burial resulted in higher nitrate retention rate than burial of red oak leaves. Leaf, but not wood, burial drove pore water to severe hypoxia and leaf treatments had higher DOC production and different DOC chemical composition than the wood and control treatments. We think that POC quality affected nitrogen processing in the sediments by affecting the quantity and/or quality of DOC and redox conditions. Our results suggest that POC burial in stream sediments can stimulate nitrogen transformation in shallow groundwater and that the rate of nitrogen removal and retention is affected by the quality of the POC.

**Conclusions/Implications/Recommendations:** POC addition stimulated nitrogen processing in shallow groundwater and the quality of the POC influenced the rates of nitrogen processing. A number of plausible mechanisms could explain how POC quantity and quality influenced N processing, including changes in DOC quantity and quality and redox status in the sediments. We think that our results have implications for the management of groundwater, groundwater-fed streams, and their associated watersheds. In particular the presence of riparian or aquatic vegetation (POC quantity), the type of plant species present (POC quality), the hydrological connection between surface water and groundwater and the availability of groundwater nitrate will all likely impact the amount of nitrogen retention and removal that occurs at the groundwater-surface water interface in lotic ecosystems. The strong link that we demonstrated between carbon availability and nitrogen processing in shallow groundwater should be considered when managing groundwater systems, streams, rivers and their watersheds.

## Related Publications

None currently. However, two manuscripts from this project have been submitted for publication and are currently in review at *Freshwater Science* (Phase I) and at *Biogeochemistry* (Phase II).

## Key Words

groundwater, carbon quality, hyporheic, groundwater-surface water interactions, stream, sediments, microbes nitrogen processing, denitrification, nitrate retention, redox

## Funding

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## Introduction

The concentration of nitrate in groundwater and surface water has increased in recent decades throughout much of the United States, including Wisconsin (Dubrovsky et al. 2010). Recent studies have shown that a substantial amount of nitrate can be removed along groundwater flow paths (e.g. Puckett et al. 2008) and in stream channels (e.g. Mulholland et al. 2008). Much of the nitrate removal in groundwater and in stream channels is due to denitrification. A better understanding of the factors that regulate denitrification and nitrate retention in groundwater is necessary to determine how ecosystems will respond to changes in nitrate loading.

The primary factors that regulate denitrification rates are nitrate availability, organic carbon supply, and redox state (Groffman et al. 2009). Organic carbon commonly serves as the energy source in denitrification and can also play a role in establishing the necessary anoxic conditions, by causing oxygen demand in sediments and saturated soils. Field experiments involving dissolved organic carbon (DOC) amendments have shown that denitrification of groundwater nitrate is frequently carbon-limited in nature (Hill et al. 2000). However, less is known about how particulate organic carbon (POC) affects denitrification rates in the field. There have been few experimental manipulations that have addressed how POC quantity affects denitrification of groundwater nitrate (Shipper and Vojvodic-Vukovic 1998).

There is less known about how organic carbon quality, particularly POC quality, affects denitrification of groundwater nitrate and nitrate retention. Several studies have shown that microbial utilization of carbon is favored when POC has low C:N ratios (e.g. Melillo et al., 1982). It is less clear how denitrifying bacteria will respond to variation in POC quality. The type of POC added to agricultural soils in the lab affected denitrification rates (Greenan et al. 2006). Denitrification rate was inversely related to the percentage of phenolic compounds in POC from wetland soils (Dodla et al. 2008). There have been no prior experimental studies that have addressed how POC quality affects groundwater denitrification rate in field settings.

There are two fundamental ways in which POC quantity can regulate denitrification at groundwater-surface water interfaces. First, because organic carbon is required by heterotrophic denitrifying bacteria, organic carbon supply can directly limit the reduction of nitrate to $N_2$. Second, organic carbon can indirectly regulate denitrification by controlling redox conditions in sediments due to the oxygen demands of heterotrophic microbes. In gaining streams nitrate concentration often declines along flow paths as groundwater moves upward through sediments (Duff et al. 2008, Stelzer et al. 2011). Based on these observations we developed and tested a conceptual model for nitrate removal in deep stream sediments in gaining stream reaches that receive oxic, nitrate-laden groundwater (Stelzer and Bartsch 2012). The model predicts that nitrate concentration in groundwater will begin to decline as the water encounters a zone with redox conditions and organic carbon supply favorable for denitrification. In a similar vein, POC quality likely has both direct and indirect effects on denitrification and other types of nitrogen transformation in sediments and soils (Fig. 1). POC quality could have direct effects on nitrogen processing by influencing the types of microbes and the carbon availability to microbes that are attached or embedded in the POC matrix. POC quality could also indirectly affect nitrogen processing by influencing the quantity and quality of DOC that is leached from the POC (Fig. 1).

