

**ABOVE- AND BELOW-GROUND LITTER MANIPULATION:
EFFECT ON RETENTION AND RELEASE OF DOC, DON AND DIN
IN THE SIKFOKUT FOREST, HUNGARY**

A Senior Scholars Thesis

by

ELIZABETH A. EVETTS

Submitted to the Office of Undergraduate Research
Texas A&M University
in partial fulfillment of the requirements for the designation as

UNDERGRADUATE RESEARCH SCHOLAR

April 2008

Major: Bioenvironmental Science

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Approved by:

Research Advisor:
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Jacqueline Aitkenhead-Peterson
Robert C. Webb

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ABSTRACT

Above- and below-ground Litter Manipulation: Effect on Retention and Release of DOC, DON and DIN in the Sikfokut Forest, Hungary (April 2008)

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The above- and below-ground litter from fallen foliage and root exudates and their decomposition has an impact on forest soil. The objective of this research project was to determine the effect of above- and below-ground litter manipulation on the retention and release of dissolved organic carbon (DOC), dissolved organic nitrogen (DON), nitrate and ammonium in the soil profile at 0-5 and 5-15 cm depths. The soils were obtained from a Long Term Ecological Research site in the Sikfokut Forest in Hungary. The site is a mature oak forest and the soil has no organic layer; the leaf litter sits directly on the A horizon. There are six treatments applied to the soil: doubling of annual leaf litter (DL), doubling of annual wood litter (DW), removal of annual leaf litter (NL), removal of roots (NR), removal of leaf and roots (NI) and control (C). Each plot is 7 x 7 m, and each soil sample taken was 15 x 15 cm square. A stock solution of leaf litter was added in different concentrations to soils from each treatment in a 50 mL centrifuge tube. Because I was investigating retention or release of carbon and nitrogen, I inverted the tube gently over a period of 2 hours prior to centrifugation and removal of supernatant. The supernatant

solution was analyzed for DOC, DON, ammonium and nitrate to determine retention and release values for each dependant upon the different treatments.

The results of the study were interesting. Dissolved organic nitrogen was neither retained nor released in the 0-5 cm layer for any treatment yet nitrate, typically considered a mobile anion showed retention and release in this layer. In the 5-15 cm layer we had no retention or release of nitrate but dissolved organic nitrogen was retained and released between the solid and solution phases.

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NOMENCLATURE

DOC	Dissolved Organic Carbon
DON	Dissolved Organic Nitrogen
DIN	Dissolved Inorganic Nitrogen
TDN	Total Dissolved Nitrogen
DL	Double Annual Leaf Litter
DW	Double Annual Wood Litter
NL	Removal of Annual Leaf Litter
NR	Removal of Roots
NI	Removal of Leaf and Roots
C	Control
IMI	Initial Mass Isotherm
RE	Retained per Unit of Soil

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CHAPTER I

INTRODUCTION

Forest ecosystems are impacted by the amount of above- and below-ground litter derived from leaf or needle fall, root exudates and the decomposition of this litter. For example, leaf litter is important in the cycling of nutrients, buffers changes in soil water and temperature (Ginter et al 1979) and hinders erosion and compaction (Geddes and Dunkerley 1999). Research investigating removal of leaf litter started in the 1800's when the removed leaf litter was used as bedding for animals or as a cover for crops. This led to the disruption of physical and chemical properties and caused destabilization of the humus layer (Krutzsch, 1869). Also it was documented that raking of leaf litter caused a greater decline in the acid-neutralizing capacity of the forest floor compared to the harvesting of trees (Glatzel 1991). The effect of this research led to the eventual replacement of deciduous forests, by less nutrient demanding coniferous forests in Europe (Jandl et al. 2002).

Leaf-litter plays an important role in forests, and its removal impacts nutrients in the leaves of living plants. This has been shown in both long and short-term studies which report deficits in foliar carbon, magnesium (McLeod et al., 1979) and phosphorous (Lopez-Zamora et al., 2001). The foliage of new needles on *Picea abies* in Eastern

This thesis follows the style of Soil Science.

Europe reflects the carbon and nitrogen content of the forest floor (Aitkenhead-Peterson et al., 2006).

Dissolved organic carbon (DOC) in the forest floor is a significant source of carbon available for retention in lower mineral soil horizons (McDowell and Likens 1988; Neff and Asner 2001). Short-term litter manipulation studies in coniferous stands show an increase of DOC production when doubling annual leaf litter (Park and Matzner 2003, 2006; Lajtha et al., 2005; Yano et al., 2005). However, removal of annual leaf litter does not always significantly effect DOC concentration and flux. The effect of above-ground litter manipulation does not impact DOC retention in Andic soils under an old-growth Douglas fir in Oregon (Yano et al., 2004) but there is some effect on the retention of dissolved organic nitrogen (DON) (Yano et al., 2004).

According to these previous studies I hypothesized that above- and below-ground litter manipulation will cause DOC, DON and dissolved inorganic nitrogen (DIN) adsorption and release to be significantly different.

CHAPTER II

METHODS

Site Description and Field Collection of Soils

The litter manipulation plots are situated in the International Long Term Ecological Research of Sikfokut forest in Hungary (47° 90' N, 20° 46' E). The forest is a mature oak forest comprising Turkey and Sessile oak species. Soils are brown earths with cambisol character. There is no organic layer in this forest and leaf litter rests directly upon the A horizon (0-5 cm). Litter manipulation at these plots started in 2000. Mean annual temperature is 9.9° C and mean annual precipitation is 601 mm. Treatments include: doubling of annual leaf litter (DL), doubling of annual wood litter (DW), removal of annual leaf litter (NL), removal of roots (NR), removal of leaf and roots (NI) and control (C). There are three replicate plots for each treatment. Plots are 7 x 7 m in area. To ensure that there is no root activity the plots were trenched to a depth of 60 cm and herbaceous vegetation is removed by hand. Soil samples from each treatment were taken during the summer of 2006 and transported back to the USA where they were air dried and sieved to 2mm. Soils were stored in brown paper bags and placed in Ziploc bags for storage.

