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# Biodegradation of dissolved organic carbon in soil extracts and leachates from a temperate forest stand and its relationship to ultraviolet absorbance

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The amount and biodegradability of dissolved organic carbon (DOC) in forest floors can contribute to carbon sequestration in soils and the release of $CO_2$-C from soil to the atmosphere. There is only limited knowledge about the biodegradation of DOC in soil extracts and leachates due to the limitations inherent in degradation experiments. Differences in the biodegradation of DOC were studied in forest soil extracts using cold and hot water and 4 mmol/L $CaCl_2$ solution and in soil leachates sampled under different conditions over a wide range of DOC concentrations. From these results, we developed a simple and rapid method for determining the biodegradable organic C in forest floors. The hot water extracts and $CaCl_2$ extracts after $CH_3Cl$ fumigation contained higher concentrations of biodegradable organic C than the cold water extracts and $CaCl_2$ extracts before fumigation, with rapid DOC degradation occurring 24–48 h after incubation with an inoculum, followed by slow DOC degradation till 120–168 h into the incubation. During a 7-d incubation with an inoculum, the variation in DOC degradation in the different soil extracts was consistent with the change in special UV absorbance at 254 nm. Relatively higher levels of biodegradable organic C were detected in soil leachates from the forest canopy than in forest gaps between April and October 2008 ($P<0.05$). Relatively lower concentrations of DOC and biodegradable organic C were observed in soil leachates from N-fertilized plots during the growing season compared with the control, with the exception of the plot treated with $KNO_3$ at a rate of 45 kg N $ha^{-1}$ $a^{-1}$. Around 77.4% to 96.3% of the variability in the biodegradable organic C concentrations in the forest floors could be accounted for by the initial DOC concentration and UV absorbance at 254 nm. Compared with the conventional inoculum incubation method, the method of analyzing UV absorbance at 254 nm is less time consuming and requires a much smaller sample volume. The results suggest that the regression models obtained using the initial DOC concentration and UV absorbance can provide a rapid, simple and reliable method for determining the biodegradable organic C content, especially in field studies involving relatively large numbers of samples.

**biodegradable organic carbon, decomposition dynamics, dissolved organic carbon, dissolved organic matter, ultraviolet absorbance, soil extracts and leachates, inoculum incubation**

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Dissolved organic matter (DOM) is important in the nitrogen and carbon cycles of forest ecosystems [1], and it plays a significant role in many aspects of forest biogeochemistry. Exports of dissolved organic carbon (DOC) from the forest floor to the mineral soil range between 100 and 400 kg C $ha^{-1}$ $a^{-1}$ [2], accounting for between 5% and 30% of the above-ground litter input in temperate mature forests [3]. However, the DOC concentrations in deep soil horizons are

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typically low and the C output from mineral soil in leachate is relatively small (5–66 kg C $ha^{-1}$ $a^{-1}$) [4–7]. The difference in the fluxes of DOC as it is transported downward in leachate along the soil profile may result in an increase in soil C sequestration, although DOC with high proportions of hydrophilic materials can be decomposed by a wide range of soil bacteria and fungi [8]. In addition to the adsorption and complexation of DOC on the soil surface, soil microorganisms are important determinants of the fate of DOC in the soil. Consequently, the biodegradability and magnitude of the DOC input under forest stands can contribute to heterotrophic respiration in the surface soil and C sequestration in the mineral soil. To quantify the contribution of DOC to the microbially mediated flux of $CO_2$ from soil to the atmosphere and C sequestration in mineral soil, the biodegradable or effective DOC pool in forest floors and its responses to different anthropogenic disturbances and environmental changes needs to be measured. Albrechtova et al. [9] reported that the variation in forest floor DOC can be estimated using foliage spectral data, which is a very promising finding with developments in remote sensing-based modelling of forest floor DOC production across different regions. Consequently, the development of rapid methods for monitoring DOC and its degradation in forest floors is very important.

The biodegradation of DOC is defined as the use of organic compounds by soil microorganisms and can be quantified by the disappearance of DOC or by the evolution of $CO_2$ [8,10]. The most widely used method to measure DOC biodegradation is batch incubations with measurements of DOC disappearance. Recent studies have developed a standard laboratory-based inoculum incubation for the measurement of DOC degradation [11], but the incubation is laborious, especially for field studies involving relatively large numbers of samples. Furthermore, the use of different microbial inoculums with such an incubation method can cause problems when comparing the results of different studies [8,12,13]. The recommended standard method is designed to determine the potentially biodegradable DOC by eliminating possible nutrient limitations and adding a microbial inoculum, but it is not suitable for studying the responses of DOC biodegradation to site specific and temporal variations of other factors controlling microbial activity. Laboratory findings related to the biodegradability of DOC require verification under field conditions [14]. Hence, a rapid, simple and reliable method for determining the biodegradation of DOC sampled *in situ* under different forest management regimes would be a useful development for studies evaluating the controls on the biodegradability of dissolved organic matter in forest floors.