Finally, POC quality could affect biological oxygen demand in sediments and soils which can affect redox status and thus, the likelihood that nitrate serves as the terminal electron acceptor during respiration.

In Phase I of this project, we tested one aspect of the Stelzer and Bartsch (2012) conceptual model by determining whether POC burial would affect denitrification and nitrate retention in deep stream sediments. We tested the following hypotheses: 1) POC burial will cause increased nitrate retention and denitrification in stream sediments and 2) POC burial will lead to conditions that are favorable for denitrification including low dissolved oxygen concentration. Phase I of our study included one of the first field experiments to assess the impacts of POC addition on nitrogen transformations at the groundwater-surface water interface.

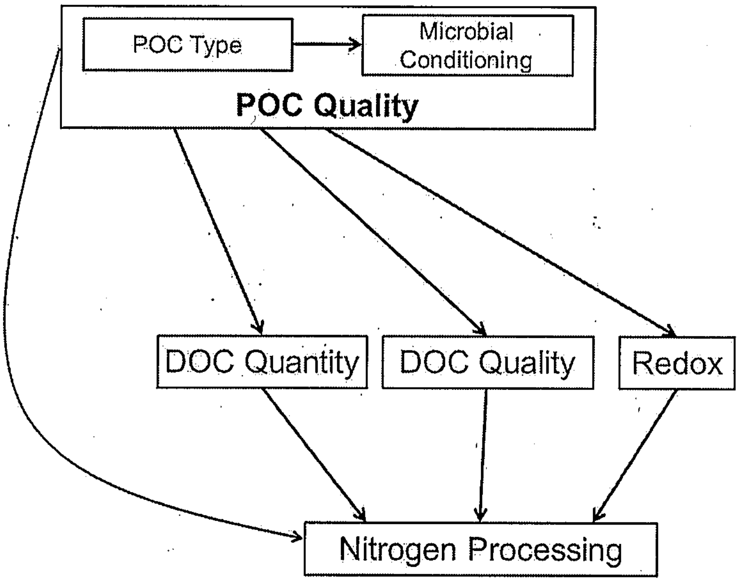

Fig. 1. Proposed model of how POC quality can influence nitrogen processing in shallow groundwater

The main objective of Phase II was to determine how the quality of buried POC affected denitrification rate, nitrate retention, and the oxygen status of pore water in sediments of Emmons Creek. We characterized the chemical composition of the POC and DOC to aid in the analysis of potential mechanisms by which POC quality could affect nitrogen processing. To our knowledge there have been no prior experimental studies that have addressed how POC quality affects denitrification and nitrate retention in field settings.

## Procedures and Methods

### Phase I

*Site Description*

Emmons Creek is a third order, groundwater-fed stream located in Central Wisconsin. Ground water in this ecoregion has high nitrate concentrations, including the groundwater associated with Emmons Creek (Stelzer et al. 2011). The dominant substrate in the wetted channel of Emmons Creek is sand (42%), followed by silt (30%) and gravel (10%). The study was conducted in a reach of Emmons Creek in southeastern Portage County.

### *Experimental Design*

We manipulated POC supply by burying red maple (*Acer rubrum*) leaves in sediments within mesocosms in Emmons Creek. There were Low (7.5 g POC) and High (15 g POC) POC treatments, a Control that consisted of combusted sand, and an Ambient treatment that consisted of untreated undisturbed sediment. The treatments were established in stainless steel cylindrical mesocosms (16 cm diameter x 36 cm long) that were inserted into the sediments (Fig. 2). We used a randomized block design and each treatment was replicated 10 times. A block consisted of a cluster of 4 mesocosms (Fig. 2). A piezometer was installed adjacent to each mesocosm cluster at a mean sediment depth of 41cm for measuring vertical hydraulic gradient. Red maple leaves from the Emmons Creek watershed were microbially conditioned by placing them in mesh bags in Emmons Creek for 19 days. Added POC was buried between two layers of combusted sand (Fig. 2).

