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REGULAR ARTICLE

Quantification and FTIR characterization of dissolved organic carbon and total dissolved nitrogen leached from litter: a comparison of methods across litter types

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Abstract
Background and aims Quantification and characterization of dissolved organic matter (DOM) leached from leaf litter in the laboratory may well depend on the method used to leach the litter. However, we lack a comparative assessment of the available methods. Here, we test how: i) four commonly used methods to leach plant litter, ii) cutting of the litter, and iii) litter species affect the quantity and composition of dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) leached using fourier transform mid-infrared spectroscopy (FTIR).
Methods We tested how soaking litter in water, dripping water over litter, and shaking litter in two different volumes of water affected leaching of both cut and whole leaves of alfalfa (*Medicago sativa*), ash (*Fraxinus excelsior*), big bluestem grass (*Andropogon gerardii*), oak (*Quercus macrocarpa*) and pine (*Pinus ponderosa*) litter. We measured DOC and TDN on the leachate to quantify how much DOM was leached by each method. We used the DOC:TDN ratio and FTIR to analyze the composition of the DOM leached.
Results The leaching method and cutting had an impact on the amount of DOM leached from the litter. The amount of DOM leached was also affected by the litter species and its interaction with leaching method and cutting. FTIR analysis identified the same main functional groups of plant litter leachates across all of the litter species. Leaching method, cutting and litter type affected the concentration of the leachate and the resolution of the FTIR spectral data but not the relative contribution of the main functional groups.
Conclusions Methods of leaching should be chosen consistently with experimental objectives and type of litter examined. The leaching method, cutting of the litter and litter species should be taken into consideration when comparing data on DOM amounts obtained from different leaching methods but the leachate consists of similar functional group components across method, cutting and litter species.

Keywords Litter decomposition · Leaching methods · Dissolved organic matter · Dissolved organic carbon · Total dissolved nitrogen · FTIR · Mid-infrared spectroscopy

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Introduction

Leaf litter decomposition is one of the main processes by which organic carbon (C) and nitrogen (N) from plant biomass enter the soil, where they can be stored long term as soil organic matter (SOM) or decomposed completely back to their mineral forms. Litter decomposition occurs through: i) catabolism of litter C and N to CO_2 and NH_4^+, ii) litter fragmentation, and iii) leaching of soluble litter and microbial components to the soil with water (Swift et al. 1979). The leaching of dissolved organic matter (DOM) from leaf litter during precipitation events plays a significant role in terrestrial biogeochemistry (Neff and Asner 2001). Leaves from different plant species have been found to leach DOM in different amounts and with different DOM chemical quality for biogeochemical reactivity in the soil (Wieder et al. 2008; Cuss and Gueguen 2013; Uselman et al. 2012). Estimates of litter dissolved organic carbon (DOC) leaching range from 6 to 39 % of litter C losses during decomposition (Qualls and Haines 1991; Magill and Aber 2000; Don and Kalbitz 2005). Most of the leaf litter soluble components are leached out soon after abscission, with anywhere from 0.08 to 2.11 % of initial dry biomass lost in a single leaching event (Cleveland et al. 2004). Thus, the initial fast phase of litter decomposition is likely dominated by leaching losses (Gimenes et al. 2013; Magill and Aber 2000; Cheever et al. 2013).

While the importance of DOM leaching is becoming more widely recognized, we still lack a thorough evaluation of the laboratory methods used to quantify it. We believe that, in order to better understand the controlling factors of litter DOM leaching across studies employing different leaching methods, it is necessary to know how the methods used to leach DOM from litter affect the quantification and characterization of leachates. Various methods for leaching litter in the laboratory have been used and are currently published, but thus far they have not been directly compared in the literature. In order to advance the study of DOM leaching from litter and to compare results across studies it is important to understand whether leaching methods affect DOM quantity and composition. Most commonly, litter leachate collection occurs in the laboratory due to the challenges of in situ field collection and the rapid decomposition of DOM generated in the litter layer (Cleveland et al. 2004; Corrigan and Oelbermann 2013). In the laboratory, DOM has been leached from litter by (1) soaking of cut (e.g., Cleveland et al. 2004; Magill and Aber 2000) and whole (Don and Kalbitz 2005; Nykvist 1962) leaves in water, (2) gentle shaking of litter in water (e.g., Wallenstein et al. 2010; Bowen et al. 2009; Fellman et al. 2013), or (3) dripping water over litter samples, in an attempt to better simulate leaching during a precipitation event (e.g., Hansson et al. 2010) . Hot water and salts can be used to extract more components from organic matter into solution, but these extracts are not directly comparable to DOM leaching and therefore are not considered in this study (Landgraf et al. 2006; Nkhili et al. 2012; Jones et al. 2012).

Soaking, shaking and dripping water over leaves to leach DOM may affect not only the quantity of DOM leached, but also the components of litter released as DOM. The amount of time that the litter is in contact with the water, the abrasiveness of that contact as well as the litter-to-water ratio could affect the DOM concentration and its suitability for chemical charactarization. Additionally, whether whole (Don and Kalbitz 2005) or cut (Magill and Aber 2000; Cleveland et al. 2004) leaves are used for leaching may affect what components are released into the water for some types of litter due to the increased surface area available for leaching (Nykvist 1962). The leaching method of choice in any one study may reflect either the need for overall high DOM concentrations for spectral or other concentration dependent analyses, or the characterization of DOM to mimic field conditions and DOM-soil interactions. An understanding of how the methods of DOM leaching in the laboratory affects the concentration and composition of DOM leachate will help inform that choice and in cross comparisons of litter leachate studies. Furthermore, an examination of how leaching methods affect DOM from a broad range of litter types will test the widespread applicability of laboratory leaching methods across different ecosystems.