Experimental Procedure and Chemical Analysis

The initial mass isotherm (IMI) method (Nordvin et al., 1986) was used to determine DOC, DON and DIN (ammonium and nitrate) adsorption to mineral soil at 0-5 and 15-30 cm. The experimentation and analysis of the 5-15 cm layer was completed by

undergraduate Whitney Taylor at the University of New Hampshire in 2006 but no statistical analysis was completed on this layer. In this approach, soils are combined with solution containing various concentrations of DOC, DON and DIN and the net release or retention of DOC, DON or DIN is measured after a brief equilibration period. The amount of DOC, DON or DIN retained per unit mass of soil (RE) is plotted as a function of the initial DOC, DON or ammonium concentration normalized to soil mass (X_I , in units of mg g soil^{-1}):

$$RE = mX_I - b$$

The slope of the line (m , the partition coefficient) describes partitioning of the solution DOC, DON or DIN between liquid and solid phases. The intercept of the regression (b) is the amount released from the soil when a solution with zero DOC, DON or DIN is added. The point at which the constituent is neither retained nor released is the null point, or equilibrium concentration, which can be used to predict concentrations in soil solution (McDowell and Wood 1984).

Stock solution used to obtain the different input concentrations of DOC, DON or DIN was made using forest floor material from the Sikfokut forest. A 50:1 ratio of leaf litter:ultra-pure water (C-free, $0.22 \mu\text{m}$ -filtered), was shaken for 24 hours. The resulting slurry was filtered sequentially through a $1.2 \mu\text{m}$ -filter to remove larger particles and then a pre-combusted Whatman GF/F (nominal pore size $0.7 \mu\text{m}$). The concentration of the stock solution was analyzed for DOC and total dissolved nitrogen (TDN) using a Shimadzu TOC-VN, for ammonium and nitrate using a Joe Westco Discrete Analyzer. DON concentrations were calculated as the difference between TDN and DIN ($\text{NH}_4\text{-N} +$

NO₃-N). The stock solution was used to make up individual solutions with six concentrations for each DOC, DON and DIN. Thirty-five mL of solution was added to each 3.5-g soil sample (10:1 ratio) in its respective centrifuge tube. The tubes were recapped, kept cool and inverted every 15 minutes for two hours. Samples were then centrifuged at 4900 g for 15 minutes at 2° C. The supernatant was filtered (syringe filter fitted with a pre-combusted Whatman GF/F filter) and analyzed for DOC, TDN, nitrate and ammonium. EPA-certified reference standards were analyzed during each run and replicates and check standards will be analyzed after every 10th sample.

CHAPTER III

RESULTS

0-5 cm Layer

Dissolved Organic Carbon

Concentration of input DOC ranged from 0 – 45 mg L⁻¹. There was considerable variation among the plots of each treatment in terms of DOC retention hence there was no significant difference in DOC concentration relative to the control in any of the treatments (Figure 1).

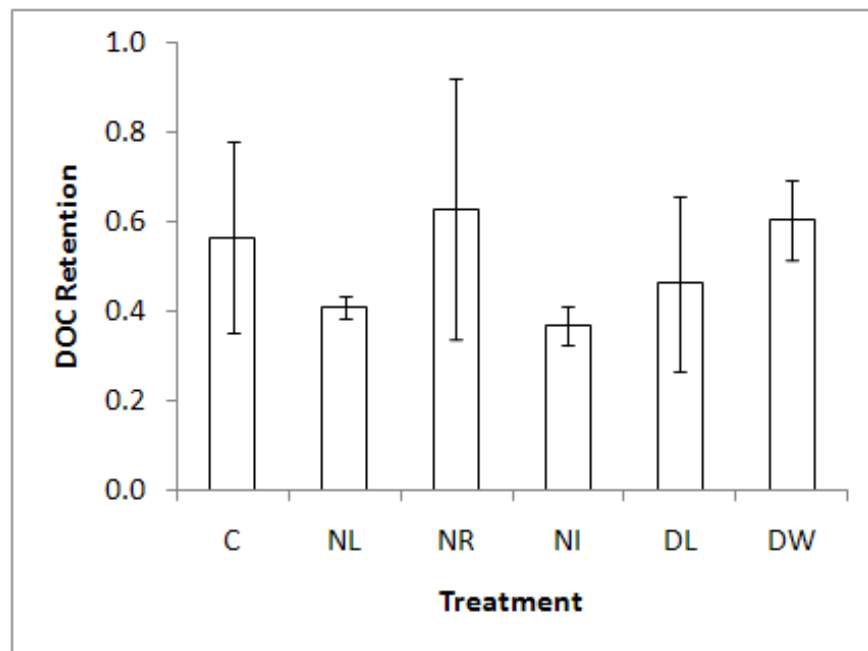


Figure 1. DOC retention in the 0-5 cm layer. Error bars are one standard deviation.

Release of DOC differed significantly ($p < 0.05$). All treatments with the exception of double wood (DW) had release of DOC that was significantly different to the control.

Those treatments with litter removed had significantly lower DOC release than the

control (Figure 2) and the double litter (DL) had significantly higher DOC release than the control (Figure 2).

