Dissolved Organic Carbon Production and Flux from Long Term Litter Manipulations in a Pacific Northwest Old-Growth Forest

by
Lucas R. Evans

A THESIS

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Honors Baccalaureate of in Science in Ecological Engineering
(Honors Scholar)

Honors Baccalaureate of Science in Sustainability
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Abstract approved:____

Kate Lajtha

Dissolved organic carbon (DOC) flux is an important mechanism to convey soil carbon (C) from aboveground organic debris (litter) to deeper soil horizons and can influence the formation of stable soil organic carbon (SOC) compounds. Aboveground litter quantity and quality was manipulated for 20-years in an old-growth Douglas fir forest under six treatments to study these relationships. DOC concentrations were measured in surface and subsurface horizons using tension lysimeters, and a hydrologic model was created to quantify water flux through the soil profile. This model, coupled with lysimeter measurements, was used to estimate annual DOC flux under the different treatment scenarios. DOC concentrations ranged from 3.0-8.0 and 1.5-2.5 mgC/L between treatments 30 and 100cm below the soil surface respectively. Aboveground detrital inputs did not have a direct linear effect on soil solution DOC; doubling the mass of aboveground leaf litter decreased observed DOC concentrations by 41%. The energetically high-quality litter accelerated metabolic rates, resulting in a "priming" effect that led to this net decrease. In contrast, the addition of aboveground woody debris increased observed DOC concentrations by 58% relative to the control. DOC temporal trends revealed that decaying root debris, coupled with the exclusion of an active rhizosphere, doubled DOC concentrations over 20 years. This debris may be a long-term source of C that is not metabolized when live roots are excluded and therefore may be a potential mechanism for the formation of stable SOC. Annual DOC flux into groundwater was small (2.7-3.7 gC/m2/year) and accounts for only 0.03-0.09% of estimated litter C at the site. Therefore, direct DOC flux from surface litter to groundwater supplies is relatively negligible in the soil C budget. However, DOC flux into surface horizons was significantly greater (73-210 gC/m2/year), equivalent to 1.2-1.9% of aboveground litter C. Therefore, DOC transport may be an important mechanism for C accumulation in shallow horizons. A greater proportion of litter C was transported annually as DOC with the addition of woody debris (1.9%), and leaf litter (1.5%), relative to the control (1.2%). This suggests that an increase in litter accumulation accelerates DOC flux relative to the total C supply, and therefore may be an important negative feedback loop on aboveground litter C storage.

Key Words: Dissolved organic carbon, soil organic carbon, litter inputs, carbon cycling.

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Honors Baccalaureate of Science in Ecological Engineering and Honors Baccalaureate of Science in Sustainability project of Lucas R. Evans presented on May 29, 2019.

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I understand that my project will become part of the permanent collection of Oregon State University, Honors College. My signature below authorizes release of my project to any reader upon request.

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Lucas Evans, Author

Introduction

Soil organic carbon (SOC) is the largest terrestrial carbon (C) sink in the biosphere and can change in relatively short geologic time scales (Pan et al. 2011; Schlesinger 1997). Approximately 45% of global forest C is stored in the top meter of soil (Pan et al. 2011), whereas the remainder is distributed in aboveground organic debris (litter), deadwood, and live biomass. Because the global SOC pool is so large, a small percent change in storage can have profound impacts on the atmospheric C pool, and hence climate change. Schimel et al. (2000) found that C flux in the U.S. from soil to the atmosphere via decomposition of organic matter, and root respiration, was approximately 10-fold greater than fossil fuel and deforestation sources combined. Therefore, it is important to understand how SOC stocks will respond to changes in the quantity and quality of forest litter. Litter C inputs are predicted to increase with climate change, but the link between litter quantity and soil C stabilization are not direct and may be ecosystem dependent (Fröberg et al. 2006; Crow et al. 2009). Therefore, it is critical to understand how the SOC pool will respond to these litter changes so future predictions of global C storage are accurate.

Dissolved organic carbon (DOC) flux can convey C from aboveground litter to deeper soil horizons and has the potential to be an important flow in the soil carbon budget. The magnitude of this flux relies on both infiltration and DOC production rates which are functions of the climatic and ecological characteristics of a region. Past studies have established that relatively little DOC produced in organic horizons gets transported to groundwater (Yano et al. 2005; K. Lajtha et al. 2005; Creed et al. 2008). Transport of DOC from surface litter through the soil profile is inhibited by direct sorption and chemical binding reactions between DOC and the soil matrix, and by direct assimilation and mineralization by soil microorganisms. Sorption-desorption reactions within the mineral matrix are substantial and may be the largest source of DOC present in deeper soil horizons (Fröberg et al. 2006). The DOC that does reach groundwater links terrestrial and aquatic ecosystems through the transport of C from soils to streams (Fellman et al. 2009). Creed et al. (2008) estimated that annual DOC flux from 33 forested catchments varied from 0.9-13.9 gC/m^2/yr. Similarly, Lajtha et al. (2005) estimated a flux of 2.0-3.8 gC/m2/yr for different aboveground litter types. These estimates reveal that a small mass of C is lost from the soil system through this pathway. However, a significant quantity of C from aboveground litter can be transported into shallow soil horizons as DOC under high leaching rates (Lee, Park, and Matzner 2018; Cleveland et al. 2004). O-horizon DOC concentrations under forest floor litters have been reported to range from 30-120 mgC/L which is an order of magnitude higher than soil solution concentrations (Solinger, 2001; Lajtha et al. 2005; Fröberg et al. 2006). These surface concentrations can result in more significant C fluxes into the mineral topsoil; Solinger et al. (2001) reported a carbon flux of 20-35 gC/m^2/yr. Flux estimates are site specific because they are a function of litter quality and quantity and meteorological conditions including temperature and precipitation (Bengtson and Bengtsson 2007; Christ and David 1996). Temperature, precipitation, and litter mass are positively related to DOC production, and hence DOC flux (Bengtson and Bengtsson 2007; Christ and David 1996; Lee, Park, and Matzner 2018).

Soil DOC concentrations can impact microbial respiration rates and the formation of stable and minerally associated organic matter (MAOM) (Córdova et al. 2018; Cotrufo et al. 2013). This is partly because respiration of SOM is limited by DOC formation rates; Bengtson and Bengtsson suggested that DOC is fully respired and replaced in solution multiple times a day (Bengtson and Bengtsson 2007). This respiration rate is limited by the degradation of litter C to DOC and then DOC utilization by microbes. Therefore, source litter quality should impact DOC concentrations and microbial substrate use. Cotrufo et al. (2013) proposed the Microbial Efficiency Matrix Stabilization theory (MEMS) to describe this relationship. The MEMS theory suggests that microbial substrate use efficiency controls the relative rate of stable SOM formation. Cotrufo suggests that labile plant constituents are the primary precursors to stable SOM formation because they are utilized most efficiently by microbes. The microbial products synthesized from C assimilation may form strong chemical bonds with the soil matrix and often dominate the stable SOM pool (Cotrufo et al. 2013). This hypothesis has been supported by both field and incubation studies: Bradford et al. (2013) established that sugars were two times more likely than amino acids to cause formation of stable SOM through DOC assimilation, similarly Cleveland et al. (2004) found that labile litter produced a greater concentration of DOC in leachate that could be utilized by microbes. However, this theory is not completely accepted as some studies have revealed inconsistencies in the conceptual framework. High quality litter can sometimes increase soil respiration greater than carbon assimilation rates, leading to a net decrease in SOM and DOC (Crow et al. 2009; Córdova et al. 2018; Sulzman et al. 2005). Furthermore, the energy available to microbes from high quality litter may allow SOM to be degraded that was previously stabilized, an effect referred to as "priming" (Sulzman et al. 2005; Crow et al. 2009). Despite no clear consensus on the mechanism for stable SOM formation, it is clear that DOC plays a key role in the formation of soil C stocks.

A long-term field study was established at the H.J. Andrews Experimental Forest, OR, USA in an old-growth coniferous forest to study the relationships connecting surface litter quality and quantity to SOM accumulation and stabilization. The Detritus Input and Removal Treatment (DIRT) plots consist of treatments that double leaf litter, double woody debris inputs, exclude litter inputs, or remove root inputs via trenching. Soil solution DOC has been measured with depth since 1999 to determine how DOC production and transport has been impacted by these litter manipulations. Past analyses have determined that DOC removal is dominated by abiotic sorption because the proportion of hydrophilic neutral DOC, which are labile to microbes, has been low (Yano et al. 2005; K. Lajtha et al. 2005). Sorption to the soil matrix, and microbial use, has retained a vast majority of produced DOC (~98%) and effectively homogenized soil solution chemistry between treatments at a depth of 100cm (Yano et al. 2005; K. Lajtha et al. 2005; Strid, Lee, and Lajtha 2016). The chemical homogenization is likely a result of preferential microbial substrate-use of labile DOC (Strid, Lee, and Lajtha 2016), while the homogenization in concentration can be attributed to the buffering capacity of sorption processes (Yano et al. 2005). This suggests that litter manipulations may take decades to result in a measurable change in DOC at depth. A greater decrease in DOC has been observed between the soil surface and 30 centimeters than between 30 and 100cm, most likely because the surface soils adsorb the larger proportion of hydrophobic DOC (Yano et al. 2005; K. Lajtha et al. 2005). Concentrations at 30cm in the first study year (1999-2000) were reported as 4.1, 2.9 and 7.5 mg C/L on average for the control

(CTL), double litter (DL), and double wood (DW) plots respectively (Yano et al. 2005). Despite higher DOC production rates in the DL O-horizon, DOC concentrations at 30 centimeters have consistently been lower than the CTL treatment as a result of microbial priming (K. Lajtha et al. 2005; Crow et al. 2009). It's estimated that the increase in above-ground detritus has accelerated C loss to respiration by 11.5-34%. This loss outweighed the higher DOC production rates, leading to a lower net soil solution concentration (Sulzman et al. 2005; Crow et al. 2009). The acceleration in DOC decomposition has not been observed in the DW treatment, suggesting that high-quality litter is required to observe a priming effect (Sulzman et al. 2005; Crow et al. 2009). Differences in DOC chemistry and concentration at 100cm were not established in these studies. Similarly, no attempt has been made to determine seasonal DOC trends for each treatment. Crow et al. (2009) found seasonal differences in respiration with the highest respiration rates observed in late spring and early summer when high temperatures and moist soils optimized microbial processes. Similar studies have reported mixed results for DOC seasonality: Solinger et al. (2001) did not observe seasonal DOC concentrations in the O-horizon or mineral soil solution, in contrast Fellman et al. (2009) observed seasonal DOC variations in soils and streams with the highest concentrations in spring and fall. Similarly, Vanderbilt et al. (2003) established that DOC had clear seasonal trends in H.J. Andrews Forest streams. DOC data now spans nearly 20-years of manipulation, re-opening the possibility to observe time-series and seasonal trends in concentration and flux at all soil depths.

