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Dissolved Organic Matter concentration and composition in the forests and streams of Olympic National Park, WA

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Abstract. Dissolved organic carbon (DOC) concentration and dissolved organic matter (DOM) character were investigated in soil water (15 and 40 cm) and streams at eleven sites in Olympic National Park. In addition, the effect of added nitrogen on soil water DOM concentration and composition was tested. Forested plots covering a gradient of precipitation, climate, slope, and aspect in Olympic National Park were fertilized with the addition of 20, 10 and zero (control) kg urea-N ha^{-1} y^{-1}. Seven sites had the two different fertilizer treatments and control plots, while the additional four sites had no fertilizer treatments. Soil water DOC concentrations ranged from 0.5 mg C/L to 54.1 mg C/L, with an average value of 14.1 mg C/L. Streams had low DOC concentrations ranging from 0.2 mg C/L to 4.4 mg C/L, with an average value of 1.2 mg C/L. DOM composition was examined with regard to molar ratios, H:C, O:C and N:C, index of unsaturation, average carbon oxidation state, and specific absorbance. Fertilizer had no consistent effect on either DOM concentration or composition across the study sites. Soil depth influenced both DOM concentration and composition. Shallow soil water DOM had greater concentrations, higher specific absorbance, a higher degree of unsaturation, and had lower molar ratios compared to deep soil water samples. Overall, changes in DOM stoichiometry and specific absorbance as a function of soil depth were consistent despite the diversity of the forested study sites sampled.

Introduction

Dissolved organic matter (DOM) is a critical link between terrestrial and aquatic cycles of carbon and nitrogen. It is the soluble fraction of organic compounds that originates from the degradation of plant litter and soil organic matter by energy-seeking microorganisms, as well as the fraction of soil organic matter that is solublized through physical and chemical leaching processes. This soluble organic matter leaches into soil water and from there is carried to aquatic ecosystems. At this point, DOM becomes part of the aquatic food web where it serves as a source of carbon and nitrogen. It also plays a role as the major light absorbing material in aquatic ecosystems where it absorbs biologically harmful UV light and helps regulate the photic zone. The heterogeneous nature of DOM makes it capable of binding to many different compounds such as metals (Schiff et al. 1990; Guggenberger et al. 1994), organic pollutants such as DDT (Jardine et al. 1989) or even silica from groundwater (Marley et al. 1989, 1992). Therefore, changes in the amount or quality of terrestrial DOM could mean increased or decreased movement of metals or pollutants downstream.

A substantial portion of the forest floor material is lost through production and leaching of DOM (Schiff et al. 1997), consequently anthropogenic induced changes that affect forest ecosystems, such as forest management practices or increased nitrogen deposition, may be observed by studying the quantity and quality of DOM. Increased nitrogen deposition may change the transformation pathway of DOM in the soil food web, by shifting the initial chemical composition of DOM starting material (plant litter, root exudates) to include more easily metabolized compounds such as amino acids or sugars. Such a shift could result in altered DOM quantity and composition in both terrestrial and downstream aquatic ecosystems (McDowell et al. 1998). Although the effect of increased nitrogen to forests has been addressed, it is still not clear how or if it will alter DOM cycling in forested ecosystems (see review by Kalbitz et al. (2000)).

In order to know how terrestrial and aquatic DOM linkages may be offset by anthropogenic disturbances such as increased N, it is critical to understand the controls of DOM concentration (measured as dissolved organic carbon, DOC) and composition in the terrestrial landscape. Among field studies, it has been consistently observed that DOC concentrations decrease with depth in the soil (Michalzik et al. 2001; Qualls and Haines 1991, 1992; Dalva and Moore 1991). The few studies that have reported N:C ratios of DOM have shown them to increase with depth in the soil column (Michalzik et al. 2001; Qualls and Haines 1991, 1992). Currently, abiotic processes are thought to control the observed changes in DOC concentrations and N:C ratios of DOM with depth, but this may depend on site and soil organic matter quality (Neff et al. 2000).

In the field, we expect material to be progressively processed as it moves from the soil surface where it originates to progressively deeper soil horizons, and/or to stream waters. Along this continuum, changes in DOM elemental ratios (N:C, H:C and O:C) should reflect processing by microorganisms, sorption to soil surfaces, or abiotic reactions. Other DOM characteristics, such as the average index of unsaturation (number of pi bonds and rings) and the carbon oxidation state, are also expected to vary with the degree of processing. Of all the aforementioned parameters, only N:C ratios have previously been reported for soil water DOM.

The objectives of this study were to:

1. investigate the effect of increased N addition on soil water DOC concentrations and DOM composition;
2. investigate differences in DOM concentration and composition between the shallow soil water, deep soil water and streams;
3. use site differences to glean preliminary insight on the controlling factors (such

as N addition, soil temperature, elevation and soil depth) of the observed changes in DOM concentration and composition across Olympic National Park.

Study site

Soil water and stream samples from Olympic National Park, WA (Figure 1) were collected for DOM analysis. Olympic National Park was chosen to be the study site for several reasons. First, it is a pristine environment with a low atmospheric nitrogen deposition rate of about 1–2 kg ha^{-1} yr^{-1} (inorganic) N (National Atmospheric Deposition Program/National Trends Network (NADP/NTN) 1997). Second, there is a large variety of ecosystem types in the park, from dry alpine slopes to a temperate rainforest, which receives 350 cm of precipitation a year on average. A total of eleven forested and four stream sites were established across the park in four regions: Deer Park, Hurricane Ridge, Elwha River valley, and the Hoh Rainforest. Table 1 lists the site characteristics for each of the forest study sites. Dominant species included Douglas-fir (*Pseudotsuga menziesii*) and western hemlock (*Tsuga heterophylla*) at most sites, subalpine fir (*Abies lasiocarpa*) at the high elevation sites, and sitka spruce (*Picea sitchensis*) and western hemlock in the rainforest.

Plot design and sample collection

Ceramic cup tension lysimeters (Model 1900 Soil Water Sampler, Soilmoisture Equipment Corp., Goleta, CA) were installed in the summer of 1999 at all eleven sites. Lysimeters were allowed to equilibrate over the winter of 1999–2000 before sampling was initiated. A tension of just over field capacity (up to 0.05 MPa) was applied to the lysimeters between sampling dates. At the Deer Park and Hoh sites, all four plots had lysimeters installed at each of two depths: the upper B horizon at 15 cm and the lower B horizon at 40 cm. The remaining four study sites (Hurricane Ridge and Elwha sites) had two lysimeters per plot at 40 cm only.