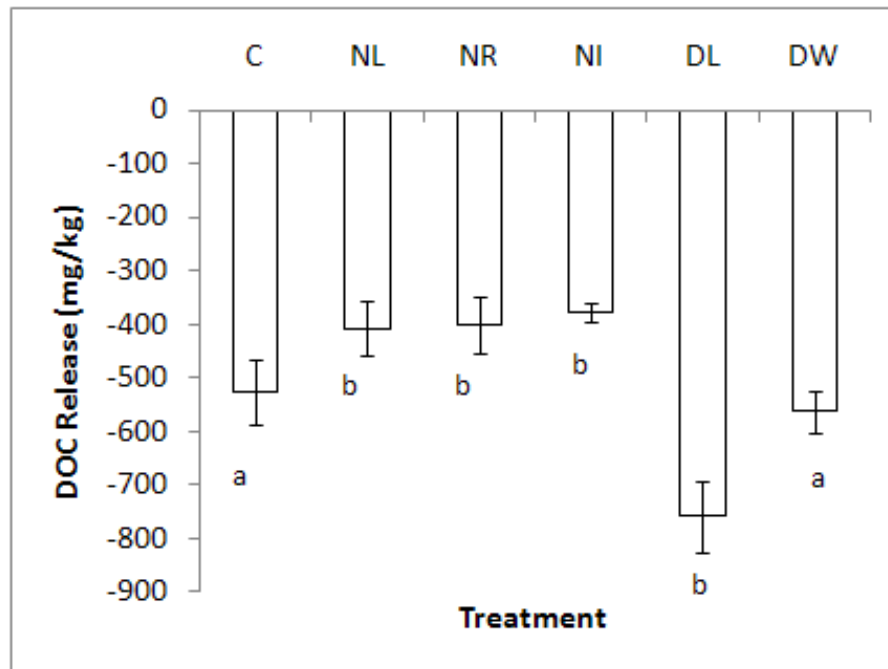


Figure 2. DOC release in the 0-5 cm layer. Error bars are one standard deviation. Different letters denote significant difference relative to the control at $p < 0.05$.

Dissolved Organic Nitrogen

Dissolved organic nitrogen (DON) was neither retained nor released in the 0-5 cm layer. Input DON ranged from 0 to 7.6 mg L^{-1} yet there was no retention in this layer. Neither was DON released from the soil solid phase into solution.

Ammonium

Input ammonium ranged from 0 – 1.6 mg L^{-1} and was retained strongly by all treatments but in the double litter and double wood treatments ammonium retention was significantly reduced relative to the control plots (Figure 3).

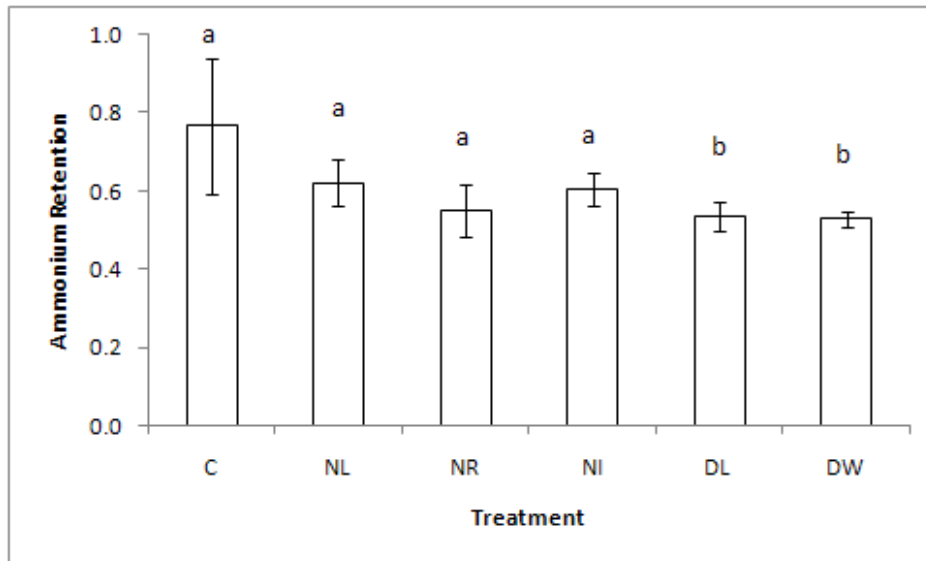


Figure 3. Retention of Ammonium in the 0-5 cm layer under different treatments. Error bars are one standard deviation. Different letters denote significant difference relative to the control at $p < 0.05$.

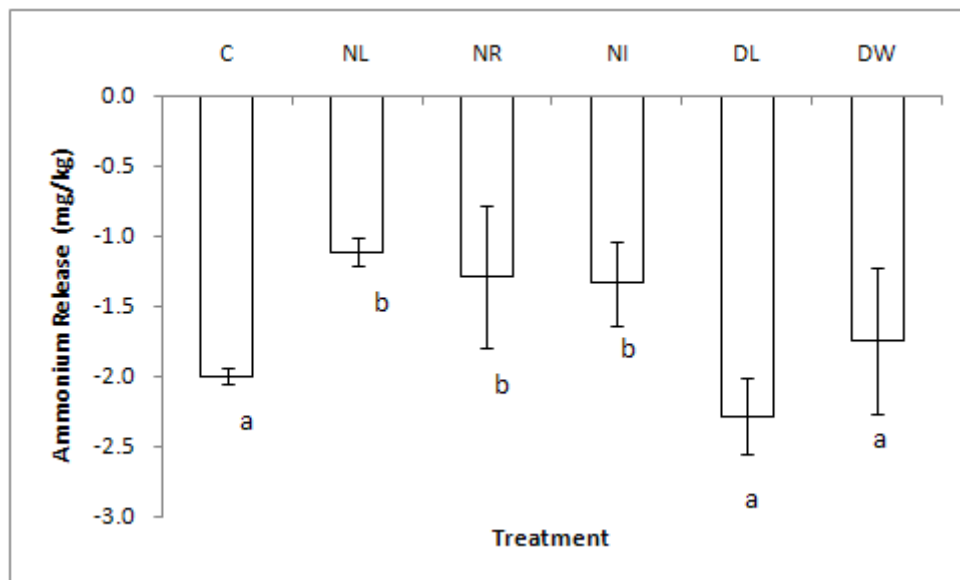


Figure 4. Release of Ammonium in the 0-5 cm layer under different treatments. Error bars are one standard deviation. Different letters denote significant difference relative to the control at $p < 0.05$.

