Land cover change affects the amount and reactivity of DOM exported from old growth and regenerating forests in headwater ecosystems

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Abstract

Headwater forest ecosystems of the western U.S. generate a large portion of the dissolved organic matter (DOM) transported across North America. Land cover changes that alter forest structure and forest species composition affect the quantity and composition of DOM transferred to aquatic ecosystems. Clear-cut harvesting effects ~1% of the forest area of North America annually, leaving most forests in varying stages of successional regrowth, and the total area of old-growth forest decreasing. The consequences of this widespread management practice on watershed carbon cycling remain unknown. We investigated the role of land cover change from old-growth subalpine forest to lodgepole pine dominated second-growth on the character and reactivity of DOM hillslope exports. We evaluated inputs of DOM from litter leachates and export of DOM collected at the base of trenched hillslopes during a three-year period (2016-2018) at the Fraser Experimental Forest in northcentral Colorado, USA. Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were higher in lateral subsurface flow draining old- versus secondary-growth forest. Fluorescence spectroscopy showed that the DOM exported from the old-growth forest was more heterogeneous and aromatic and that proteinaceous, microbially processed DOM components were more prevalent in the second-growth forest. Biological oxygen demand (BOD) assays revealed much lower microbial metabolism of both DOM inputs from litter leachate and subsurface exports from old-growth forest. Old-growth and second-growth forests are co-mingled in managed ecosystems, and our findings demonstrate that the influence of species composition on DOM inputs can affect the reactivity of DOM transferred from terrestrial to aquatic ecosystems.

Introduction

Dissolved organic matter (DOM) derived from vegetation and soils accounts for up to 90% of the carbon (C) found in surface water (Aitkenhead-Peterson, McDowell, & Neff, 2003) and DOM character regulates microbial C processing in aquatic environments (Benner, 2003; D'Andrilli, Junker, Smith, Scholl, & Foreman, 2019; Lennon & Pfaff, 2005). While encompassing less than 1% of the global land surface, inland waters regulate carbon cycling from landscape to continental scales (Battin et al., 2009; Cavallaro et al., 2018; Cole et al., 2007; Raymond et al., 2013), and headwater streams drain 70% of North American land area (Colvin et al., 2019). Changes to forest land cover that affect terrestrial DOM character will influence aquatic ecosystem metabolism (Cawley, Yamashita, Maie, & Jaffe, 2014; Lajtha & Jones, 2018; Williams, Yamashita, Wilson, Jaffe, & Xenopoulos, 2010), and deciphering these relationships are crucial for predicting C cycling in inland waters.

Vegetation responses to land management can alter watershed hydrology and biogeochemistry for decades to centuries (Chantingy, 2003; Lee & Lajtha, 2016; Rhoades, Hubbard, & Elder, 2017; Troendle & King, 1985; Wilm & Dunford, 1948). In the mountain ranges of the American West, where the short growing season and

cold, dry climate limit forest regrowth, the consequences of timber harvesting on snowpack, streamflow, and nutrient export as well as on potential wildfire behavior and susceptibility to insect outbreaks are long-lasting (Stottlemyer & Troendle, 1999; Rhoades et al., 2017; Wilm & Dunford, 1948). Timber harvesting decreases canopy interception and evapotranspiration resulting in more snowpack water storage, and increased soil moisture and streamflow (Troendle & King, 1985) with effects that remain measurable for more than 50 years. Timber harvesting also shifts the species composition of these forests and their development trajectory for a more than a century (Collins, Rhoades, Hubbard, & Battaglia, 2011; Lotan & Perry, 1983).

Harvest of subalpine forests comprised of subalpine fir (*Abies lasiocarpa*), Engelmann spruce (*Picea engelmannii*) and lodgepole pine (*Pinus contorta*) typically regenerate into lodgepole pine-dominated stands (Collins et al., 2011). While the effects of land cover, age, and species change on terrestrial nutrient cycling and organic matter decomposition are well studied (Chantigny, 2003 for a review), their consequences on adjacent aquatic ecosystem C cycling are not. The chemical characteristics of litter in regenerating forests alter hillslope DOM inputs relative to those in old-growth forest (Beggs & Summers, 2011; D'Andrilli et al., 2019), likely regulating the reactivity of DOM exported to streams at watershed scales. The highly polyphenolic and protein-like DOM released from lodgepole pine litter in regenerating stands, for example, is more reactive than that generated by old-growth, mixed-conifer forests (Beggs & Summers, 2011; Yavitt & Fahey, 1984 & 1986). Connecting the effects of land cover type on the reactivity of DOM inputs from the forest floor to the reactivity of hillslope DOM exports will increase understanding of the effects of land cover change on watershed-scale C cycling.