Because warm summer temperatures stimulate microbial degradation of litter, I hypothesized that soluble byproducts would accumulate during the dry summer months and only be flushed out with the beginning of fall rains. This would result in the highest observed DOC concentrations in a "first flush" event in Fall, followed by a gradual decline throughout the sustained rainfall and low temperature wet season. DOC concentrations in litter exclusion treatments were hypothesized to decline over time and have the lowest mean values of all treatments because microbially-accessible C pools would be depleted and not renewed by new litter inputs. In contrast, microbial processing of the added litter in the DL and DW treatments was expected to increase DOC production rates, leading to the highest observed concentrations. But, I hypothesized that priming-induced consumption of DOC could counter this effect, especially with high quality litter additions in the DL treatment. This priming effect could accelerate DOC utilization more rapidly than DOC production in the DL treatment and therefore result in an equilibrium concentration lower relative to the control. While I expected measurable differences in DOC between treatments 30cm belowground, this was not predicted at 100cm. DOC concentrations are buffered by sorption to the mineral matrix and microbial processing of the soluble materials as soil water is transported deeper into the mineral horizon. Thus, I hypothesized that sorption of DOC to mineral binding sites, coupled with microbial respiration and assimilation, would effectively homogenize DOC concentrations between treatments at 100cm. Finally, I hypothesized that annual water flux would be similar for each treatment because the high hydraulic conductivity (Ks) of the site would make runoff negligible for all treatments, despite any decrease in infiltration rates from litter removal. Therefore, DOC flux at each depth was predicted to be a direct function of concentration and would transport a very small proportion of total C into groundwater. Given these hypotheses, the primary objectives of this study were: (1) to determine DOC response to litter quality and quantity manipulations and assess temporal and seasonal DOC trends and (2) to estimate the relative importance of annual DOC flux in the soil carbon budget.

The study was completed in two stages, first DOC concentrations were measured at the DIRT site under the six litter manipulations for 19 years (1999-2017) and only during the seasonally wet periods (October-May). Second, a hydrologic model was created using Hydrus-1D: a PC-PROGRESS numerical modeling software to estimate water flux through the soil profile using daily meteorological data, soil hydraulic properties, and characteristics of local vegetation. The water flux computation allowed the quantification of DOC flux at multiple depths within the soil profile.

Methods

Study Site

This study was conducted at the H.J. Andrews Experimental forest located in Oregon's Western Cascades. The experimental forest was founded in 1948 by the US Forest Service and it is now a hub for long term ecological research (LTER). H.J. Andrews is part of the National Science Foundation funded LTER network and offers an extensive database of meteorological and ecological observations from its inception to the present. Mean annual precipitation is 2080 mm yr-1 and mean temperature at the forest headquarters is 9.4^{o} C (averaged from 1999-2014). Over 70% of annual precipitation occurs during a "wet period" between November and March (Sollins et al. 1980).

The study site for the detrital input removal treatment (DIRT) experiment was established in an undisturbed old growth Douglas fir (*Pseudotsuga menziesii)* and Western Hemlock (*Tsuga heterophylla*) forest. Within the forest, aboveground litter inputs have been manipulated since 1997 (44^{o}15' N, 12^{o}10' W, 726 m elevation). Other significant species include Western Red Cedar (*Thuja plicata*), Vine Maple (*Acer circinatum*), Big-Leaf Maple (*Acer macrophyllum*), Red Huckleberry (*Vaccinium parvifolium*) and Oregon Grape (*Mahonia aquifolium*). The soil surface is covered with mosses and a diverse community of ground cover species. The soil is derived from volcanic parent material and classified as coarse loamy mixed mesic Typic Hapludands (Yano et al. 2005; Spears and Lajtha 2004; K. Lajtha et al. 2005). Small areas of Andic Dystrudepts and Vitrandic Dystrudepts also underlie the treatment plots.

Six litter treatments were established at the DIRT plots in 1997, these treatments include the addition of coarse woody debris and leaf litter, and the exclusion of litter and roots by screening and trenching (Table 1). Each treatment was replicated three times (n=3) and assigned random plot locations. Not all plots were sampled consistently after the first three years of the study period because it was not always possible to extract soil water from each lysimeter. Therefore, some data points only rely on one or two plots (n=1), these data were averaged over the course of the study period to remove plot variability. The plots are approximately 10 × 15m and include trees and other natural features. Trees and all other live vegetation were removed from the no input (NI) and no root (NR) treatments during site creation. There is some variability in size due to natural obstacles and space constraints. Litter was excluded from the NL and NI treatments using 1mm mesh screening to collect all falling debris. All litter material was initially removed and kept bare on these plots. Litterfall from the NL plots has been systematically collected and transferred to the DL plots. In early stages of the study, litter was collected 4–5 times per year: at the end of the dry season, twice or more during the wet season (November–March), and at the beginning of the dry season (K. Lajtha et al. 2005). In the past ten years litter has been collected and transferred on an annual basis during the dry season. This method of removal does allow some DOC flux into the NL and NI plots because the organic debris is not immediately removed from the screens. However, this flux is minimized because the mass of source litter is greatly reduced.

Table 1. *DIRT plot treatments at H.J. Andrews LTER site.*

Treatment	Method
Control (CTL)	*Normal litter inputs.*
Double Litter (DL)	*Aboveground needle/leaf litter is double by adding litter removed from NL plots.*
Double Wood (DW)	*Aboveground wood inputs are doubled by adding large shredded wood debris based on measured input rates of woody debris fall.*
No Litter (NL)	*Aboveground litter inputs are excluded from plot.*
No Roots (NR)	*Roots are excluded with impenetrable barriers extending from the soil surface to the top of the C horizon.*
No Inputs (NI)	*Aboveground inputs are prevented as in NL plots; belowground inputs are prevented as in NR plots.*

A mix of decomposed woody debris and shredded chips (5–20 cm in length) of Douglas-fir wood with a ratio of decomposed woody debris to intact woody debris of 4:1 have been added every other year to the double wood treatment plots (K. Lajtha et al. 2005). The mass of this addition was estimated to be equal to falling wood debris in the control plots. Logs were obtained from a local mill and were chipped by Rexius of Eugene, Oregon. Roots were excluded from the NR and NI treatments using an impermeable barrier at the plot boundaries as deep as the C-horizon (K. Lajtha et al. 2005).

Water Sample Collection

Five Prenart Superquartz tension lysimeters were installed at a 30^{0} angle in each treatment plot in 1997 to extract water samples from the soil matrix. Two of the lysimeters were installed to a depth of 100cm and three were installed at 30cm in each plot according to the method described by Lajtha et al. (1999). The lysimeters were sampled on a monthly basis during the first three years of the DIRT study and were subsequently sampled multiple times per year until 2008. The consistency of sampling events decreased as the study progressed and no samples were obtained in 2009-2013. Lysimeter sampling resumed from 2014-2017 but was limited to two sampling events in 2014 and a single sampling event in the years 2015-2017. Not all Lysimeters or plots were sampled during every sampling event because soil water content was too low for sampling or the lysimeter was not able to maintain a negative suction force. Near the end of the study some lysimeters were terminated because of this. Samples were only collected during the wet season (October-May) when sufficient soil moisture was available for water extraction. To minimize soil disturbance, all samples were collected within 72 hours of tension being applied to the lysimeters. After extraction, samples were stored on ice and transferred to Oregon State University where they were frozen until analysis. Initial experiments conducted in the early 2000s established that tension lysimeter samples did not need to be filtered before storage. However, 2014-2017 samples were filtered before analysis using GF/F Whatman fiberglass filter paper (0.7 micrometer nominal pore size) because suspended solids were observed in the leachate.

A single zero tension lysimeter was installed at the bottom of the O-horizon in DW, DL, and CTL plots in 2000 (K. Lajtha et al. 2005). These lysimeters were installed by placing a 20cm × 20cm plastic container at the soil surface after carefully removing an identically sized O-horizon quadrant. This O-horizon material was then placed back directly on top of the lysimeter. O-horizon leachate was collected from these lysimeters during the wet season of 2000-2001 and analyzed for dissolved organic carbon (DOC) as described by Lajtha et al. (2005). The results from this study were used to extrapolate DOC concentrations between the soil surface and the 30cm lysimeters.