Forested plots in Olympic National Park were fertilized five times with the addition of 20, 10, and zero (control) kg urea-N ha^{-1} y^{-1} during both the 1999 and 2000 growing seasons (Table 2 and Figure 2). The seven sites at Deer Park and the Hoh had the two different fertilizer treatments as well as two control plots each, while the additional four sites each had two plots each with no fertilizer treatments. Soil water samples were collected approximately monthly (June, July, August, November). Sample collection required sufficient water in the lysimeters for a sample and conditions (e.g., no snow) that allowed access to the sites. Often there was enough soil water collected for DOC analysis but not enough for elemental analysis. Consequently, many more soil water samples (≈ 230) yielded DOC concentration values than were available for elemental analysis (≈ 120 values for CHNO).

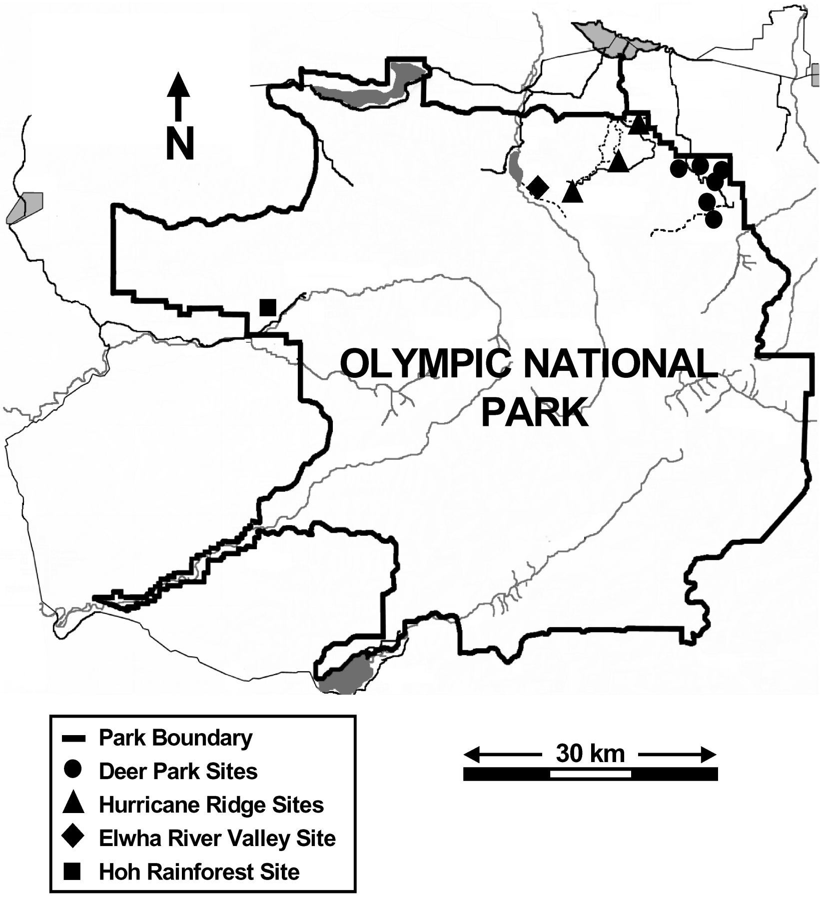


Figure 1. Map of Olympic National Park.

Volumes of soil water with sufficient DOM for elemental analysis were collected from each plot approximately twice during the period of June-October of 2000.

Stream water was collected by dip sampling from four streams, East Twin Creek (Hoh Rainforest), Siebert Creek (Deer Park), Wolf Creek (Hurricane Ridge), and Heart O' the Hills (Elwha River valley). For each of the four streams, duplicate samples (about 3.4 L) were collected twice in June and once in July, August and October. A total of 33 stream samples were analyzed for DOC concentration and DOM composition.

Table 1. Site Characteristics.

Site	Code	Treatment	Dominant Overstory	Elevation (m)	Aspect	% Slope	Stand Age (yr)	Precipitation* (cm/yr)	Soil Texture			
									% Sand	% Silt	% Clay	% Rock
Deer Park Low South	DLS	C1 C2 F1 F2	DF WH	710	133	33	79	50	44	35	21	53
Deer Park Low North	DLN	C1 C2 F1 F2	DF WH	669	23	14	78	50	60	28	12	33
Deer Park Mid South	DMS	C1 C2 F1 F2	DF WH	955	173	35	221	50	63	20	17	54
Deer Park Mid North	DMN	C1 C2 F1 F2	DF WH	898	7	52	205	50	67	20	13	52
Deer Park High South	DHS	C1 C2 F1 F2	DF SF	1423	158	71	76	50	52	30	18	63
Deer Park High North	DHN	C1 C2 F1 F2	SF PSF	1450	337	58	73	50	54	33	13	54
Hurricane Ridge Low	HRL	C1 C2	DF WRC	568	358	22	216	76	63	23	14	54
Hurricane Ridge Mid	HRM	C1 C2	DF	1075	146	60	> 302	95	69	20	10	71
Hurricane Ridge High	HRH	C1 C2	SF DF	1383	140	37	206	127	82	5	13	48
Elwha, Whiskey Bend	ELW	C1 C2	DF	480	262	40	183	178	48	31	21	69
Hoh, East Twin Creek	Hoh	C1 C2 F1 F2	SS WH	175	–	0	216	350	52	33	15	0

DF = Douglas-fir; WH = Western Hemlock; SF = Subalpine Fir; PSF = Pacific Silver Fir; WRC = Western Red Cedar; SS = Sitka Spruce. C1 and C2: Duplicate control plots, no fertilizer added. F1: 10 kg urea-N ha^{-1} yr^{-1}. F2: 20 kg urea-N ha^{-1} yr^{-1}. For site codes, L = low, M = mid, and H = high elevation; N and S refer to north and south aspect. Mean percentages of sand, silt, and clay are proportions of the non-rock soil fraction. *precipitation data was obtained for the region for Deer Park only, no site specific precipitation data was available.

Table 2. DOC Concentrations, Soil Respiration and Soil Temperature.

Site	Mean Percent DOC Decrease^{+}	Seasonal CO_2 Flux* (μmol CO_2 m^{-2} s^{-1})	Soil Temp* (1 cm) (°C)
Hoh	82	0.63	7.6
DLS	63	0.72	6.3
DMN	39	0.54	5.3
DHS	38	0.52	4.6
DMS	32	0.58	6.4
DHN	30	0.54	3.4
DLN	25	0.55	5.7

+ Average percent loss of DOC between 15 and 40 cm, relative to amount in 15 cm; *From Kane et al. (2002)

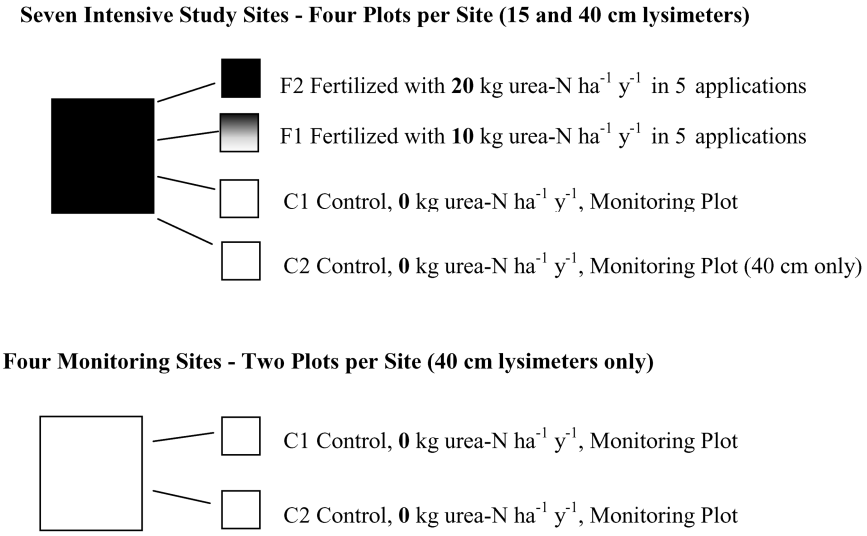


Figure 2. Plot design schematic.