The consequences of land cover and tree species shifts on watershed-scale DOM dynamics benefit from techniques that differentiate DOM reactivity of hillslope inputs and exports. Analysis of fluorescing components of DOM (FDOM) has become a relatively routine, low-cost approach to characterize the relative abundance of biologically reactive organic molecules (Smith et al. 2018). Correlation between fluorescing and non-fluorescing DOM molecules of similar chemical structures and biological reactivities expands the utility of FDOM as an index of overall DOM quality (Stubbins et al., 2014). Optical characterization can be used in conjunction with heterotrophic microbial oxygen consumption assays to identify which DOM components are microbially created or utilized (D'Andrilli et al., 2019).

In this study we compare DOM inputs from litter leachate and the concentration, character and biological reactivity of DOM exports in subsurface flow between adjacent old-growth subalpine and second-growth pine forests. Early work at these sites documented that clear cut harvesting resulted in lasting increases in snow accumulation, subsurface discharge, and nutrient export (Reuss, Stottlemyer, & Troendle, 1997; Starr, 2004; Troendle & Reuss, 1997). The amount of DOC exported from forests is determined by O horizon C content, with old-growth forests exporting more DOC compared to regenerating ones (Cawley et al., 2014; Lajtha & Jones, 2018; Pacific, Jensco, & McGlynn, 2010). However, DOC reactivity is affected by C character and processing within subsurface flow paths (Lehmann & Kleber, 2015). Because old-growth forests generally have deeper O-horizons composed of more recalcitrant DOM (Jandle et al., 2007; Johnson, Johnson, Huntington, & Siccama, 1991), we expect greater flux but lower biological reactivity of DOC from these forests. Conversely, we hypothesize that DOM exported from pine-dominated, second-growth forest to contain proportionally more biologically reactive DOM typical of the water-soluble proteins and polyphenolics found in pine needle litter (Beggs & Summer, 2011). Western conifer forests often require a century to regrow to their pre-harvest stand structure (Burns & Honkala, 1990), so the extent of old-growth forest cover has decreased during more than a century of timber harvesting and most forests are in intermediate stages of recovery (Anderson-Teixeira et al., 2013; Hurtt et al., 2011; McDowell et al., 2020). This study evaluates how forest change following clear-cut harvesting alters the reactivity of DOM from the landscape to aquatic ecosystems.

Methods

Site description

This research occurred at the Fraser Experimental Forest (FEF), 137 km west of Denver, Colorado. Mean

annual temperature at FEF headquarters (elevation 2725 m) is 0.5 °C, and ranges from -10 °C in January to 12.7 °C in July. Mean annual precipitation is 610 mm (range 427-902 mm; 1981-2010), nearly two-thirds of which falls as snow from October to May. Annual inorganic N deposition averaged 2.6 kg N ha^{-1} over the last decade (Argerich et al., 2013). The Fraser area was extensively glaciated and FEF is underlain by metamorphosed rock, most commonly biotite schist and hornblende or calc-silicate gneiss (Shroba, Bryant, Kellogg, Theobald, & Brandt, 2010). Soils are skeletal, sandy loam Dystric and Typic Cryochrepts (Alstatt & Miles, 1983) with 20-30% gravel and 30-50% cobble-sized materials. To evaluate the role of forest cover on hillslope hydrologic processes, including quantifying the timing and amount of subsurface flow, paired trenched hillslopes were installed in 1979. The paired hillslopes are adjacent to each other on a glacial moraine that is underlain at 3 m by a clay aquaclude (Reuss, Stottlemyer, & Troendle, 1997; Troendle & Reuss, 1997). Subsurface flow is collected by slotted PVC pipe installed at 4 m depth in lined trenches at the base of the hillslopes and piped to individual gauging stations (HS flumes) where water level is recorded using Druck pressure transducers and Campbell CR-1000 data loggers at 10-minute intervals. These water level data are then converted to subsurface flow rates using known relations between water depth and discharge (Bos, 1989). In December 1984, after five years of pretreatment calibration of subsurface flow rates, a 3-ha section of one of the trenched hillslopes was clear cut harvested, while the other was left intact.

Old-growth subalpine forests at FEF are comprised of equal proportions of lodgepole pine (*Pinus contorta*), subalpine fir (*Abies lasiocarpa*) and Englemann spruce (*Picea engelmannii*), respectively 32%, 34%, and 34% of overstory basal area (Supp. Info: T1). Low-stature shrubs (*Vaccinium scoparium, V. myrtillus, V. caespitosum, Shepherdia canadensis*) form a dense understory beneath the coniferous overstory (Popovich, 1993). The clear-cut hillslope has regenerated into a pine-dominated second-growth stand (82% of overstory basal area) with 9 cm mean diameter and 4 m mean height (Supp. Info: T1). Mountain pine bark beetles (*Dendroctonus ponderosae*) preferentially attack larger trees (Rhoades et al., 2017), and in a recent outbreak beetles killed 18% of the old-growth basal area but did not infest the second-growth stand.