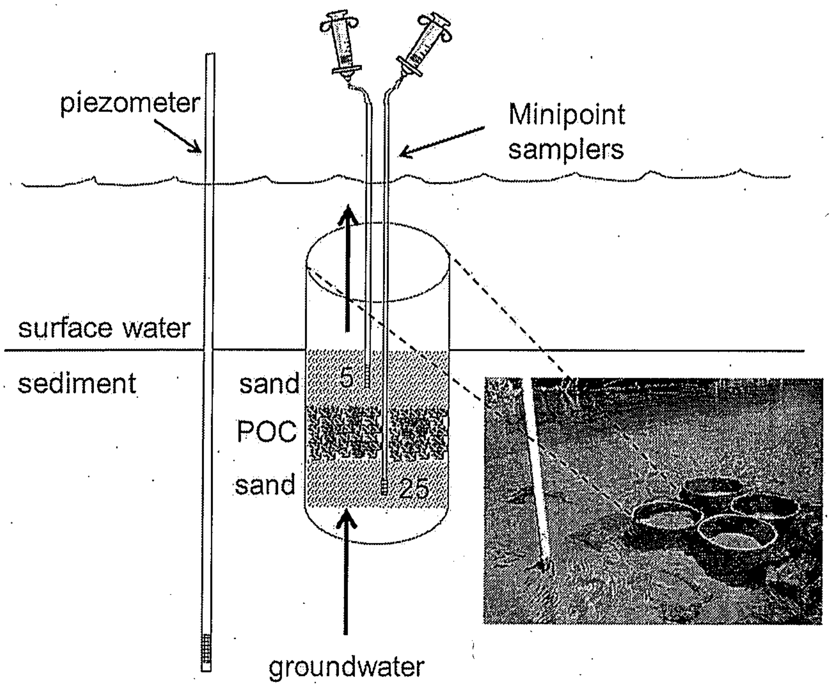

Fig. 2 Schematic diagram of mesocosms used in the experiments in Phase I and II

### *Hydrology*

Vertical Hydraulic gradient was measured to confirm that upwelling was occurring at each mesocosm cluster (Dahm et al. 2006). Groundwater velocity was measured at the end of the experiment using NaCl injections in 24 mesocosms. Minipoint samplers (Duff et al. 1998) were used for the velocity measurements. NaCl solution was added to a Minipoint at a sediment depth of 25 cm. Specific conductivity of the pore water at 5 cm was measured and the time at which peak or plateau conductivity occurred was used to determine the mean travel time. Groundwater velocity was calculated by dividing the distance between the injection depth and sampling depth by the mean travel time.

### *Nitrate and Total Dissolved Nitrogen Retention*

Water samples for nitrate, ammonium, dissolved organic nitrogen (DON), and DOC were collected from Minipoint samplers installed in each mesocosm at 25 and 5 cm sediment depths (Fig. 2).

We determined nitrate retention two different ways. First, we compared the nitrate concentrations in pore water at 25 and 5 cm sediment depths. We also compared ammonium, DON, and total dissolved nitrogen concentrations between 25 and 5 cm depths. Second, for a subset of mesocosms (17 total), we calculated the nitrate fluxes (mg $NO_3$-N/h) at 25 and 5 cm sediment depths using the following equation:

Solute flux = V x A x C (eq. 1)

where
V is groundwater velocity, A is cross sectional area of the mesocosm, C is solute concentration

We then determined the net retention rate for nitrate by calculating the difference between the nitrate fluxes at 25 and 5 cm.

### *Denitrification*

Denitrification was measured with the acetylene block method in the laboratory (Richardson et al., 2004) and with a membrane-inlet mass spectrometry (MIMS)-based method in the field (Kana et al. 1994). Sediment was collected from each mesocosm on Day 23 for the acetylene block denitrification assays. Acetylene block incubations commenced the following day. Incubation vessels were made anoxic by a series of evacuation by vacuum and helium addition to the head space. Immediately after addition of 20 ml of acetylene (time zero), vessels were placed on a shaker in the incubator. Head space gas was sampled with a syringe at 30-min intervals during the 90-min incubations.