Chemical Analysis

Water samples were analyzed for DOC through Pt-catalyzed high-temperature combustion using a Shimadzu TOC analyzer. Different models of Shimadzu analyzers were used throughout the study including the Shimadzu TOC-5000A or TOC-V CSH analyzer for 1999-2007 samples, and TOC-VSCH for 2014-2017 samples. 2014-2017 sample analysis was completed at Oregon State Universities' Institute for Water and Watersheds (IWW) Collaboratory. A five-point calibration curve was created at the start of each run using high purity TOC standards with a stock concentration of 1000 ppm. Every ten samples were verified using a DI blank and 1 ppm Agilent TOC check standard. The calibration curve had a range of 0.2-5.0ppm DOC, any results outside of these bounds were re-analyzed. Dilutions were necessary for many of the 30cm lysimeter samples, these dilutions were prepared with high precision pipettes. Any DOC that was contributed from DI water during dilutions was excluded from the final reported concentration. Glucose check standards and DI blanks were used for DOC analysis completed before 2014.

Data Analysis and Statistics

All DOC data was averaged by plot and then within treatment for each sampling date before any analysis. Graphical and statistical comparisons were completed using R version 3.5.2. The DOC seasonal and time series trends for each treatment were assessed first. DOC data was sorted by month and averaged using all years of data to determine if monthly DOC trends were present over the entire study period. This analysis revealed that summer months (June and July) were outliers compared to all other sampling months. These months were also not consistently sampled because the soil was too dry to extract a water sample in most years. Therefore, July and June sampling events were excluded from all further averaging and statistical analysis. Treatments were also categorized by season (fall, winter, spring) where fall includes months 10-12, winter includes months 1-3, and spring includes months 4-5. A single variable ANOVA analysis was used to compare DOC results by month and season. Time-series trends were assessed using the mean DOC concentration by year for each treatment. Data was averaged annually to ensure an equal weight for each year, this was necessary because years did not contain the same number of observations. A linear regression was utilized to determine if trends were significant. We considered a trend to be significant if $p < 0.05$ and a best fit line was included in any plot that met this requirement.

These analyses revealed no temporal or seasonal trends for almost all treatments; therefore, it was appropriate to average all data over the study period without considering temporal or seasonal impacts on the sampling date. The mean DOC concentration for each treatment was computed to compare treatment effects. As a result of inconsistent sampling frequencies, each treatment has a different number of total observations that range from (n=25 to n=33). A single-variable ANOVA analysis was used for group comparisons of the mean DOC concentration versus treatment at 30 and 100cm and for comparison of Ks versus treatment. A single variable t-test was also computed between the CTL and all other individual treatments at both depths. Single variable t-tests were also performed to compare treatments to each other (i.e. DW vs. DL). Differences were considered significant when $p<0.05$ but were also noted when $p<0.10$ for all analyses.

Hydrus Model Implementation

A computational simulation was completed using an open-source numerical modeling software (PC-Progress HYDRUS-1D) to estimate the cumulative and annual water flux out of each treatment at multiple soil depths. The simulated water flux and measured DOC concentrations were used to approximate annual DOC flux. Hydrus-1D is a public domain Windows-based modeling environment for analysis of water flow and solute transport in variably saturated porous media (Simunek et al. 2013). The software uses a one-dimensional finite element model that solves the Richards-Richardson flow equation to simulate the movement of water, heat and solutes.

The major processes included in this simulation included water flow in a heterogenous soil profile, root water uptake, and evapotranspiration calculated using the Penman-Monteith equation. Daily meteorological data was accessed from the HJ Andrews experimental forest PRIMET station from 1/1/1999 to 12/31/2014 and includes daily precipitation, solar radiation, wind speed, relative humidity and air temperature. The PRIMET station is located approximately four kilometers from the DIRT site. Boundary conditions were defined at the mineral soil surface, and at 150cm in depth. The upper boundary condition was specified as an “Atmospheric Boundary Condition with Surface Runoff”, the lower boundary condition used was “Free Drainage”. This lower boundary condition assumes that the water table is deep in the soil profile and plays no part in the system hydrology. This assumption was made because the study site is located at a high elevation hillslope far from the closest stream.

A soil profile was created in the simulation from the soil surface to 150cm and was cut into three horizons with unique hydraulic properties informed by past soil surveys. Soil horizons were split at 0-20cm (A-horizon), 20-70cm (B-horizon) and 70-150cm (C-horizon). Each horizon had a specified Ks, tortuosity, porosity and residual water content (Table 2). The Van-Genutchen hydraulic model was used to simulate soil water flux using these hydraulic properties. Ks at the soil surface was directly measured in the field using a single ring permeator and the B and C horizon conductivity was informed by Web Soil Survey and the Hydrus built-in database (Simunek et al. 2013; NRCS 2017). Ks decreased with depth significantly based on this web resource. The other variables were estimated using the known soil texture of each horizon and the associated properties provided by the software.

Table 2. *Soil hydraulic properties by horizon. The properties were estimated using the Hydrus built-in database for the known soil textures. Ks was directly measured in the A-horizon and estimated from texture and depth in the B/C horizons.*

Horizon	Residual Water Content	Porosity	Ks (cm/day)	Tortuosity	Depth (cm)	Texture
A	0.078	0.43	400	0.5	0-20	Loam
B	0.065	0.41	106	0.5	20-70	Sandy Loam
C	0.065	0.41	25	0.5	70-150	Sandy Loam

The Feddes root uptake model was utilized to predict root water uptake. This model is a simplified representation of root dynamics and was developed to simulate crop yield and water uptake in limiting situations (Feddes, Kowalik, and Zaradny 1978). Root water uptake is split into three categories: 1) non-optimal uptake because of low soil moisture 2) optimal root water uptake and 3) non-optimal uptake because of soil saturation. Input parameters that define these regions include the pressure at wilting point, the range of soil potential for optimal uptake, and soil porosity. These parameters were estimated from a default dataset for deciduous fruit trees. This was the best alternative to coniferous tree data which was unavailable in the literature. Roots were included in the soil profile from 0-100cm, which is usually the lower extent for Douglas fir trees (McMinn 1963). Root density decreased dramatically with soil depth, with approximately 50% of total root mass in the top 20cm of soil.

After the core model was created in Hydrus, the simulation was computed from 1/1/1999 to 12/31/14 (5844 days) for each DIRT treatment using an initial water content at field capacity. The maximum specified time-step was 0.01 days. Each DIRT treatment received the same input data and hydraulic properties except for measured differences in Ks (Table 3). Transpiration and root water uptake were excluded from the NI and NR plots.

Table 3. *Hydrus methods by treatment. Each treatment functioned using the same core model with some manipulations depending on treatment type. The Ks value is the measured A-horizon saturated hydraulic conductivity, the B/C conductivities were unchanged.*

Treatment	Ks (cm/day)	Method
CTL	400	*Core model.*
DL	400	*Same as CTL.*

DW	700	*Same as CTL.*
NL	300	*Same as CTL.*
NR	300	*Root water uptake and ET excluded.*
NI	300	*Root water uptake and ET excluded.*

Model Validation

A closer examination of the Hydrus model results was necessary to confirm that physical processes were accurately represented. This systematic evaluation was completed in the following steps: (1) model mass balances were confirmed, (2) annual soil water content was analyzed throughout a randomly chosen year (3) hand calculations were completed using Darcy's law and the latent heat of water to validate flux and maximum root water uptake, (4) a sensitivity analysis was completed on four model variables.

The water mass balance confirmed that precipitation was equivalent to the sum of all outflows (root water uptake, evaporation, runoff and infiltration). The model reported an annual average precipitation of 208cm, negligible runoff and evaporation, and root water uptake of 76.4cm for the core model (Table 4). The sum of all water outflows equaled the input precipitation. Runoff was essentially negligible in the simulation because the input Ks greatly exceeded the maximum daily precipitation rate, this caused all precipitation to be infiltrated. Direct evaporation was minimal because water did not pond on the surface, so root water uptake and transpiration became the mechanism for atmospheric water loss.

Table 4. *Hydrus model predicted annual average water flux (cm) for all hydrologic flows. These values are from the core model.*

Water Mass Balance Results (cm)	
Runoff	0.01
Root Water Uptake (ET)	76.4
Evaporation	0.75
Precipitation	208

Soil water content results were assessed at 100, 50 and 10 centimeters below the soil surface during the second year of simulation. Seasonal trends were compared to known precipitation and measured water contents at the H.J. Andrews forest. The water content plot at these observation nodes revealed that the moisture ranged between the specified residual and saturated moisture contents and followed expected seasonal trends (Figure 1). At 100cm in the soil profile, volumetric moisture content was buffered from seasonal changes and declined slowly from a moisture content of approximately 0.33 to a minimum of 0.16 at the end of summer. Observations nodes at 50 and 10cm had more temporal variability which reflects the fast response these surface horizons had to infiltrating storm events. Root water uptake demand was also higher at these depths which drove the steep decline in moisture during the summer months. Even in winter months, the moisture content at 50 and 10cm was consistently lower than at 100cm and never reached saturation because the high input hydraulic conductivity quickly infiltrated precipitation. The surface soils had a relatively constant moisture content during the wet season that hovered near field capacity (0.2-0.25).

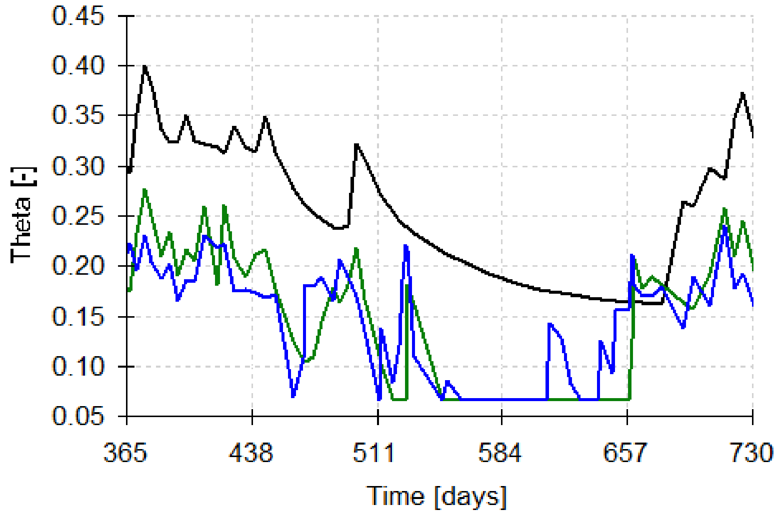

Figure 1. *Soil water content during the second year of simulation at 100, 50, and 10cm. The black line is 100cm, blue is 50cm and green is 10cm.*

The model output also established that root water uptake and surface flux followed seasonal trends corresponding to winter rain and summer radiation. Root water uptake was highest in the summer and peaked at a little over 1 cm/day (Figure 2). The maximum possible evaporation was calculated using the highest input summer radiation (~ 30 MJ/m2/day), and the latent heat of water. Thos atmospheric flux was estimated to be 1.2cm/day which is essentially equal to the Hydrus predicted peak root water uptake (Figure 2).