Sampling and analysis

Lateral subsurface flow begins mid to late April at the start of snowmelt, increases to the peak in early to mid-May, then declines and halts by mid to late June (Fig 1a, Troendle & Reuss, 1997). We collected hillslope export samples as lateral subsurface flow at the base of each hillslope (i.e., at the piped trenches) weekly in 2016-2018 and analyzed for nutrients, dissolved organic carbon (DOC), total dissolved nitrogen (TDN), and DOM chemical characteristics. These samples were collected in pre-combusted (heated for 3 hours at 500 0C) amber glass bottles then filtered through 0.7 μm pore-size glass fiber filters (Millipore Corp, Burlington, MA) within 24 hours of collection and analyzed for DOC, TDN, and DOM fluorescence spectroscopy. We collected DOM reactivity samples in 250 mL pre-combusted amber glass bottles, then filtered through (GF/C Whatman®, 1.2 μm effective pore size) filters within 3 hours of collection to remove bacterial grazers. DOC and TDN were determined using a Shimadzu TOC-V_{CPN}total organic carbon analyzer, with 2M HCl addition before analysis to remove mineral C (Shimadzu Corporation Columbia, MD). Detection limits for DOC and TDN were 50 μg L^{-1}.

The chemical character of DOM exported from each hillslope (i.e., lateral subsurface flow) was analyzed using UV-Visible fluorescence spectroscopy on a Horiba Scientific Aqualog (Horiba-Jobin Yvone Scientific Edison, New Jersey, US). Sample C concentration was standardized to 5 mg C L^{-1} using deionized water (>18 m). Ultraviolet absorbance was analyzed at the 254 nm wavelength. Excitation emission matrices (EEMs) fluorescence scans were completed from 240 nm-600 nm excitation and emission wavelengths, with 3 nm band-pass, 3 nm increments, and 3 second integrations. Scans were blank corrected using deionized water and corrected for inner filter effects (Kubista, Sjoback, Eriksson, & Albinsson, 1994). First- and second-order Rayleigh scattering were masked (10 nm width masking), and samples were normalized by the area of the deionized water Raman scattering peak (Lawaetz & Stedmon, 2009).

$SUVA_{254}$ (L mg $C^{-1}m^{-1}$) was calculated by dividing ultraviolet absorbance at 254 nm by DOC concentration (Weishaar et al., 2003). Humification index (HIX) was calculated as the area of spectra collected at 254 nm emission under 435-480 nm excitation divided by the area under the peak 300-345 nm emission (Zsolnay,

Baigar, Jimenez, Steinweg, & Saccomandi, 1999). Fluorescence Index (FI) was calculated as the ratio between emission at 470 nm and 520 nm at 370 nm excitation (McKnight et al., 2001; Cory & McKnight, 2005). Fluorescence regional integration modelling (FRI) was applied to EEMs data to account for areas of increased fluorescence not identified by standard EEMs indices (Chen, Westerhoff, Leenheer, & Booksh, 2003). Briefly, EEMs scans were separated into five regions associated with distinct DOM chemical components. Total fluorescence intensity for each region was divided by total intensity of the entire scan, resulting in percentages of total FDOM derived from each DOM chemical component region.

We sampled the organic (O horizon) and mineral (A horizon; 10 cm depth) soil layers at ten locations distributed across the old-growth and second-growth hillslopes (n=20). O horizon material was sampled within a 30 cm by 30 cm quadrat and total O-horizon depth was measured in each quadrat corner. O horizons were separated into litter and duff layers as follows (FIA 2019): needles and recognizable plant material (< 6 mm diameter) were classified as litter, while unrecognizable, fragmented material between the litter and mineral soil layers was considered duff. There was no measurable duff in the second-growth forest. Mineral soil (0-10 cm depth) was collected using a 7.5 cm diameter corer after the O horizon was removed. Mineral soils had rocks, mosses, and lichens removed and were then sieved to 2 mm. We determined gravimetric soil moisture after drying a subsample at 105 0C for 48 hours. Organic horizon samples were well mixed and moss, lichen, and rocks were removed by hand. Organic horizon samples were dried for 6 days at 60 0C and the total mass was recorded. Total bulk density for mineral and organic soils was calculated by dividing the dry weight of the total soil (including > 2 mm fraction in mineral soils) by the sample volume. A subset of each sample was dried for 48 hours at 60 0C, then ground and analyzed for total C and N by dry combustion (LECO 1000 CHN analyzer, LECO Corporation, St. Joseph, MI, USA). Total C and N pools were calculated by multiplying C and N concentrations by the associated O- and A-horizon masses. Water extractable soil organic matter (WEOM) samples were created by leaching a litter layer sample from each hillslope's O-horizon to replicate DOM inputs (Sparling, Vojvodic-Vukovic, & Schipper, 1998). Litter samples were air dried for 3 days then 10 g subsamples were steeped in 50 mL of DI (>18 m) at 70 0C for 18 hours, shaken and filtered through 0.7 μm pore size glass fiber filters.

