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Biodegradable dissolved organic carbon in forest soil solution and effects of chronic nitrogen deposition

Y. Yano^{a,*}, W.H. McDowell^a, J.D. Aber^b

^aDepartment of Natural Resources, University of New Hampshire, Durham, NH 03824, USA

^bComplex Systems Research Center, University of New Hampshire, Durham, NH 03824, USA

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Abstract

Using a flow-through bioreactor, biodegradable dissolved organic carbon (BDOC) in forest floor solution was determined for a red pine (*Pinus resinosa*) plantation and a naturally regenerated mixed hardwood forest (dominant species: *Quercus velutina*, *Q. rubra*, *Acer rubrum*, *Fagus grandifolia*, *Prunus serotina*). The forests have received chronic-N fertilization at three different rates (0, 50, 150 kg N ha⁻¹ year⁻¹) since 1988. Both BDOC concentration and %BDOC [% of dissolved organic carbon (DOC)] were significantly higher in the summer (18–20 mg C l⁻¹, ≈30%) than spring or fall for both forest types. The BDOC depletion that was hypothesized to occur with chronic-N application and N saturation was not found in either stand. Instead, for the hardwood stand, BDOC concentration increased with N application, probably due to increased BDOC production in the forest floor with increases in available N. The strong negative relationship between litterfall mass and both BDOC concentration ($r^2 = 0.36$) and %BDOC ($r^2 = 0.60$) for the hardwood stand suggests that the primary source of BDOC in the hardwood forest floor is recent photosynthate allocated to fine roots for their growth, and organic compounds released from them (e.g., exudates and mucilage) rather than the leachate of freshly fallen autumn litter. A significant but weaker positive relationship between BDOC and ambient temperature for both stands suggests that the decomposition of forest floor organic matter is an additional source of BDOC in the forest floor. The production of non-biodegradable DOC (NBDOC) in the forest floor was not sensitive to ambient temperature or the chronic-N treatment. This suggests that an abiotic process, such as chemical equilibration between forest floor and forest floor solution, is responsible for the concentration of non-labile DOC. High %BDOC in throughfall (50–75%) for both stands suggests that throughfall is possibly an additional source of BDOC in the forest floor. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

The forest floor plays an important role in the carbon and nitrogen dynamics of forest ecosystems (Schimel and Firestone, 1989; Qualls et al., 1991). It is viewed as a source of C or N for plants and soil microorganisms (Pritchett and Fisher, 1987) as well as a sink for C and N that enter the forest floor (McDo-

well and Likens, 1988; Aber and Melillo, 1991; Qualls et al., 1991). The C and N cycles in forest floors are linked through the processes of N assimilation, N mineralization, denitrification and organic matter (OM) decomposition (Van Miegroet et al., 1990; Vitousek, 1994; Eviner and Chapin, 1997). The balance between these processes is important in forest soils since most of the nutrient demands by forest vegetation are supplied by decomposing organic matter in the soil (Schlesinger, 1997). In addition, the balance of these processes strongly controls the N retention capacity of forest ecosystems. However, the dynamics of N in forest soils are still not well understood due to the complexity of interactions between the C and N cycles. It

* Corresponding author. Department of Forest Science, Forest Sciences Laboratory, Oregon State University, Corvallis, OR 97331-501, USA. Tel.: +1-541-737-2244; fax: +1-541-737-1393.

E-mail address: yanoy@fsl.orst.edu (Y. Yano).

is known that heterotrophic microbes in soils play important roles in N dynamics, and that their metabolism is often restrained by the availability of C in the soil (Johnson and Edwards, 1979; Davidson and Swank, 1987; Starr and Gillham, 1993). Aber et al. (1989, 1993) have hypothesized that biodegradable C in forest soil will decrease when available N in the soil increases, due to an increase in the microbial demand for C to assimilate N.

Dissolved organic carbon (DOC) is the primary form of C that is transported from forest floor to mineral soils. The biodegradable fraction of this DOC is believed to serve as both an energy source and a potential source of N (as DON) to heterotrophic microorganisms (Qualls and Haines, 1992). In general, the leaching of freshly fallen litter and the decomposition of forest floor OM are thought to be major sources of BDOC in forest soils (e.g., McDowell and Likens, 1988; Guggenberger et al., 1994; Jandl and Sollins, 1997). Organic compounds released from roots (e.g. exudates, mucilage and mucigel) are known to be highly biodegradable (Smith, 1976; Paul and Clark, 1996) and could also be a potential BDOC source, however, little is known about their contribution to BDOC production in forest floors. We hypothesized that the amount of BDOC found in forest floor solution should be positively related to both ambient temperature, which controls microbial metabolism, and litterfall mass if the decomposition of forest floor OM and the leaching of fresh litterfall are the major sources of BDOC in the forest floor. Alternatively, if the contribution of the roots to BDOC production is significant in the forest floor, we should observe a significant association between BDOC and season.