The chemical and structural characteristics of DOC in terrestrial and aquatic ecosystems are considered to be the most intrinsic factor controlling DOC biodegradability and are closely related to the extent and rate of DOC biodegradation in laboratory incubations. The spectroscopic properties of DOC such as UV absorbance and fluorescence can be used to characterize the dissolved humic substances in the soil and aquatic environment and their responses to changes in anthropogenic and natural conditions [15,16]. The UV absorption is a successful predictor of the aromatic C content of humic acids in soil and the aromaticity of DOC in soil solution and water samples [17,18]. The aromatic and hydrophobic structures of DOC that decrease its biodegradability can also be assessed by analyzing UV absorbance [10,19,20]. These results suggest that measuring both the UV absorbance and the DOC concentration would provide a rapid and useful method for determining the amount of biodegradable DOC in soil extracts and leachates sampled *in situ* under different forest management regimes.

The objectives of this study were to: (i) examine the dynamics of DOC biodegradation in forest soil extracts and compare the results with the UV absorbance of extracts at 254 nm following a laboratory-based inoculum incubation; and (ii) combine the UV data from forest soil extracts and leachates sampled under different forest management regimes and from forest gaps to develop a calibration model for estimating the biodegradation of DOC sampled *in situ* from forest ecosystems by considering a wide range of DOC concentrations and different environmental disturbances.

## 1 Materials and methods

### 1.1 Preparation of soil extracts and leachates from field experiments

To study the biodegradation of forest floor DOC and its relationship to UV absorbance, a portion of soils and leachates sampled in 2008 from field experiments under a Korean pine and broadleaf mixed forest in the Changbai Mountains, northeast China, were used. The conditions in this forest stand, and soil properties were reported by Xu et al. [7,21]. Soil leachates were collected at a soil depth of 15 cm from different forest management plots (e.g. C and N amendments, removal of litter) using purpose built zero-tension lysimeters attached to a funnel (12.5 cm in diameter).

In July 2006, we established an N-fertilized field experiment with four replicates, which was supplemented with a relatively low dose of added N in 2007. Aqueous solutions of N sources including $(NH_4)_2SO_4$, $NH_4Cl$ and $KNO_3$ were sprayed on the ground of four individual plots in equal monthly doses during the tree growing period at rates equivalent to 2.25 and 4.5 g N per $m^2$ each year, and with each application we also applied the equivalent of 5.0 mm rainfall. Tap water was added to the non-fertilized plot (control) without and with (blank) the removal of litter. The rates of N application correspond to equal and double the amount of local atmospheric wet N deposition at present [22]. A glucose addition treatment was also included in the field experiments, with glucose solution sprayed on the

ground at a rate equivalent to 64 kg C $ha^{-1}$ $a^{-1}$ in the same manner described for the N-fertilized experiments. This rate of glucose addition corresponds to approximately 25% of the amount of litter-C delivered to the soil annually, taking into account the amount of C released by litter decomposition annually and the $CO_2$ evolution involved [23,24]. Due to the higher concentrations of biodegradable dissolved organic C (BDOC) in soil leachates under the forest canopy than in forest gaps, three different forest gaps (diameter >10 m) and corresponding canopy zones were selected and zero-tension lysimeters were installed at a soil depth of 15 cm in each zone. Four replicate lysimeters were installed in each zone. Forest management regimes and forest gaps can result in differing soil conditions and soil microbial activities, which may affect the biodegradability and concentration of DOC in forest soils and leachates.

The top layer of the forest soils in each plot under different forest management regimes were sampled (in duplicate) during the tree growing period using a soil auger (3.3 cm in diameter and 10 cm in height), with the samples used for laboratory experiments. Sieved fresh soil was frozen at −20°C for no more than 2 months prior to measurement of the biodegradable DOC. To study the differences in DOC biodegradation in forest soil extracts, part of the soil samples from the field experimental plots were initially incubated at 25°C for 1 week, and then separately extracted with cold water and hot water (soil∶solution, 1∶5, w/w) at 80°C, and with a 4 mmol/L $CaCl_2$ solution (1∶5, w/w) both before and after $CH_3Cl$ fumigation. The low concentration $CaCl_2$ extraction provided a reasonable estimate of the DOC content in soil pore water [25], with a minimal effect on microorganisms due to the low osmotic pressure. All soil extracts were centrifuged at 7000× *g* for 10 min, and filtered through a cellulose-acetate membrane filter (0.45 μm pore size). The filtrate was immediately used for measurement of DOC biodegradation. Soil leachate samples from different forest management regimes and forest gaps were filtered through a cellulose-acetate membrane filter (0.45 μm pore size) and stored at −20°C prior to measurement of DOC biodegradation.