In this study, we will quantify and characterize DOM leached by different methods for a variety of litter types. DOM quantity is measured as DOC and total dissolved nitrogen (TDN) concentration in the leachate, while characterization is described based on the DOC:TDN ratio and functional groups of the compounds leached using fourier transform mid-infrared (FTIR) analysis. FTIR is a sensitive and inexpensive method for analyzing the chemistry of organic matter, and has been used successfully to study the functional group distribution in decomposing litter (Gallo et al. 2005), and extensively on freeze-dried aqueous extracts of soils and litters (He et al. 2011a, b, 2012; Kaiser and Ellerbrock 2005; Kaiser et al. 2007; Peltre et al. 2011). FTIR is particularly useful to gain information regarding the aromaticity of DOM (Strobel et al. 2001), or in detecting spectral features in the extracts such as 3,400 cm^{-1} O–H/N–H stretching, 2,936 cm^{-1} aliphatic C–H stretching, the 1,605 cm^{-1} band for aromatic C=C vibrations, COO− stretching, and/or H-bonded C=O in conjugated ketones, and the peak at 1,070 cm^{-1} for polysaccharide-like absorbance (He et al. 2009). However, correlation of these features with DOC and TDN concentrations would help in providing an ecological interpretation of the spectral features. Water extracts are thought to contain the labile organics from environmental samples, so the FTIR data from this fraction has also been used to observe changes in aliphatic and proteinaceous functional groups during composting (He et al. 2011a). FTIR analysis thus provides an informative characterization of the composition and reactivity of litter leachate, and can improve our understanding of how the leaching method, cutting of litter and litter species affects the functional composition and potential fate of DOM leached (Oren and Chefetz 2012).

Our main research question was, how do the leaching method, cutting and litter types affect DOM leaching quantity and chemical composition across a range of litter types? We tested four leaching methods, on both cut or whole litter samples, from five plant species ranging in C:N ratios. By comparing dripping water over litter vs. soaking litter in water, we tested the effect of time of water and litter contact. By comparing soaking litter in water vs. shaking litter in water, we tested the effect of abrasion. We tested the effect of litter-to-water ratio by shaking the litter in two different volumes of water. Finally, we tested the effect of cutting by leaching whole and cut leaves in three of the four methods. We used FTIR and DOC:TDN to assess the chemistry of leachates. We hypothesized that, 1) the leaching method and cutting of litter affects the amount of DOM leached from the litter, with shaking and cutting dissolving more components than dripping and whole leaf leaching, 2) leachate chemistry is not affected by leaching methods, but litters with a higher C:N ratio will leach less DOM overall and contain more C-rich compounds such as carbohydrates and aromatics, and 3) the leaching methods with better extraction efficiency will result in a more concentrated extract that in turn will result in FTIR spectra with better band definition and spectral quality. By addressing this question we hope to provide a basis for comparison of DOM studies across different laboratories and studies.

Methods

Litter samples

For this study we used five litter types: alfalfa (*Medicago sativa*), ash (*Fraxinus excelsior*), big bluestem grass (*Andropogon gerardii*), oak (*Quercus macrocarpa*) and pine (*Pinus ponderosa*), representing a range of litter quality in terms of %N, C:N ratios and % lignin (Table 1). We collected the leaf and needle litters in the fall of 2011 as freshly senesced litter that had not hit the ground and had not been rained on since abscission. However, mature alfalfa leaves were standing and still green when collected. Abscised ash leaves and pine needles were collected from separate raised litter traps. Senesced, standing big bluestem was hand cut from a native tallgrass prairie. Senesced oak leaves were shaken off of a tree and collected in a litter trap. We removed all stems and petioles from the litter. We pooled the litter samples by species and air-dried them. We ground three subsamples from each litter pool for elemental analysis as described below.

Leaching methods

We tested four leaching methods, each on four replicate sub-samples of the air-dried litter samples, for all litter types, with either whole or cut litter. All cut litter samples were cut into 1 cm×1 cm pieces, or 1 cm lengths (pine needles and bluestem grass blades), and homogenized. A blank, with no litter, was also added to each method to account for any background C and N on our equipment. For the 'Soak' method, 1 g of cut or whole litter was soaked in 70 ml of deionized water in an acid washed 250 ml beaker for 1 h. For the 'Shake 70 ml' and 'Shake 30 ml' methods, 1 g of cut or whole litter was placed in an acid washed 250 ml beaker with 30 or 70 ml deionized water and shaken for 1 h on an orbital shaker at 1 rpm. The DOM from the Soak, Shake 70 ml and Shake 30 ml methods were collected by filtering the samples over a 20 μm ash free (Whatman #41) filter and freezing it at −5 °C. For the 'Drip' method, 1 g of cut or whole litter was placed on an acid washed funnel fitted with a 20 μm ash free (Whatman #41) filter and 70 ml of deionized water was slowly dripped evenly over the

Table 1 Initial litter carbon, nitrogen, C:N and lignin concentrations

Litter Type	%C	%N	C:N	% Lignin
Alfalfa (*Medicago sativa*)	44.1 (0.026)	4.09 (0.012)	10.8 (0.035)	5.63 (0.345)
Ash (*Fraxinus excelsior*)	46.5 (0.608)	0.884 (0.012)	52.6 (0.139)	10.03 (0.109)
Bluestem (*Andropogon gerardii*)	44.1 (0.049)	0.478 (0.003)	92.2 (0.637)	8.42 (0.522)
Oak (*Quercus macrocarpa*)	47.6 (0.038)	1.32 (0.010)	34.0 (0.286)	18.80 (0.219)
Pine (*Pinus ponderosa*)	52.3 (0.085)	0.413 (0.007)	126.9 (2.120)	24.39 (0.302)

Values are means of three laboratory replicates, with standard errors in parentheses

entire sample at a rate of 23 ml/min. The DOM from the Drip method was also frozen at −5 °C until further analysis. In these four methods, we tested how the length of time of litter and water contact (Drip vs. Soak), the abrasiveness of contact (Soak vs. Shake) and the litter to water ratio (Shake 30 ml vs. Shake 70 ml) affected DOM leaching. Additionally for the Soak, Shake 70 ml and Drip methods we compared DOM leaching of both whole and cut litters for all litter species, except alfalfa, whose leaves are already approximately 1 cm×1 cm. Only cut (other than whole alfalfa leaves) litter was used in the Shake 30 ml method because this volume of water was not great enough to cover the entire whole leaf samples.