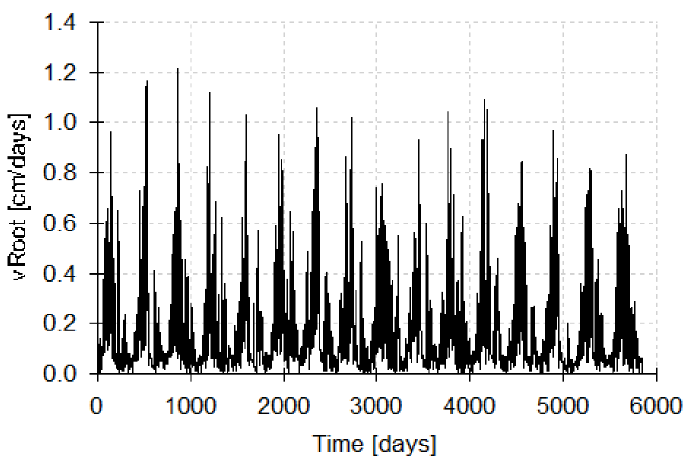

Figure 2*. Actual root water uptake (cm/day) during simulation. The peaks correspond to summer radiation and minimums are during the winter season.*

Hand calculations were completed using Darcy's law to assess the accuracy of the soil hydraulic results on smaller timescales. A day during the first year of simulation was selected when negative soil pressures began to increase (t= 123 days). The pressure and water content from the surface, and at 50cm, were taken from the model and used to calculate the Darcy flux (cm/day) from each observation point. The flux at the surface was assumed to be equal to the daily precipitation on that day (2.8cm). All hand calculations were completed using a Ks that was a function of moisture content from the Van-Genutchen hydraulic model. The effective conductivity was used for calculations involving more than one soil horizon. Hydraulic calculations showed that the model surface flux was slightly smaller (2.7cm) than the input precipitation (2.8cm). The flux at the other observation's nodes (10, 50, 100cm) had errors ranging from 0-5%. Flux was overestimated at 10 and 50cm but underestimated at 100cm.

A sensitivity analysis was completed on four parameters with the most uncertainty, these included (1) the "free drainage" lower boundary condition, (2) effective Ks (3) Feddes root water uptake wilting point pressure and (4) leaf area index. These variables were perturbed by ±25% and the model was computed with the new input value. Absolute change (%), relative sensitivity, and absolute sensitivity were tabulated for annual average flux at 10 and 100cm. The lower boundary condition was changed to "Constant Water Content" with a specified water content of 0.33. This was the average water content at 150cm in the unaltered model.

The perturbed input variables had little impact on the predicted annual average water flux at 10 and 100cm (Table 5). The -25% perturbation in effective Ks resulted in an increase of 1.53% and 2.67% in flux at 10 and 100cm respectively. This was the largest change observed from any perturbation. The perturbation in LAI resulted in a change in flux of <1%. Similarly, the Feddes wilting point pressure changed flux by 1.16% at 10cm. The lower boundary condition alteration decreased the flux at 100cm by 1.66%. These results are all within the range of expected error that can compound in a numerical model ran for approximately 60,000-time steps and establish that the simulation was primarily driven by the input daily meteorological data which controlled precipitation and root water uptake rates.

Table 5. *Sensitivity analysis results on the four input parameters. Shaded values correspond to the sensitivity of annual flux 10cm belowground, non-shaded values are 100cm annual flux. Parameters were perturbed by ± 25%, the value on the left is the result of +25% and the result on the right is -25% for each parameter.*

Sensitivity	**Effective Ks (cm/day)**		**Leaf Area Index**		**Wilting Point Pressure (cm)**		**Lower B.C.**
	+25%	-25%	+25%	-25%	+25%	-25%	Constant Moisture
Relative	0.03	-0.06	0.03	-0.02	-0.02	-0.05	NA
Absolute	0.03	-0.06	0.48	-0.36	0.00	0.00	NA
% Change	0.74	**1.53**	0.63	0.48	-0.48	**1.16**	0.16
Relative	-0.03	-0.11	-0.03	-0.03	-0.01	-0.02	NA
Absolute	-0.02	-0.08	-0.44	-0.36	0.00	0.00	NA
% Change	-0.79	**2.67**	-0.79	0.65	-0.22	0.58	**-1.66**

Field Methods

Saturated hydraulic conductivity was directly measured at the study site using a single ring permeator. Measurements were taken at randomly chosen plot locations where the mineral soil surface could be easily accessed. Natural obstacles including fallen logs, decaying wood or root channels were avoided to ensure consistent measurements. Avoiding all uncharacteristic regions was not possible, so outliers greater than 1.5x the interquartile range, were removed from the dataset. Each plot was sampled at nine locations (n=9) resulting in a total of 27 observations per treatment.

Permeators consisted of plastic rings with a diameter of 9.5 or 5.4cm that were driven into the soil to a depth of ~2cm. The 5.4cm diameter rings were used when soil conductivities were too high to be measured accurately with the 9.5cm ring. All O-horizon material was removed prior to infiltration testing. Infiltration rates were measured by adding a known volume (100ml) to the ring and recording the time it took for the water level to drop below the top of the soil surface. This procedure was repeated until a minimum of 1 liter was infiltrated or until a steady state infiltration rate was observed.

The Ks was calculated from field data using Darcy's law and the Green and Ampt model assumption. This model has been used extensively in hydrology to predict infiltration rates and assumes that (1) infiltration is driven by a constant wetting front potential and (2) all soil pores are filled behind the wetting front (Green and Ampt 1911). The depth of the wetting front was determined from the known volume of water added, an assumed soil porosity of 0.45, and the soil water content estimated from field observations and historical data. Edge effects from horizontal spreading were not eliminated in the field by a double ring, so the wetting front was modeled as a half-ellipse with the vertical-axis two times greater than the horizontal axis. The wetting front potential was determined using the field estimated soil water content and the Hydrus created soil water characteristic curves from the Van-Genuchten hydraulic model. The average aboveground water depth from infiltration testing exerted a pressure head at the soil surface.

DOC Flux Calculations

The average DOC concentrations at 30cm and 100cm were multiplied by the Hydrus predicted water flux for each treatment to obtain a DOC mass flux. Surface DOC flux was also estimated in this way. DOC surface concentration data was available from measurements completed by Lajtha et. al (2005) that utilized zero-tension lysimeters above the plots. This data was only available for the DW, DL and CTL treatments. The DOC flux 10cm belowground was determined from a linearly interpolated concentration. These mass flux values were compared to an estimated litter mass above each treatment. Litter mas was measured by collecting all organic material in a 0.015 square meter quadrant from each plot. This material was dried in an oven at 60° Celsius for 24 hours and then was weighed. The average mass per treatment was used to estimate total litter mass. This was converted to total litter C utilizing previous data reported by Yano et al. (2005) that characterized treatment litter composition (%C content). The calculated aboveground litter C stocks, and the estimated DOC flux, were used to determine the relative mass of C lost from litter through DOC flux annually and was reported as a percent of total litter C.

Results

Seasonal and Temporal Trends

The DOC dataset in this study spans 19 years (1999-2017) and has sampling dates from 8 months. Greater variability was observed in early winter and late fall for nearly all treatments (Figure 1 and 2). These months also had the smallest number of sampling events. There were no significant trends in DOC concentration by month or season for the CTL, DW and NI treatments from the ANOVA analysis ($p > 0.10$). Monthly differences were detected for the NL and NR treatments ($p= 0.034$ and 0.022 respectively), but when data was grouped by season there were no significant differences ($p= 0.087$, 0.193 respectively). These results do not indicate that there were clear seasonal trends, but they do demonstrate that some months had distinct DOC concentrations. The DL treatment was the only manipulation that had significantly different monthly ($p=0.0028$) and seasonal ($p=0.017$) DOC results. A single variable t-test revealed that the fall season had an average DOC concentration approximately 1.5ppm higher compared to winter and spring ($p = 0.027$ and 0.029 respectively).

DOC concentrations were plotted by treatment and year to assess the temporal trends of litter manipulation. A linear regression revealed that DOC concentrations at 30cm did not have trends for almost all treatments during the study period ($p >> 0.05$) (Figure 3). The NR manipulation was the only exception; this treatment nearly doubled in DOC over the 20-year timespan and had a strong linear regression ($p=0.00035$, $R^2=0.749$). The NI treatment, which also has roots excluded, did not exhibit this trend. No treatments experienced temporal trends in DOC at 100cm (Figure 4).