### 1.2 Measurement of DOC biodegradation and UV absorbance

To study the DOC biodegradation dynamics in soil extracts, soil samples from the N-fertilized and control plots were extracted with cold and hot water (80°C), and with a 4 mmol/L $CaCl_2$ solution before and after $CH_3Cl$ fumigation. These soil extracts were incubated in 60 mL tubes at 25°C in the dark for 7 d. The tubes were shaken by electrical stirring at 12 h intervals throughout the incubation period. The extracts were then inoculated with 1 mL of an inoculation suspension per 100 mL of extracts. This inoculum was prepared by shaking a mixture of 25 g of forest topsoil incubated at a water capacity of 60% for two weeks at 20°C with 50 mL of 4 mmol $L^{-1}$ $CaCl_2$ solution for 30 min, followed by centrifugation at 7000× *g* for 10 min. It was then used as the inoculation suspension. This method, which analyzed samples without added nutrients, aimed to compare the differences in the DOC biodegradation and UV absorbance of the soil extracts [11]. Three replicate incubation tubes for each extract were sub-sampled at 0, 6, 12, 18, 24, 48, 72, 96, 120 and 168 h after the incubation began to determine the changes in DOC concentrations and special UV absorbance at 254 nm (SUVA, a measure of aromaticity) of the extracts. The DOC concentrations and UV absorbance at 254 nm were measured using a TOC/TN-analyzer (Shimadzu TOC-$V_{CSH}$/TN, Kyoto, Japan) and a Unic 2800A spectrophotometer with a 1-cm path-length cell, respectively.

The specific ultraviolet absorbance at 254 nm was calculated from the UV absorbance at 254 nm divided by the DOC concentration (mg C $L^{-1}$) and the path length of the quartz cell of the spectrophotometer (cm), and is expressed as L $mg^{-1}$ C $cm^{-1}$. The amount of mineralized C (defined as the biodegradable dissolved organic C, BDOC) was calculated as the difference in DOC concentrations before and after incubation, providing the DOC mass loss during the incubation. The biodegradation of the inoculum C was determined from a control sample containing ultra pure water, inoculum and nutrients and subtracted from the other samples. A glucose solution with and without added N and P (at a C∶N∶P ratio of 100∶10∶1) was used to test the function of the microbial community.

To quantify the relationships between the BDOC and DOC concentrations of forest soil extracts and leachates and the UV absorbance at 254 nm, three replicates of each soil extract and leachate sample were placed in 60 mL tubes, inoculated and incubated at 25°C in the dark for 5 d, as described above. The DOC concentrations of the samples before and after the incubation were measured using a TOC/TN-analyzer (Shimadzu TOC-$V_{CSH}$/TN). The initial UV absorbance of the samples at 254 nm was measured using a Unic 2800A spectrophotometer with a 1-cm path-length cell.

### 1.3 Calculations and statistical analysis

The means and standard errors of the DOC and BDOC concentrations and the UV absorbance at 254 nm for all samples were calculated. Linear regressions of the DOC concentration in the soil extracts or glucose solutions against the incubation time were performed to calculate the rates of DOC biodegradation. Significant differences in the rates of DOC biodegradation between different soil extracts were identified at the $P < 0.05$ level using paired-sample *t*-tests. The soil extract and leachate data were used to derive a robust calibration model for estimating the BDOC concentration in forest floors by using the initial DOC concentration and UV absorbance at 254 nm before incubation.

## 2 Results

### 2.1 Changes in DOC biodegradation in soil extracts and glucose solutions

Approximately 120 h after the start of the inoculum incubation, more than 80% of the glucose-C with added N and P had been mineralized (Figure 1(a)). The mineralization of the inoculum carbon with added N and P was negligible in the control samples (ultrapure water, inoculum and nutrients). Thus, approximately 5 d were required for the inoculum incubation method to determine the BDOC concentration.

The DOC concentrations in the cold water extracts and $CaCl_2$ extracts of forest soils were much lower than those in the hot water extracts and $CaCl_2$ extracts after $CH_3Cl$ fumigation (Figure 1(b),(c)). The hot water extracts and $CaCl_2$ extracts after $CH_3Cl$ fumigation also contained higher concentrations of biodegradable DOC, with an initial rapid degradation in the first 24–48 h of the incubation, followed by a slow degradation rate to 120–168 h (Figure 1(b),(c) and Table 1). In the first 24 h after the incubation started, the DOC biodegradation rates in the $CaCl_2$ extracts of fumigated soils and in the glucose solution with added N and P were negligible, and were significantly lower than the rates in the hot water extracts ($P<0.05$) (Figure 1(b),(c) and Table 1). The initial short-term lag phase for DOC mineralization in the $CaCl_2$ extracts of fumigated soils and glucose solution was probably due to the initial limited number and activity of microorganisms. Overall, compared with the other soil extracts, the hot water extracts of forest soils had higher biodegradable DOC concentrations.