Biological oxygen demand assays were performed to assess the reactivity of DOM inputs (WEOM litter leachates) and DOM exports (lateral subsurface flow). DOM input incubations were diluted and standardized to 30 mg C L^{-1} to mirror C concentrations of DOM export incubations. Each DOM input sample was inoculated with 88 mL of water from an adjacent stream (Sparling et al. 1998), and export DOM was incubated with microbes that occurred naturally in the subsurface flow. Relationships in reactivity between input and output DOM should be considered tentative correlations, as different microbial cultures were used in litter and subsurface leachate incubations. Experimental controls, which only contained the stream water microbial culture with no additional DOM and analytical controls which only contained DI (>18 m) were incubated simultaneously with the oxygen demand assays. Microbial dissolved oxygen consumption (mg O_2 L^{-1}) was continuously measured (15 second intervals) using Oxy-4 probes (PreSens, Precision Sensing GmbH, Regensburg, Germany). Input and export samples were incubated in the dark at 20 0C and the hourly oxygen consumption rate (mg O_2 L^{-1} hr^{-1}) was averaged for each sample over the 24-hour incubation period. No samples approached hypoxic conditions (<4 mg O_2L^{-1}). Oxygen consumption rates were normalized per gram of C to remove any bias of C quantity on consumption rate. Post-incubation samples were filtered to remove microbial biomass (Nucleopore®, polycarbonate filter, 0.2 μm). Concentration of C mineralized (Δ mg C L^{-1}) was calculated as the difference between measured pre- and post- incubation DOC concentrations, then converted to loss as CO_2(ΔCO_2, in mg CO_2 L^{-1}), under the assumption all CO_2 produced by incubations was removed from the sample with HCl addition during C measurement. The respiratory quotient (RQ), was calculated by dividing C lost to respiration (ΔCO_2, in mg CO_2L^{-1}) by the change in O during the incubation (ΔO_2 in mg O_2 L^{-1}).

DOC and TDN concentrations, and FDOM indices in subsurface flow exports were compared between old-growth and second-growth land cover types using Welch-Satterthwaite unequal variance t-test with significance assigned at $p < 0.01$ (R Core Team, 2019). Regional percentages for FDOM components (FRI modelling) in export samples were logit transformed before statistical analyses to remove biases inherent in

proportional data. We compared input nutrient concentrations and pools in organic and mineral horizon soils, along with comparisons of oxygen consumption and respiratory quotients in DOM input and export samples using the Student's t-tests for parametric data, with significance assigned at $p < 0.01$ (t.test function, R Core Team, 2019). The data that support the findings of this study are available from the corresponding author upon reasonable request.

Results

C and N inputs to the hillslope from soil O horizons

The old-growth hillslope contained 6, 4, and 11 times more O horizon mass, total C, and total N content compared to the adjacent second-growth stand, respectively (Table 1). The C:N ratio of the old-growth total O horizon was roughly half that of the second-growth hillslope. Mass and C content of litter layers were similar between hillslopes but the N concentration was twice as high in litter on the old-growth hillslope. The C concentration of the uppermost mineral soil (10cm depth) was 1.3 times higher in the second-growth forest but N concentration did not differ (Table 1).

C and N exports in lateral subsurface flow

Lateral subsurface flow from the second-growth forest started earlier, ran later, and generated nearly twice the total runoff compared to the old growth forest (Fig 1a, Table 2). Concentrations of DOC and TDN exported in lateral subsurface flow from the old-growth forest were 5 and 3 times higher than those from the second-growth forest and C:N (DOC/TDN) was also higher in old-growth exports (Table 2, Fig 1b and c), likely resulting from direct leaching of O-horizon and transport through shallow, subsurface flowpaths. Peak DOC and TDN concentrations for the old-growth forest export occurred during the mid-May peak in subsurface runoff and then declined (Fig 1). In contrast, the DOC and TDN concentrations in lateral subsurface flow exported from the second-growth hillslope were uniformly low throughout the flow period. Annual subsurface flux of C and N were 10 and 8 times higher from the old-growth hillslope (Table 2).

Characteristics of DOM exported in hillslope lateral subsurface flow

Indices of aromaticity ($SUVA_{254}$) of old-growth DOM export were 14% higher (3.46 vs. 3.00) and humification index (HIX) was nearly twice that of DOM exported from the second-growth forest (12.24 vs. 6.63). The DOM exported from the old-growth hillslope had a mean fluorescence index (FI) of 1.14 compared to 1.52 from the second-growth hillslope. Fluorescence regional integration modelling (FRI) confirms the patterns shown by the EEMs indices (Fig 2). FDOM corresponding to amino acids (RI and RII) and microbial exudates (RIV) were higher in exports from the second-growth hillslope. Conversely, plant-derived fulvic- and humic-like hydrophobic organic acids (RIII and RV) were higher in exports from the old-growth hillslope.