Our primary objective was to investigate whether the amount of BDOC in forest floor solution is affected by the chronic addition of N. We also investigated the relationships between BDOC in forest floor solution and litterfall input or ambient temperature, as well as the relationship between BDOC and chemical composition (i.e. C-to-N ratio) in forest floor solution.

2. Materials and methods

2.1. Study site

The study was conducted at the chronic-N study plots at the Harvard Forest in central Massachusetts (42°30' N, 72°10' W; Aber et al., 1993). Average maximum temperature is -1.3°C in January and 26°C in July. Elevation of the plots is from 220 to 410 m. Annual precipitation averages 109 cm and is distributed fairly evenly throughout the year. However, the summer of 1995, when all forest floor solution samples were collected for this study, experienced a particularly

dry period (Goulden et al., 1996). Total precipitation in August of that year was 4.8 cm or 46% of normal. In 1988, two adjacent forests were chosen to study the effects of increased N deposition: a ≈70-year-old even-aged planted red pine (*Pinus resinosa*) stand and ≈55-year-old naturally-regenerated mixed hardwood stand, which is dominated by black and red oak (*Quercus velutina*, *Q. rubra*), red maple (*Acer rubrum*), black cherry (*Prunus serotina*) and American beech (*Fagus grandifolia*). Soils in both stands are Entic Haplorthods; the soils are stony to sandy loams formed from glacial till (Aber et al., 1993). The forest floor (organic horizon) is 4.6 cm deep in the pine stand and 6.5 cm deep in the hardwood stand (Magill et al., 1997). Other characteristics of the soils are reported in detail by Magill et al. (1997).

Nitrogen has been applied six times per year as dissolved NH_4NO_3 using backpack sprayers, from early May through late September (approximately once per month), since 1988. The amount of water added to soil through this N application was equivalent to 0.002 cm rainfall. The treatments are control (no N added), low-N (50 kg N ha^{-1} year $^{-1}$), and high-N (150 kg ha^{-1} year $^{-1}$). The treatment plots are 30 m × 30 m. The pine and hardwood stands are located on the opposite sides of a ridge, and in each stand, the control plot is located upslope of the N-treatment plots. Average pH of forest floor solution after 8 years of N application ranged between 3.7 and 3.8 for all N-treated plots, and 4.1 and 4.0 for control plots.

2.2. Sample collection and preservation

In 1995, forest floor solution was collected from zero-tension lysimeters (ZTL) originally installed in 1992 (Currie et al., 1996). Each treatment plot had 5 ZTL except the low-N and high-N plots in the hardwood stand (4 ZTL per plot), where one originally installed ZTL had been discarded in previous years due to disturbance by mice. After each rain event, the 1000 ml HDPE (high density polyethylene) bottles were replaced with a new acid-washed set. Normally, the samples were collected within 30 h of the end of a rain event. In the case of a series of intermittent rain events, a collection was made after the last event. Throughfall (TF) samples were also collected for two rain events in September 1996. TF collectors were built with HDPE funnels (14 cm diameter) and 1000 ml bottles that were lined with a plastic bag. The collectors were placed approximately 1 m from each ZTL (i.e., 5 TF collectors per plot). The details of TF collection have been described by W.S. Currie (unpublished Ph.D. thesis, University of New Hampshire, 1995). TF samples were collected the day after each rain event. The immediate collection of water samples presumably minimized BDOC degradation in the field. All samples

were transported on ice (approximately 2 h) to the University of New Hampshire where the solution in each bottle was immediately weighed, bulked for each plot, filtered through ashed (8 h at 500°C) Whatman GF/F glass-fiber filters (Whatman International, Maidstone England), and the pH measured. Samples were frozen in HDPE or polypropylene storage bottles until analysis. The equipment was acid washed before use.

2.3. Chemical analysis

All ZTL samples were first thawed, and then analyzed for DOC (total dissolved C – dissolved inorganic C) with high temperature platinum-catalyzed combustion (Shimadzu TOC-5000 HTCO carbon analyzer; Shimadzu Scientific Instruments, Columbia, MD). Nitrate was measured using the hydrazine sulfate reduction method (Technicon Method 782-86T) and $\text{NH}_4\text{-N}$ was determined using the Berthelot reaction method (Technicon Method 780-86T). Total dissolved N (TDN) was measured using high-temperature Pt-catalyzed combustion and a chemiluminescent NO detector (Merriam et al., 1996). Since $\text{NO}_2\text{-N}$ in the ZTL solution was negligible (Currie et al., 1996), dissolved organic N (DON) was calculated as:

$$[\text{DON}] = [\text{TDN}] - [\text{NO}_3\text{-N}] - [\text{NH}_4\text{-N}]$$

Detection limits were $180 \mu\text{g l}^{-1}$ for both $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$. Because DON is calculated by difference, values sometimes fell below 0 mg l^{-1} . DON values below 0 mg l^{-1} were obtained for approximately 2% of all samples analyzed; a value of 0 mg l^{-1} was assigned for these samples.