Methods

Sample preparation and storage

All samples were pre-filtered with Gelman 0.2-micron nylon filters. Samples were then frozen until the concentration step. Between individual procedures, samples were refrigerated, except after dialysis when they were frozen in preparation for freeze-drying. During concentration and dialysis, which were preformed at room temperature, samples were kept covered and out of direct light. After concentration, samples were acidified with HCl (about two drops) to pH 2–3 in order to remove inorganic carbon (carbonates).

Laboratory

For an in-depth description and evaluation of the methods used for this study, see Cory (2001). Only a brief description of the methodology will be presented here. Both stream and soil water samples were concentrated by tangential flow filtration (TFF). A Millipore TFF unit consisting of a regenerated cellulose ester membrane, with a surface area of 0.23 m^2 and a nominal molecular weight cut-off (MWCO) of 1000 Daltons (1-kDa) was used. MasterFlex C-flex and Pt-cured silicone tubing were used in conjunction with a peristaltic pump and the TFF unit. Initial water volumes ranged from about 250 mL to 2 L for soil water samples. All stream samples had initial volumes of about 3.4 L. Both soil water and stream samples had retentate (final) volumes between 100–130 mL (a volume practical for dialysis). Concentration factors ranged from 2 to 30, with the stream samples dominating the higher end of that range. Concentration results indicated high DOM recovery (about 85%) in the retentate for the soil water samples, while approximately half of the DOM was lost from the stream samples. Low DOM recoveries may be due to loss of lower molecular weight material which passes through the TFF membrane, or to breakthrough of higher molecular weight DOM over the long times (≈ 9 hours for 3 L) required to process stream samples with very low DOM concentrations. Between each sample, the membrane was exhaustively flushed with MilliQ (MQ) water and stored in 0.1 N NaOH.

In addition to the high recoveries of soil water DOM, there is further evidence that the method used to concentrate DOM did not fractionate the soil water DOM. A near perfect linear relationship between specific absorbance of the original and retentate soil water DOM suggests that fractionation during concentration of at least the light-absorbing portion of DOM was very low (data not shown; see Cory (2001)). The stream samples had low recoveries of DOC as mentioned above, and the graph of original vs. retentate specific absorbance showed that the retentate had higher specific absorbance than the original sample. This result was interpreted as loss of low molecular weight material which is not retained by TFF.

After concentration, samples were dialyzed to remove inorganic ions. Spectra-Por cellulose ester membranes (3.1 mL/cm) with a MWCO of 500 Daltons were used for dialysis. A dialysis membrane containing the concentrated sample was placed inside a 20-L plastic bucket of MQ water. MilliQ water was replaced five times for each sample and at least two hours passed between each replacement of MQ water. Samples were generally left dialyzing overnight, so that total dialysis time averaged 24 hours. DOM loss during dialysis, measured by absorbance and DOM concentrations, was low (less than 10%).

DOM concentrations were measured on the original, filtered water sample (before the samples went through any of the isolation procedures described above) as dissolved organic carbon (DOC). For the soil water samples, DOC was measured as total organic carbon (TOC) by high temperature catalytic oxidation on a Shimadzu 5000A TOC Analyzer (Shimadzu Corporation, Japan). For DOC measurements on stream samples, an OI Analytical Model 1010 Wet Oxidation Total Organic Carbon Analyzer (College Station, TX) was used. Stream samples had very low concentrations, making it difficult to obtain accurate measurements. Replicate DOC measurements for the stream samples indicated an analytical error of 16%. Absorbance was measured on original water samples in a 1-cm cell at 300 nm with a Hewlett Packard 8452A Diode Array Spectrophotometer. Each concentrated, purified and freeze-dried DOM sample was analyzed twice with an elemental analyzer (Carlo-Erba NA 1500 NC, CE Elantech, Lakewood, NJ), once for C, H, N, and once for O measurement. For oxygen measurement on the elemental analyzer, samples are pyrolyzed in helium and oxygen-containing gases are quantitatively converted to carbon monoxide in the presence of nickelized carbon heated to approximately 1070 °C. Suwannee River natural organic matter (International Humic Substance Society, http://www.ihss.gatech.edu/) was used as a standard for C, H, N and O values on the elemental analyzer.

Calculations and statistical analysis

Elemental analysis data give an average empirical formula for each sample, thus allowing calculation of molar ratios, index of unsaturation, and average carbon oxidation state. The molar ratios N:C, H:C, and O:C were determined.

The index of unsaturation, number of rings and pi bonds per mole of organic molecules, is expected to be related to the light absorption of DOM; that is, a higher degree of conjugated rings and pi bonds should increase light absorption. To calculate index of unsaturation for DOM, a complex heterogeneous mixture, Perdue (1984) presented the following equation:

$$\text{Unsaturation}\quad(\text{mmol/mol}\quad\text{DOM}) = C + N/2 - H/2 + 1000/Mn \tag{1}$$

This equation requires elemental analysis data for C, H, N and an assumption of the average molecular weight (Mn) of the DOM. An average molecular weight of 1000 g/mol was chosen for DOM. Assuming an average molecular weight is acceptable and does not cause large errors because the index of unsaturation is relatively insensitive to the molecular weight term, Mn (Reuter and Perdue 1984). For example, changing the Mn term from 1000 to 5000 g/mol changes the index of unsaturation by only about 3–6%. A larger source of error in unsaturation values is possible contamination by water. The presence of water, from incomplete removal during the freeze-drying process, would result a lower index of unsaturation by adding extra hydrogen; thus reported numbers should be considered minimum values. Thermogravimetric analysis indicated that the soil water samples had no measurable water contamination (data not shown); however, only a few samples were analyzed by this method. Although the presence of oxidized sulfur, which was not measured, could increase the index of unsaturation, there is less sulfur in organic matter than nitrogen (Urban et al. 1999). Therefore, error from not including sulfur is probably minimal.