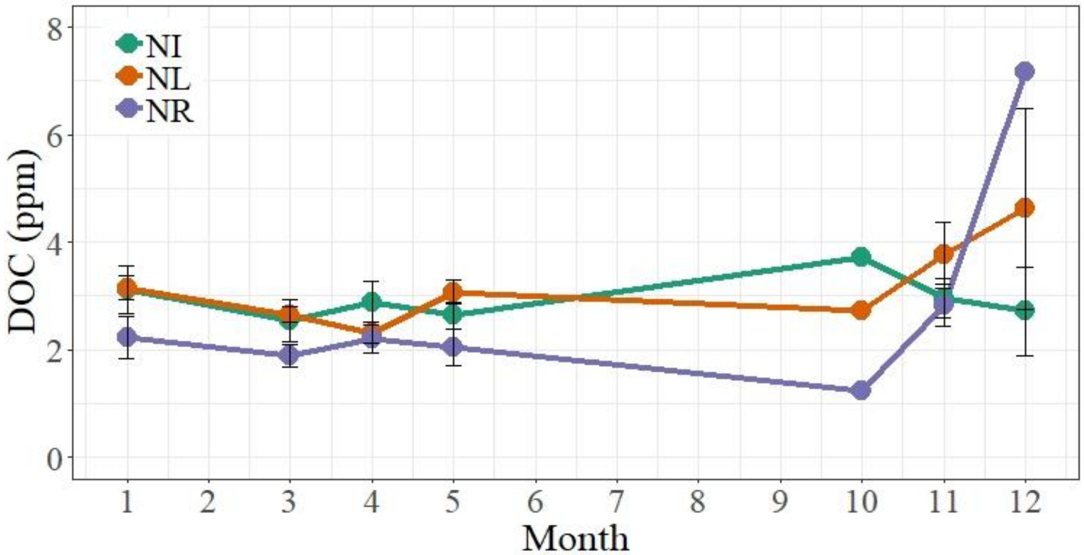

Figure 3. *Litter exclusion treatment (NI/NR/NL) average monthly 30cm DOC results (± SE) using all sampling dates. No results are presented from June-September because soil moisture contents were too low for consistent water extraction.*

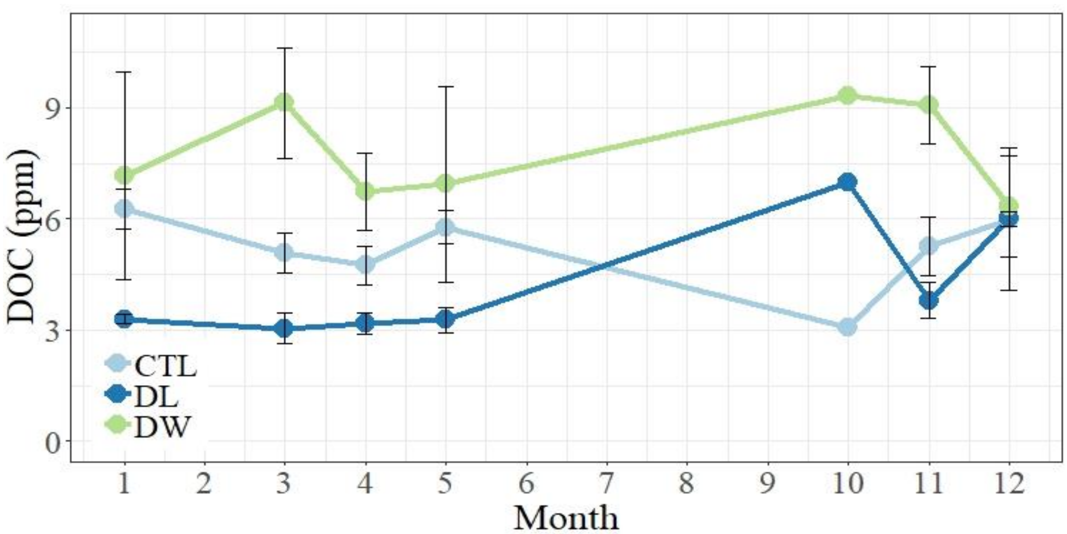

Figure 4. *Control and litter addition treatment (DW/DL) average monthly 30cm DOC results (± SE). No results are presented from June-September because soil moisture contents were too low for consistent water extraction.*

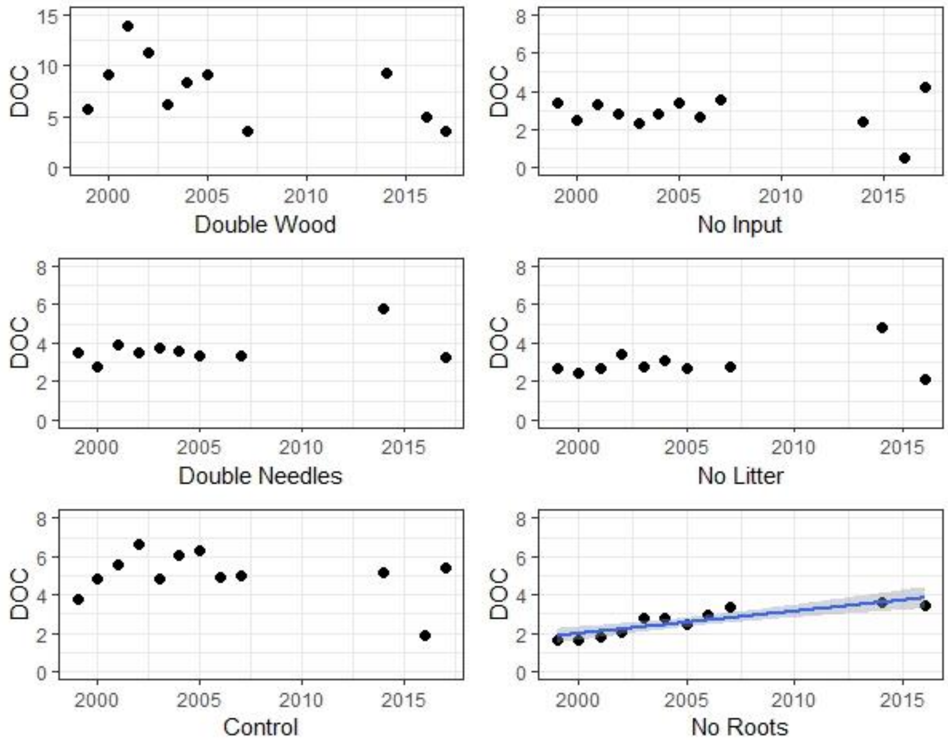

Figure 5. *Temporal DOC trends at 30cm by treatment. Data is averaged by year and trendlines are included when p < 0.05. DW treatment is on a different y-scale.*

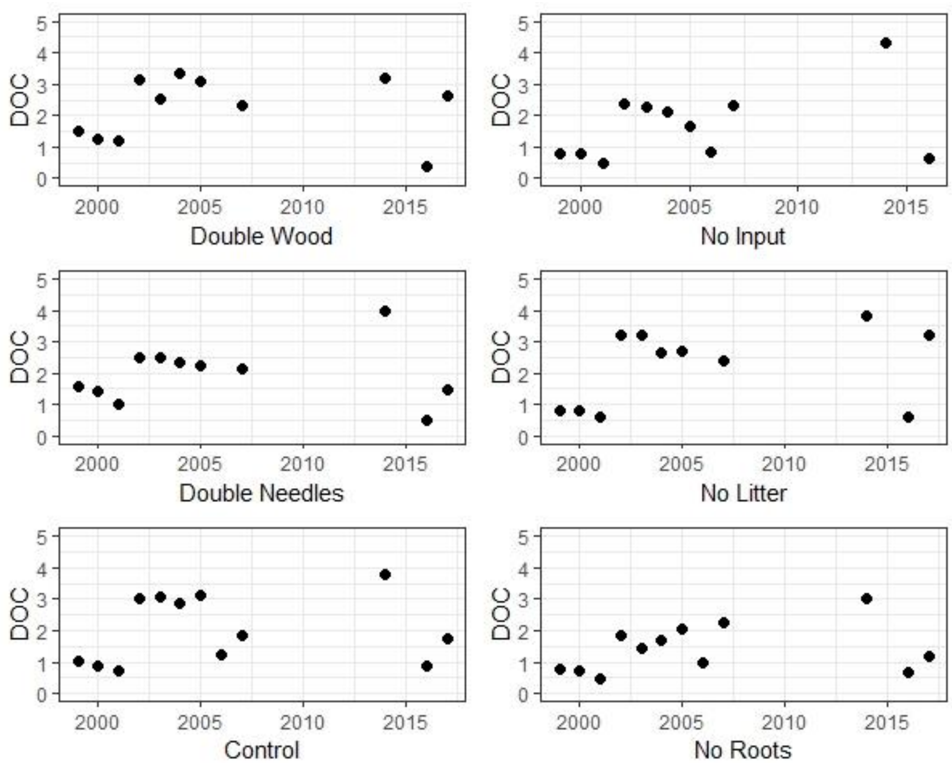

Figure 6. *Temporal DOC trends at 100cm by treatment. Data is averaged by year and trendlines are included when p < 0.05.*

DOC Concentration and Flux

DOC concentrations averaged over the entire study lifespan revealed significant differences ($p < 0.001$) between the litter treatments at 30cm from an ANOVA analysis (Figure 7). DOC was approximately 2.5ppm higher in the DW treatment relative to the CTL treatment and was also significantly different from a single variable t-test ($p < 0.001$). Despite the addition of litter in the DL treatment, the average DOC concentration at 30cm was more than 1ppm lower than the CTL treatment ($p < 0.001$). The NI, NL and NR treatments had the lowest DOC concentrations which were all approximately 2.5ppm lower that the CTL treatment ($p < 0.001$ for all three treatments). These exclusion treatments were not different from each other ($p > 0.10$). Differences in the mean DOC concentrations at 100cm were less evident than at 30cm and groups were not statistically different from the ANOVA test ($p = 0.07$) (Figure 8). In contrast to the 30cm results, the DL DOC was the same as the CTL at this depth. The DW treatment had the highest DOC concentration but was not significantly different than the CTL treatment. No treatments were significantly different than the CTL, but the NR treatment approached this threshold ($p = 0.06$). The DW and NR treatments were the only manipulations that were significantly different from each other at this depth ($p = 0.003$).