2.4. BDOC analysis

Biodegradability of DOC was measured using a flow-through bioreactor (Yano et al., 1998). The bioreactor was modified for soil solutions, which are often limited in volume, from a flow-through method previously developed for drinking water or for stream water by Frias et al. (1992) and modified by Kaplan and Newbold (1995). Continuous flows of two types of forest floor extracts (biosoup) which had been amended with inorganic N, P, S and K were used to make two types of bioreactors: pine and hardwood. Biosoup was made by extracting approximately 300 g of frozen forest floor, collected adjacent to the control plots, with 2000 ml of filter-sterilized, low-C DI water (Milli-Q ultra-pure water) at room temperature in the dark for 2 to 3 days with occasional stirring. Details of the design and operation of the bioreactors are described by Yano et al. (1998).

Each frozen ZTL sample was thawed, filtered through a rinsed Durapore filter (pore size $0.22 \mu\text{m}$,

Millipore), then diluted to 10 mg C l^{-1} after being amended with inorganic nutrient salts (N, P, S and K). Inlet and outlet solutions were sampled three times each. Percent BDOC in the diluted sample was calculated as:

$$\% \text{BDOC} = 100 \times [\text{mean inlet DOC (mg l}^{-1}) - \text{mean outlet DOC (mg l}^{-1})] / \text{mean inlet DOC (mg l}^{-1})$$

BDOC concentration (mg l^{-1}) in the original forest floor solution sample was then calculated as:

$$\text{BDOC (mg l}^{-1}) = \text{original DOC in forest floor solution (mg l}^{-1}) \times [\% \text{BDOC} / 100]$$

Non-biodegradable DOC (NBDOC) was calculated as:

$$\text{NBDOC (mg l}^{-1}) = \text{DOC (mg l}^{-1}) - \text{BDOC (mg l}^{-1})$$

All ZTL samples were tested on the reactor type that corresponded to the sample being analyzed (i.e. the pine ZTL samples were tested on the pine type bioreactor). Percent BDOC values obtained by this method were consistent over the study period: repeated analysis of reference forest floor solution samples showed that the SD around the mean values were not greater than 5% for both types of bioreactor (Yano et al., 1998).

2.5. Statistical analysis

The chronic-N addition treatments are not replicated within each stand. Because no serial correlation was found in DOC, BDOC and %BDOC measurements with a Durbin–Watson test (SAS institute, 1994) and each measurement within the plot was considered to be independent, we applied a time-for-space substitution (Currie et al., 1996) to all measurements within the plots. All measurements were grouped into three seasons: spring (April to May), summer (June to August), and fall (September to November). Two-way analysis of variance (ANOVA) for the N-treatment rate and season was performed for all variables within each stand. Relationships between BDOC and either ambient temperature (T), DOC-to-DON ratio (CN) and litterfall mass (L) were analyzed using simple and multiple linear regressions. None of the independent variables (T , CN and L) interacted with each other. Maximum temperature for the day of a rain event (source: daily weather observation using NOAA maximum thermometer at the weather station of Harvard Forest, MA) was used as ‘ambient temperature’ for each forest floor solution sample. Litterfall was collected approximately monthly between May and November from five fixed baskets per plot (Magill et al., unpublished data). For regression analyses, which

include litterfall as an independent variable, BDOC values were pooled for the litterfall collection intervals and the means for each interval were used. For the means of forest floor solution collected between any two litterfall collection days, litterfall data for the later day were used. Statistically significant differences were those with $P < 0.05$.

3. Results

3.1. DOC

In all plots, maximum DOC concentrations were observed in summer (Fig. 1). The means in summer (72.1 ± 4.8 SE mg l^{-1}) and in fall (66.6 ± 3.9 SE mg l^{-1}) were significantly higher than in spring for the pine plot (Table 1). In the hardwood stand, the mean DOC concentration in the summer (60.0 ± 3.5 SE mg l^{-1}) was significantly higher than in the other two seasons. Overall, means of DOC for each season were higher in the pine stand than in the hardwood stand (Table 1).

