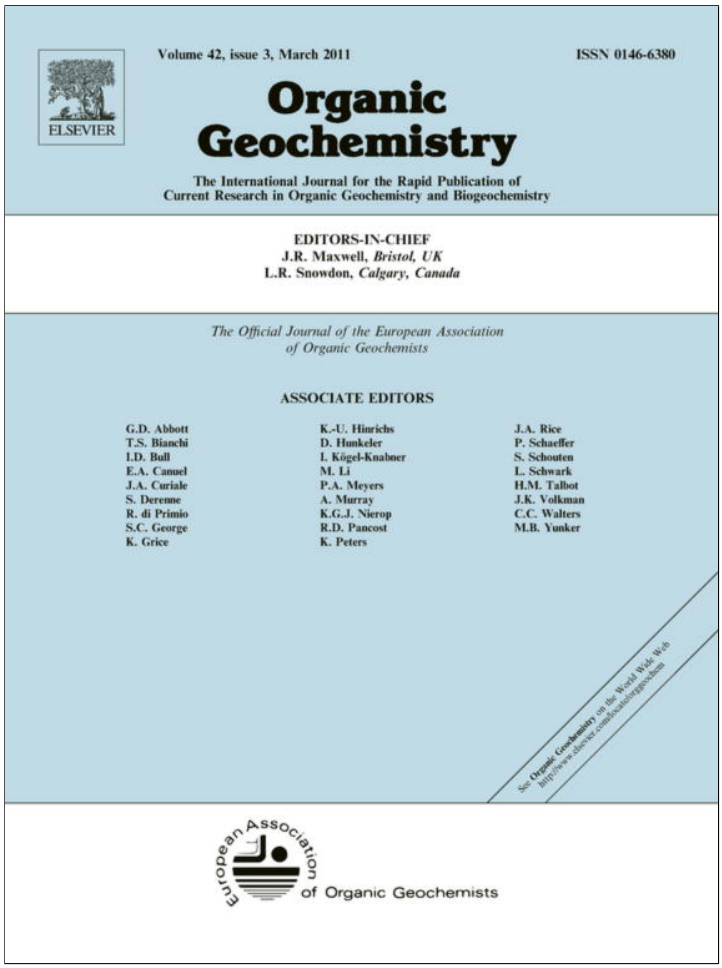

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The role of biodegradation and photo-oxidation in the transformation of terrigenous organic matter

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ABSTRACT

Microbial and photochemical decomposition are two major processes regulating organic matter (OM) transformation in the global carbon cycle. However, photo-oxidation is not as well understood as biodegradation in terms of its impact on OM alteration in terrigenous environments. We examined microbial and photochemical transformation of OM and lignin derived phenols in two plant litters (corn leaves and pine needles). Plant litter was incubated in the laboratory over 3 months and compositional changes to OM were measured using nuclear magnetic resonance (NMR) and gas chromatography–mass spectrometry. We also examined the susceptibility of soil organic matter (SOM) to ultraviolet (UV) radiation. Solid-state ^{13}C NMR spectra showed that *O*-alkyl type structures (mainly from carbohydrates) decreased during biodegradation and the loss of small carbohydrates and aliphatic molecules was observed by solution-state ^{1}H NMR spectra of water extractable OM from biodegraded litters. Photochemical products were detected in the aliphatic regions of NaOH extracts from both litter samples by solution-state ^{1}H NMR. Photo-oxidation also increased the solubility of SOM, which was attributed to the enhanced oxidation of lignin derived phenols and photochemical degradation of macromolecular SOM species (as observed by diffusion edited ^{1}H NMR). Overall, our data collectively suggests that while biodegradation predominates in litter decomposition, photo-oxidation alters litter OM chemistry and plays a role in destabilizing SOM in soils exposed to UV radiation.

1. Introduction

As the primary decomposition process on land (Singh and Gupta, 1977), biodegradation has been extensively studied with regard to its impact on terrigenous organic matter (OM) transformation (Melillo et al., 1989; Baldock et al., 1997; Berg, 2000; Grandy and Neff, 2008). By comparison, photo-oxidation of OM is well documented in aquatic systems (Kieber et al., 1989; Moran and Zepp, 1997; Opsahl and Benner, 1998; Osburn et al., 2001; Dalzell et al., 2009) and is considered to play a vital role in litter decomposition in arid and semi-arid regions (Austin and Vivanco, 2006; Gallo et al., 2006; Day et al., 2007). However, photochemical transformation of terrigenous OM, in particular, soil organic matter (SOM), remains poorly understood (Rutledge et al., 2010). Although ultraviolet (UV) radiation does not penetrate significantly into soil due to protection by litter layer and soil mineral components (Skjemstad et al., 1993), photo-oxidation of SOM may be important in agriculture soils after crop residues are removed from soil surfaces or in erosion affected soils where part of SOM is exposed to solar radiation. With increasing UV radiation due to ozone depletion (Madronich et al., 1998), it is important to understand photochemical transformation of plant litters and native OM in soil.