Release of ammonium from the soil solid phase was significantly reduced in the no litter (NL), no roots (NR) and no input (NI) treatments relative to the control plots (Figure 4).

Nitrate

Nitrate, considered a conservative or mobile anion showed retention and release in the 0-5 cm layer and so mass isotherms were able to be produced to quantify retention and release. Input nitrate ranged from 0 to 37.8 mg L⁻¹. Nitrate was the dominant species of nitrogen in our input solution.

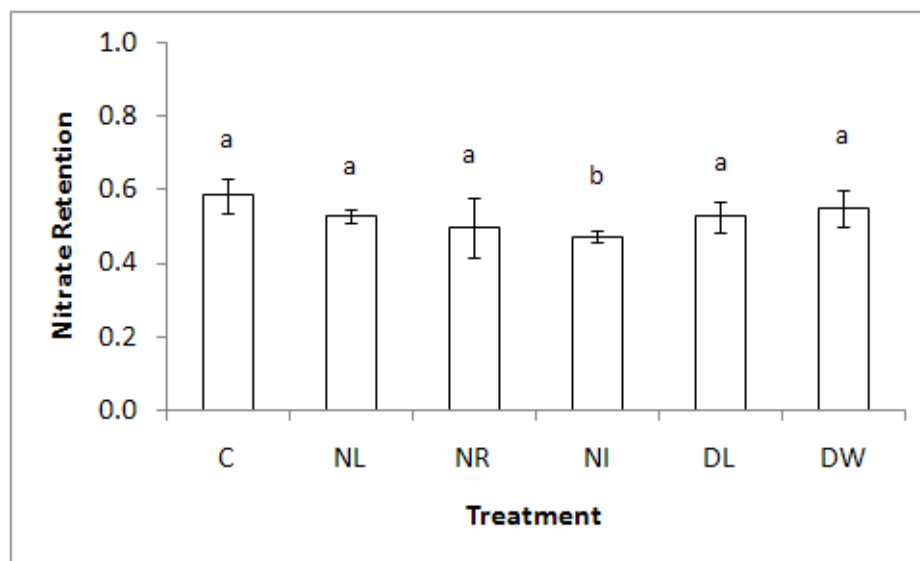


Figure 5. Nitrate retention in the 0-5 cm layer under different treatments. Error bars are one standard deviation. Different letters denote significant difference relative to the control at $p < 0.05$.

Retention of nitrate was significantly reduced in the no input (NI) treatment relative to the control plots but the other treatments were not significantly different from the control in their ability to retain nitrate ($p > 0.05$).

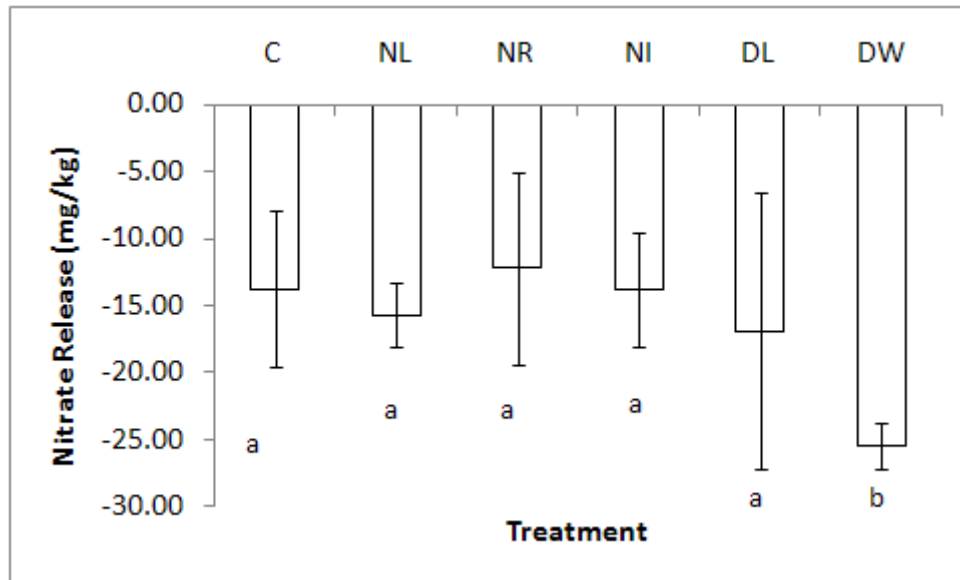


Figure 6. Nitrate release in the 0-5 cm layer under different treatments. Error bars are one standard deviation. Different letters denote significant difference relative to the control at $p < 0.05$.

We had significantly higher nitrate release from the double wood (DW) treatment compared to the control (Figure 6) but the other treatments did not show a significantly different nitrate release to the control.

5-15 cm Layer

Dissolved Organic Carbon

Input DOC to this layer ranged 0 – 60 mg L⁻¹. There was a very high variation in the amount of DOC retained among the replicates from the DL soil. As a consequence there was no significant difference in DOC retention among treatments relative to the control plots (Figure 7). Release of DOC from the solid phase to solution was affected by treatment. All treatments with the exception of double annual leaf litter released less DOC than the control (C) plots (Figure 8).