An N-treatment effect was found for the hardwood

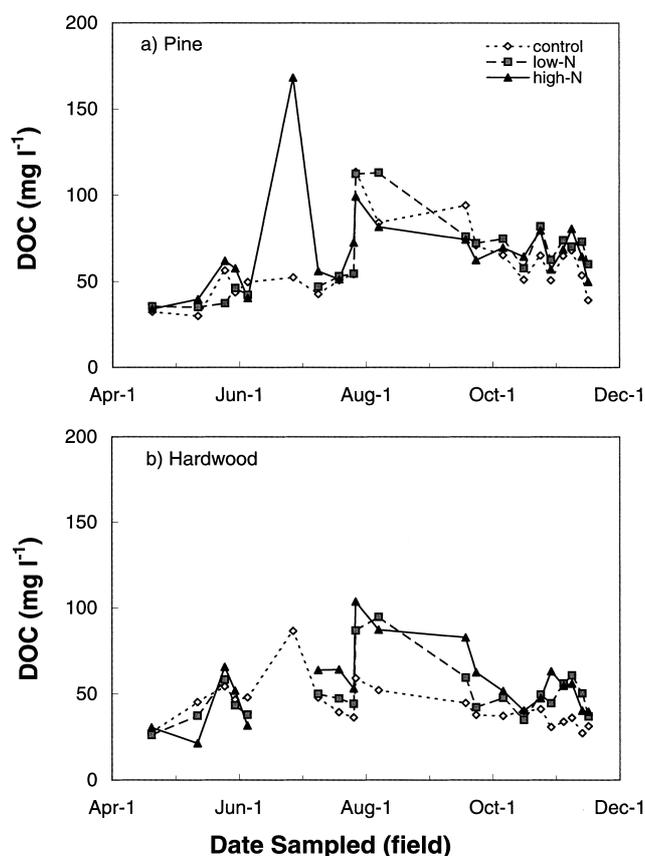


Fig. 1. DOC concentration over time in the pine stand (a) and hardwood stand (b) at Harvard Forest. All samples were collected in 1995.

Table 1

Least square means of DOC, BDOC, %BDOC and NBDOC grouped by season^a

Stand type	DOC type	Season		
		Spring	Summer	Fall
Pine	DOC	42.54a (6.13)	72.05b (4.75)	66.63b (3.88)
	BDOC	10.12a (1.94)	19.77b (1.33)	11.77a (1.06)
	%BDOC	23.37ab (2.74)	30.14a (1.88)	17.41b (1.49)
	NBDOC	32.03a (6.81)	53.06b (4.66)	54.86b (3.71)
Hardwood	DOC	42.31a (4.42)	60.04b (3.51)	46.04a (2.79)
	BDOC	9.13a (1.64)	16.77b (1.13)	7.87a (0.90)
	%BDOC	23.05a (2.28)	28.61a (1.56)	16.61b (1.24)
	NBDOC	30.12a (4.24)	43.27b (2.91)	38.17ab (2.31)

^a Bold letters refer to significant differences within each DOC type. Values in parentheses show 1 SE.

stand. DOC increased with N-treatment showing a significant difference between the control and high-N plots (42.5 ± 3.4 SE mg l^{-1} for control, 55.5 ± 3.5 SE mg l^{-1} for high-N; Table 3). A similar trend was observed for the pine DOC, but differences among the N-treatments were not statistically significant (Table 2).

3.2. BDOC and NBDOC

BDOC concentrations in forest floor solution increased from spring to summer, then decreased toward winter in both stands (Fig. 2). When means were compared for each season, summer BDOC concentrations (19.8 ± 1.3 SE mg l^{-1} for pine, 16.8 ± 1.1 SE mg l^{-1} for hardwood) were significantly higher than those of spring or fall in both stands (Table 1). Additionally, a significant N-treatment effect was found on BDOC concentration for the hardwood forest floor solution; the difference was significant between the control BDOC (8.84 ± 1.2 SE mg l^{-1}) and

Table 2

Least square means of DOC, BDOC, %BDOC and NBDOC grouped by N-treatment^a

Stand type	DOC type	N-treatment ($\text{kg-N ha}^{-1} \text{y}^{-1}$)		
		0	50	150
Pine	DOC	55.34a (4.75)	61.00a (4.86)	64.88a (4.75)
	BDOC	12.99a (1.39)	15.50a (1.45)	15.16a (1.30)
	%BDOC	23.35a (1.96)	23.30a (2.05)	24.27a (1.84)
	NBDOC	42.73a (4.86)	47.56a (5.09)	49.67a (4.57)
Hardwood	DOC	42.47a (3.42)	50.39ab (3.51)	55.53b (3.51)
	BDOC	8.84a (1.15)	11.53ab (1.23)	13.40b (1.13)
	%BDOC	20.40a (1.59)	23.87a (1.71)	24.01a (1.57)
	NBDOC	32.51a (2.96)	37.34a (3.17)	41.72a (2.91)

^a Bold letters refer to significant differences within each DOC type. Values in parentheses show 1 SE.