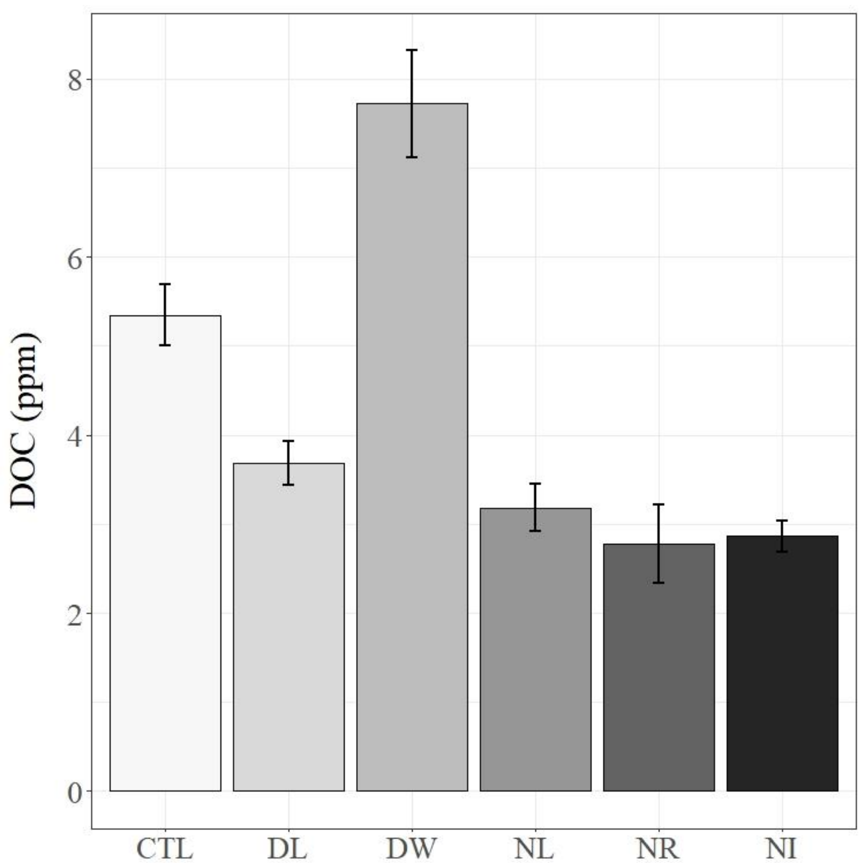

***Figure 7**. Average 30cm DOC concentration ($\pm SE$) by treatment for all sampling events. A cumulative average was taken because no clear seasonal or time series trends were discovered for most treatments. Groups were statistically different from an ANOVA analysis (p< 0.001).*

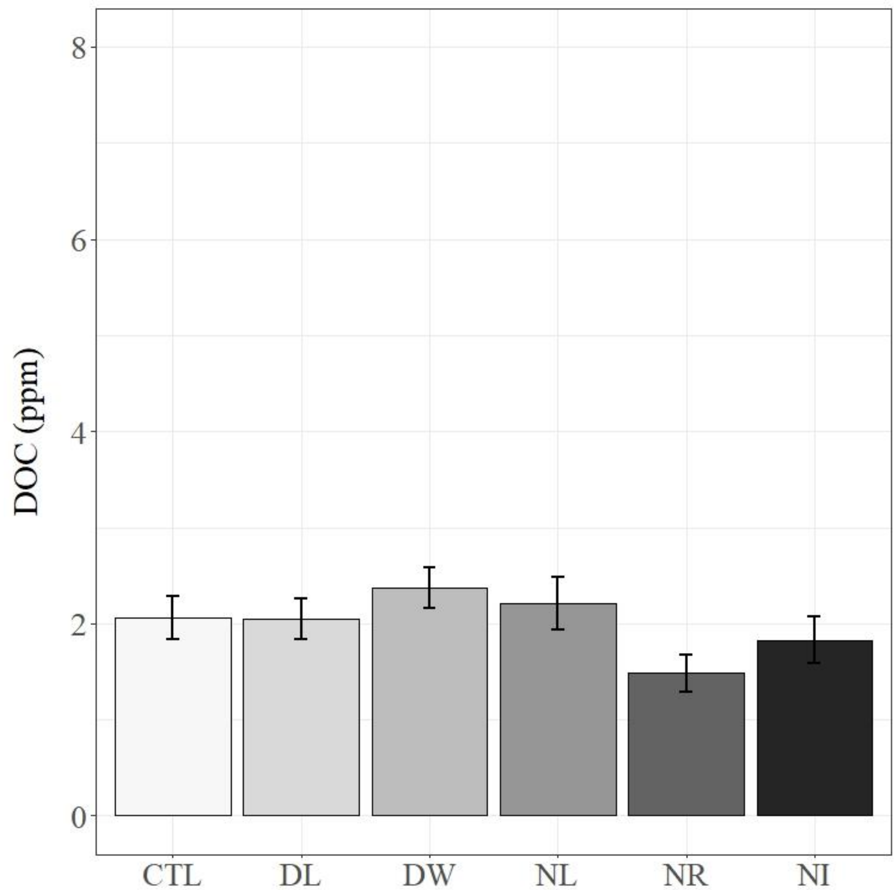

Figure 8*. Average 100cm DOC concentration ($\pm SE$) by treatment for all sampling events. Treatments were nearly statistically different from an ANOVA analysis (p = 0.06).*

The Hydrus simulation created a physically accurate representation of the site hydrology that was able to predict annual water flux at multiple soil depths for all treatments. There were no differences in water flux between treatments at the soil surface, but a higher flux was observed deeper in the soil profile for the NI and NR treatments (Table 6). The annual average water flux for the core model was 208, 156 and 150 cm/m^2/yr at the soil surface, 30cm, and 100cm respectively. Despite the different Ks input values for each treatment annual water fluxes at all depths were similar for treatments that included roots. Ks field measurements revealed significant differences in infiltration rates among treatments ($p < 0.001$). The DW treatment had the highest Ks that was about 300cm/day greater than the CTL treatment ($p = 0.023$) (Figure 8). The Ks of the NI manipulation was approximately 100cm/day less than the CTL treatment ($p = 0.041$). The NL treatment was not significantly different than the CTL or the NI manipulation ($p = 0.25$, 0.41 respectively).

Table 6. *Annual average water flux predicted by Hydrus simulation at three depths reported in cm/year. Flux was predicted for all treatments.*

Annual Average Water Flux (cm)						
Depth (cm)	CTL	DW	DL	NL	NI	NR
0	208	210	208	209	207	207
30	156	156	156	156	207	207
100	130	130	130	130	205	205

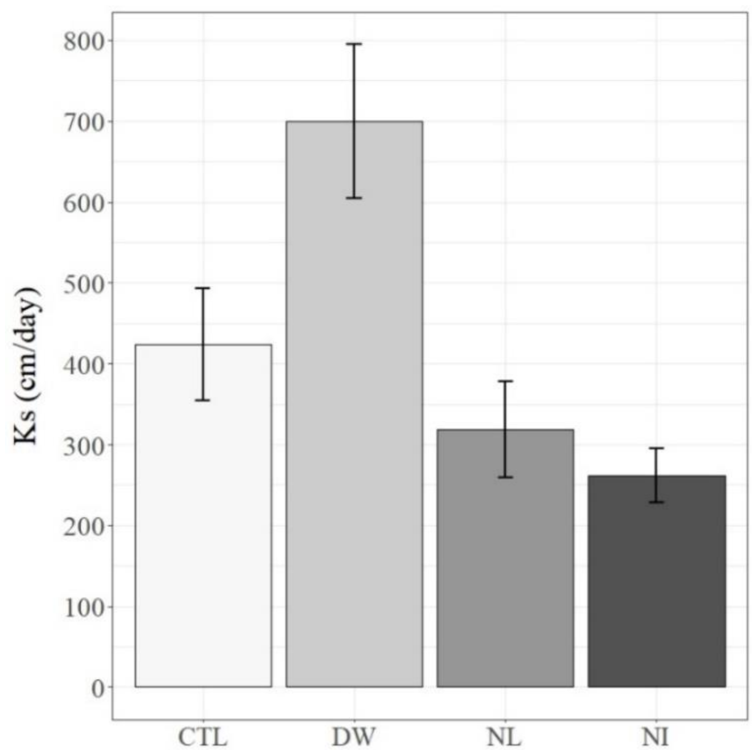

Figure 9. *Average saturated hydraulic conductivity (Ks) (±SE) from single ring permeator measurements reported in cm/day. Treatment groups were statistically different ($p < 0.001$).*

The estimated water flux (Table 6) and DOC concentration data (Table 7) were used to quantify DOC mass flux out of each treatment. DOC flux into the soil surface ranged between 73-210 gC/m^2/yr for the CTL, DW and DL treatments. This flux decreased drastically as water progressed through the mineral horizons with a range of 2.7-3.7 gC/m^2/yr at 100cm. This flux was considered to be the DOC entering groundwater supplies. The DW treatment experienced a DOC mass flux nearly three times higher than the CTL treatment at the surface and in shallow soil horizons (Table 9). This flux was effectively homogenized deeper in the soil profile and stabilized near the same value as the CTL at 100cm. The DL treatment had an estimated surface flux that was approximately 40% higher than the CTL treatment, but this flux decreased more rapidly with depth than the CTL treatment and was 45% lower than the CTL at 30cm. Surface

DOC data for the litter exclusion treatments were not available. These treatments experienced a DOC mass flux approximately 75% less than the CTL at 30cm. The highest flux at 100cm was experienced by the NI treatment because this treatment had a greater predicted water flux as a result of root water uptake being excluded, and its concentration was slightly higher than the NR manipulation. The DL and CTL treatments had a lower estimated DOC flux into groundwater than all three litter exclusion treatments (NL/NR/NI).