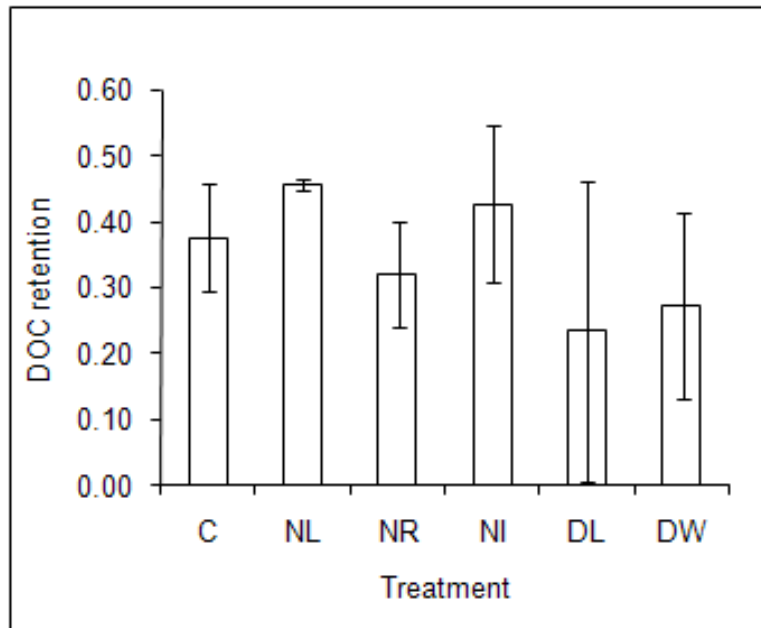


Figure 7. Retention of DOC in soils under different above- and below-ground litter manipulation. Error bars are one standard deviation.

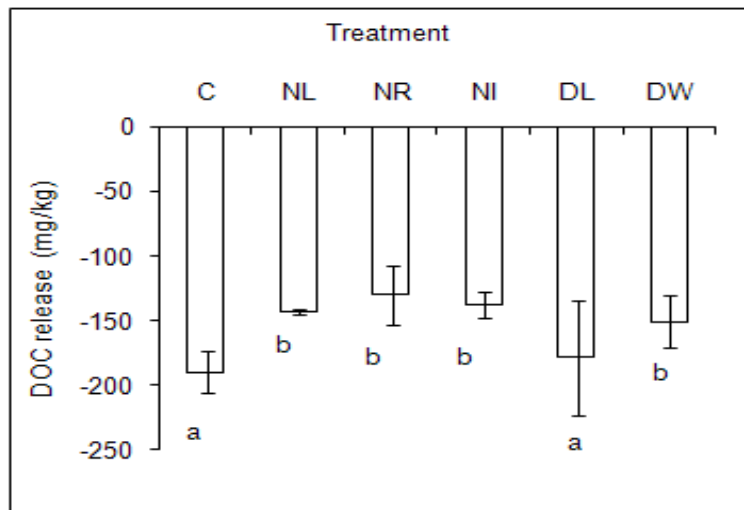


Figure 8. Release of DOC. Error bars are one standard deviation. Different letters mean significant difference relative to the control plots at $p < 0.05$.

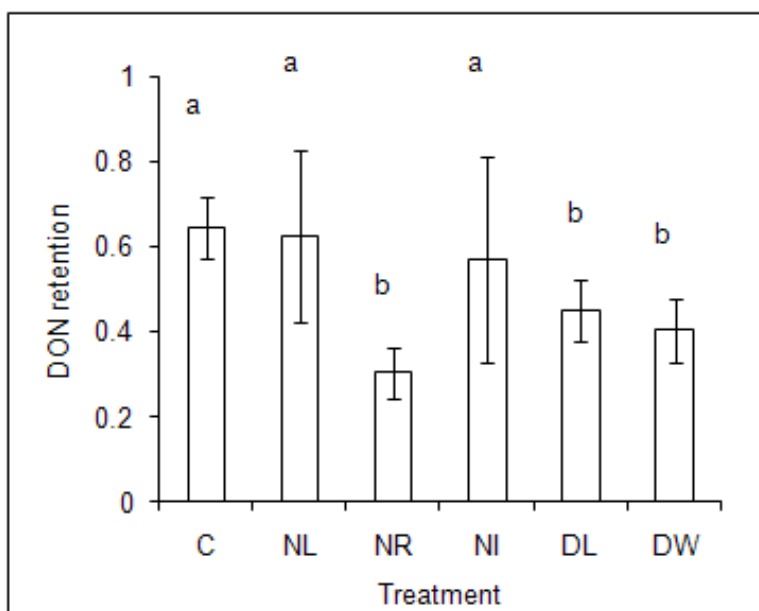


Figure 9. Retention of DON. Error Bars are one standard deviation. Different letters mean a significant difference relative to the control plots at $p < 0.05$.