Chemical analysis

We analyzed all initial litter samples for %C and %N on a solid-state elemental analyzer (LECO Tru-SPEC, St. Joseph, MI). We measured % lignin content of the litters as the mass that was resistant to digestion in 73 % sulfuric acid, according to the Van Soest and Wine (1968) acid detergent fiber digestion method. All leachate samples were thawed and analyzed on a Shimadzu TOC analyzer (Shimadzu TOC 5000) for DOC and TDN. Leachates were prepared for Mid-infrared spectroscopy (FTIR) analysis by adding 0.5 g KBr to 250 µl of leachate, then freeze-drying the mixture. The dried leachates, in KBr, were scanned using a Digilab FTS 7000 spectrometer (Varian, Inc., Palo Alto, CA) with a Pike AutoDIFF sampler (Pike Technologies, Madison, WI). The scans were done on the mid-infrared (FTIR) from 4,000 to 400 cm^{-1}, 4 cm^{-1} resolution, and each spectrum was the result of 64 co-added scans.

Data analysis

We tested the effect of leaching method, cutting and litter type on the concentration of C (DOC) and N (TDN) in the leachate by means of a generalized linear mixed model. We included leaching method, cutting, litter type and all interactions as categorical fixed effects. Due to the large variation in DOC and TDN concentrations between litter types and leaching methods, we applied a log-transformation to the data to homogenize variance and make pairwise comparisons. We checked for normality of the data and applied the Tukey-Kramer method for multiple comparisons of pairwise differences. In all cases, we used Type III tests of fixed effects. A direct comparison of Drip vs. Soak, Soak vs. Shake 70 ml, and Shake 70 ml vs. Shake 30 ml methods was done using a paired *t*-test by pairwise comparison of the methods within each litter type and cutting status. We carried out all the above analyses using SAS® software version 9.3.

We used GRAMS version 9.1 software with the GRAMS IQ package (Thermo Fisher, Woburn, MA) to perform the spectral averaging and Principal Components Analysis (PCA) of the FTIR spectra, as well as the Pearson correlation coefficient (R) between the FTIR spectral data and total DOC and TDN concentrations. We centered all means before the PCA analyses. We tested the effect of leaching method, cutting and litter type on the leachate composition, or relative contribution of different FTIR peaks, by examining the ratios of bands at 3,350, 2,920, 1,605 and 1,070 cm^{-1}. The spectra were corrected by standard normal variate before the band ratios were calculated. We included all combinations of these ratios to determine overall DOM chemistry differences by means of a multivariate generalized linear mixed model. We used the Wilks' Lambda multivariate measures and Tukey's HSD post-hoc testing for pairwise comparisons using SAS® software version 9.3. The data met our tests for homogeneity of variance.

Results

Method effects on DOM leaching from litter

The leaching method, cutting and litter type all had significant main effects and significant interactive effects on DOC and TDN concentrations in the leachate (Fig. 1; $p<0.005$). The leaching method affected the total concentration of DOC and TDN obtained from all of the different litter types (Fig. 1): the Shake 70 ml method resulted in the highest DOC and TDN leaching across all litter types, followed by the Soak, Shake 30 ml, and Drip treatments. The Drip treatment leached significantly less DOM than the other methods, across all litter types. DOM from the Drip method had a significantly lower DOC:TDN for bluestem and pine, but not for ash, oak or alfalfa.

Paired t-tests were used to isolate the effect of method on DOM leaching. We tested the effect of time of water contact in the Drip vs. Soak comparison. Soaking the litter for 1 h in water leached significantly more DOM from all of the litter types than dripping the same amount of water over the litter ($p<0.0001$), indicating that the amount of time the litter is in contact with the water does affect the quantity of DOM leached. We tested the abrasiveness of water contact in the Soak vs. Shake 70 ml comparison. The Shake 70 ml method had significantly higher DOC values ($p=0.0046$) but not significantly different TDN values ($p=0.0963$) across all litter types. This indicates that abrasion had a stronger effect on DOC leaching than TDN leaching, likely due to the higher amount of C leached. We tested the effect of the water to litter ratio in the Shake 70 ml vs. Shake 30 ml comparison. The Shake 70 ml method yielded significantly more DOC and TDN from the cut litter than the Shake 30 ml method ($p<0.0001$) across all cut litter types, demonstrating that we leached more DOM with more water. However the Shake 30 ml treatment, with a higher litter to water ratio, produced a more concentrated leachate solution, which was better for a clear FTIR analysis (Fig. 2).

The average spectra for the uncut samples from the four leaching methods are shown in Fig. 2. The 30 mL Shake had higher quality spectra than the rest of the leaching methods, while the Drip treatment had less spectral quality in agreement with its low DOM concentrations (Figs. 1 and 2). The spectra from the Drip treatment show that overall absorbance is low, with relatively little spectral information present in the leachates from all litter species (Fig. 2). The 30 ml shake had pronounced spectral features at 3,320–3,120 2,950–2,870, 1,600, and 1,070 cm^{-1} bands (Fig. 2). These bands are assigned to polysaccharides and cellulose-like compounds (~1,070 cm^{-1}), amide C=O stretch, aromatic C=C stretch, carboxylate C-O stretch and/or conjugated ketone C=O stretch (~1,605 cm^{-1}), aliphatic C-H stretch (~2,950–2,870 cm^{-1}), and OH/NH stretch (~3,320 cm^{-1}) (Stewart 1996; Socrates 1994). The alfalfa extract, with its higher DOM leaching (Fig. 1) produced better quality spectra with the Soak and 70 mL shake methods relative to the other species. The Drip spectra do not resolve the alfalfa leachate from the ash and pine leachates (Fig. 2), even though the alfalfa leachate had much higher DOM (Fig. 1).