Table 7. *Average DOC concentrations by depth for all treatments. Concentrations at 10cm were calculated from linear interpolation between the surface and 30cm. The surface concentrations for CTL, DW, DL treatments were estimated from measurement at the field site completed in 2005 (Lajtha et. al).*

DOC Concentration (mg/L)						
Depth	*CTL*	*DW*	*DL*	*NL*	*NI*	*NR*
0	35	100	50	-	-	-
10	25	69	35	-	-	-
30	5.3	8.4	3.7	3.2	2.9	2.8
100	2.1	2.4	2.0	2.2	1.8	1.5

Table 8. *Calculated DOC flux at multiple soil depths for all treatments. Flux was calculated using average DOC concentrations reported in Table 8 and the computed average annual water flux from the HYDRUS simulation.*

DOC Flux ($g/m^2/yr$)						
Depth (cm)	*CTL*	*DW*	*DL*	*NL*	*NI*	*NR*
0	73	210	104	-	-	-
10	46	130	63	-	-	-
30	8.3	13.1	5.7	5.0	5.9	5.7
100	2.7	3.1	2.7	2.9	3.7	3.0

Discussion

Seasonal and Temporal Trends

I hypothesized that because warm summer temperatures stimulate microbial degradation of litter, soluble C byproducts would accumulate during the dry summer months that would only be flushed out at the beginning of the fall rains. These products would result in elevated DOC concentrations during the preliminary rainfall events in Fall, followed by a gradual decline throughout the wet season. The DL treatment was the only manipulation with clear seasonal trends to support this hypothesis. Fall month DOC was on average 1.5ppm higher than concentrations measured in winter and spring under this manipulation. Therefore, the high-quality litter source must have accumulated C products in summer months, and during deciduous leaf-death in fall, that were flushed out throughout the early wet season. This seasonal trend is consistent with most prior studies which have reported the highest DOC concentrations in fall during a hydrologic flush event and in spring just before peak snowmelt (Boyer et al. 1997; Vanderbilt, Lajtha, and Swanson 2003; Laudon, Köhler, and Buffam 2004; Fellman et al. 2009). Boyer et al. (1997) discovered that DOC in tension lysimeters peaked in fall during the first precipitation events and in early spring during the preliminary snowmelt event. Stream DOC responded similarly, but had a delayed peak concentration associated with the conveyance time of DOC from the soil surface to stream water. Incubation leaching experiments have similarly showed that DOC production is highest during initial leaching events and curtails with an asymptotic nature with sustained flushing (Christ and David 1996; Cleveland et al. 2004; Lee, Park, and Matzner 2018). These established relationships support the hypothesis that DOC concentrations would decrease throughout the wet season as the consistently high precipitation rates depleted C sources, and the low winter temperatures prevented microbial degradation of litter. This trend was not experienced by any treatment, suggesting that litter sources were able to sustain a steady DOC production rate in winter despite low temperatures and high rates of water flux.

Seasonal DOC trends were not observed in treatments other than the DL manipulation which contradicts the hypothesis that warm summer temperatures would stimulate microbial degradation of litter and accumulate soluble C byproducts during the dry summer months. DOC seasonality in soil solution has been elusive to measure in past studies; Solinger et al. (2001) did not detect any differences between months or seasons for soil solution or O-horizon DOC. I suggest two potential mechanisms for this result (1) there may not have been a sufficient quantity of accumulated C products from the prolonged summer drought period to detect a hydrologic flush event in the other treatments, and (2) seasonal trends did occur, but were experienced almost entirely in macropores and preferential flow paths that were not sampled by the tension lysimeters. The first explanation would be possible if labile C products were respired as rapidly as they were produced during the warm summer conditions. I believe it's more likely that the second mechanism occurred because it is consistent with the expected litter leaching response supported by numerous field and incubation experiments. This would also explain why more studies describe DOC seasonality in stream water, in contrast to soil solution, which has been difficult to characterize (Solinger, Kalbitz, and Matzner 2001; Fröberg et al. 2006). Preferential flow paths can be a significant pathway for infiltration in forest soils, especially for sandy soils under sustained unsaturated conditions (Kung 1990a, 1990b; Ritsema et al. 1993; Hagedorn and Bundt 2002). Kung (1990) suggested that the Ks of the uppermost soil layer is the largest predictor for the formation of "funneled" flow. There are three pieces of evidence that strengthen the likelihood of this phenomenon at the H.J. Andrews study site. First, the soils in question have a relatively sandy texture (loam-sandy loam) and the Hydrus model predicted that the soil profile remained unsaturated during 17 years of simulation. This suggests that if a preferential flow path was created, it would be persistent throughout the entire study period (Kung 1990a, 1990b). Second, infiltration testing discovered very high surface conductivities and revealed that preferential flow occurred during some tests because a notable portion of soil remained dry after more than 15cm of water flux. Third, the litter addition treatments have a thick organic horizon which can facilitate the distribution of water to preferential flow paths in the underlying mineral horizons (Ritsema et al. 1993). The prevalence of preferential flow can have large implications for the magnitude of DOC flux; Hagedorn and Bundt (2002) found that compared to the rest of the matrix, SOC was as much as 3.5 times greater adjacent to preferential flow paths. The higher SOC concentrations are likely a result of greater DOC transport rates through these channels. There is a high likelihood that preferential flow channels transport a significant quantity of infiltrated water at this site, and that this water sustains high DOC concentrations with depth because mineral and microbial interactions are limited.

The preferential flow mechanism may explain why seasonal solute trends were not observed, and it implies that soil water stored in the preferential flow pathways may have a small residence times relative to the rest of the soil matrix. This has been suggested by Kirchner (2003) who described the apparent double paradox between streamflow response to storm events and the resulting stream chemistry. In many small catchments, stream flow responds promptly to storm events, but the released water has a dampened concentration of isotopic tracers suggesting that the released water is "old" water stored in the basin. However, this response can have a very different chemical signature depending on the magnitude of discharge. Because of this, Kirchner (2003) suggests that catchments may have different stores of water with unique residence times that are released at different seasonal cycles or with specific storm magnitudes. It's likely that preferential flow channels at this study site are one of these proposed "stores" of water that have unique chemical signatures relative to the rest of the soil matrix.

For most treatment types, including the NI and NL manipulations, there were no observed DOC trends with time 30cm belowground once lysimeter measurements began. The first lysimeter measurements were taken in 1999, two years after plot manipulation began, so the difference in DOC between treatments must have been a result of a rapid response to manipulated litter inputs during the first two years of manipulation. This was then sustained throughout the study period for all manipulations except the NR treatment. This refutes the hypotheses that DOC in litter exclusion treatments would decline over time as the most labile microbially-accessible C pools were depleted and not renewed by litter inputs. It seems that even after 20-years of manipulation the microbially accessible C pools have not been depleted sufficiently to cause a decreasing DOC concentration.

The NR treatment was the only manipulation that displayed a significant trend with time; this trend also had a surprisingly strong correlation for a natural system ($R^2 = 0.749$). This finding may have serious implications for SOC storage and DOC dynamics and has not been previously observed at the study site (Yano et al. 2005; K. Lajtha et al. 2005; Crow et al. 2009). Increased DOC from the NR treatment may be explained by the different proportion of high:low quality C accessible to microbes in the soil profile from root death. By trenching the NR plots, all soil-rhizosphere interactions were essentially stopped. These interactions greatly influence the activity and composition of soil microbial communities (Rovira 1969; Philippot et al. 2013). The root-exudates from the rhizosphere are a high-quality carbon source that influence the distribution and metabolic rates of microbes (Rovira 1969). Depletion of these high-quality C products within the soil matrix may have inhibited metabolism of DOC conveyed from O-horizon litter in the NR plots. DOC products from the soil surface that were not metabolized would eventually accumulate, resulting in the observed increasing DOC trend. Furthermore, dead root biomass could have simultaneously degraded within the mineral horizons and been another source for the increasing quantity of DOC. The NI plots, which also have roots excluded, would have similarly depleted the high-quality SOC sources. But in contrast to the NR plots, there were no additions of DOC from aboveground litter in the NI treatment. This may explain why NR DOC doubled during the study period while the NI plots stayed relatively constant. These results indicate that DOC concentrations (and therefore microbial metabolism) may be intricately linked to presence of an active rhizosphere. When the rhizosphere is removed, but aboveground litter inputs are sustained, C sources cannot be utilized by microbial communities and DOC concentrations increase. SOC similarly may not be utilized effectively by microbes in this situation, therefore this may be a mechanism to increase SOC concentrations within mineral horizons.

There were no trends detected at 100cm for any of the treatments. This is likely a result of homogenization of DOC in the mineral matrix through sorption and metabolic processes that filter out labile and hydrophobic DOC sources (Yano et al. 2005; K. Lajtha et al. 2005). The magnitude of C entering the soil annually from DOC flux is orders of magnitude less than the quantity of C already stored on mineral binding sites, therefore it's not surprising that a temporal trend could not be detected at this depth. Yano et al. (2005) suggested that the equilibrium DOC concentrations are likely controlled by C stored in the mineral matrix to a greater degree than the concentration of surface inputs. Furthermore, Fröberg et al. (2006) found that a majority of DOC in subsurface B-horizons is derived from the horizon itself. These findings suggest that aboveground litter manipulations have little influence on DOC deep in the soil profile.