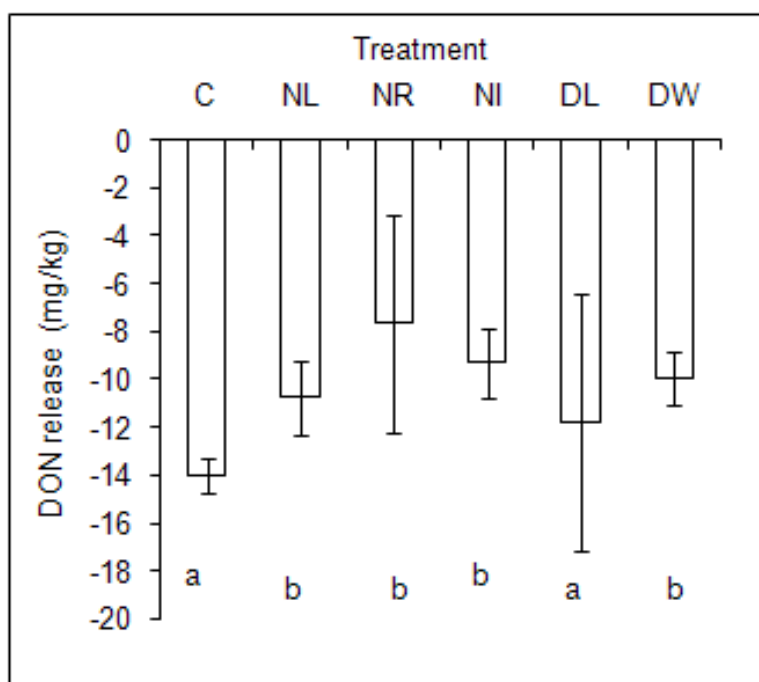


Figure 10. Release of DON from the soil solid phase to solution. Error bars are one standard deviation. Different letters are a significant difference at $p < 0.05$.

Dissolved Organic Nitrogen

Input solution DON ranged between 0 – 3 mg L⁻¹. There was significantly reduced retention of DON in the no roots (NR), double litter (DL) and the double wood (DW) plots relative to the control plots ($p < 0.05$; Figure 9). There was a significant reduction in the release of DON into soil solution in all treatments with the exception of double litter relative to the control plots. DON release was not significantly different between the double litter and control plots ($p > 0.05$; Figure 10). Because there was no evidence of DON retention or release in the 0-5 cm layer we were unable to compare the two layers.

Ammonium

Input ammonium ranged from 0 – 3.1 mg L⁻¹ to the 5-15 cm layer. Ammonium was the most strongly retained of all molecules tested in this layer but only the no input (NI) treatment retained significantly more ammonium than the control ($p < 0.05$; Figure 11). The variance in ammonium release was large in the control plots (Figure 12). Only the no roots (NR) treatment had significantly reduced ammonium release relative to the control ($p < 0.05$).

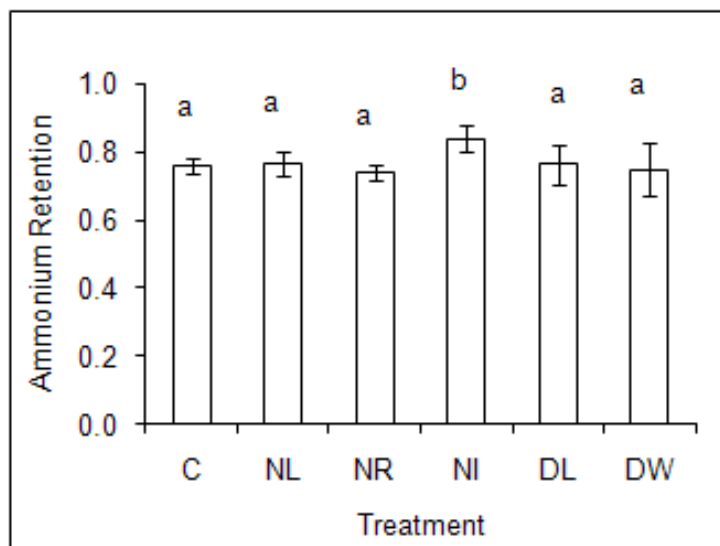


Figure 11. Retention of ammonium in soils under different above- and below-ground litter manipulation. Different letter above bar indicates a significant difference ($p < 0.05$) from control.

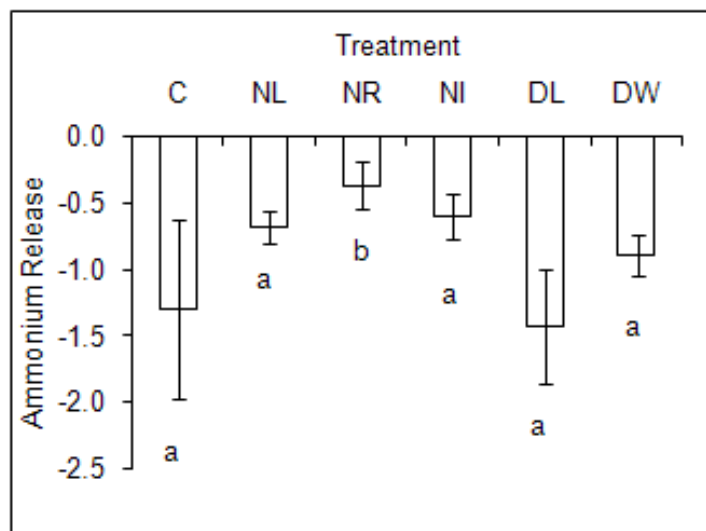


Figure 12. Ammonium release (mg/kg) from the solid to solution phase under different above- and below-ground litter manipulation. Different letters indicate significant difference from control at $p < 0.05$. Error bars are one standard deviation.

Nitrate

It was impossible to construct nitrate isotherms because nitrate appeared to be neither retained nor released in the 5- 15 cm layer in these soils.