Indices of FDOM chemical character (FI and HIX) were similar in exports from the two hillslopes at the onset of subsurface flow but diverged over time (Fig 3a and 3b). Across the subsurface flow season, FI decreased by half the possible range in the old-growth hillslope. The proportion of complex organic acids in FDOM exports (based on HIX), increased 3-fold during the flow period. In contrast, the chemical character of FDOM exported from the second-growth hillslope remained relatively uniform (Fig 3a and 3b).

Reactivity of litter DOM inputs and lateral subsurface flow DOM exports

Patterns of biological reactivity in DOM inputs in litter leachate between hillslopes matched those of DOM exports in subsurface flow. DOM inputs and exports on the second-growth hillslope had dissolved oxygen consumption rates more than twice those from old-growth, reflective of higher DOM reactivity (Fig 4). Similarly, the average respiratory quotient of DOM inputs from second-growth was 5 times higher than inputs to old-growth DOM (1.24, compared to 0.18 for old-growth, Table 2).

Discussion

Forest recovery following timber harvesting and wildfire often shifts mixed-species subalpine forests to pine-dominant stands (Collins et al., 2011), and in our study, this transition from old-growth to second-growth

forest decreased hillslope DOM export by 89% (Tables 1 and 2, Fig 1). The amount of C contained within the O horizon determines the amount of DOM available for microbial processing and subsurface transport (Lee, Park, & Matzner, 2018; Nave, Vance, Swanston, & Curtis, 2010). As seen elsewhere we found higher O-horizon C stock, DOC concentrations and annual export in the old-growth compared to the second-growth hillslopes (Chatterjee, Vance, Pendall, & Stahl, 2008; Chatterjee, Vance, & Tinker, 2009). In spite of less C contained in mineral soils of old-growth forests, the higher C exported from them underscores the importance of subsurface transport of C from soil O horizons. Our findings suggest that land cover shifts following forest disturbance (McDowell et al., 2020) and subsequent harvesting is likely to decrease the amount of C exported to headwater streams.

The chemical character and biological reactivity of DOM inputs from organic horizons also responded to the land cover change (Supp. Info: T1, Fig 4). The transition from old- to second-growth forest induced a change from recalcitrant DOM inputs to more reactive non-humic and labile compounds such as proteins, small polyphenolics, and carbohydrates released by pine litter (Beggs & Summer, 2011; Berg & McClaugherty, 2014; Reckhow, Singer, Malcom, 1990; Yavitt & Fahey, 1984). The higher respiratory quotient (RQ, $\Delta CO_2/\Delta O_2$) and dissolved oxygen consumption rate of input DOM in the second-growth forest reflect this change in chemical composition and bioavailability (Fig 4) and suggest that labile proteins or carbohydrates are more readily utilized than less reactive components common to old-growth DOM (Berggren & Del Giorgio, 2012). As opposed to the thin pine needle litter of the second-growth pine stand, the deep, stratified, O horizon of the old-growth forests generate less reactive DOM (Table 1).

The forest cover changes altered the reactivity of subsurface DOM exports. Pine litter produces biologically reactive DOM (Fig 4) that is subject to microbial transformation as it infiltrates into soils and moves along subsurface flowpaths (Fig 2 and 3). The greater prevalence of microbially processed DOM we found in second-growth hillslope exports agreed with numerous other studies of DOM composition and export after post-harvest and subsequent forest change (Cawley et al., 2014; Lee & Lajtha, 2016; Williams et al., 2010; Yamashita, Kloeppel, Knoepp, Zausen, & Jaffe, 2011). In contrast, the DOM exported from old-growth forest was less biologically reactive (Fig 3b, Fig 4a), consistent with fluorescence indicators of DOM recalcitrance and complexity (Fig 3a and 3b) and respiratory quotients of litter leachate (Supp. Info: T1, Fig. 4).

We found that DOM reactivity changed in distinct ways in the two forest types as it moved along subsurface flowpaths (Fig 4). The thick O horizons in the old-growth forest released more C, though its recalcitrant, aromatic composition limited the degree of subsurface DOM processing (Fig 4). In contrast, the more reactive DOM of the second-growth forest changed substantially downslope. Both our FRI modelling and earlier work (Cordova et al., 2018; Yano, Lajtha, Sollins, & Caldwell, 2005) demonstrate the conversion of labile DOM from litter into microbial biomass and release of increasingly labile compounds downslope (Fig 2). The highly reactive nature of second-growth DOM inputs may also stimulate turnover of C stored in mineral soil (Evans, Pierson, & Lajtha, 2020; Kuzyakov, 2010) and agrees with studies that indicate that microbially processed C may contribute to vertical C movement from surface litter into the mineral soil profile (Cordova et al., 2018; Cotrufo, Wallenstein, Boot, Denef, & Paul, 2013).