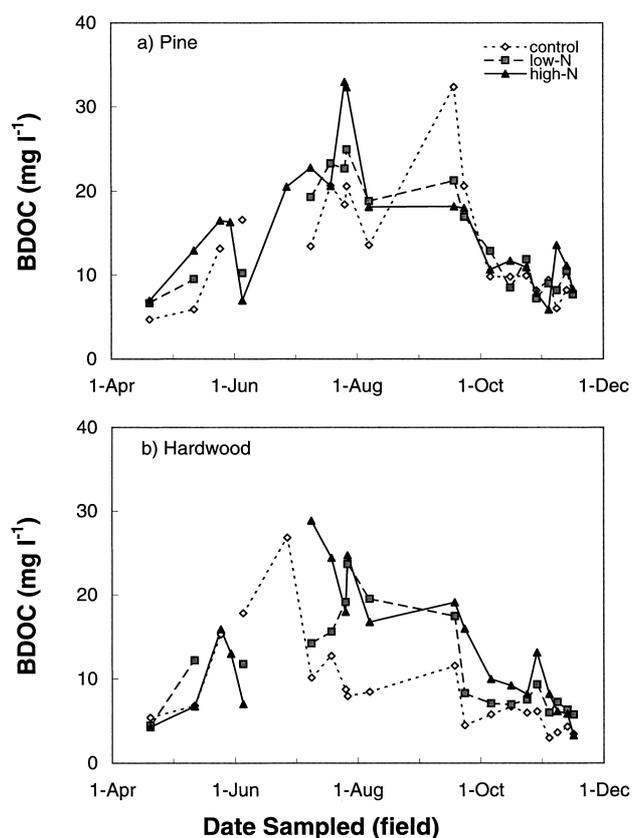


Fig. 2. BDOC concentration over time in the pine stand (a) and hardwood stand (b) at Harvard Forest. All samples were collected in 1995.

high-N BDOC ($13.4 \text{ mg} \pm 1.1 \text{ SE l}^{-1}$; Table 2). However, no significant effect of N-treatment was observed for the pine forest floor solution (Table 2).

Highest %BDOC values were observed in summer (Fig. 3), and the seasonal effect was significant, with higher %BDOC in summer than fall in both stands (Table 1). There was no significant effect of N-treatment on the %BDOC values for either stand (Table 2). Percent BDOC values in throughfall samples collected in September 1996 were higher than any %BDOC values in forest floor solution samples in both stands (Table 3).

Non-biodegradable DOC (NBDOC) was calculated for each forest floor solution sample. A significant difference in means of DOC found between the hardwood control plot and high-N plot disappeared when means of NBDOC were compared (Table 2).

3.3. Regression models predicting BDOC

For both pine and hardwood stands, simple linear regression models indicated significant positive relationships between BDOC concentration and ambient temperature, as well as between %BDOC and temperature (Table 4). Litterfall had the greatest and

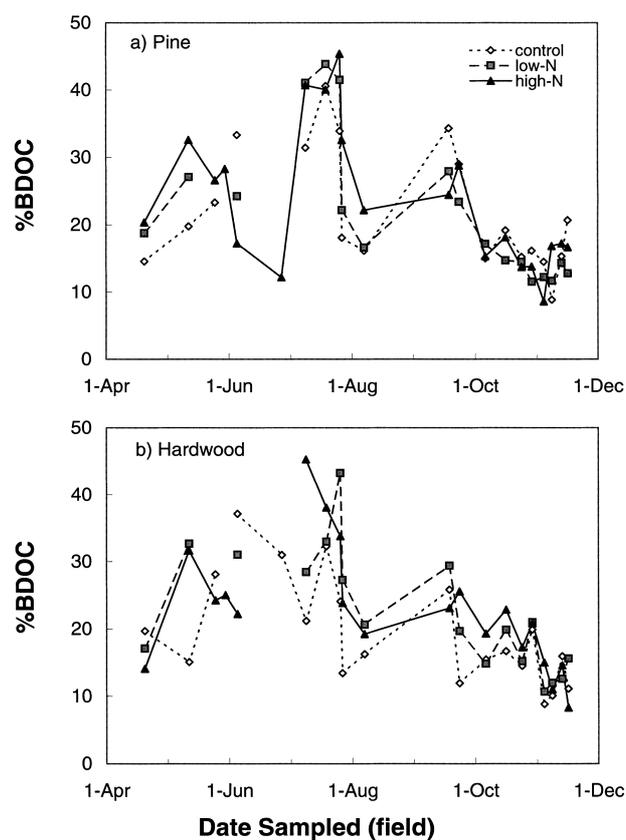


Fig. 3. %BDOC over time in the pine stand (a) and hardwood stand (b) at Harvard Forest. All samples were collected in 1995.

negative effect on both BDOC concentration and %BDOC for the hardwood forest floor solution (Table 5). A simple linear regression model for litterfall explained approximately 60% of the variation in %BDOC (Table 5, Fig. 4b). When temperature was incorporated into the model, it explained approximately 70% of the variation in %BDOC (Table 5). On the other hand, BDOC did not show any significant relationships with litterfall in pine forest floor solution (Fig. 4a). Carbon-to-nitrogen ratio of forest floor solution was negatively associated with both BDOC concentration and %BDOC values (Tables 4 and 5). Only 8–10% of variation in both BDOC concentration and %BDOC can be explained by C-to-N ratio of the solution (Tables 4 and 5).