Nitrous oxide ($N_2O$) in the vials was analyzed on a Hewlett-Packard Model 5890 gas chromatograph. Denitrification potential was calculated as the rate of $N_2O$ production. Subsamples of sediments for organic matter determination were collected and stored at -20 °C.

Pore water samples were collected on Day 14 of the experiment at 25 and 5 cm depths from the Minipoints (Fig. 2) using syringes for the in situ (MIMS-based) denitrification measurements. Samples were transferred to test tubes fitted with ground-glass stoppers and preserved using ZnCl solution. $N_2$ and argon concentrations were measured using MIMS (Kana et al. 1994). $N_2$ fluxes at 25 and 5 cm sediment depths were calculated based on equation 1. Denitrification rate was calculated as net $N_2$ production which was determined as the difference in $N_2$ flux between 25 and 5 cm depths.

### *Dissolved Oxygen*

Dissolved oxygen was measured in the deep groundwater by pumping water from the piezometer adjacent to each mesocosm cluster into a flow cell in which a dissolved oxygen probe was inserted. Dissolved oxygen in the pore water was measured at 5 cm sediment depth in each mesocosm with a Microelectrodes dissolved oxygen electrode.

### *Solute analysis*

Nitrate concentration was measured with a Dionex ICS-1000 ion chromatograph. Ammonium concentration was measured colorimetrically after Solóranzo (1969). Total dissolved nitrogen (TDN) and dissolved organic carbon (DOC) concentrations were measured with a Shimadzu TOCV Carbon Analzyer with TNM Nitrogen Module.

## Phase II

The experimental design and methods for characterizing POC and DOC quality in Phase II are described below. The other methods used in Phase II were similar or identical to those used in Phase I.

### *Experimental Design*

We established four POC quality treatments in sediments within mesocosms in Emmons Creek as follows: northern red oak (hereafter red oak) (*Quercus rubra*) leaves, red maple (*Acer rubrum*) leaves, red maple wood and a control. Identical quantities of POC (15 g) were added to the mesocosms for the leaf and wood treatments (Fig. 2). We used a randomized block design and each block consisted of a cluster of four mesocosms to which treatments were randomly assigned. Each treatment was replicated 11 times.

Red oak and red maple leaves and small red maple twigs were collected from live trees and cut into small pieces. The POC was air dried and then placed in mesh bags in Emmons Creek for microbial colonization. After colonization the POC was added to the mesocosms (Day 1, June 4, 2012). The control mesocosms only received combusted sand.

### *Organic Carbon Quality*

POC quality was described by the C:N ratio and %N of initial POC, POC on Day 1 of the experiment, and POC on Day 24 when sediments were collected from the mesocosms for the acetylene block incubations. POC quality was also assessed by percent lignin and lignin:N ratios for POC collected on Day 1. POC was analyzed for total C and N using a CE Instruments NC 2100 elemental analyzer. POC was analyzed for lignin using the acid detergent method (AOAC 1990). DOC quality was assessed using fluorescence excitation emission matrix (EEM) spectroscopy on water samples collected from mesocosms at 5 and 25 cm on Days 8 and 22.

## **Results and Discussion**

### Phase I

### *Hydrology*

Vertical hydraulic gradient was consistently positive at all mesocosm cluster locations, which suggests that upwelling conditions prevailed throughout the experiment. Groundwater velocity ranged from 1.5 to 3.2 cm/h (grand mean of 2.3 cm/h).

### *Dissolved Oxygen*

Deep groundwater collected from the piezometers was oxic. Mean DO in deep groundwater was 6.4 mg/L and ranged from 5.4 to 7.8 mg/L. POC addition had strong effects on DO concentration at 5 cm sediment depth where conditions approached anoxia (< 0.2 mg O2/L) for both POC treatments (one-way ANOVA $F_{3,36} = 27.9$, $P < 0.001$).