The average carbon oxidation state of each sample was also calculated from empirical formulae. For an empirical formula of $C_wH_xN_yO_z$, the average carbon oxidation state is given by Equation 2. Unlike the index of unsaturation, average carbon oxidation states are not affected by water contamination. The average carbon oxidation state may range from −4 (methane) to +4 (carbon dioxide).

$$\text{Oxidation}\quad \text{state} = (-x + 2z + 3y)/w$$

Analysis of variance (ANOVA) was used to evaluate the effects of site, depth, and N-treatment on the measured DOM characteristics (e.g., concentrations, molar ratios), with values for different sample dates considered as repeated measures. Significant P values for main effects and interactions are included in the figures presented in the results section; data from N-treated and control plots are pooled in these figures. Because depth was the most consistently important variable in explaining observed responses, it was also examined within each site. Significant differences between depth means within sites were tested using ANOVA for depth, N-treatment and collection date. Significant differences between depths within a site are indicated by an asterisk (*) in subsequent figures. Mean values for each plot and sample collection date were used in the analyses, and all statistical tests were performed using PROC GLM in SAS (Version 6.0). Significance was accepted at the 0.05 level of probability.

Results

Soil water DOC concentrations

Statistical analysis revealed that site, depth, date and nitrogen treatment (N-treatment) were all significant variables for DOC concentration differences. There was significant interaction between site and N-treatment. Figure 3 shows mean DOC concentrations by depth at every site. DOC concentrations decreased significantly with depth at every site where both soil depths were sampled. DOC concentrations ranged from 0.5 to 54.1 mg C/L, with an overall mean of 14.1 mg C/L.

Unlike depth, the effect of nitrogen addition on DOC concentrations across all sites was not clear. For example, the Hoh rainforest, Deer Park low-north (DLN) and low-south (DLS) (all low elevation sites) had control plot DOC concentrations consistently higher than fertilized plots in the 15 cm lysimeters, while the opposite trend occurred at Deer Park mid-north (DMN). This inconsistent trend was reflected in the significant interaction between site and fertilizer level in the statistical analysis ($P < 0.0001$). Furthermore, there were significant differences between control plot (C1 vs. C2) DOC concentrations at some of the monitoring sites where N was not added.

Soil water DOM composition

Statistical differences for DOM composition were analyzed in the same manner as DOC concentrations. It can be seen in Figure 4 that for N:C and H:C molar ratios, both depth and site were significant factors in the model, while only site was sig-

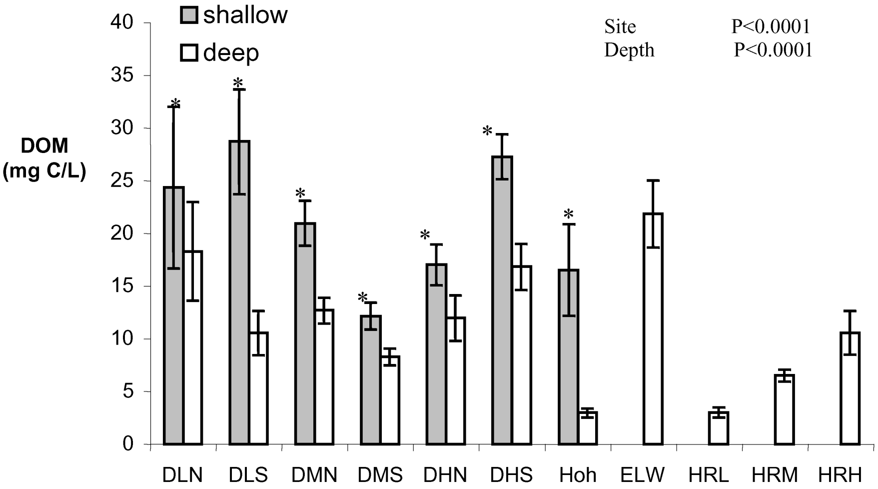


Figure 3. Mean DOC concentrations (mg C/L) by depth at each study site. Sites at ELW, HRL, HRM, HRH had only deep lysimeters. Error bars represent one standard error of the mean. Shallow = 15 cm lysimeter; Deep = 40 cm lysimeter. Sites with an asterisk (*) exhibit significant differences between mean DOC concentrations with depth ($P < 0.05$).

nificant for O:C molar ratios. At almost every site, H:C and N:C molar ratios increased with depth. Although depth was not a significant main effect for the O:C ratios, five out of seven sites showed slightly increased O:C ratios with depth.

Mean specific absorbance decreased with depth at every site (Figure 5a; absorbance at 300 nm per mg C). Figure 5b shows the results for the calculation of index of unsaturation (number of rings and pi bonds) from elemental analysis. The depth trends for mean specific absorbance and the index of unsaturation are similar. Average carbon oxidation state was different the other parameters in that it showed no consistent or significant depth effect across all the sites (data not shown). The oxidation state varied only minimally; 95% of the values were in the range of −1.4 to 0.50.

Stream DOM

Due to the low recovery and high fractionation of stream DOM during the concentration procedure, only those parameters measured on the original (whole water) stream samples are presented here. Stream DOC concentrations were low relative to the soil water samples, ranging from 0.2 to 4.0 mg C/L with an average of 1.2 mg C/L. As can be seen from the slope of absorbance versus DOC concentration in Figure 6, the specific absorbance of stream DOM falls between that of shallow and deep soil water DOM.

In Figure 6, the intercept provides a measure of the fraction of non-absorbing compounds (Vodacek et al. 1997), such as sugars or carbohydrates, which comprise DOM. Solving the equation of the line for zero absorbance, stream DOM has 0.089