The composition of DOM exported from the old-growth hillslope shifts over the course of the snowmelt season (Fig 3b and c), with a decline in relatively labile byproducts of microbial processing and an increase in recalcitrant compounds. This pattern suggests the presence of distinct DOM sources in this forest type. The DOM transported early in the snowmelt season may be comprised of labile metabolic byproducts that accumulated during overwinter microbial transformation of recalcitrant organic compounds from old-growth litter (Schmidt et al., 2007). After this initial flush, export DOM may originate from unprocessed compounds leached directly from the recalcitrant O horizon (Fig 3b). In contrast, the DOM exported from the second-growth forests was consistently labile (Fig 3c). This uniform compositional pool may result from the absence of a recalcitrant source of litter inputs and the continual turnover of C between labile litter and microbial biomass.

Conclusion

Forested headwater streams drain much of the land area in North America (Colvin et al., 2019) and are a major contributor of C to the atmosphere (Cavallaro et al., 2018). Regeneration of pine stands following harvest of old-growth forests is a common land cover change in the western United States (Collins et al., 2011) that will have lasting consequences for the quantity and composition of DOM exported from forest hillslopes and watershed C cycling. We found that the DOM exported from second-growth hillslopes was five times more reactive than that from old-growth, though the total amount of C released was ten times lower. As such, export of bioavailable C from second-growth was roughly half of that released from old-growth forest, reducing the amount of DOM available to stream microbes. The slow recovery of these forests reinforces the persistent effects of land cover change on DOM composition and reactivity in headwater ecosystems.

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Table 1. Soil horizon mass, carbon (C) and nitrogen (N) pools, C and N concentrations, and C:N ratios in organic and mineral (0-10cm) soil horizons on adjacent old-growth and second-growth forest hillslopes at the Fraser Experimental Forest, CO. Values are means with standard errors (n=10 per hillslope type). Within each depth, * and ** denote differences at $p<0.01$ and <0.001 using a Student's parametric t-test.

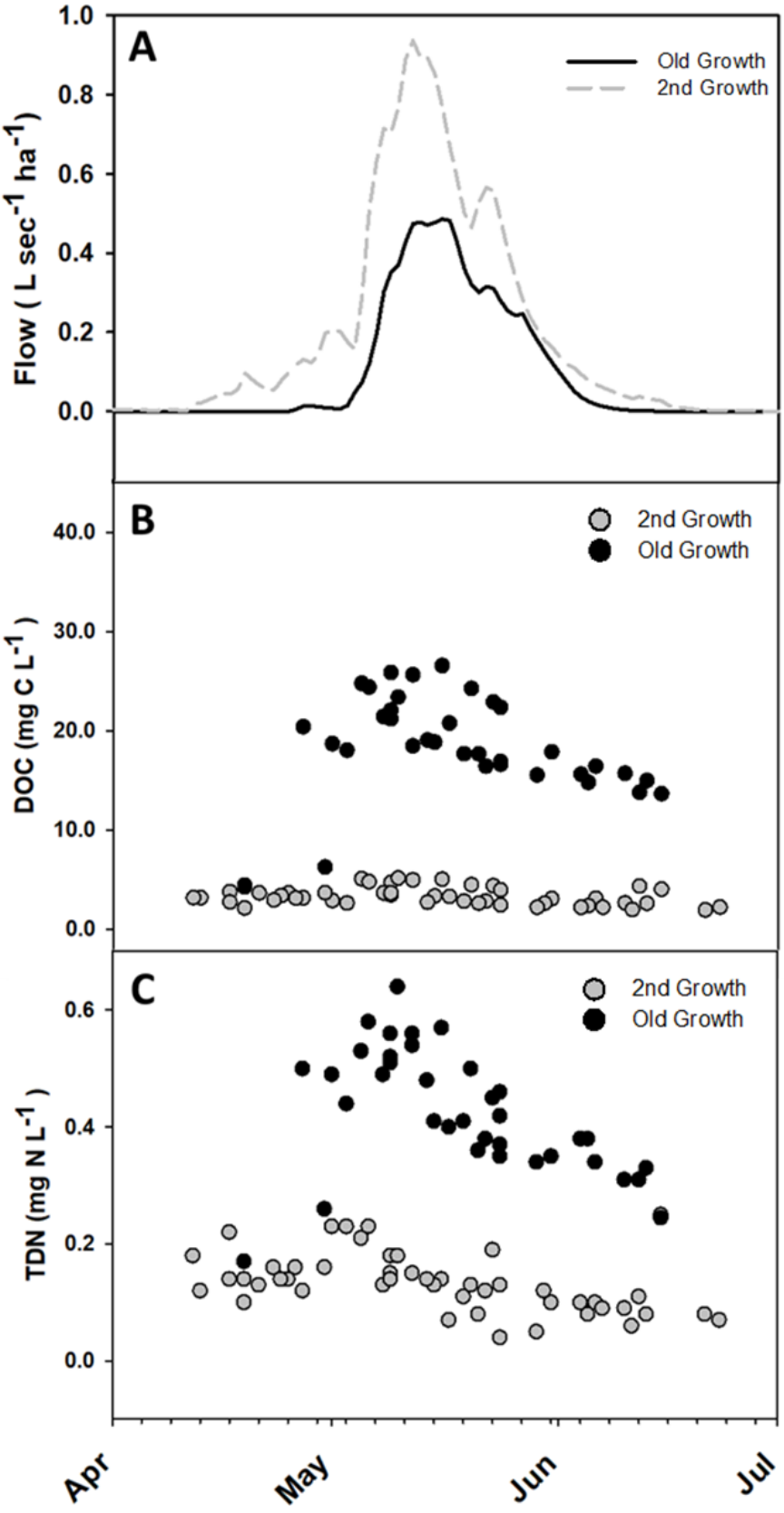
Table 2: Discharge, dissolved organic carbon (DOC), total dissolved nitrogen (TDN) concentrations and annual fluxes in subsurface flow from adjacent old-growth and second-growth hillslopes at the Fraser Experimental Forest, CO. Values are means with standard errors for the 2016, 2017, and 2018 snowmelt seasons. Sample numbers for DOC and TDN were n=36 for the old-growth and n=48 for second-growth. Differences between hillslope treatments are noted by ** at p<0.001 using a Welch-Satterthwaite non-parametric t-test.