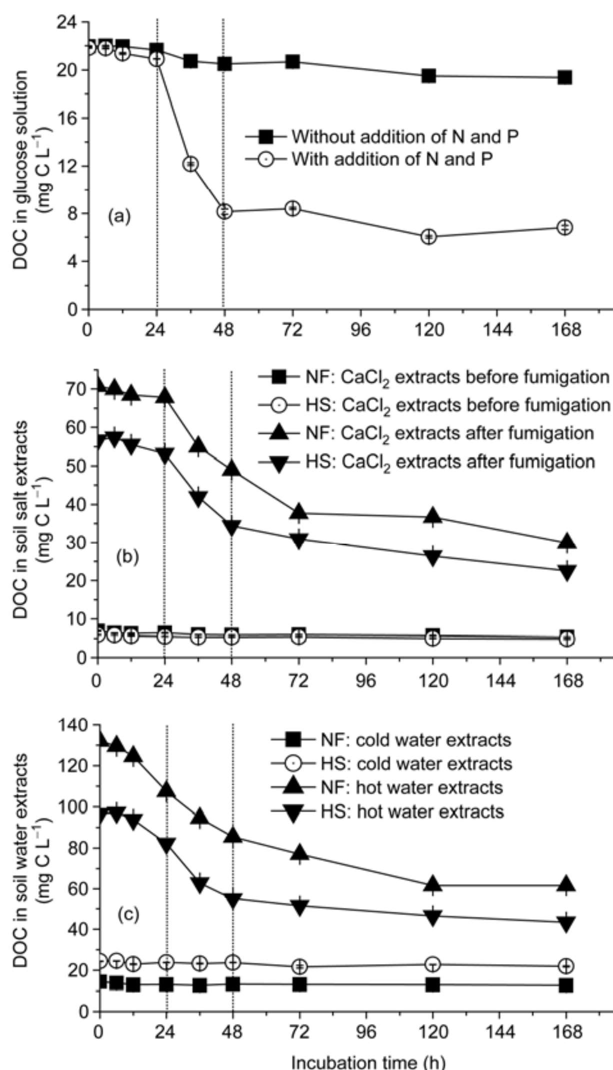


**Figure 1** Changes in DOC concentrations in (a) glucose solution with and without added N and P; (b) $CaCl_2$ soil extracts before and after $CH_3Cl$ fumigation; and (c) cold and hot water (80°C) soil extracts during the 168-hour incubation. NF and HS represent mixtures of non-fertilized and experimentally fertilized forest topsoil plots, respectively. Error bars represent the standard error of three replicates.

### 2.2 Changes in SUVA in soil extracts and glucose solutions

The cold water extracts and $CaCl_2$ extracts of the soils had initial higher SUVA values compared with the hot water extracts and $CaCl_2$ extracts of fumigated soils, but changes in the SUVA values of the cold water and $CaCl_2$ extracts during the 7-d incubation were negligible (Figure 2(a)–(c)). However, the SUVA values of the hot water extracts and $CaCl_2$ extracts of fumigated soils increased significantly between 24 and 72 h after the start of incubation and then remained relatively high (Figure 2(b),(c)).

The largest decrease in the biodegradability of DOC in the hot water extracts and $CaCl_2$ extracts of fumigated soils was consistent with the period of increased SUVA at 254 nm during the 7-d incubation (Figures 1 and 2). These results indicate an enrichment of the aromatic content during the biodegradation of DOC. Surprisingly, the cold water soil extracts had relatively high SUVA values (Figure 2(c)), suggesting that cold water soil extracts may not be good indicators of soil C bioavailability. The glucose solution without added N and P had consistently low SUVA values during the incubation, while the SUVA values of the glucose solution with added N and P significantly increased due to degradation after an initial 24 h delay (Figure 2(a)).

### 2.3 Relationships between BDOC, DOC and UV absorbance for soil extracts and leachates

As shown in Table 1, the DOC and BDOC concentrations in the hot water extracts and $CaCl_2$ extracts after $CH_3Cl$ fumigation were much larger than those in soil leachates collected at 15 cm depth. The BDOC concentrations were significantly higher in soil leachates under the forest canopy than those in forest gaps between April and October 2008 ($P<0.05$), but there were no significant differences in the DOC concentration between the two types of site (Table 1). Somewhat lower DOC and BDOC concentrations were

**Table 1** Slopes ($K$), coefficients of determination ($R^2$) and significance levels ($P$) for linear regressions of the DOC concentration (mg C $L^{-1}$) in soil extracts or glucose solutions against the incubation time (h)[a)]

| Soil extracts or solutions | Regression coefficients during different incubation periods | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0–24 h | | | 24–48 h | | | 48–168 h | | |
| | $K$ | $R^2$ | $P$ | $K$ | $R^2$ | $P$ | $K$ | $R^2$ | $P$ |
| Glucose solution: with added N and P | −0.039 (0.004) | 0.968 | 0.011 | −0.640 (0.100) | 0.952 | 0.099 | −0.025 (0.014) | 0.403 | 0.224 |
| NF: $CaCl_2$ extracts after fumigation | −0.123 (0.021) | 0.921 | 0.027 | −0.724 (0.146) | 0.887 | 0.038 | −0.158 (0.013) | 0.980 | 0.007 |
| HS: $CaCl_2$ extracts after fumigation | −0.143 (0.023) | 0.926 | 0.025 | −0.910 (0.062) | 0.991 | 0.044 | −0.090 (0.006) | 0.987 | 0.005 |
| NF: Hot water extracts | −0.825 (0.175) | 0.876 | 0.042 | −0.908 (0.093) | 0.979 | 0.065 | −0.329 (0.002) | 0.999 | 0.004 |
| HS: Hot water extracts | −0.694 (0.098) | 0.942 | 0.019 | −1.185 (0.285) | 0.890 | 0.150 | −0.096 (0.004) | 0.996 | 0.001 |

a) NF and HS represent mixtures of non-fertilized and experimentally fertilized forest topsoil plots, respectively. The standard error of three replicates is shown in parentheses.