PCA analysis was used as a dimension reduction technique to identify the spectral differences between the leaching methods (Fig. 3a) and the litter type (Fig. 3b). Litter type was the main source of variation between the spectral data, with leaching method having a secondary influence. Component 1 explains 85.2 % of the variation in the spectral data between the samples and shows the differences between the Alfalfa and the rest of the litter types. Component 2 explains 10.7 % of the variation and helps to discern between the Drip treatment, and the rest of the leaching methods (Fig. 3c). The Shake 30 ml and 70 ml treatments had a tendency for low component 2 scores, and are mostly separated from the Drip treatment. Loadings indicate that this is due to higher absorbance in the Shake 30 ml and 70 ml treatments at 1,067, 1,607, 2,950–2,870, and 3,320 cm^{-1} (Fig. 2). The highest combined component 1 and component 2 scores fall in the Drip treatment (Fig. 3a), and loading values (Fig. 3c) are consistent with reduced organic absorbance bands due to the lower concentration of DOM in the Drip leachates (Fig. 1). The DOM FTIR band ratios of 3,350:2,920;

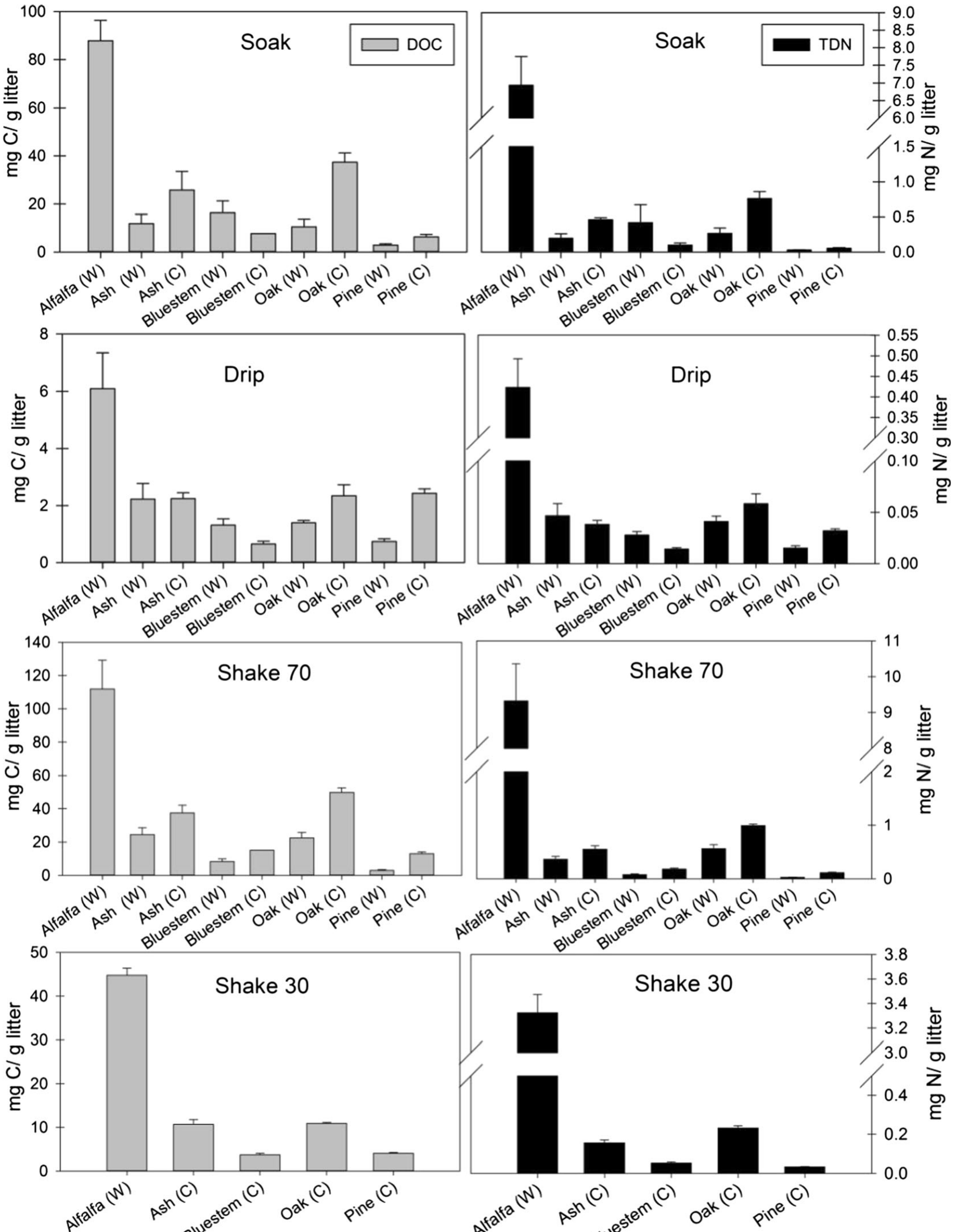

Fig. 1 Dissolved organic carbon (DOC, *left axis*) and total dissolved nitrogen (TDN, *right axis*) in leachates from the Soak, Shake 70 ml, Drip, and Shake 30 ml leaching methods, for the five plant litter species. (*W*) indicates whole leaf treatments and (*C*) indicates cut leaf treatments. Error bars are standard error ($n=4$). Only cut leaves were used in the Shake 30 ml method, due to the fact that the 30 ml volume of water did not cover the entire whole leaf samples

3,350:1,070; 2,920:1,070; 2,920:1,605; 1,605:1,070; 3,350:1,605 cm^{-1} showed no significant multivariate or pairwise differences between leaching method or cutting ($p>0.05$).