DOC Concentration and Flux

Although there were not striking temporal or seasonal DOC trends for most treatments, the mean DOC concentrations at 30cm were clearly different between treatments when all sampling dates were averaged. This suggests that treatment manipulations impacted DOC concentrations rapidly within the first two years of implementation before lysimeter samples were taken. The manipulations then sustained the altered DOC concentrations throughout the remainder of the study period, essentially reaching a steady state. I hypothesized that these steady state DOC concentrations would be greater in the litter addition treatments (DW and DL), but that this could be confounded by priming-induced consumption of high-quality products in the DL treatment. The mean DOC concentration for the DL treatment was 43% lower than the CTL (3.7 versus 5.3 mgC/L) despite higher surface C inputs. This provides strong evidence that priming occurred in the DL plots as hypothesized. These findings are consistent with Yano et al. (2005) who reported a DL DOC concentration 41% lower than the CTL. While belowground concentrations were lower, the DOC input at the surface was higher for the DL treatment because source litter was doubled. Lajtha et al. (2005) established that surface DOC concentrations were approximately 15 mgC/L greater than the CTL treatment. Assuming this aboveground DOC concentration, the rate of DOC consumption was approximately 56% greater between the surface and 30cm under the DL treatment relative to the CTL, hence the priming effect. This accelerated consumption is greater than the 11.5-34% increase in reported C loss to respiration from priming reported previously (Sulzman et al. 2005; Crow et al. 2009). The priming effect indicates that microbial metabolic rates increased with the addition of the labile C source. Multiple studies have established that these increased metabolic rates from the addition of labile C material can accelerate respiration more rapidly than carbon assimilation rates (Cordova 1994; Sulzman et al. 2005; Crow et al. 2009). If the increased respiratory C losses outweigh C storage via the formation of stable SOC products, then the observed priming effect provides evidence that disputes the MEMS hypothesis proposed by Cotrufo et al. (2013). Under these conditions, the addition of a labile C source would result in a net decrease in SOC storage instead of higher rates of stable SOC formation.

Wood derived DOC is generally more recalcitrant in soil solution because it has a high C:N ratio, has a greater proportion of hydrophobic compounds, and can decrease soil solution pH (Spears and Lajtha 2004; Yano et al. 2005). These qualities can make the DOC products less accessible to microbes and require a higher activation energy for degradation. This is likely why DOC concentrations were highest in the DW treatment. Because wood-derived DOC is less efficiently incorporated into microbial biomass, the MEMS theory would suggest that DW DOC will result in a slower formation rate of stable SOC (Cotrufo et al. 2013). However, the net gain or loss of SOC depends on the relative magnitude of C loss to respiration (Bradford et al. 2013), and it's been established that respiratory C loss is lower for the DW treatment relative to DL at this site (Sulzman et al. 2005; Crow et al. 2009). Similarly, Cordova et al. (2018) found that respiratory losses outweighed the increase in formation rates for high-quality litter types, resulting in a net decrease in soil C storage. The DW treatment had a mean DOC concentration more than two times greater than the DL treatment, providing evidence that the litter material was much more recalcitrant in solution. Therefore, because respiration is inhibited by the low-quality wood litter in the DW treatment, I suggest that wood derived C may be a more important factor for DOC concentrations and SOC storage relative to labile leaf litter at this site.

The lower DOC concentrations in the litter exclusion treatments were consistent with the hypothesis that decreasing C inputs would deplete degradable and soluble C sources over time and result in the lowest mean DOC value. These treatments (NL/NR/NI) had an average concentration approximately 55% lower than the CTL. The elimination of aboveground litter and root C inputs in the NI treatment, had the same effect as eliminating just one of those factors in the NR and NL treatments. This suggests that the dominant DOC source in these plots was desorption of already stored C products from the soil matrix. Sorption-desorption reactions have been proposed as the dominant mechanism to control soil solution chemistry in subsurface soil horizons previously (Yano et al. 2005; Fröberg et al. 2006). Fröberg et al. (2006) found that DOC chemistry in these horizons was distinct from surface DOC sources, suggesting that the soil matrix itself was the origin of DOC. Root exudates did not appear to have a significant direct contribution to DOC because there was no detectable difference between the NL treatment, which had live roots, and the NI or NR treatments which did not. Similarly, litter additions on the NR plots did not have an independent effect on the mean DOC concentrations relative to the NI treatment.

Soil solution DOC decreased less drastically from 30 to 100cm than from the soil surface to 30cm and was effectively homogenized between treatments despite 20-years of active litter manipulation. Although there was not a significant difference between treatment groups, there is still a strong likelihood that the treatments have different DOC concentrations given that the ANOVA analysis approached the 0.05 criteria ($p=0.06$). The significant difference between the DW and NR treatments provides further evidence that DOC concentrations have begun to respond to the aboveground perturbations. Distinct DOC concentrations have not been reported at this depth previously (Yano et al. 2005; K. Lajtha et al. 2005; Strid, Lee, and Lajtha 2016) and it's been suggested that it may take decades of manipulation to observe a response. This is most likely due to the cumulative buffering effect caused by sorption of hydrophobic DOC in upper soil horizons, and microbial substrate use of labile DOC components throughout the soil profile (Lajtha et al., 2005). The ability of the soil profile to buffer DOC concentrations indicates that deep mineral horizons are essentially unimpacted by short-term changes in aboveground litter production.

Lysimeter DOC concentrations, coupled with the Hydrus model, revealed that DOC flux into groundwater was small (2.7-3.7 $g/m^2/year$), and relatively negligible relative to the 4,040-11,000 grams C/m^2 estimated to be stored in the different aboveground litter manipulations at the study site. This annual flux accounts for only 0.03-0.09% of total litter C. The estimated DOC flux is consistent with the 0.9-13.9 $gC/m^2/yr$ reported for similar forested catchments (Creed et al. 2008) and falls within the range of the 2.0-3.8 $gC/m^2/yr$ estimated by Lajtha et al. at the study site in 2005. The DOC flux estimation assumes that preferential flow paths are negligible. As discussed previously, there are a number of factors that make this unlikely, including the high soil Ks, the thick O-horizon typical at the site, and field observations of preferential flow. Therefore, the actual DOC transport below 100cm could be much greater than this estimate. Despite having slightly lower concentrations, the NI and NR treatments had the highest rates of DOC flux below 100cm because annual infiltration was greatest in these plots with the absence of root-water uptake. Therefore, the removal of live root biomass effectively increased C transport from the terrestrial forest ecosystem into groundwater and streams.

Conveyance of DOC into the surface soil horizons was much greater than at 100cm; annual flux was two orders of magnitude higher (73-210 $g/m^2/yr$) and accounted for 1.2-1.9% of total litter C. This is greater than the 20-35 $g/m^2/yr$ estimated by Solinger et al. (2001). A greater proportion of DW (1.9%) and DL (1.5%) litter was removed through this mechanism relative to the 1.2% loss in the CTL treatment. This implies that C transport from the surface to shallow soil horizons accelerates when aboveground litter supplies increase. Because respiration of SOM is limited by DOC formation rates and transport (Bengtson and Bengtsson 2007), this accelerated conveyance likely aids in the priming effect experienced in the DL treatment. The accelerated C loss associated with higher litter inputs may also partially explain why soil C storage has not increased linearly with additional aboveground C inputs at the H.J. Andrews study site. Most soil samples used to characterize the SOC at the site have been taken to a depth of 10cm. This may not accurately characterize SOC stocks between treatments because DOC is preferentially transported below this depth in the litter addition treatments (DL/DW).

The high Ks of the on-site soils prevented all annual runoff in the Hydrus model, which would be another pathway for litter C transport. Because DOC concentrations are two orders of magnitude higher at the surface, relative to 100cm belowground, DOC flux in runoff could easily result in a greater C loss compared groundwater flux at sites with a different hydrologic regime. Therefore, the observed 100cm/day Ks decrease in the NI plots, could potentially increase off-site C transport rates. In contrast, the addition of woody debris nearly doubled infiltration rates, suggesting that aboveground wood debris have the potential to decrease off-site DOC flux in overland flow. The drastic increase in Ks indicates that wood litter must play an important role in facilitating macropore creation and ensuring soil structure. Therefore, woody debris should be sustained on the soil surface if reduced runoff and erosion are desired. The clear changes in soil infiltration rates between treatments indicates that manipulating aboveground litter inputs can have direct hydrologic impacts. Removing litter will decrease the proportion of precipitation that infiltrates, while the addition of wood should increase this proportion.

The DOC flux approximations show that direct DOC transport from surface litter to groundwater supplies is negligible in the soil C budget relative to aboveground litter C stocks at this site. Therefore, DOC flux out of forest soils likely does not have notable implications on landscape level C stocks. However, flux into shallow soil horizons accounted for as much as 1.9% of total aboveground C annually and is likely an important mechanism for the increased metabolic rates experienced in the DL treatment. Furthermore, higher litter input rates accelerated the proportion of litter C lost to DOC annually. Therefore, DOC flux may act as a negative feedback loop for SOC and litter C storage under future scenarios that have increased litterfall rates.

Conclusion

Aboveground detrital inputs do not always have a direct linear effect on soil solution DOC. Greater inputs do not result in higher concentrations when energetically high-quality leaf litter induces microbial priming. But, wood derived DOC is resistant to priming and does seem to increase proportionally with litter inputs. DOC temporal trends revealed that decaying root debris, coupled with the exclusion of an active rhizosphere, doubled DOC concentrations over 20 years. Therefore, decaying root debris may be a long-term source of C that is not metabolized when live roots are excluded, and may be a potential mechanism for the formation of stable SOC.
Annual DOC flux into groundwater is negligible in the soil C budget relative to aboveground litter C supplies (<0.01% of total litter C). But, annual DOC flux into surface horizons is significant (1.2-1.9%) and is an important mechanism for C accumulation in these horizons. A greater proportion of litter C was transported as DOC with the addition of woody debris (1.9%), and leaf litter (1.5%), relative to the control (1.2%). This suggests that an increase in litter accumulation accelerates DOC flux relative to the total C supply, and therefore may be an important negative feedback loop on aboveground litter C storage.

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