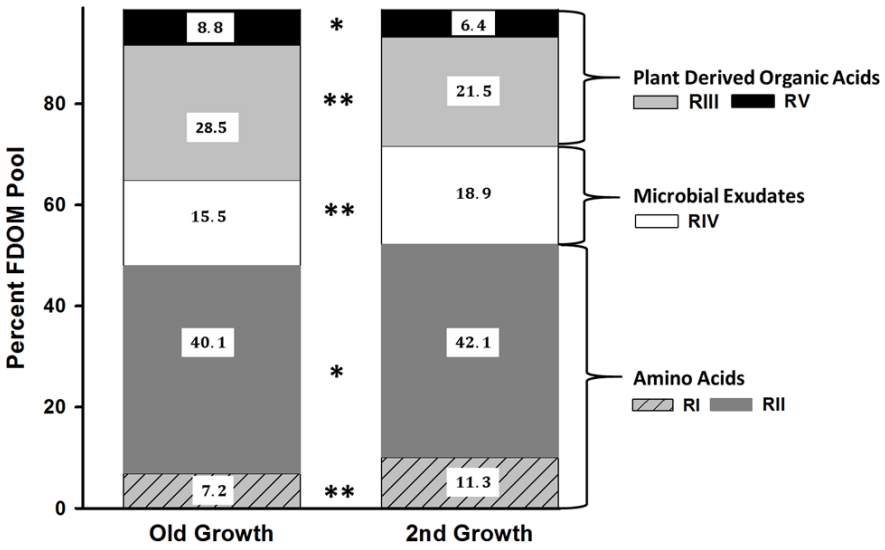
Figure 1. Average daily subsurface flow (A) from adjacent old-growth and second-growth hillslopes at the Fraser Experimental Forest for 2016, 2017 and 2018. Dissolved organic carbon (B) and total dissolved nitrogen (C) in subsurface flow (old-growth n = 36; second-growth n = 48).