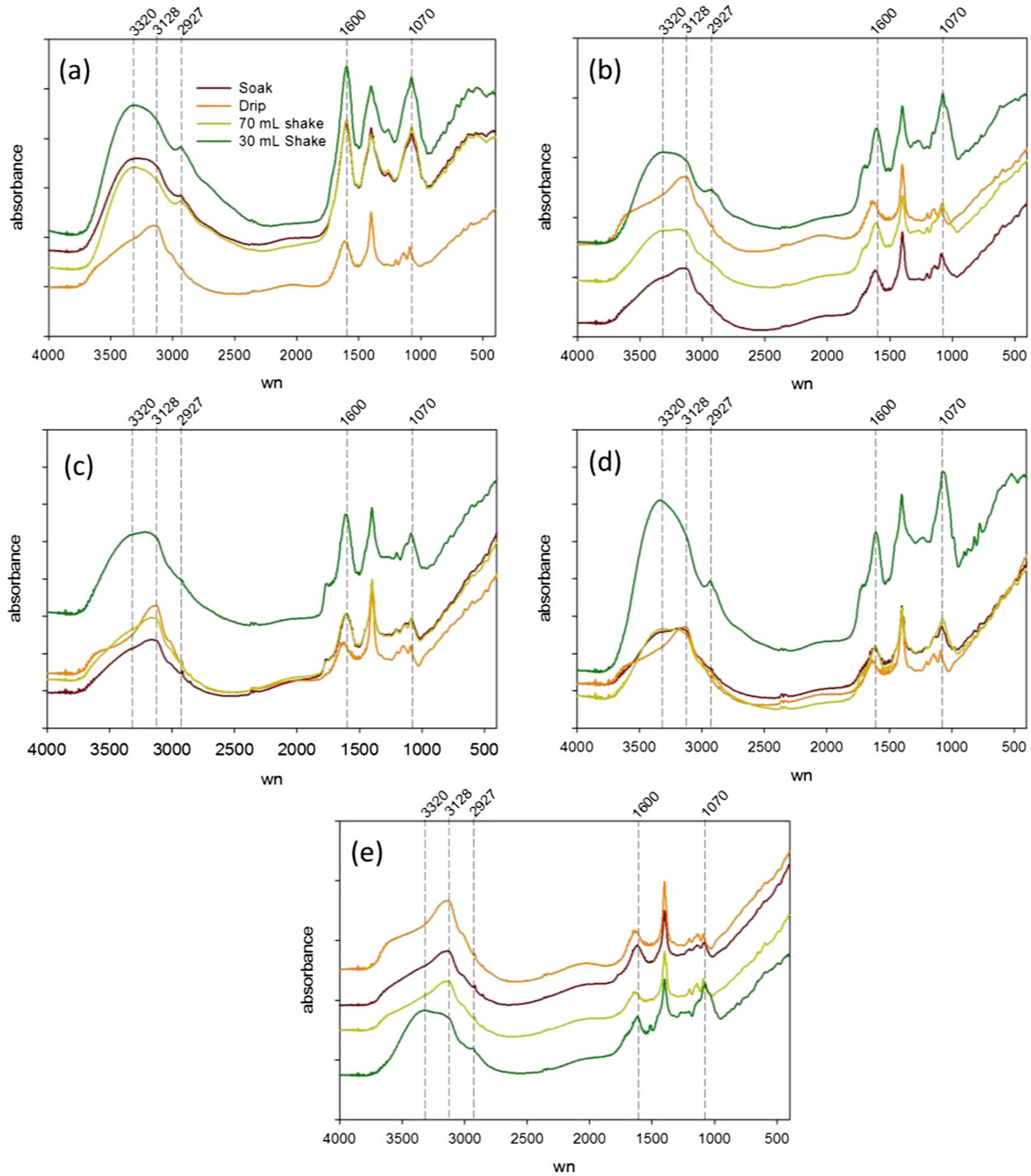

Fig. 2 Spectral averages of the four dissolved organic matter (*DOM*) leaching methods for each plant litter species (**a**) alfalfa, (**b**) ash, (**c**) bluestem, (**d**) oak, and (**e**) pine. Whole samples only, no controls. Only the uncut samples were used for the averages. Note that absorbance is unit-less, and spectra were stacked when necessary to improve the visualization of the spectral differences between the averages

Cutting effects on DOM leaching from litter

Cutting had significant main and interactive effects with litter type on DOC and TDN concentrations in the leachate (Fig. 1; $p<0.0001$). In general, cutting of the litter increased DOC and TDN concentrations, except in bluestem, which saw no effect of cutting on DOM concentrations. DOM from the cut pine litter had a higher DOC:TDN than whole pine litter ($p=0.0001$), but not for the other litter types ($p>0.05$). A comparison of the cut and uncut Shake 70 ml extracts showed that the cut extracts had increased absorbance at the 3,320, 1,605, and 1,070 cm^{-1} spectral bands (data not shown).