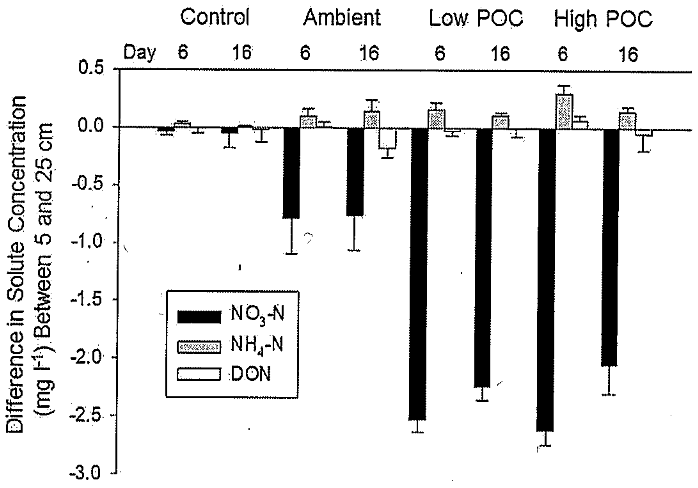

Fig. 3 Change in groundwater solute concentration (mean, SE) between 5 and 25 cm sediment depths in Phase I experiment

### *Nitrogen Retention*

Groundwater concentrations of nitrate, ammonium, and DON at 25 cm in the mesocosms did not differ among treatments (one-way ANOVAs $P > 0.14$) while DOC concentrations at 25 cm were low but different among treatments (one-way ANOVA $F_{3,75} = 3.03$, $P = 0.034$). POC addition had strong effects on nitrate retention, when expressed as the difference in concentrations between 25 and 5 cm depths (one-way ANOVA $F_{3,75} = 71.99$, $P < 0.001$, Fig. 3). The Low and High POC treatments had mean decreases of 2.39 and 2.33 mg $NO_3$-N/L between 25 and 5 cm. Overall, the magnitude of net nitrate retention was much larger than the magnitude of net ammonium production (Fig. 3).

POC addition also had strong effects on nitrate retention rate (one-way ANOVA $F_{3,13} = 29.43$, $P < 0.001$, Fig. 4). Nitrate retention rate was highest in the POC treatments (means of 1201 and 1109 mg $NO_3$-N $m^{-2}$ $d^{-1}$ for the Low and High POC treatments), intermediate in the Ambient treatment (581 mg $NO_3$-N $m^{-2}$ $d^{-1}$) and negligible in the Control treatment (24 mg $NO_3$-N $m^{-2}$ $d^{-1}$, Tukey $P < 0.05$).

*Organic Carbon*
POC addition increased net DOC production between 25 and 5 cm depths (one-way ANOVA $F_{3,75} = 15.45$, $P < 0.001$) but only the High POC treatment mean was significantly different from the Control (Tukey $P < 0.001$).

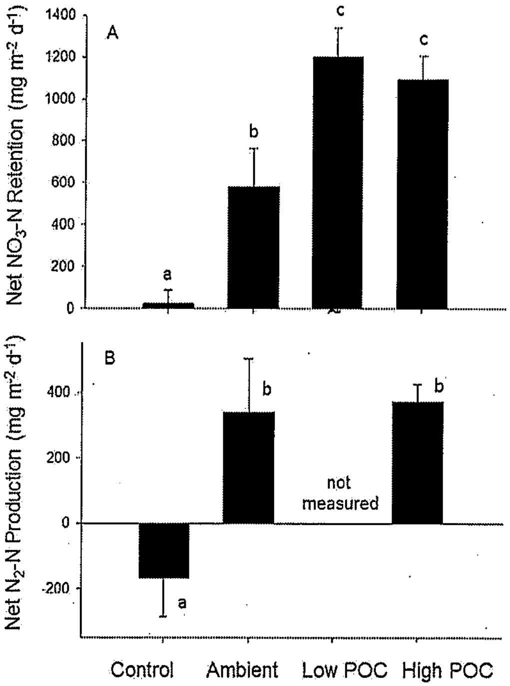

Fig. 4. Net $NO_3$-N retention and net $N_2$-N production (in situ denitrification) rates in groundwater in Phase I.

*Denitrification*
POC addition increased denitrification potential measured using the acetylene block method (one-way ANOVA $F_{3,36} = 13.25$, $P < 0.001$). POC addition also increased in situ denitrification rate (net $N_2$ production) (one-way ANOVA $F_{2,11} = 7.44$, $P < 0.01$, Fig. 4). However, unlike for denitrification potential, net $N_2$ production did not differ between the High POC (371 mg $N_2$-N $m^{-2}$ $d^{-1}$) and Ambient (340 mg $N_2$-N $m^{-2}$ $d^{-1}$) treatments (Tukey $P = 0.981$).