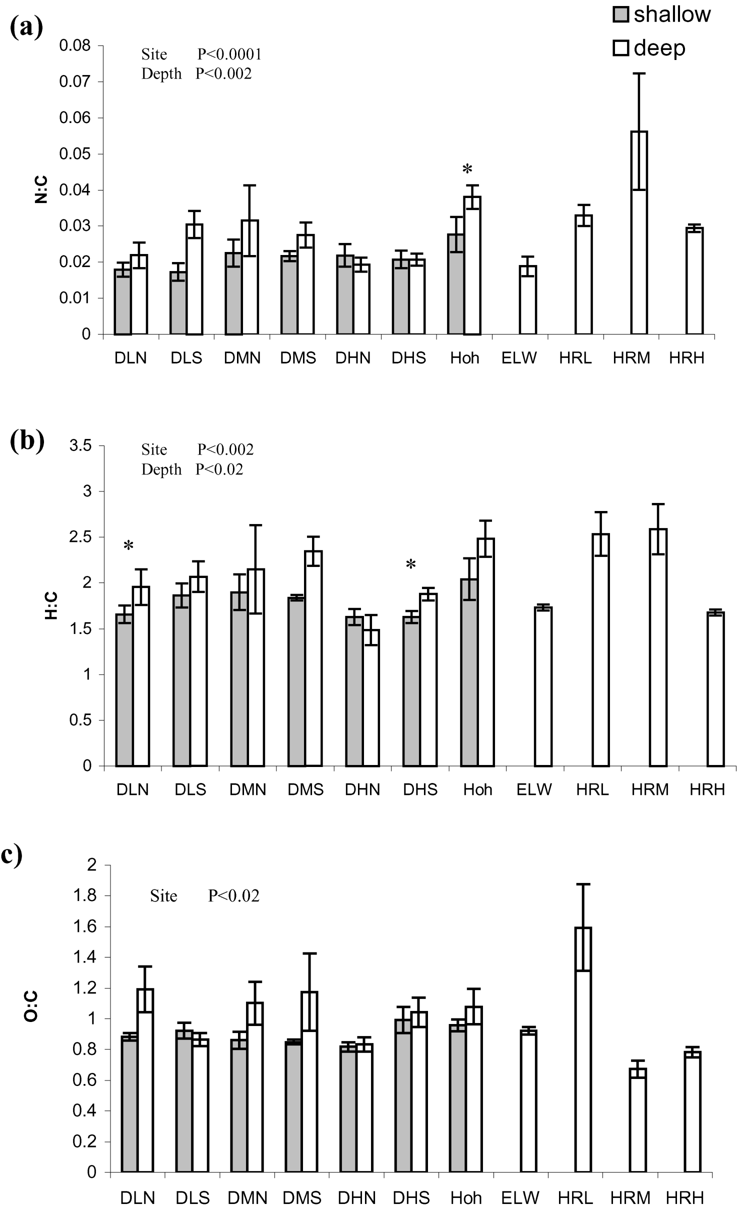


Figure 4. Mean molar ratios by depth at each study site (a) N:C (b) H:C (c) O:C Error bars represent one standard error of the mean. Sites with an asterisk (*) exhibit significant differences between mean molar ratios with depth ($P < 0.05$).

mg C/L that is non-absorbing at 300 nm, as opposed to 1.7 mg C/L for the shallow soil water DOM and 0.33 mg C/L for the deep soil water DOM. Average total DOM

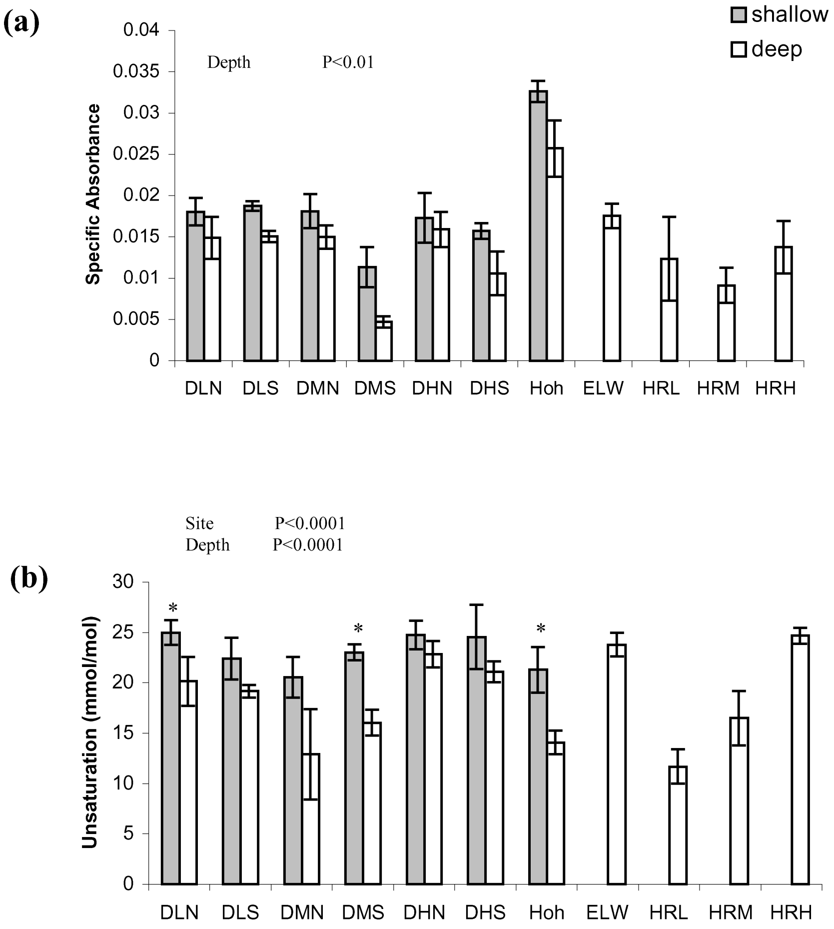


Figure 5. (a) Mean specific absorbance at 300 nm by depth for each site (absorbance/mg C). (b) Mean index of unsaturation by depth for each site (mmol of unsaturation per mole of DOM). Error bars on both graphs represent standard error. Sites with an asterisk (*) exhibit significant differences with depth ($P < 0.05$).

concentrations for the stream, shallow and deep soil water were about 1.2 mg C/L, 21.2 mg C/L and 11.1 mg C/L respectively. Consequently, stream and shallow soil water DOM have similar percentages of non-absorbing DOM (eight and nine percent respectively) compared to deep soil water DOM, which only has about 3% non-absorbing DOM.