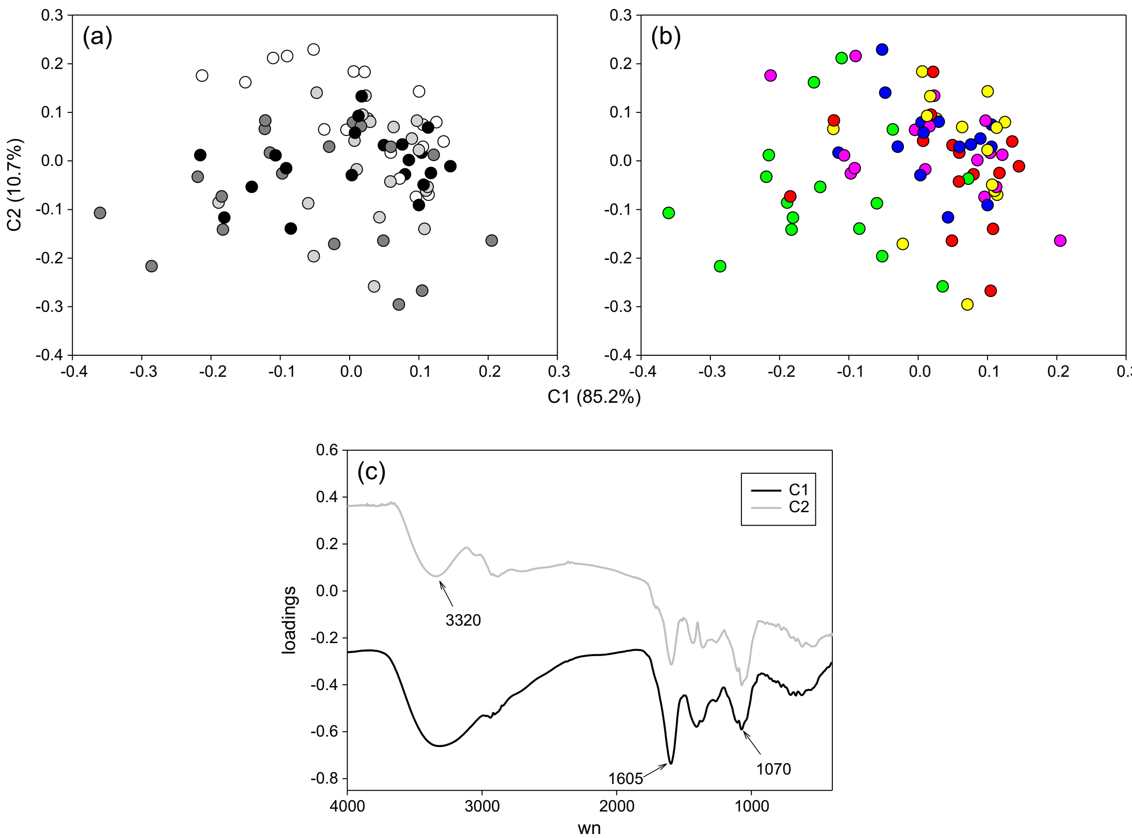

Fig. 3 Principal Component Analyses (*PCA*) of the FTIR spectral data from the litter leachates, only whole litter were included, no controls. Data are the same for the two upper panels: (**a**) Coded by extraction method, *white* is Drip, *light gray* is Shake 70 ml, *black* is Soak, and *dark gray* is Shake 30 ml. (**b**) Coded by plant species, *yellow* is oak, *red* is ash, *pink* is pine, *blue* is bluestem and *green* is alfalfa. Panel (**c**) reports component loadings for the PCA

Litter species effects on DOM leaching

Litter type had significant main and interactive effects on DOC and TDN concentrations in the leachate ($p<0.0001$), with the low C:N litter (e.g., alfalfa) having higher DOC and TDN concentrations overall than litters with higher C:N ($p<0.0001$; Table 1; Fig. 1). Additionally, the DOC:TDN of the leachate reflected the C:N of the litter type ($p<0.0001$). DOC and TDN leaching did not vary significantly with % lignin content of the litter types, except when alfalfa was included, which had the lowest % lignin content and by far the highest DOC and TDN leaching (Table 1; Fig. 1). PCA analysis of the FTIR spectra according to plant species separates the alfalfa spectra from the rest of the species (Fig. 3b), in agreement with the DOC and TDN data (Fig. 1). Species treatment averages from the Shake 30 ml treatment (Fig. 2) confirm the PCA results (Fig. 3b and c), showing that the organic spectral bands at 3,320, 1,605, and 1,070 cm^{-1} are strongest in the alfalfa DOM, and least in the Pine. The DOM FTIR band ratios of 3,350:2,920; 3,350:1,070; 2,920:1,070; 1,605:1,070; 3,350:1,605 cm^{-1} showed no significant multivariate or pairwise differences between litter types ($p>0.05$).

Correlation of DOC and TDN concentration and mid infrared spectral data

We determined the correlation coefficients across the mid infrared spectral range for the DOC and TDN concentrations of the DOM from the Shake 70 ml treatment (Fig. 4). The Shake 70 ml method was chosen for this analysis given that it has relatively high DOM concentrations (Fig. 1), yielding high resolution FTIR spectra (Fig. 2) as well as a full complement of cut and whole samples resulting in a higher number of available data points to build a correlation compared to the Shake 30 ml method. The bands that correlated better with

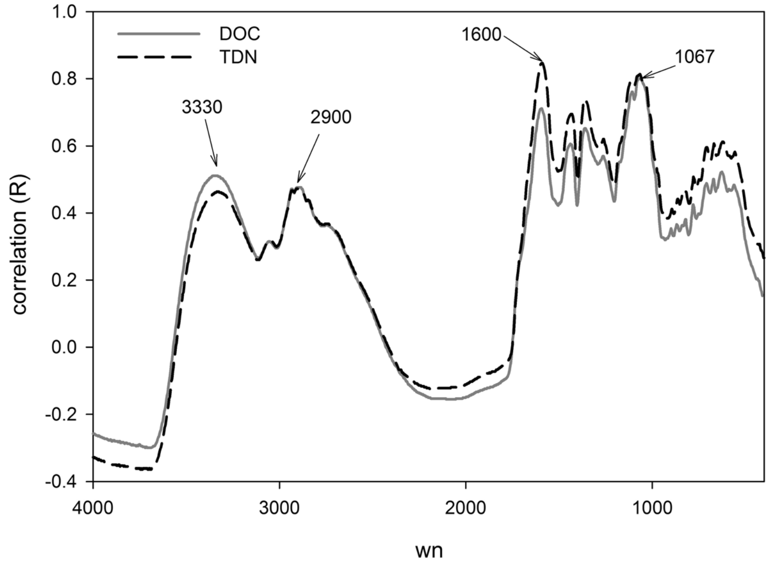

Fig. 4 Correlation coefficients for the FTIR absorbance data and total DOC and TDN in the DOM of the Shake 70 ml treatment

DOC and TDN include all the main bands in the litter spectra, namely 3,330, 2,900, 1,600, and 1,067 cm^{-1} (Fig. 4). Absorbance at 3,010 and 3,110 cm^{-1} form inverted peaks in the correlation spectra. These bands fall within the aromatic C-H stretch region and our analysis indicates that they are less related to DOC and TDN concentrations than the four main spectral bands.