Table 3
%BDOC of throughfall samples collected in 1996. All samples in the same plot were pooled before analysis

Date sampled (1996)	BDOC (%)	
	Pine stand	Hardwood stand
9 September	75.7	69.5
24 September	47.9	59.7

Table 4
Regression equations predicting BDOC (mg l⁻¹ or % of total DOC) in forest floor solution^{a, b}

Stand	Regression model	<i>r</i> ²	<i>n</i>
Pine	BDOC (mg l ⁻¹) = 0.53 (<i>T</i>) + 5.01	0.232***	58
	BDOC (mg l ⁻¹) = -0.103 (<i>CN</i>) + 17.8	0.096*	52
	BDOC (mg l ⁻¹) = 0.54 (<i>T</i>) - 0.116 (<i>CN</i>) + 8.89	0.336***	52
	%BDOC = 0.67 (<i>T</i>) + 10.9	0.187**	58
	%BDOC = -0.136 (<i>CN</i>) + 27.4	0.083*	52
	%BDOC = 0.81 (<i>T</i>) - 0.156 (<i>CN</i>) + 14.0	0.349***	52
Hardwood	BDOC (mg l ⁻¹) = 0.55 (<i>T</i>) + 1.45	0.285***	58
	%BDOC = 0.63 (<i>T</i>) + 10.6	0.211***	58
	%BDOC = -0.132 (<i>CN</i>) + 26.3	0.079*	57
	%BDOC = 0.74 (<i>T</i>) - 0.156 (<i>CN</i>) + 14.4	0.362***	57

^a Regression models for unpooled data. Independent variables are maximum daily temperature (*T*) and DOC-to-DON ratio in forest floor solution (*CN*). Only models with significant relationships are shown.

^b *Represent significance at *P* < 0.05, ***P* < 0.01, ****P* < 0.001.

4. Discussion

4.1. Biodegradability of DOC

Our finding of a seasonal pattern for %BDOC (highest values in summer) is consistent with the results of Qualls and Haines (1992), who measured BDOC in forest floor solution collected from a hardwood forest at Coweeta Hydrologic Laboratory, North Carolina. Using a batch culture method, they found that biodegradable DOC was ≈30% of total DOC in a single August forest floor solution sample, and ≈25% in a single February sample. On the other hand, Boyer and Groffman (1996) found no seasonal changes in the proportion of BDOC in water-extractable organic C in hardwood forest soils. The median %BDOC which they found using a batch culture method was ≈12%, considerably lower than our results (Table 1). The lower BDOC reported by Boyer and Groffman (1996) may be due to differences in the chemistry of soil solution collected in the field and that which is physically extracted in the lab. Lawrence and David (1996) found that DOC concentration and the ratio of DOC to inorganic N in solution varied con-

siderably in a comparison of lysimeter solution and lab extracts of forest floor.

Greater evapotranspiration in summer itself could concentrate soil solutions and result in higher BDOC values. However, this evapotranspiration effect was not the major cause for the higher BDOC concentrations in the summer in our study, because %BDOC values were also the greatest in the summer (Table 1, Fig. 3).

Release of NBDOC from the forest floor appears to be mainly controlled by abiotic chemical equilibrium between soil solution and extractable soil C pool. The difference in DOC concentrations which we observed between the control and high-N hardwood plots (Table 2) was due to a difference in BDOC, since there was no difference in NBDOC between those plots (Table 2). Additionally, the strong seasonal pattern found for BDOC in both stands (Table 1) disappeared (pine stands) or became weaker (hardwood stands) for DOC due to the weak seasonal changes for NBDOC (Table 1).

4.2. N-saturation and BDOC

Contrary to our hypotheses, concentrations of

Table 5
Regression equations predicting BDOC (mg l⁻¹ or % of total DOC) in forest floor solution^{a, b}

Stand	Regression model	<i>r</i> ²	<i>n</i>
Hardwood	BDOC (mg l ⁻¹) = -0.007 (<i>L</i>) + 17.5	0.364*	15
	BDOC (mg l ⁻¹) = 0.268 (<i>T</i>) - 0.006 (<i>L</i>) + 12.1	0.396*	15
	%BDOC = 0.949 (<i>T</i>) + 5.50	0.340*	15
	%BDOC = -0.011 (<i>L</i>) + 31.5	0.604***	15
	%BDOC = 0.567 (<i>T</i>) - 0.009 (<i>L</i>) + 20.0	0.710***	15

^a Regression models for BDOC data pooled by litterfall collection periods. Independent variables are (*T*) and total litterfall (kg ha⁻¹) for the collection period (*L*); the effect of (*CN*) is not significant. Only models with significant relationships are shown.