*Discussion*
We think POC addition increased nitrate retention and denitrification due to two primary mechanisms. First, POC addition caused a sharp decline in dissolved oxygen concentration in the sediments. Because deep groundwater (41 cm sediment depth on average) was oxic we assumed that groundwater at 25 cm depth was also oxic. This suggests that the POC addition caused a dramatic shift from oxic to severe hypoxic conditions as groundwater passed through the layers of leaves. This shift towards anoxia likely promoted denitrification. Second, POC addition provided a source of particulate and dissolved organic carbon for heterotrophic bacteria which they could use to support assimilatory and dissimilatory nitrate reduction (Burgin and Hamilton 2007). These two mechanisms are probably linked and we were not able to compare the relative importance of each mechanism for nitrate removal given our experimental design.

Comparison of the net nitrate retention rates and in situ denitrification rates revealed that denitrification could account for about 30 to 60% of the total nitrate removal . This estimate of the contribution of denitrification to nitrate removal is probably conservative, because our results suggest that gross $N_2$ consumption occurred based on the mean $N_2$ loss in the Control treatment. The $N_2$ loss could have been due to nitrogen fixation (Fulweiler et al. 2007) or $N_2$ offgassing as groundwater moved through the sediments. Thus, regardless of the influence of $N_2$ consumption on the reported denitrification rates, denitrification played an important role in nitrate removal.

<u>Phase II</u>
*Hydrology*
Vertical hydraulic gradient was positive (mean 0.092, range 0.024 to 0.203) at all mesocosm clusters.

Groundwater velocity measurements ranged from 1.3 to 3.3 cm/h among mesocosms with a mean of 2.3 cm/h.

*Organic Carbon*
The POC types differed in chemical composition. Leaves had a lower mean C:N ratio and higher mean %N than red maple wood on Day 24 (one-way ANOVA $P < 0.001$, Tukey $P < 0.001$). Red oak leaves had slightly higher %N on Day 24 than red maple leaves (Tukey $P = 0.005$). The data suggest that the C:N ratio of leaves decreased and %N increased during microbial colonization prior to the start of the experiment (data not shown). Percent lignin and lignin:N ratios were 24 and 47 for red maple wood, 39 and 29 for red maple leaves and 38 and 30 for red oak leaves on Day 1.

POC quality had strong effects on net DOC production (rmANOVA, $F_{3,40} = 8.84$, $P < 0.001$, Fig. 5). The leaf treatments had higher DOC production than the control (Tukey $P < 0.01$) and the red maple leaf treatment had higher DOC production than the red oak leaf treatment (Tukey $P < 0.001$). The red maple leaf treatment (Tukey $P < 0.001$), but not the red oak leaf treatment (Tukey $P = 0.052$), had higher DOC production than the red maple wood treatment.

PARAFAC analysis of EEMs generated from shallow pore water samples resulted in three components that could be characterized by different excitation-emission spectra (Fig. 6). A plot of Components 1 and 2 suggested that DOC chemical composition at 5 cm depth differed among treatments, particularly between the leaf treatments and the non-leaf treatments (data not shown).

*Dissolved Oxygen*
POC quality strongly affected dissolved oxygen concentration at 5 cm sediment depth (one-way ANOVA $F_{3,84} = 26.91$, $P < 0.001$). Burial of red maple and red oak leaves lead to severe hypoxia (means of 0.11 and 0.29 mg $O_2$/L respectively).

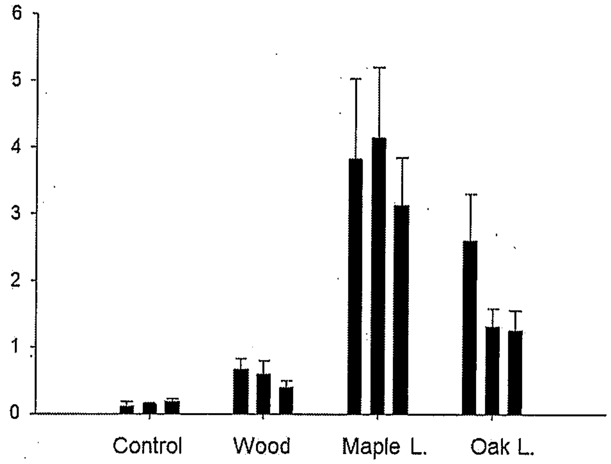

Fig. 5. Dissolved organic carbon production (difference in concentrations between 5 and 25 cm depth) in Phase II