Discussion

Leaching method and cutting

Our results demonstrate the importance of considering the leaching method, cutting of the litter, and litter species when interpreting the results of laboratory litter leaching studies. Confirming our first hypothesis, our results show that the leaching method of choice significantly affects the amount of DOM released from plant litter (Fig. 1). Since soaking, shaking and dripping have all been used to attempt to quantify DOM availability, but none actually measures DOM leaching in situ, estimates of DOM leaching using these methods in the laboratory must be considered in the context of how much DOM each method leaches. For example, the relatively high DOC concentrations reported by Don and Kalbitz (2005) and Wieder et al. (2008) were obtained from litter soaked for 24 h, rather than the 1 h soaking used in this study and by Cleveland et al. (2004). Although this length of time was not tested in our experiment, we did find time of litter-water contact to have a statistically significant impact on the amount of DOM leached, and this could help to explain differences in DOM availability across these studies. Magill and Aber (2000) report relatively low DOM concentrations in their leaching study, but they also were attempting to simulate rainfall by dripping water through their litter samples with very little time for litter-water contact. Our results show that the difference between their DOM quantities and others can be mechanistically explained by the difference in leaching methods applied. Additionally, the litter-to-water ratio differs across our study and others, and must be taken into consideration when comparing DOM estimates across studies as demonstrated by the comparison of the Shake 30 ml and Shake 70 ml treatments.

Cutting of the litter had a confounding effect on the amount and DOC:TDN ratio of DOM leached by each method with interactions by litter types (Fig. 1). Cutting of the litter likely increases DOM leaching due to the increased surface area available for water-litter contact. Cutting may not have affected the bluestem grass blades as strongly as the other litter species because they are long and thin compared to the broad leaves of oak and ash, so cutting may have a weaker effect on grasses. Cutting may have affected the DOC:TDN ratio for pine litter, but not the other species, due to the thick cuticle waxy coating on pine needles, which protects the needles from drying out and was broken by cutting, allowing for more C-rich compounds such as carbohydrates to be leached (Eglinton and Hamilton 1967). In their study, Don and Kalbitz (2005) report that sycamore maple (*Acer pseudoplatanus* L.) had the highest DOC leachate concentration out of the five species in their study, however these were also the only leaves that were cut prior to leaching. Our results suggest that DOM extractions of cut and uncut litters should not be directly compared. However, cutting of the litter provided a more concentrated solution for spectral analysis.

Our results indicate that studies focused on optimizing spectral resolution by employing a leaching strategy, such as using cutting and shaking, do not necessarily misrepresent DOM characterizations according to FTIR analysis as compared to less aggressive techniques such as dripping or soaking (Fellman et al. 2013; Wallenstein et al. 2010). The effect of method on the DOC:TDN ratio within litter type was consistent across methods, except for the lower DOC:TDN for the Drip method for pine and bluestem litters. The Drip pine and bluestem DOM samples contained very little C and N overall (Fig. 1), which may have confounded the DOC:TDN results. The Drip method produced a relatively dilute leachate that resulted in limited FTIR resolution of organic spectral bands, which may have generated the observed discrepancy between the DOC:TDN results and the FTIR spectra for the Drip pine and bluestem . PCA analysis of the FTIR spectra according to leaching method (Fig. 3a) separates the drip method from the other leaching methods, due primarily to the lower concentration of DOM leached by this method. The Drip treatment, however, had the advantage that it mimics field conditions in which rainfall drips through the litter layer and mobilizes the DOM into the soil.

The methods that included litter-water contact for 1-h (Soak, Shake 70 ml and Shake 30 ml) resulted in spectra with more defined spectral bands. Regardless of method, the resulting FTIR spectra are relatively simple with only 4 major spectral features, which are proportionally similar across methods. These results confirm those of Gressel et al. (1995) who found that infrared spectra of pine litter extracts had the most defined peaks at 3,350, 1,610, 1,410, and 1,070 cm^{-1}. This is likely a result of the leaching process, which fractionates the complex leaf litter chemistry into the limited set of soluble components. The DOC concentration in the extracts ranged from 15 mg L^{-1} in the bluestem drip treatment to 444 mg L^{-1} in the alfalfa 70 mL shake treatment. The spectral data suggest that a leachate DOC concentration closer to 444 mg L^{-1} is necessary to produce good spectroscopic results unless measures are taken to concentrate the sample before scanning.

Across all methods, our FTIR analysis shows higher absorbance at four major bands across most of the leachate samples at 3320 (OH/NH stretch), 2950–2870 (aliphatic C-H stretch), 1605 (amide C=O stretch, aromatic C=C stretch, carboxylate C-O stretch and/or conjugated ketone C=O stretch), and 1,070 cm^{-1} (cellulose-like compounds) (Stewart 1996; Socrates 1994). The peak at 1,400 cm^{-1} can be explained by several functional groups including CH_3 bending modes of methyl groups, stretching C-N, deformation N-H, and deformation C-H (Movasaghi et al. 2008). This band at 1400 cm^{-1} has also been observed in the aqueous extracts of pine litter and assigned to COO- stretching, aliphatic CH_2 and CH_3 deformation, and C-O stretching of phenolic OH (Gressel et al. 1995). However this peak also shows in the spectra from our blanks and does not correlate strongly with DOC or TDN concentrations (Fig. 4), indicating that it is partly an artifactual peak and not entirely of litter origin.

FTIR band ratios can be used as a semi-quantitative indicator of differences in organic matter quality based on different proportional contributions of the various functional groups to the leachate chemistry (Calderon et al. 2006; Gressel et al. 1995). Although the same major bands were found in all of the DOM samples, we needed to test whether the methods and cutting leached out different relative amounts of each functional group due to the mechanisms of shaking, soaking, dripping and cutting. In examining all of the band ratios between peaks 3,350, 2,950–2,870, 1,605 and 1,070 cm^{-1}, we did not find any statistically significant differences between the leaching methods or cutting. This further demonstrates that although the leaching methods and cutting are mechanistically different in the quantity of DOM they leach from litter, they do not differ in the relative functional group composition of what is leached out.