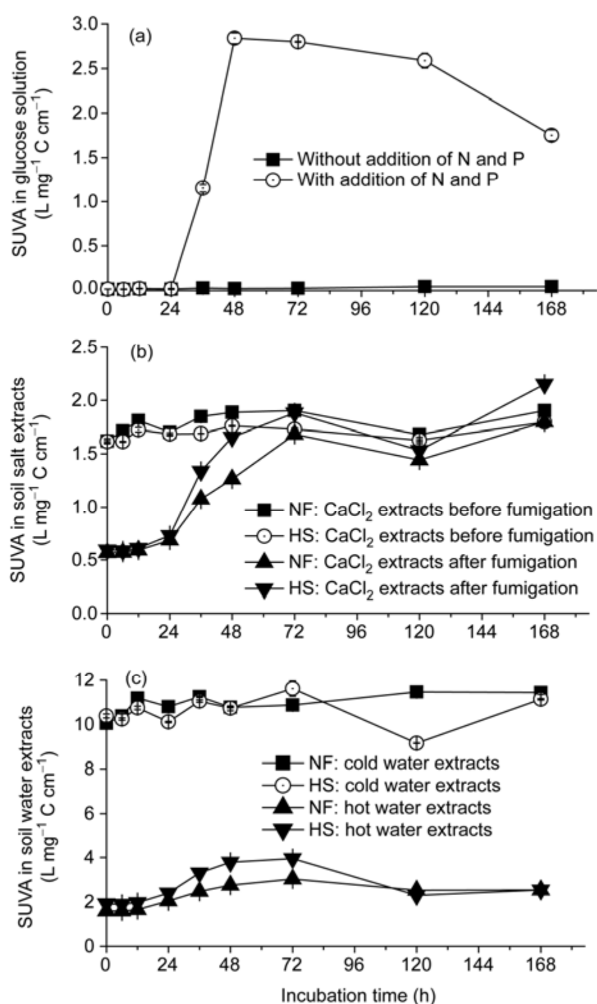


**Figure 2** Changes in SUVA values in (a) glucose solution with and without added N and P; (b) $CaCl_2$ soil extracts before and after $CH_3Cl$ fumigation; and (c) cold and hot water (80°C) soil extracts during the 168-h incubation. NF and HS represent mixtures of non-fertilized and experimentally fertilized forest topsoil plots, respectively. Error bars represent the standard error of three replicates.

observed in the soil leachates from the N-fertilized plots during the growing seasons compared with the control, except for the $KNO_3$-treated plot (45 kg N $ha^{-1}$ $a^{-1}$) and the October samples (Table 1).

For a wide range of DOC concentration, between 62% and 97% of the variability in the DOC concentrations of these soil extracts and leachates could be accounted for by the UV absorbance at 254 nm (Figure 3(a)). The decrease in DOC concentration after incubation was considered to be the BDOC and was linearly correlated with the UV absorbance at 254 nm of the extracts prior to incubation (Figure 3(b)). Between 28% and 78% of the variability in the BDOC concentrations of these soil extracts and leachates could be accounted for by the UV absorbance (Figure 3(b)). When the initial DOC concentration was incorporated into the UV-based regressions as a covariate, the coefficients of determination increased and the standard errors of the estimates decreased. Consequently, a robust calibration model for estimating BDOC concentrations can be recommended for the forest soil extracts and leachates as follows:

If the initial DOC concentration is more than 50 mg $L^{-1}$,

$$y=-(0.674\pm0.159)+(0.012\pm0.003)a+(0.007\pm0.008)b, \quad (1)$$
$$R^2=0.774,\ n=28,\ P<0.0001$$

and if the initial DOC concentration is less than 50 mg $L^{-1}$,

$$y=-(0.069\pm0.031)+(0.037\pm0.002)a+(0.038\pm0.030)b, \quad (2)$$
$$R^2=0.963,\ n=33,\ P<0.0001$$

where $y$ is the BDOC concentration in the forest soil extracts or leachates (mg C $mL^{-1}$), and $a$ and $b$ represent the initial DOC concentration (mg C $mL^{-1}$) and UV absorbance at 254 nm ($cm^{-1}$) of the soil extracts or leachates, respectively.

## 3 Discussion

Approximately 70% of the glucose-C was degraded in 2–3 d in this study while Qualls and Haines [26] and Bourbonniere and Creed [27] reported 66% and 75% degradation of glucose-C in the 7 and 5 d of their studies, respectively. Bowen et al. [28] reported that the DOC mass loss after 70 d