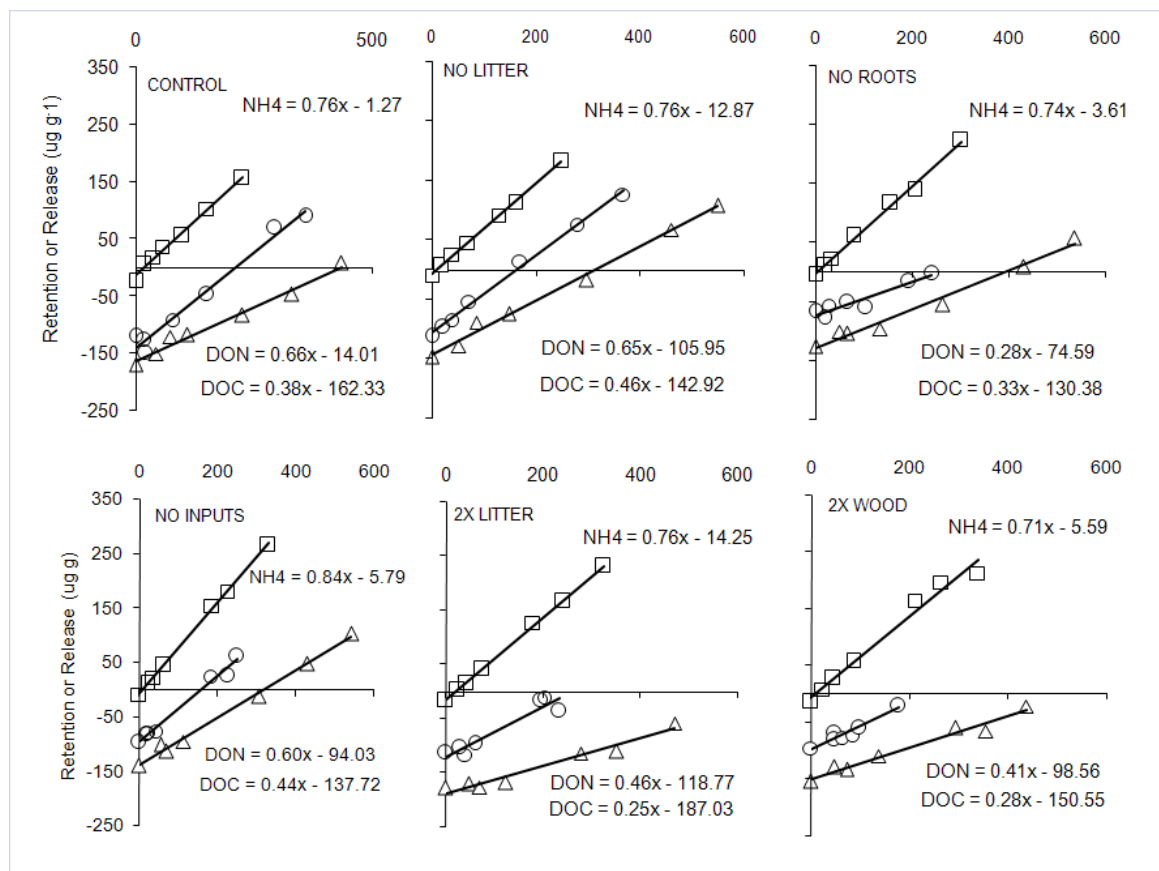


Figure 13. Mass isotherms of DOC, DON and ammonium in the 5-15 cm layer of soils undergoing above- and below-ground litter manipulation.

Ammonium in the input solution is retained much more strongly in the soil than is DON or DOC in all treatments (Figure 13). DON is retained more strongly than DOC in all treatments with the exception of the no roots (NR) treatment (Figure 13).

Differences between Layers

There was a significant difference in retention of DOC when comparing the 0-5 and 5-15 cm layer ($p < 0.05$). The no roots had significantly higher retention in the 5-15 cm layer and the double wood had significantly lower DOC retention in the 5-15 cm layer relative to the 0-5 cm layers (Table 1). Release of DOC was significantly higher in the 0-5 cm than the 5-15 cm layer for all treatments (Table 1). Only the no litter treatment had significantly different equilibrium DOC between layers where the equilibrium DOC was higher in the 0-5 cm layer than the 5-15 cm layer (Table 1).

Table 1. Retention, release and equilibrium DOC between layers. Different letters between layers indicate a significant difference at $p < 0.05$.

	Layer (cm)	Retention (unitless)	Release (mg/kg)	Equilibrium (mg/L)
Control	0-5	0.56 ^a	527.1 ^a	104.3 ^a
	5-15	0.38 ^a	189.9 ^b	51.8 ^a
No Litter	0-5	0.41 ^a	407.7 ^a	105.9 ^a
	5-15	0.46 ^b	143.1 ^b	32.0 ^b
No Roots	0-5	0.63 ^a	400.9 ^a	82.0 ^a
	5-15	0.32 ^a	130.1 ^b	43.5 ^a
No Inputs	0-5	0.39 ^a	378.5 ^a	100.0 ^a
	5-15	0.43 ^a	137.7 ^b	71.5 ^a
Double Litter	0-5	0.47 ^a	759.1 ^a	231.9 ^a
	5-15	0.23 ^a	178.7 ^b	180.1 ^a
Double Wood	0-5	0.53 ^a	562.9 ^a	102.0 ^a
	5-15	0.27 ^b	150.8 ^b	64.8 ^a

There was a significant difference in ammonium retention between layers 0-5 cm and 5-15 cm ($p < 0.01$) for all treatments but not the control. Ammonium retention was significantly higher in the 5-15 cm layer compared to the 0-5 cm layer. Release of ammonium was also significantly higher in the 5-15 cm layer compared to the 0-5 cm layer for all treatments ($p < 0.01$) but not the control plots (Table 2). Equilibrium

ammonium was significantly reduced in the 5-15 cm layer compared to the 0-5 cm layer in all treatments but not the control (Table 2).