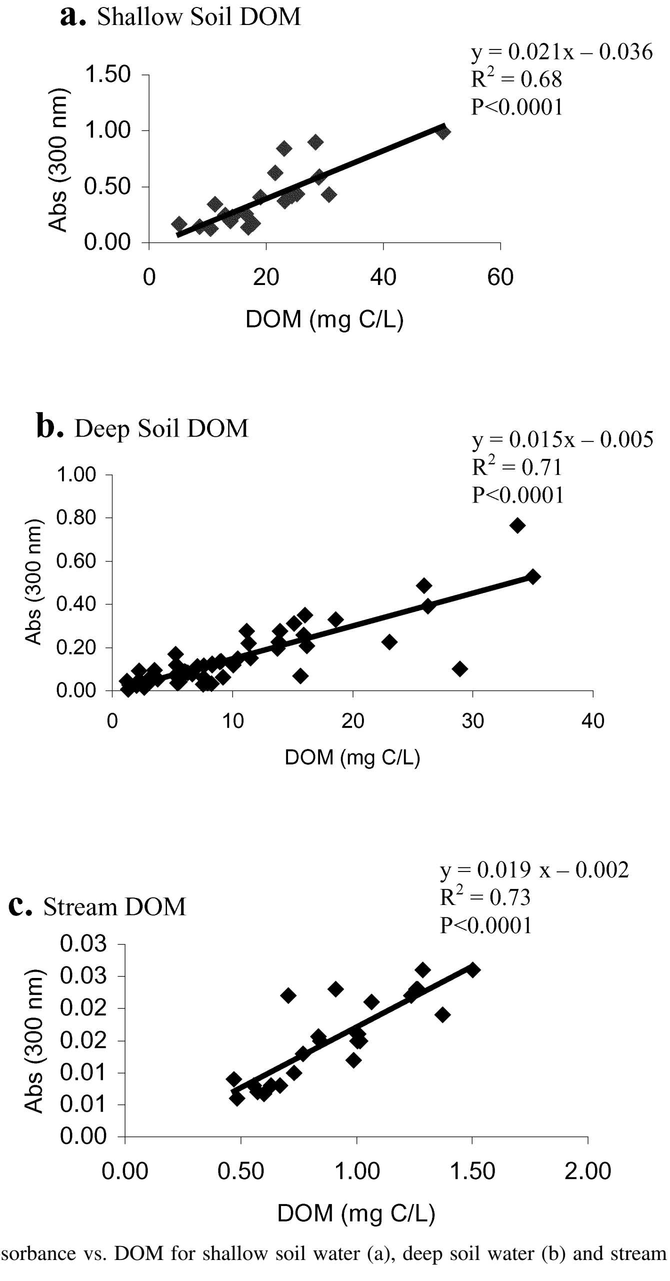


Figure 6. Absorbance vs. DOM for shallow soil water (a), deep soil water (b) and stream samples (c).

Discussion

Controls on DOC concentrations

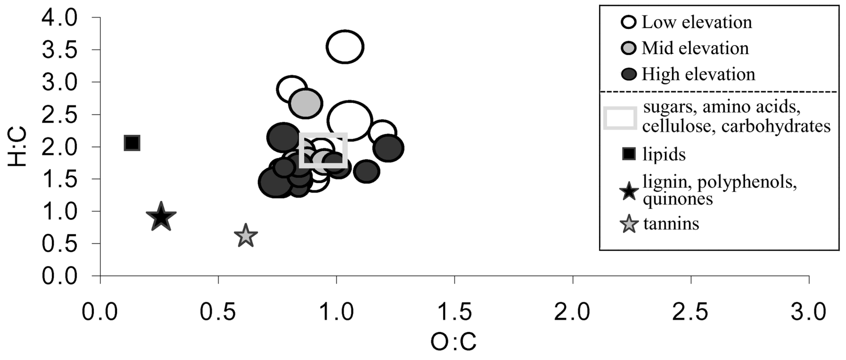
DOC concentration decreased with depth in the soil at all sites, across all ecosystem types. Decreasing DOC concentration with soil water depth is consistent with results from other studies (McDowell and Likens 1988; McDowell and Wood 1984; Qualls and Haines 1991; Dalva and Moore 1991; Michalzik et al. 2001). Microbial utilization of DOM could explain the decreasing concentrations of DOC from shallow soil water to deep soil water. However, many studies suggest that sorption of DOM to mineral surfaces is more important than decomposition in mineral soils for decreasing DOC concentrations with soil depth (McDowell and Likens 1988; Kalbitz et al. 2000; Solinger et al. 2001; Currie et al. 1996). Furthermore, Qualls and Haines (1992) specifically investigated the importance of decomposition on DOM removal with soil depth. They concluded that biological decomposition of soil water DOM was too slow to account for the decrease in concentration with depth. They theorized that decreased concentrations with depth must be due to quick initial adsorption followed by slow biological mineralization. However, Michalzik et al. (2001) found that throughfall provides easily decomposable carbon and nitrogen compounds and suggested that it may serve to promote decomposition processes in the soil. Results from lab studies where DOM is separated into various components (throughfall vs. soil, etc) and inoculated with bacterial cultures may not accurately reflect in-situ decomposition processes, where there are both complex mixtures of organic matter and diverse communities of bacteria and fungi.

A companion study of soil temperature and soil respiration at seven of the study sites (Kane et al. 2002) provided data to test for relationships between DOC concentration, soil respiration, and soil temperature (Table 2). At four sites, the mean decrease in DOC concentration with depth ranged from 25% to 39%, while much larger decreases of 82% and 63% were observed at the Hoh Rainforest and DLS, respectively. The Hoh and DLS also had higher seasonal CO_2 fluxes than the other sites, suggesting that microbial utilization of DOC may have been important at these two sites in addition to abiotic removal mechanisms.

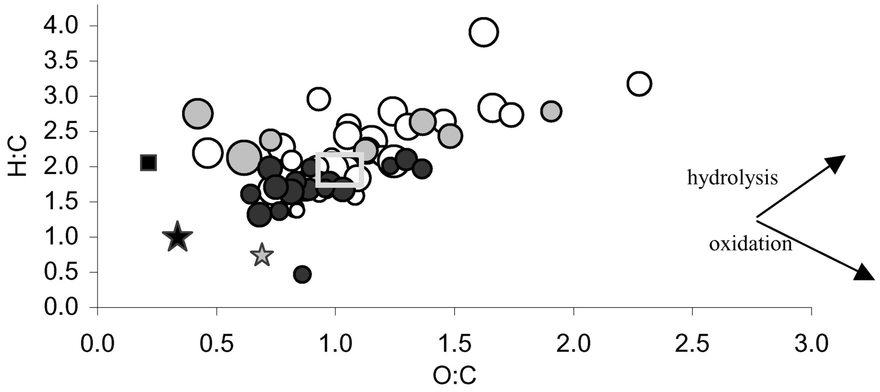
Controls on DOM composition

The stoichiometry of DOM isolates and of compounds characteristic of plant litter, such as lignins, tannins, polyphenols, cellulose, carbohydrates, amino acids, sugars, and lipids have been plotted in Figure 7. These compounds comprise some of the initial compounds eventually transformed into DOM

(Stevenson 1994). Figure 7a clearly shows that the majority of the shallow DOM lies near the square representing the molar ratios of sugars, amino acids, and carbohydrates, while the deep soil water samples display a wider range of molar ratios. Both shallow and deep DOM have H:C ratios that are higher than those of lignin, polyphenols, tannins and quinones, and O:C ratios higher than lipids. It may be that the latter compounds are either hydrolyzed or broken up into more hydrolys-



a. Shallow soil DOM



b. Deep soil DOM

Figure 7. (a) Shallow soil water DOM (b) Deep soil water DOM. On both graphs, the y-axis depicts the H:C molar ratio; x axis depicts the O:C molar ratio. Elevation is indicated by the shading of the symbols: open symbols, low elevation; grey symbols, mid elevation; dark symbols, high elevation; symbols size corresponds to the N:C molar ratio. N:C molar ratios can not be compared directly between shallow soil water (a) and deep soil water (b) because bubble size range was normalized for each data set. Other symbols indicate molar ratios of model compounds. Arrows in (b) indicate the direction of change predicted from hydrolysis and oxidation reactions.

able pieces, and incorporated into DOM by the time they reach 15 cm, the depth of the shallow lysimeters. McKnight and Aiken (1998) describe the breakdown process of lignin and other plant compounds as characterized by oxidation and the resulting addition of carboxylic and other oxygen containing groups, which could explain the wider range of O:C ratios for the DOM compared to lignin, lipids, and other compounds commonly found in plant litter.