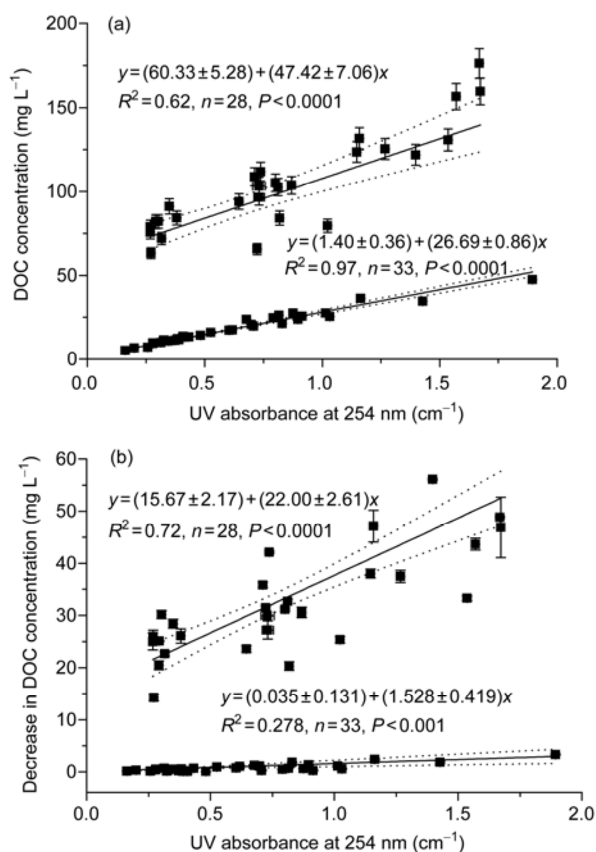


**Figure 3** Relationships between (a) the initial DOC concentrations of soil extracts and leachates; and (b) the decrease in DOC concentrations during the 5-d incubation, against the initial UV absorbance at 254 nm before the incubation. The solid lines represent linear regressions, while the dash lines show the upper and lower 95% confidence limits.

incubation was equivalent to 96% of the initial DOC in the glucose solution culture. These different glucose degradation rates can be attributed to different inoculums and incubation conditions. Normally, a glucose solution with added N and P is used to assess the ability of a microbial community for DOC degradation. More than 70% degradation of glucose-C after a short-term incubation indicates good microbial effectiveness of the inoculums in determining the biodegradable organic C in soil extracts and leachates.

The hot water extracts had relatively higher concentrations of biodegradable organic C compared with the other soil extracts, with no lag phases at the start of the incubation (Figure 1(c)). This can be explained by the fact that hot water extracts contain substantial amounts of labile and bioavailable C such as carbohydrates [29–31]. Hot water extractable C is considered to be one of the most sensitive indicators of soil C availability under different forest sites and management regimes [31–34]. Chloroform fumigation of soil lyses the cells of soil microorganisms and makes their contents partially extractable by $CaCl_2$ solution. The release of soil components of microbial origin due to fumigation [35] can result in a relatively low SUVA in the fumigated soil $CaCl_2$ extract (Figure 2(b)), and initial DOC biodegradation in these extracts was delayed for 24 h probably because of the initially limited number and activity of microorganisms. However, within 24–48 h after the start of incubation there were no significant differences in the rates of DOC degradation between the hot water extracts and the $CaCl_2$ extracts of fumigated soils (Table 1). Unfortunately, the microbial mechanisms involving the biodegradation of DOC in soil extracts are unclear at present. The differences in the characteristics of DOC biodegradation (Figure 1 and Table 1) for the different extracts suggest different responses by the microbial communities to different labile organic C compositions. Due to a lack of information, we were not able to understand the role of bacteria and fungi in DOC biodegradation in soil leachates sampled from different forest stands and seasons.

A significant portion of the C input from above-ground litter can be translocated as DOC, but the degradability of DOC and its role as a substrate for microbial metabolism is still unclear. Our studies showed that 20%–50% of the DOC in the forest soil extracts during the 5-d incubation was biodegradable (Table 2), which is consistent with previous studies that have reported DOC degradation within the range from 10% to 88% measured over periods ranging from a few days to months [10,36]. Less than 10% of the DOC present in forest soil leachates was biodegradable after the 5 day incubation (Table 2), which is in agreement with the results reported by Qualls and Haines [26] and by Jandl and Sletten [37]. However, the proportion of biodegradable DOC in soil solutions was lower than that reported by other researchers [12,38], who identified that 10%–30% of the dissolved organic matter in soil solutions was microbially degradable. The different rates of DOC degradation mainly result from the different sources of DOC, specific site conditions and experimental approaches.

The biodegradable DOC concentration in forest soil leachates is determined by the balance between the production and consumption of BDOC in forest floors. The higher BDOC concentrations in soil leachates under the forest canopy than in forest gaps in the growing season (Table 2) resulted from microbial activities associated with fine root structures. The amount of organic compounds released from roots is greater in the growing season [39], and the rates of root elongation increase with increased temperature [40] and intensity of photosynthesis [41]. Consequently, the difference in the fine root biomass between the forest canopy and forest gaps supports the idea that roots are one of the main sources of BDOC in soil leachates. The inputs of N into forest floors and the microbial use of N may lead to a decrease in the biodegradable soil C concentration, due to increased microbial demand for C to assimilate the N [42,43]. However, unlike the plots treated with ammonium-N, there were higher DOC and BDOC concentrations in soil leachates from the $KNO_3$-treated plots in the growing seasons than from the control (Table 2). This is consistent with the