Table 2. Retention, release and equilibrium NH₄-N between layers

	Layer (cm)	Retention (unitless)	Release (mg/kg)	Equilibrium (mg/L)
Control	0-5	0.77 ^a	2.0a	0.27a
	5-15	0.76 ^a	1.3a	0.16a
No Litter	0-5	0.62a	1.1a	0.18a
	5-15	0.77b	0.7b	0.09b
No Roots	0-5	0.55a	1.3a	0.23a
	5-15	0.74b	0.4b	0.05b
No Inputs	0-5	0.61a	1.3a	0.22a
	5-15	0.84b	0.6b	0.07b
Double Litter	0-5	0.53a	2.3a	0.43a
	5-15	0.76b	1.4b	0.19b
Double Wood	0-5	0.52a	1.7a	0.34a
	5-15	0.75b	0.9b	0.12b

CHAPTER IV

DISCUSSION, SUMMARY, AND CONCLUSION

Disturbance to a forest floor either by removing or adding leaf litter, adding woody debris or removing root input can have a significant effect on carbon and nitrogen dynamics in different soil layers.

Stock Input Solution

We used the same input solution (leaf litter in ultra-pure water at a 1:50 litter:water ratio) for both soil layer studies. We did this so that we could compare the effect on retention and release of carbon and nitrogen in the two soil layers. Stock was prepared from leaf litter after drying it in 2006 for the 5-15 cm layer. Fresh stock was prepared again using the dried leaf litter in 2007 for the 0-5 cm layer. However, we noted that that the stock solution chemistry was different in terms of nitrogen species yet the same for DOC.

Total dissolved nitrogen increased 8-fold in the solution extracted in 2007 compared to that extracted in 2006. Of the stock solution extracted in 2006 the dominant N-species was DON followed by ammonium-N and nitrate-N. In the solution extracted in 2007 nitrate-N was the dominant N-species followed by DON and ammonium-N.

Concentrations of ammonium-N were significantly higher and nitrate-N and DON significantly lower in 2006 extractions.

Mass loss of leaf litter leading to increased N is a common phenomenon (REFS). It may be then that our comparisons between layers of retention and release of N-species are

compromised by this radical change in concentration and N-composition of our stock solution.

In the field, older leaf litter combines with fresh leaf litter and is leached with throughfall inputs to provide an input solution to underlying soil layers. Unless labeled C or N is used in stock solution it is unknown whether all the input solution is retained and the soil then releases C and N that has been retained on soil minerals or whether solution infiltrating underlying layers is a mixture of initial input solution plus C and N released from soil minerals.

Dissolved Organic Carbon

Dissolved Organic Carbon (DOC) is used to describe the thousands of dissolved compounds and molecules found in soil and surface water that derive from organic materials such as decomposed plant, animal and fecal matter. This organic material is broken down to a particle size of < 0.45 μm and although termed 'dissolved' is merely that material which passes through a filter with a nominal pore size of 0.45 μm . Some of the DOC molecules have recognizable chemical structures such as soluble carbohydrates, proteins, fats, tannins and humic acid.

Although the concentration of stock DOC was unchanged in our 2006 and 2007 extracts it is possible that forms of carbon within the DOC were altered. Nevertheless, treatment did not significantly affect retention of DOC in the 0-5 cm layer but it did affect DOC release. Treatments with above- and below-ground litter removed released significantly

less DOC than the control. Doubling of annual leaf litter resulted in significantly greater DOC release relative to the control but doubling of woody debris and no impact on DOC release. In the 5-15 cm layer again DOC retention is not affected by litter manipulation and similar to the 0-5 cm layer, release of DOC is significantly reduced in the treatments with no litter inputs relative to the control. The doubling of annual leaf litter does not impact DOC release in this lower layer as it did in the 0-5 cm layer but by doubling woody debris release of DOC in this treatment at 5-15 cm is significantly less than the control.

Most studies investigating retention and release of DOC test soils at much lower depths than we did in this study and are interested in the soil minerals on which the DOC adsorbs. Other studies use the mass isotherm method to determine whether DOC or DON is retained or released more strongly.

No other study has investigated this type of litter manipulation treatment on C and N retention and release. This is likely due to the sheer amount of work involved which has necessitated that soil layers are examined during the summer months when more time is available for the experiment itself and the analyses needed. For example 14 soil samples are needed for each treatment plot to produce to mass isotherms and each treatment has 3 replicates. In addition three different chemical analyses are run on the supernatant of each of soil samples.

Our values for DOC retention, which ranged from 0.37 to 0.63 in the 0-5 cm layer and from 0.23 to 0.46 in the 5-15 cm layer are within the range of most reported studies. Values for DOC release are extremely high for the 0-5 cm layer compared to its underlying layer.

Dissolved Nitrogen Species

We examined the dynamics of ammonium, nitrate and organic nitrogen in 0-5 and 5-15 cm layers under different litter manipulations. Our results were very interesting.

Dissolved organic nitrogen was neither retained nor released in the 0-5 cm layer for any treatment yet nitrate, typically considered a mobile anion showed retention and release in this layer. In the 5-15 cm layer we had no retention or release of nitrate but dissolved organic nitrogen was retained and released between the solid and solution phases.

Retention of N-species followed the order ammonium > nitrate > DON in the 0-5 cm and ammonium > DON > nitrate in the 5-15 cm layer. Equilibrium values of N-species were concentrations expected to find in soil solution in the field. Nitrate-N equilibrium values ranged from 2.3 to 4.7 mg/L in the control and double wood plots respectively in the 0-5 cm layer. Ammonium-N equilibrium values ranged 0.18-0.43 mg/L in the no litter and double litter treatments respectively in the 0-5 cm layer and 0.05-0.19 mg/L in the no roots and double litter plots respectively in the 5-15 cm layer.

No other study has performed an extensive study on retention and release of C and N in soil layers under many different litter manipulation treatments. The study has advanced our knowledge on the activities of DON and nitrate in surface soils under forest stands.

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