Litter chemistry and DOM composition

In this study, the C:N of the source litter was a strong inverse predictor of DOM availability ($p<0.001$), as we predicted in our second hypothesis. However, in another study by Cleveland et al. (2004), C:N of the litter did not predict initial DOC and TDN leaching. These confounding results indicate that the predictability of DOM leaching across different litter species may be controlled by something more complex than C to N stoichiometry, such as litter structural composition. Lignin content has been shown to be an important regulator of DOM leaching from litter in previous studies (Kalbitz et al. 2006; Klotzbucher et al. 2011), but we did not find any correlation between % lignin content and DOC or TDN leaching across litter types (R^2<0.01 for both DOC and TDN). However, the objective of our study was to compare the DOM leaching from fresh litters whereas lignin has been shown to be more important for controlling DOM leaching during the later phases of decomposition when leaching rates are slower (Klotzbucher et al. 2011).

PCA analysis of the FTIR spectra according to plant species separates the alfalfa spectra from the rest of the species (Fig. 3b). This separation between litter species is mainly due to concentration of the leachate, which is strongest in the alfalfa DOM, and least in the pine. The same four bands, namely 3,330, 2,900, 1,600, and 1,067 cm^{-1}, appear across all litter species and leaching methods, and are merely subdued in the Drip treatment. These bands should be regarded as the main features of soluble organics in litter as characterized by FTIR analysis. An examination of the FTIR band ratios of 3,350:2,920; 3,350:1,070; 2,920:1,070; 1,605:1,070; 3,350:1,605 cm^{-1} showed no significant differences between the relative contributions of these bands across litter types. The separation in the PCA therefore is mainly due to differences in the absorbance at these four bands, which are strongly correlated with DOC and TDN (Fig. 4).

The correlation analysis between FTIR absorbance and DOC or TDN concentrations (Fig. 4) are valuable in confirming that the bands at 3,330, 2,900, 1,600, and 1,067 cm^{-1} are well correlated to soluble C and N from litter. DOC and TDN followed very similar patterns of R scores, which reached 0.84 for TDN at 1,600 cm^{-1} (Fig. 4). The higher R score for TDN at 1,600 cm^{-1} suggests that this peak might be due in part to amide I absorbance caused by proteinaceous material in the DOM leachates (Gressel et al. 1995). In contrast, correlation at 3,330 cm^{-1} was higher for the DOC than the TDN, indicating that this band could be proportionately more due to phenolic OH than to NH. Absorbance in the OH/NH region between 3,690 and 3,300 cm^{-1} is a common feature of DOM extracts and the light fraction of soil (Gressel et al. 1995; Strobel et al. 2001; Gallo et al. 2005; Calderon et al. 2011). The close agreement between the DOC and TDN may be due to the fact that the soluble bands detected in the extracts are mostly C, H, and/or O containing bands, with the possible exception of 1,600 cm^{-1} and the bands at 3,400–3,100 cm^{-1}, which correlate highest with TDN concentrations. The low correlation results, however, suggest that absorbance at 1,600 cm^{-1} is mostly due to OH in the leachates, whereas the high correlation between the 3,330 cm^{-1} band and DOC, and the 1,600 cm^{-1} band and TDN provide some clues toward resolving the identity of the compounds at these wn's.

The best correlations between FTIR absorbance and DOC and TDN concentrations include the major peaks in the spectra of the litter leachate (Fig. 2), suggesting that all spectral features in the leachate of our samples were of organic origin. Previous studies of DOM leachate have identified silicate bands, possibly because of the presence of soil minerals in litter leachate (Gallo et al. 2005). In our study however, efforts were taken to avoid soil contamination in the litters before the assay.

The five different litter types ranged in litter quality in terms of % N and % lignin content. However, the band ratios between peaks 3,350, 2,950–2,870, 1,605 and 1,070 cm^{-1}, were not significantly different between litter types. This demonstrates that the functional group chemistry of the soluble components of fresh litters contributed the same relative amounts to DOM across a broad range of litter types. Alfalfa, for example had the most concentrated DOM and thus the highest absorbance and spectral quality for FTIR (Figs. 1 and 2). However, this more concentrated leachate and high absorbance failed to reveal any major differences between the relative functional group concentrations of alfalfa in comparison to the other litter types, as revealed by the band ratio analysis. The ratios of these different functional groups are likely to change with decomposition stage (Calderon et al. 2006; Gressel et al. 1995). The phenolic and proteinaceous character of litter DOC can be affected by the type of litter and its degradation stage (Kiikkila et al. 2012). Infrared spectroscopy should be an accurate enough method to detect fluctuations in phenolic and polysaccharide composition in DOC (Calderon et al. 2006), however our band ratio analysis did not detect large differences between the extracts from the different litter types. It is possible that Nuclear Magnetic Resonance would have allowed for detection of small variations in the chemical composition that were not detected by FTIR. The identification of litter leachate functional group chemistry can be used to further understand how the initial flush of DOM from fresh litter to the soil may interact with the mineral soil and soil microbes to form SOM (Kaiser and Guggenberger 2000; Oren and Chefetz 2012).

Conclusions

We found that the litter to water ratio, the time of contact between the litter and water, and to a lesser extent the abrasiveness of litter and water contact all had statistically significant effects on the amount of DOM leached from the five litter types tested. Additionally, cutting and litter species also had statistically significant main and interactive effects on the amount, but not the FTIR characterization, of DOM leached from fresh litter. These results provide a mechanistic explanation for why leaching method, cutting of the litter, and litter species must be taken into consideration when comparing estimates of DOM availability within and across laboratory studies. The relationships between DOM availability, lignin content and fresh litter C:N found here may not always apply at all stages of litter decomposition, and is an important area for future research. Furthermore, FTIR spectroscopy revealed that the same four major spectral features were observed across all methods and litter types. However, the intensity of the spectral bands was highest in the litter with the highest DOM (alfalfa), and in the leachates obtained by the higher yielding leaching methods (30 and 70 mL Shake). Based on this and other studies it becomes apparent that the functional groups associated with wn's 3,330, 2,900, 1,600 and 1,067 cm^{-1} are the main soluble features of undecomposed litters across a range of litter types. Now that these main features have been identified to contribute consistently to fresh litter leachate chemistry across a broad range of litter types and leaching methods, further investigations of the fate of DOM in the soil can focus on these four features based on their high correlation with leachate DOC and TDN concentrations.

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