Figure 7 shows a trend towards hydrolysis as opposed to oxidation reactions (as indicated by the arrows in Figure 7), which might explain the lack of a consistent depth effect on the average oxidation state. Lack of a consistent depth trend suggests that microbial oxidation of DOM is not solely responsible for the observed depth changes in DOM stoichiometry. It is also possible that oxidation of DOM components occurs primarily before the DOM reaches 15 cm; in that case depth differences would not have been observed.

Although there is no consensus on the relative importance of sorption versus microbial uptake as controls on DOM loss with soil depth, the observed differences in DOM composition with depth at each site agree well with an abiotic (sorption) interpretation. As DOM moves with the soil water, HMW and hydrophobic fractions would be preferentially sorbed relative to the hydrophilic fraction (Qualls and Haines 1992; Kalbitz et al. 2000; Kaiser et al. 2001). The hydrophobic fraction of DOM consists of humic substances, which have higher molecular weights, lower O:C and H:C ratios (Stevenson 1994), and higher specific absorbance, or color (Chin 1997; Zepp and Schlotzhauer 1981), compared to lower molecular weight fulvic acids. Preferential sorption of a HMW, hydrophobic fraction of DOM is consistent with the observed decreases in specific absorbance and unsaturation, and the increased H:C and O:C molar ratios with depth. In addition, the observed concurrent decrease of the index of unsaturation and specific absorbance with depth agrees with results presented by Kaiser et al. (2002). In that study, upon passage through mineral soil at a pine forested site, subsoil porewater DOM was found to contain more aliphatic carbon and less aromatic carbon relative to the forest floor seepage DOM.

The increased N:C molar ratios with depth is consistent with findings from Qualls and Haines (1991, 1992) and Kaiser and Zech (2000), Solinger et al. (2001), where N:C was measured as the ratio of concentrations of DON/DOC. These authors all suggested the reason for the increased N:C ratios is preferential sorption of the hydrophobic fraction relative to the N-enriched hydrophilic fraction of DOM. In addition, the increased N:C ratio with depth concurs with the hypothesis put forward by McGill and Cole (1981) and Qualls and Haines (1992): soil microorganisms do not selectively hydrolyze N compounds, instead N mineralization is tied to C mineralization.

Elevation differences in DOM composition are apparent in Figure 7. If the trend towards higher molar ratios with an increase in soil depth is indicative of a greater degree of abiotic and/or biotic processing, then low elevation DOM is more processed relative to the mid and high elevation DOM. This trend is observed in the shallow samples, and is accentuated with depth. This suggests that at the high elevation sites, DOM was not processed as quickly or as thoroughly as at lower elevation (warmer) sites. Similar elevation trends at both depths in Figure 7 suggest microbial processing of DOM, regulated in part by temperature, is responsible for at least some of the changes in DOM composition as it moves through the soil. However, abiotic reactions, such as nucleophilic or electrophilic addition reactions are also temperature dependent.

Fertilizer effect

No clear effect of nitrogen added as fertilizer was seen in either DOM concentration or DOM composition. Variability in DOM concentration and composition among sites and season were greater than nitrogen effects. It is possible that the absence of any effect was due to the length of the study. Sites were only fertilized for two growing seasons with small quantities of total N, and only samples collected during the second season were analyzed. However, other long and short term N fertilization studies have also produced ambiguous results. After seven years of nitrogen addition to pine and hardwood stands in the Harvard Forest, Currie et al. (1996) found no significant difference in DOC concentrations, but DON concentrations and ratios of DON:DOC did increase significantly. Furthermore, Currie et al. observed different patterns of response to N amendments at the pine versus the hardwood stands. In another long term field study by Neff et al. (2000), no influence of N fertilization on DOC or DON fluxes was found, but there was a significant site and N fertilizer interaction for the flux of DON. In a short term (15 week) study by Magill and Aber (2000), inorganic N was added to leaf litter and then leachate DOC concentrations were measured. In that study, differences in DOC concentration were attributed primarily to a diverse litter chemistry and not to N treatment. Based on the previous studies, there is evidence that response of DOM to N fertilization varies considerably by site. Neff et al. (2000) suggested that this variation may be a function of the amount of nitrogen in soil organic matter, rather than short term microbial processes.

The Hoh Rainforest showed differences in DOM concentration and composition between the fertilized and control plots. Shallow depth control plots had DOC concentrations significantly higher ($P < 0.008$) than fertilized plots, and the control samples were much more colored than fertilized plots. The greater degree of color was reflected by a higher mean index of unsaturation ($P < 0.003$) for the DOM in the shallow depth control plots vs. fertilized plots at the Hoh rainforest. Fertilized plots had higher mean H:C and N:C ratios, and higher average carbon oxidation states. Like DOC concentration and index of unsaturation, differences in molar ratios and oxidation state between control and fertilized plots at the Hoh were significant only for the shallow samples ($P < 0.01$, 0.04, and 0.03 respectively). The fertilizer appears to have altered the way DOM was processed at the Hoh, although effects of altered processing did not persist in the deeper soil water samples. Longer and more comprehensive studies are required to determine whether these differences were due to inherent variation between the plots before fertilizer addition began. However, based on the possible site-specific response to the fertilizer at the Hoh plus the significant interaction between N-treatment and site, our results tentatively support the conclusions of Currie et al. (1996) and Neff et al. (2000): response to added nitrogen varies by site.

Stream DOM

Data presented here shows that the stream DOM is more similar in character to the shallow soil water DOM than to the deep soil water DOM. This supports the idea that stream DOC in headwater environments is dominated by inputs from the upper soil horizon (Brooks et al. 1999), and that DOC lost from the upper soil solution to the lower horizons never enters the stream (McDowell and Likens 1988).

Conclusions and implications

Depth was the most important variable controlling DOC concentrations and DOM composition across Olympic National Park. The observed changes in concentration and composition of DOM with depth were most consistent with abiotic (sorption and adsorption) processes in the soil, with possible enhancement by microbial action at a few sites. Several previous studies have also shown a preferential accumulation of dissolved organic nitrogen in the deeper soil water. The fate of this deep soil water DOM could play a role in both nitrogen and carbon retention in forests; more work is needed to identify the organic nitrogen compounds in these deeper soil horizons. If there is a response of N amendments on DOM dynamics, it appears to be very site-specific. Sorption of DOM may buffer any impact on downstream aquatic ecosystems, however long term effects probably depend on the fate of the deep soil water DOM (whether or not it percolates into the streams). Results from this study and others cited above suggest that the next steps are to identify site characteristics that may control the varying response of forests to increased N addition.