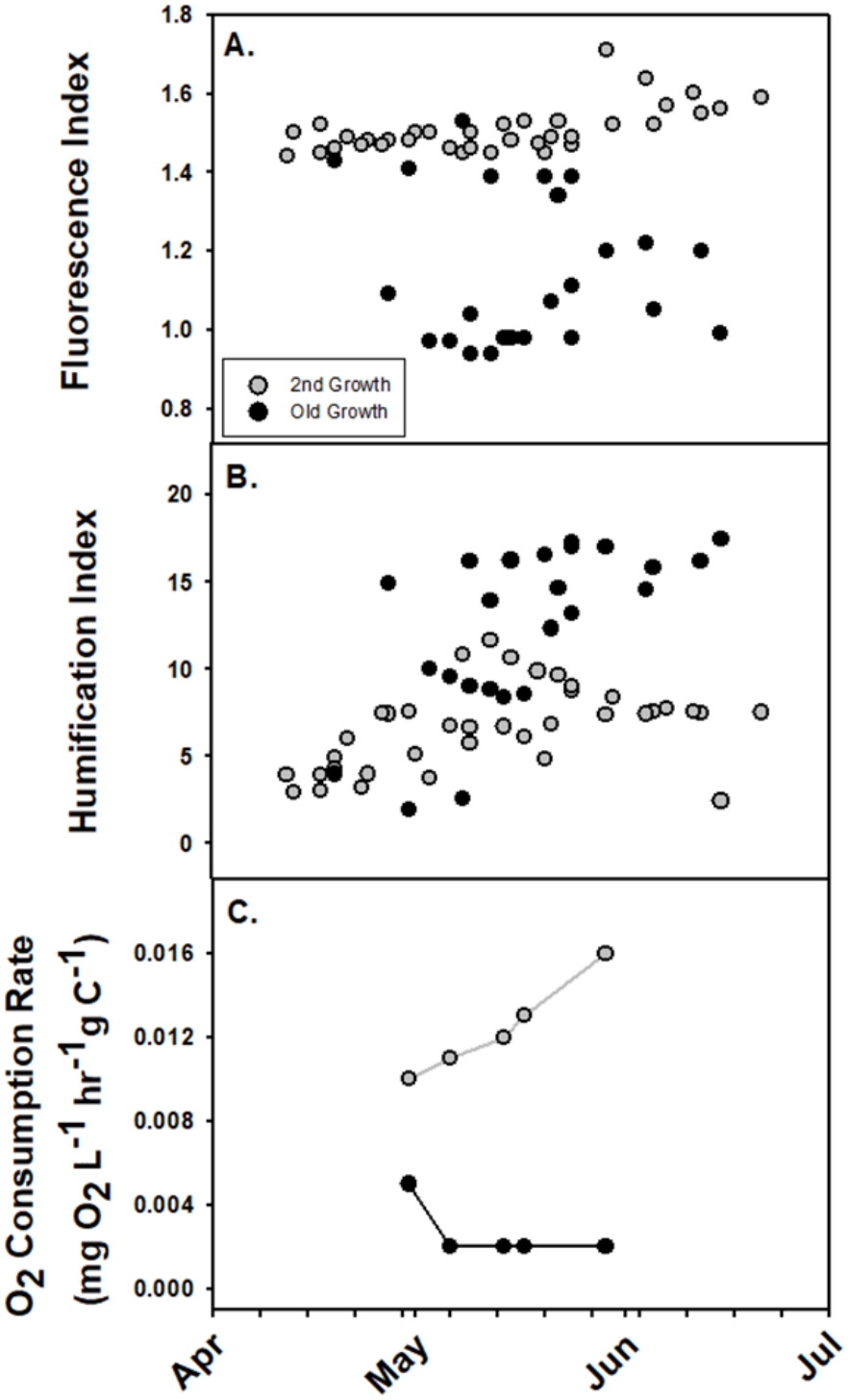
Figure 2. Components of fluorescing DOM (FDOM) in subsurface flow differentiated by fluorescence regional integration (FRI) modeling. Values are means from 2016, 2017 and 2018 for adjacent old-growth (n=23) and second-growth (n=35) hillslopes. Differences between FRI regions (RI-RV) were identified on logit transformed data at * and ** at p<0.01 and 0.001 using a Welch-Satterthwaite non-parametric t-test.

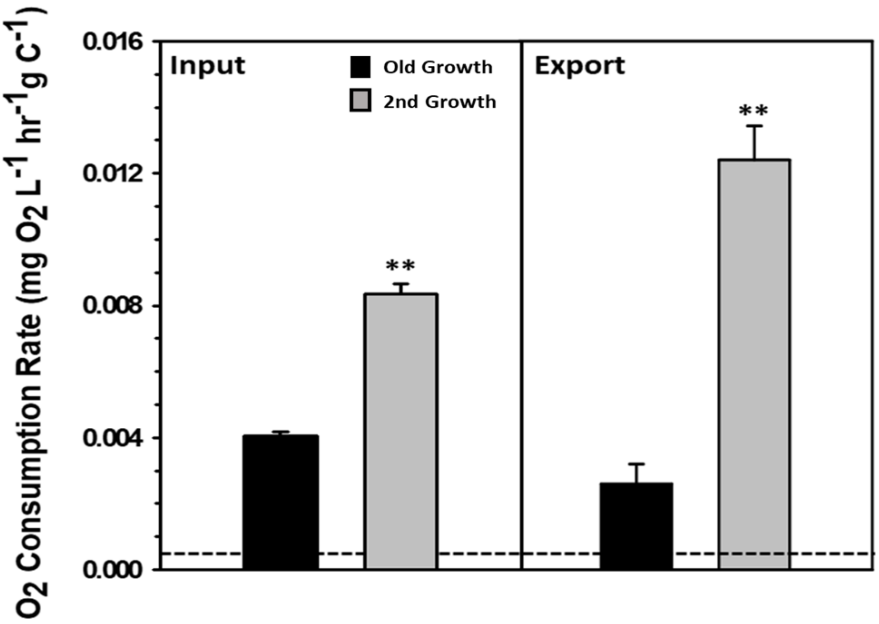
Figure 3. DOM quality and reactivity in subsurface leachate for adjacent old-growth and second-growth hillslopes. DOM quality examined through fluorescence index (A), and humification index (B) for samples collected during 2016, 2017, and 2018 flow periods (n=23 for second growth and n=35 for old-growth). Biological oxygen demand assays of DOM reactivity report oxygen consumption during the 2018 flow period (C).

Figure 4. Reactivity measured as oxygen consumption rates for DOM extracted from O-horizon litter inputs and subsurface flow exports (n=10 per each hillslope treatment with standard error bars). The dashed line shows oxygen consumption for a sample of the native microbial culture without hillslope DOM added (experimental control). Differences between hillslope treatments are noted by ** at p <0.001 using a Student's parametric t-test.









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