^b *Represent significance at *P* < 0.05, ***P* < 0.01, ****P* < 0.001.

BDOC increased or remained constant with N application (Table 2). Because heterotrophic microbial incorporation of inorganic N into soil OM is assumed to be the major process of N retention, Aber et al. (1993) hypothesized that the decreased N retention (as % of N input) in the pine high-N plot from the first 3 years (95%) to the first 6 years (85%) was caused by severe depletion of biodegradable C in the soil. Since the hardwood high-N plot retained 78% of inorganic N inputs (681 kg N ha^{-1}) in non-extractable soil pools during the first 6 years of the chronic-N addition and the pine high-N plot has shown indications of N-saturation since late 1989 (Magill et al., 1997), we expected to see the depletion of BDOC and DOC in both plots, with an especially strong effect in the pine plot. However, our results show the opposite, with no change in BDOC in the pine plot and increased BDOC in the hardwood high-N plot (Table 2).

Biodegradable DOC concentration in soil solution is determined by the balance between the production and consumption of BDOC in the forest floor. Thus, a decrease in microbial BDOC consumption could have resulted in the increased BDOC concentrations that we

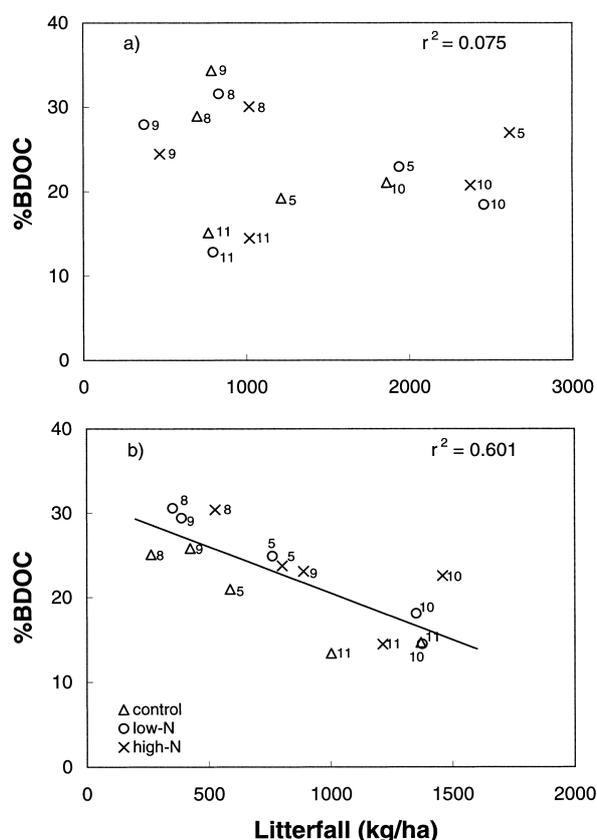


Fig. 4. The relationship of %BDOC and litterfall biomass for pine stand (a) and hardwood stand (b). Means of %BDOC data pooled by litterfall collection periods were used. Numbers next to the symbol indicate months where each litterfall collection period was set (e.g. May = 5, August = 8).

observed. Increases in available N in soil following a clear-cut or N fertilization may cause a shift of soil microbial communities from C economy (heterotrophic) to N economy (autotrophic) resulting in a greater net nitrate production (Sollins and McCorison, 1981). Data on the overall effects of N application on microbial activity at our site are inconclusive. Microbial respiration of sieved forest floor samples (i.e. respiration of soils without fine roots) collected in 1995 was significantly greater in the hardwood control than the pine control, and greater in the control plots than high-N plots for both stands (R. D. Boone, personal communication). These results, when combined with our observation of increased BDOC concentrations with N addition in the hardwood stand support the community-shift hypothesis (i.e., shifting toward N economy \rightarrow decrease in BDOC consumption \rightarrow decrease in soil microbial respiration and increase in BDOC concentration). However, with this hypothesis, we cannot explain the strong inorganic N retention into soil OM in the hardwood high-N plot (78% of inorganic N inputs) without inorganic N losses from the mineral soil (Magill et al., 1997). All of these can be explained if BDOC production increased with the chronic-N addition, and microbial activities associated with fine roots (i.e. mycorrhizal fungi and microbes associated with the rhizoplane or rhizosphere), which increased with the chronic-N addition in the hardwood stand and were removed prior to the soil microbial respiration measurement, were responsible for the retention of N measured in the field (Aber et al., 1998). Alternatively, significant N retention in both stands after 6 years of chronic-N addition ($\geq 85\%$ of total N input; Magill et al., 1997) without a decline in BDOC concentration in the forest floor provides support for abiotic N retention.