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References

Brooks P.D., McKnight D.M. and Bencala K.E. 1999. The relationship between soil heterotrophic activity, soil dissolved organic carbon (DOC) leachate, and catchment-scale DOC export in headwater catchments. Water Resources Research 35: 1895–1902.

Chin Y.-P. 1997. Binding of Pyrene to Aquatic and Commercial Humic Substances: The Role of Molecular Weight and Aromaticity. Environmental Science and Technology 31: 1630.

Cory R.M. 2001. Method development and analysis of dissolved organic matter in the soil water and streams of Olympic National Park. Michigan Technological University, Houghton, pp 90.

Currie W.S., Aber J.D., McDowell W.H., Boone R.D. and Magill A.H. 1996. Vertical transport of dissolved organic C and N under long-term N amendments in pine and hardwood forests. Biogeochemistry 35: 471–506.

Dalva M. and Moore T.R. 1991. Sources and sinks of dissolved organic carbon in a forested swamp catchment. Biogeochemistry 15: 1–19.

Guggenberger G., Glasser B. and Zech W. 1994. Heavy metal binding by hydrophobic and hydrophilic dissolved organic carbon fractions in a Spodosal A and B horizon. Water, Air and Soil Pollution 72: 111–127.

Jardine P.M., Weber N.L. and McCarthy J.F. 1989. Mechanisms of dissolved organic carbon adsorption on soil. Soil Science Society of America Journal 53: 1379–1385.

Kaiser K. and Zech W. 2000. Sorption of dissolved organic nitrogen by acid subsoil horizons and individual mineral phases. European Journal of Soil Science 51: 403–411.

Kaiser K., Guggenberger G., Haumaier L. and Zech W. 2002. The composition of dissolved organic matter in forest soil solutions: changes induced by seasons and passage through the mineral soil. Organic Geochemistry 33: 307–318.

Kaiser K., Guggenberger G. and Zech W. 2001. Isotopic fractionation of dissolved organic carbon in shallow forest soils as affected by sorption. European Journal of Soil Science 52: 585–597.

Kalbitz K., Solinger S., Park J.-H., Michalzik B. and Matzner E. 2000. Controls on the dynamics of dissolved organic matter in soils: a review. Soil Science 165: 277–304.

Kane E.S., Pregitzer K.S. and Burton A.J. 2002. Soil respiration along environmental gradients in Olympic National Park. Ecosystems (in press).

Magill A.H. and Aber J.D. 2000. Dissolved organic carbon and nitrogen relationships in forest litter as affected by nitrogen deposition. Soil Biology & Biochemistry 32: 603–613.

Marley N.A., Bennett P., Janecky D.R. and Gaffney J.S. 1989. Spectroscopic evidence for the organic diacid complexation with dissolved silica in aqueous systems-I. Oxalic Acid. Org. Geochem. 14: 525–528.

Marley N.A., Gaffney J.S., Orlandini K.A., Picel K.C. and Choppin G.R. 1992. Chemical characterization of the size-fractionated humic and fulvic materials in aqueous samples. The Science of the Total Environment., 113.

McDowell W.H. and Wood T. 1984. Podzolization: Soil Processes Control Dissolved Organic Carbon Concentrations in Stream Water. Soil Science 137: 23–32.

McDowell W.H. and Likens G.E. 1988. Origin, composition, and flux of dissolved organic carbon in the Hubbard Brook Valley. Ecological Monographs 58: 177–195.

McDowell W.H., Currie W.S., Aber J.D. and Yano Y. 1998. Effects of chronic nitrogen amendment on production of dissolved organic carbon and nitrogen in forest soils. Water Air and Soil Pollution 105: 175–182.

McGill W.B. and Cole C.V. 1981. Comparative aspects of cycling of organic C, N, S and P through soil organic matter. Geoderma 26: 267–286.

McKnight D.M. and Aiken G.R. 1998. Sources and Age of Aquatic Humus. In: Hessen LJT D.O. (ed.), Ecological Studies. Vol. 133. Springer-Verlag, New York, pp. 9–37.

Michalzik B., Kalbitz K., Park J.-H., Solinger S. and Matzner E. 2001. Fluxes and concentrations of dissolved organic carbon and nitrogen – a synthesis for temperate forests. Biogeochemistry 15: 173–205.

Neff J.C., Hobbie S.E. and Vitousek P.M. 2000. Nutrient and mineralogical control on dissolved organic C, N and P fluxes and stoichiometry in Hawaiian soils. Biogeochemistry 51: 283–302.

National Atmospheric Deposition Program/National Trends Network (NADP/NTN) 1997. Wet Deposition in the United States 1996. Illinois State Water Survey, Champaign, IL, USA.

Perdue E.M. 1984. Analytical constraints on the structural features of humic substances. Geochimica et Cosmochimica Acta 48: 1435–1442.

Qualls R.G. and Haines B.L. 1992. Biodegradability of dissolved organic matter in forest throughfall, soil solution and stream water. Soil Sci. Soc. Am. J. 56: 578–586.

Qualls R.G. and Haines B.L. 1991. Geochemistry of dissolved organic nutrients in water percolating through a forest ecosystem. Soil Sci. Soc. Am. J. 55: 1112–1123.

Reuter J.H. and Perdue E.M. 1984. A chemical structural model of early diagenesis of sedimentary humus/proto-kerogens. Mitt. Geol.-Palaont. Inst. 56: 249–262.

Schiff S.L., Aravena R., Trumbore S.E. and Dillon P.J. 1990. Dissolved organic carbon in forested watersheds – a carbon isotope approach. Water Resourses Research 26: 2949–2957.

Schiff S.L., Aravena R., Trumbore S.E., Hinton M.J., Elgood R. and Dillon P.J. 1997. Export of DOC from forested catchments on the Precambrian shield of central Ontario: clues from C-13 and C-14. Biogeochemistry 36: 43–65.

Solinger S., Kalbitz K. and Matzner E. 2001. Controls on the dynamics of dissolved organic carbon and nitrogen in a Central European deciduous forest. Biogeochemistry 55: 327–349.

Stevenson F.J. 1994. Humus Chemistry, Genesis, Composition, Reactions. 2nd edn. John Wiley and Sons, Inc., New York.

Urban N.R., Ernst K. and Bernasconi S. 1999. Addition of sulfur to organic matter during early diagenesis of lake sediments. Geochimica et Cosmochimica Acta 63: 837–853.

Vodacek A., Blough N.V., DeGrandpre M.D., Peltzer E.T. and Nelson R.K. 1997. Seasonal variation of CDOM and DOC in the Middle Atlantic Bight: Terrestrial inputs and photooxidation. Limnology and Oceanography 42: 674–686.

Zepp R. and Schlotzhauer P.F. 1981. Comparison of photochemical behavior of various humic substances in water: III. Spectroscopic properties of humic substances. Chemosphere 10: 479–486.