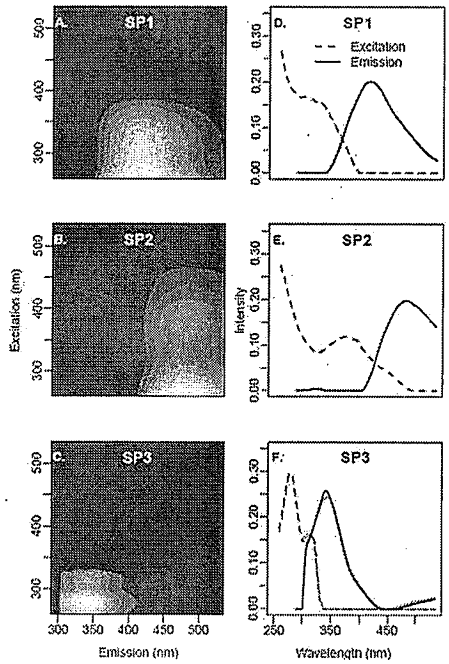

Fig. 6. Components of the PARAFAC analysis of EEMs from pore water samples collected at 5 cm sediment depth. A-C. Excitation and emission contour plots of components SP1-3. Light colors indicate greater fluorescence. D-F. Excitation and emission line models with relative peak intensity on the y-axis.

*Nitrogen Retention*

POC quality strongly affected nitrate retention, when expressed as the difference in concentrations between 25 and 5 cm depths (rmANOVA $F_{3,40} = 32.15$, $P < 0.001$). The two leaf treatments had higher nitrate retention than the red maple wood treatment, which in turn had higher nitrate retention than the control (Tukey $P < 0.05$).

Net nitrate retention rate was also affected by POC quality (rmANOVA $F_{3,21} = 33.50$, $P < 0.001$, Fig. 7). Red maple and red oak leaf treatments had higher net nitrate retention rates than the red maple wood and control treatments (Tukey $P < 0.001$). In addition, the mesocosms receiving red maple leaves had a higher net nitrate retention rate (mean 1406 mg $NO_3$-N $m^{-2}$ $d^{-1}$) than the red oak leaf mesocosms (mean 965 mg NO3-N $m^{-2}$ $d^{-1}$) (Tukey $P < 0.001$, Fig. 7).

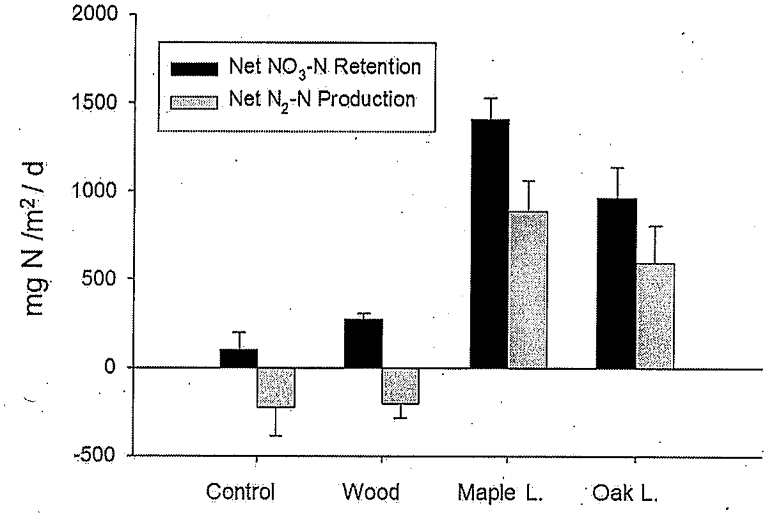

Fig. 7. Mean (+SE) net $NO_3$-N retention rates and net $N_2$-N production rates for the control, red maple wood, red maple leaf and red oak leaf treatments. Net NO3-N retention rates are grand means based on Days 4, 12, and 19 of the experiment and net N2-N production rates are grand means based on Day 8 and Day 22 in Phase II.