**Table 2** Initial DOC concentrations and UV absorbances at 254 nm for soil extracts and leachates, and the change in DOC concentrations after 5-d incubation[a)]

| No. | Treatments | $UV_{254}$ ($cm^{-1}$) | Initial DOC | Decrease in DOC |
|---|---|---|---|---|
| | | | (mg/L) | |
| 4 mmol/L $CaCl_2$ soil extracts after $CH_3Cl$ fumigation | | | | |
| 1 | 9Blank | 0.303(0.001) | 82.13(0.51) | 30.18(1.50) |
| 2 | 9Control | 0.349(0.024) | 91.07(0.31) | 28.44(1.70) |
| 3 | 9H$KNO_3$ | 0.268(0.010) | 78.94(0.94) | 25.02(3.24) |
| 4 | 9H$NH_4Cl$ | 0.271(0.009) | 63.20(0.42) | 14.23(1.21) |
| 5 | 9H$(NH_4)_2SO_4$ | 0.292(0.004) | 81.90(0.22) | 20.47(1.71) |
| 6 | 9L$KNO_3$ | 0.316(0.004) | 71.97(1.17) | 22.70(0.90) |
| 7 | 9L$NH_4Cl$ | 0.269(0.013) | 75.57(1.65) | 25.99(2.41) |
| 8 | 9L$(NH_4)_2SO_4$ | 0.293(0.011) | 82.12(0.88) | 25.10(0.24) |
| 9 | 9Glucose | 0.381(0.019) | 84.27(1.09) | 26.10(2.74) |
| 10 | 10Blank | 0.712(0.010) | 108.76(0.06) | 35.84(1.06) |
| 11 | 10Control | 0.869(0.017) | 103.76(0.98) | 30.59(2.05) |
| 12 | 10H$KNO_3$ | 0.801(0.001) | 105.19(0.55) | 31.20(0.62) |
| 13 | 10H$NH_4Cl$ | 0.819(0.012) | 84.13(0.27) | 20.32(1.64) |
| 14 | 10H$(NH_4)_2SO_4$ | 0.731(0.046) | 96.67(1.83) | 27.22(3.46) |
| 15 | 10L$KNO_3$ | 0.730(0.052) | 103.22(0.30) | 29.98(0.12) |
| 16 | 10L$NH_4Cl$ | 0.646(0.015) | 94.01(0.37) | 23.61(1.21) |
| 17 | 10L$(NH_4)_2SO_4$ | 0.812(0.013) | 102.73(0.67) | 32.72(0.86) |
| 18 | 10Glucose | 0.738(0.025) | 111.82(0.12) | 42.09(0.49) |
| Soil hot water extracts at 80°C | | | | |
| 19 | 8Blank | 1.268(0.006) | 125.35(0.45) | 37.46(2.36) |
| 20 | 8Control | 1.147(0.001) | 123.60(0.00) | 38.03(1.69) |
| 21 | 8H$KNO_3$ | 1.570(0.018) | 156.50(0.30) | 43.65(2.25) |
| 22 | 8H$NH_4Cl$ | 1.023(0.016) | 79.58(0.71) | 25.36(1.21) |
| 23 | 8Glucose | 1.536(0.015) | 130.80(0.90) | 33.30(0.93) |
| 24 | 9Blank | 1.668(0.010) | 176.20(0.10) | 48.85(0.45) |
| 25 | 9Control | 1.158(0.005) | 131.65(0.15) | 47.10(6.12) |
| 26 | 9H$KNO_3$ | 1.673(0.000) | 159.60(0.90) | 46.88(11.64) |
| 27 | 9H$NH_4Cl$ | 0.723(0.014) | 65.58(0.26) | 31.46(0.81) |
| 28 | 9Glucose | 1.398(0.006) | 121.90(0.40) | 56.10(0.33) |
| Soil leachates collected using zero-tension lysimeters | | | | |
| 29 | 4ForestGap | 0.327(0.002) | 11.34(0.11) | 0.20(0.01) |
| 30 | 4ForestIn | 0.816(0.001) | 26.28(0.13) | 0.66(0.05) |
| 31 | 5ForestGap | 0.372(0.002) | 11.28(0.01) | 0.14(0.02) |
| 32 | 5ForestIn | 0.313(0.001) | 9.80(0.03) | 0.63(0.00) |
| 33 | 7ForestGap | 0.915(0.003) | 25.58(0.17) | 0.24(0.32) |
| 34 | 7ForestGap | 0.383(0.006) | 12.07(0.11) | 0.52(0.38) |
| 35 | 7ForestIn | 0.830(0.004) | 21.17(0.13) | 1.82(0.12) |
| 36 | 7ForestIn | 0.896(0.002) | 23.80(0.26) | 1.30(0.12) |
| 37 | 10ForestGap | 0.482(0.001) | 14.23(0.17) | 0.01(0.05) |
| 38 | 10ForestIn | 0.698(0.005) | 20.61(0.10) | 1.05(0.39) |
| 39 | 4Blank | 0.345(0.000) | 10.88(0.25) | 0.47(0.11) |
| 40 | 4Control | 0.433(0.002) | 13.36(0.09) | 0.64(0.02) |
| 41 | 4H$KNO_3$ | 0.408(0.002) | 13.62(0.05) | 0.17(0.51) |
| 42 | 4H$NH_4Cl$ | 0.279(0.000) | 9.29(0.02) | 0.33(0.10) |
| 43 | 4H$(NH_4)_2SO_4$ | 0.200(0.001) | 6.65(0.02) | 0.25(0.15) |
| 44 | 5-6Blank | 0.525(0.001) | 15.87(0.11) | 0.86(0.37) |
| 45 | 5-6Control | 0.618(0.002) | 17.40(0.05) | 0.98(0.56) |
| 46 | 5-6H$KNO_3$ | 1.014(0.002) | 27.58(0.30) | 1.09(0.28) |
| 47 | 5-6H$NH_4Cl$ | 0.280(0.002) | 9.73(0.15) | 0.46(0.08) |
| 48 | 5-6H$(NH_4)_2SO_4$ | 0.707(0.001) | 19.66(0.09) | 0.24(0.20) |
| 49 | 5-6L$KNO_3$ | 0.407(0.000) | 13.04(0.10) | 0.04(0.16) |
| 50 | 5-6L$(NH_4)_2SO_4$ | 0.324(0.004) | 11.18(0.21) | 0.12(0.08) |
| 51 | 7-8Blank | 0.604(0.002) | 17.21(0.32) | 0.70(0.35) |
| 52 | 7-8Control | 1.427(0.001) | 34.63(0.31) | 1.78(0.45) |
| 53 | 7-8H$KNO_3$ | 1.894(0.002) | 47.47(0.09) | 3.30(0.35) |
| 54 | 7-8H$NH_4Cl$ | 1.032(0.002) | 25.42(0.27) | 0.54(0.21) |
| 55 | 7-8H$(NH_4)_2SO_4$ | 0.391(0.002) | 11.52(0.09) | 0.01(0.03) |
| 56 | 7-8L$KNO_3$ | 0.257(0.002) | 7.08(0.02) | 0.11(0.03) |
| 57 | 7-8L$NH_4Cl$ | 0.162(0.001) | 5.11(0.02) | 0.09(0.01) |
| 58 | 10Control | 0.791(0.002) | 24.62(0.25) | 0.48(0.15) |
| 59 | 10H$KNO_3$ | 1.163(0.005) | 36.16(0.26) | 2.38(0.34) |
| 60 | 10H$NH_4Cl$ | 0.876(0.000) | 27.58(0.06) | 0.61(0.09) |
| 61 | 10H$(NH_4)_2SO_4$ | 0.678(0.001) | 23.68(0.12) | 1.21(0.08) |