The significant difference in DOC between the control and the high-N plots for the hardwood stand (Table 2) is not consistent with results obtained for forest floor solution collected in 1994, when no significant difference in DOC concentration was found across N-treatment rates (Currie et al., 1996). This could be because the hardwood forest floor has changed slowly with chronic-N application, and the year when all ZTL samples for this study were collected (1995) was the first time that the potential change exceeded a threshold expressing an apparent change in DOC concentration in forest floor solution. In fact, McDowell et al. (1998) found that N application caused a significant change in DON concentrations for the period of 1993–1996, with high values first appearing in 1995. Magill et al. (1997) found that the net N-mineralization rate in both organic and mineral soils of the hardwood high-N plot had increased gradually since 1991, although no apparent changes in the chemistry of soil solution collected below the mineral hor-

izon were observed. All these results suggest that the activity or composition of the forest floor microflora might have been changing gradually with chronic-N addition.

4.3. Origin of BDOC

Below-ground inputs via roots (e.g., exudates, mucilage, and mucigel) appear to have primary control on BDOC in the forest floor. Single and multiple regression models for litterfall mass and temperature do not support the hypothesis that leaching of freshly fallen autumn litter is the major source of BDOC. Plant roots are known to excrete highly labile compounds, such as carbohydrates and amino acids (Smith, 1976; Paul and Clark, 1996; Eviner and Chapin, 1997). The amount of organic compounds released from roots is greater in the growing season (Paul and Clark, 1996), and the rates of root elongation increase with increased temperature (Tryon and Chapin, 1983) and the intensity of photosynthesis (Horváth et al., 1980). Differences in fine root mass among the N-treatments at our study site after 4 years of N application (Magill et al., 1997) are consistent with the idea that roots drive BDOC concentrations in soil solution. A significantly larger fine root mass in the hardwood high-N plot (713 kg ha^{-1}) than in the control plot (427 kg ha^{-1}) was associated with a greater concentration of forest floor BDOC in the hardwood high-N plot than the control plot (Table 2). No significant difference in fine root mass was found for the pine stand (332 kg ha^{-1} for the control and 308 kg ha^{-1} for the high-N plot; Magill et al., 1997), nor was there any significant difference in BDOC concentrations with the N application (Table 2).

A strong negative relationship between BDOC and litterfall for the hardwood stand across the N-treatment (Table 5) also suggests a significant contribution of recently photosynthesized C to BDOC in forest floor solution. The amount of litterfall would be a good indicator for the physiological state of forest vegetation, because litterfall is closely associated with environmental stress, and thus with the rate of photosynthesis. For instance, many plants respond to temperature and drought stress by senescence (Aber and Melillo, 1991). Carbon allocation of trees is controlled by physiological processes, such as photosynthesis, and aboveground and belowground net primary production are thought to be positively associated with each other in forest ecosystems (e.g., Nadelhoffer et al., 1985; Zak et al., 1994). Lower litterfall in the growing season (i.e. greater aboveground biomass during the growing season) observed in our study site would, therefore, indirectly indicate greater allocation of photosynthate below ground as fine roots and root

exudates during that time. The strong negative relationship between BDOC and litterfall found only for the hardwood forest floor solution (Table 5, Fig. 4) presumably reflects the stronger influence of season on the rate of photosynthesis for the hardwood (deciduous) stand than the pine (evergreen) stand, where photosynthesis occurs throughout most of the year.

Microbial breakdown of soil OM and microbial cells into smaller OM is thought to be a process that adds DOC to soil solution (Guggenberger et al., 1994; Huang et al., 1998). Because cell walls and cell membranes of microbes contain soluble carbohydrates that are known to be highly biodegradable (e.g., glucose and N-acetylglucosamine; Myrold, 1998), microbial breakdown of OM may also add BDOC to soil. Our data cannot distinguish the relative importance of microbial modification of OM on the total BDOC production, because contrary to litterfall (or fine roots), temperature is a variable that would influence both production and consumption of BDOC.

The extremely high %BDOC values in throughfall (Table 3) suggest that throughfall might also have an influence on BDOC in forest floor solution. Qualls and Haines (1992) found a greater proportion of biodegradable DOC in August throughfall ($\approx 60\%$) than that in forest floor solution ($\approx 30\%$). At Hubbard Brook Experimental Forest, NH, McDowell and Likens (1988) also found a greater proportion of carbohydrates in throughfall than in forest soil solutions (17.7% of total DOC for throughfall, 3.9–5.7% for soil solutions). Alternatively, much of the BDOC in throughfall could be consumed by free-living soil microbes in the forest floor as water moves through it (McDowell and Likens, 1988; Qualls and Haines, 1992) and thus may not be as important as BDOC produced in the forest floor.

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