*Denitrification*

Denitrification potential measured by the acetylene block method was elevated relative to the control for the leaf treatments but not for the wood treatment (one-way ANOVA $F_{3,38} = 6.34$, $P = 0.001$, Tukey $P < 0.05$ for comparisons between control and leaf treatments). Similarly, POC quality affected in situ denitrification rate as measured by MIMS (one-way ANOVA $F_{3,42} = 15.57$, $P < 0.001$, Fig. 7). Red maple and red oak leaf treatments had higher net $N_2$ production than the control and red maple wood treatments (Tukey $P < 0.01$), which had negative rates.

*Discussion*

POC quality had strong effects on nitrogen processing in that red maple and red oak leaf treatments resulted in much higher nitrate retention and denitrification rates than the red maple wood treatment. In addition, burial of red maple leaves resulted in higher nitrate retention rate, but not higher in situ denitrification rate, than burial of red oak leaves. As illustrated in our conceptual model (Fig. 1) we think that there are several mechanisms that could have caused POC quality to affect nitrogen processing, including direct effects of POC chemical composition on microbes associated with the POC, and indirect effects of POC chemical composition on microbes through changes in the quantity and quality of DOC and changes in redox status. We think that all of these mechanisms are plausible as there were differences in POC chemical composition, DOC production, DOC chemical composition, and pore water dissolved oxygen concentration among the POC treatments. Denitrification accounted for a substantial amount of the nitrate removal (about 60%) for the leaf treatments. Carbon availability and favorable redox conditions are necessary, but not sufficient, conditions for heterotrophic denitrification (Tiedje 1982). For example, production of labile DOC would not be expected to stimulate denitrification if redox conditions were not favorable for nitrate to serve as the preferred electron acceptor in respiration. We think it is likely that increases in DOC production and severe hypoxia in the leaf treatments both lead to higher nitrogen processing than in the wood treatments. The higher amount of DOC production in the red maple leaf treatment compared to the red oak leaf treatment or differences in the DOC quality between these treatments may have contributed to the higher nitrate retention rate for the red maple leaves than the red oak leaves. Previous studies have shown that plant species identity can affect rates of organic matter breakdown and nitrogen processing (e.g. Mehring and Maret 2011).

## Conclusions and Recommendations

POC addition stimulated nitrogen processing in shallow groundwater and the quality of the POC influenced the rates of nitrogen processing. A number of plausible mechanisms could explain how POC quantity and quality influenced N processing in the groundwater, including changes in DOC quantity and quality and redox status in the sediments. We think that our results have several implications for the management of groundwater, groundwater-fed streams, and their associated watersheds. In particular, the presence of riparian or aquatic vegetation (POC quantity), the type of plants species present (POC quality), the hydrological connection between surface water and groundwater and the availability of groundwater nitrate will all likely impact the amount of nitrogen retention and removal that occurs at the groundwater-surface water interface in lotic ecosystems. The strong link that we demonstrated between carbon availability and nitrogen processing in shallow groundwater should be considered when managing groundwater systems, streams, rivers and their watersheds.

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## Appendix A

### Manuscripts in Review

Stelzer, R.S., Scott, J. T., L.A. Bartsch, T. Parr. 2013. Particulate organic matter quality influences nitrate retention and denitrification in stream sediments: evidence from a carbon burial experiment. In Review, *Biogeochemistry*

Stelzer, R.S., Scott, J. T., L.A. Bartsch. 2013. Buried particulate organic carbon stimulates denitrification and nitrate retention in stream sediments at the groundwater-surface water interface. In Review, *Freshwater Science*

### Invited Presentations

Stelzer, R.S., Scott, J.T. Bartsch, L.A. and Parr, T. 2013. The influence of particulate organic carbon quality on nitrogen transformation in deep stream sediments. Abstract of an oral presentation in special session entitled "Advances in groundwater and surface-groundwater interactions research" at Society for Freshwater Science Annual Meeting, Jacksonville, FL.

Stelzer, R.S., Scott, J. T., L.A. Bartsch. 2012. Buried particulate organic carbon stimulates denitrification and nitrate retention in stream sediments. Abstract of an oral presentation in special session entitled "Into the benthos: new insights into how sediment processes affect aquatic ecosystems" at Society for Freshwater Science Annual Meeting, Louisville, KY.

Stelzer, R.S., University of Wisconsin-Madison, Department of Entomology, 2012

Stelzer, R.S., University of Wisconsin-Madison, Molecular and Environmental Toxicology Center, 2012

Stelzer, R.S., State of Wisconsin Groundwater Coordinating Council, 2011