a) The initial numbers for each treatment indicate the sampling months for soil samples and soil solution mixtures; H and L represent 45 and 22.5 kg N $ha^{-1}$ $a^{-1}$ for N-fertilized plots, respectively; ForestGap and ForestIn represent plots in forest gaps and beneath the forest canopy, respectively.

results reported by Liu and Greaver [44], who found that the soil DOC concentration was significantly increased (by 116%) by the addition of $NO_3^-$. Similarly, Evans et al. [45] found that the addition of $NaNO_3$ consistently increased the soil DOC concentration while the addition of $NH_4^+$ salts tended to decrease DOC at their 12 study sites. They hypothesized that the change in soil DOC was probably associated with the effects of different N forms on soil acidification. Consequently, the change in soil properties such as acidity and the responses of fine root growth and litter decomposition to N amendments [12,46–48] could have a significant impact on the DOC and BDOC concentrations in soil leachates. Further research is required to explain how these factors can affect the concentration and biodegradation of DOC in soil leachates sampled under N-fertilized forest floors.

The characteristics of DOC with aromatic and hydrophobic structures can be assessed by the UV absorbance [10, 18–20]. For a wide range of DOC concentrations, the different UV absorbance at 254 nm could explain 62% and

97% of the variation in DOC concentrations in soil extracts and leachates, respectively (Figure 3(a)). This result supports our hypothesis that a UV-based method can rapidly determine the amount of DOC in soil extracts and leachate sampled under forest floors, especially for field studies involving relatively large numbers of samples. The soil hot water extracts and $CaCl_2$ extracts of fumigated soils had high levels of DOC mineralization, and differed markedly from the other soil extracts due to their greater initial specific UV absorbance values (Figures 1 and 2). In several studies, the extent of DOM degradation was inversely related to the UV absorbance and the size of the hydrophobic fraction [26,37, 49,50]. It seems reasonable to assume that the differences in the biodegradation of DOC in soil extracts and leachates were caused by differences in the DOC composition. The strong correlation between the UV absorbance at 254 nm for the soil extracts and leachates, and the biodegradation of DOC supports the findings of Kalbitz et al. [10] and confirms the suitability of a spectroscopic method such as UV absorbance to rapidly estimate the biodegradability of DOC in forest floors.

In summary, the hot water soil extracts had relatively high biodegradable organic C contents and can be considered a good indicator of soil C bioavailability. The UV absorbance at 254 nm in combination with the initial DOC concentration could provide a rapid, simple and reliable method for determining the biodegradable organic C in forest soil extracts and leachates. As site-specific factors such as vegetation, land use and the seasonality of meteorological parameters can affect DOM composition and its biodegradability, a wide range of DOM sources sampled from different sites should be considered before any such UV-based method for determining the biodegradable organic C is used for all soil extracts and leachates.

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