As the second most abundant component of terrigenous plant residues, lignin plays a key role in regulating plant litter decomposition (Melillo et al., 1982; Taylor et al., 1989), humic substance formation (Ertel and Hedges, 1984; Kiem and Kögel-Knabner, 2003) and dissolved organic matter (DOM) production from terrigenous sources (Kalbitz et al., 2006). Phenolic compounds, including lignin, absorb heavily in the UV range and previous studies have reported that UV or solar radiation induces lignin loss in plant litters (Gehrke et al., 1995; Day et al., 2007). Moreover, photo-oxidation reduces the molecular size of lignin or DOM (Opsahl and Benner, 1998; Osburn et al., 2001; Lou and Xie, 2006), which potentially increases the biodegradability of DOM (Lindell et al., 1995; Wetzel et al., 1995; Moran and Zepp, 1997; Rosenstock et al., 2005). It is not known whether similar photochemical processes operate in SOM, which may have significant implications for soil carbon sequestration and management (Rutledge et al., 2010).

This study examines and compares the molecular level transformation of plant litters in a 3 month laboratory simulated biodegradation and photo-oxidation experiment. In particular, we focused on OM solubility and lignin components because they are considered to be sensitive to microbial and photochemical decomposition

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processes (Moorhead and Callaghan, 1994; Lou and Xie, 2006; Hilli et al., 2008). Lignin derived phenols were extracted by CuO oxidation from the water extractable OM (WEOM) and water extracted residues (WERs) of litter samples and analyzed by gas chromatography–mass spectrometry (GC–MS), whereas bulk OM chemical composition was examined by solid-state ^{13}C and solution-state ^{1}H nuclear magnetic resonance (NMR) spectroscopy (Table 1). We also investigated the susceptibility of SOM to UV radiation and changes in macromolecular SOM species (i.e., relatively large OM components or stable aggregate species) were measured by diffusion edited ^{1}H NMR (Simpson et al., 2007a). The objectives of this study were to compare the molecular level transformation of terrigenous OM and lignin derived phenols during microbial and photochemical decomposition, to assess OM solubility during decomposition and to examine the susceptibility of SOM to photo-oxidation. We hypothesize that photo-oxidation may increase the solubility of terrigenous OM by reducing the number of macromolecular species and by increasing lignin oxidation, hence stimulating carbon loss from terrigenous ecosystems.

2. Materials and methods

2.1. Litter and soil samples

Two litter samples, two mineral soils and a peat soil were selected for the laboratory simulation of biodegradation and photo-oxidation based on their varying OM and lignin composition. The litter samples were corn (*Zea mays* L.) leaves grown in a greenhouse at McGill University, Quebec, Canada and loblolly pine (*Pinus taeda* L.) needles collected from the Duke Forest near Chapel Hill, NC, USA. The two litter samples differ in OM solubility and lignin contents (Taylor et al., 1989), which facilitates the investigation of microbial and photochemical alterations of litters with different OM quality. Mineral soils included a sandy loam Humic Gleysol soil (referred to as Soil M; pH 6.1) cropped with corn from the Macdonald Campus Farm in Ste-Anne-de-Bellevue, Quebec (Whalen et al., 2008) and a pristine grassland soil collected near the Agriculture and Agri-Food Canada Research Station near Lethbridge, Alberta (referred to as Soil L; pH 6.4–6.8; classified as Brown Chernozem). The particle size distribution of the mineral soils is provided in Table 2. The surface layer of both soils is potentially subject to solar or UV radiation due to crop residue removal or thin litter layer coverage, so photochemical transformation of SOM may be important in these soils. A Florida peat soil (Peat) purchased from the International Humic Substance Society (IHSS; Minneapolis, MN) was also included to examine photochemical transformation of OM without mineral interactions. All samples were air dried upon collection and kept in the dark. Corn leaves and pine needles were cut into small pieces (~6 mm in diameter) and soil samples were passed through a 2 mm sieve, ground (<100 μm), and homogenized before experiment. A subset of air dried litter and soil samples were kept in glass jars in the dark as experimental controls. The control samples of litters and mineral soils were sub-sampled (6 g of litter or 120 g of mineral soil) and analyzed together with the biodegraded and photo-oxidized samples on days 0, 30, 60 and 90 of the experiment.

Table 1
Summary of analyses and organic matter (OM) properties under investigation.

Sample fractions	Analyses	OM properties under investigation
Bulk litter or soil	Total organic carbon (TOC)	Degradation of TOC
	^{13}C NMR	Composition of bulk OM
Water extractable OM (WEOM)	Water extractable organic carbon (WEOC)	OM solubility
	Lignin derived phenols	Lignin solubility and degradation
	^{1}H NMR (litter samples only)	Composition of soluble OM
Water extracted residues (WERs)	Lignin derived phenols (litter samples only)	Lignin degradation
NaOH extracts	^{1}H NMR	Composition of NaOH extractable OM (more sensitive than bulk OM)

Table 2
Organic carbon (OC) content of corn leaves, pine needles, soil and peat samples on day 90 and soil particle size distribution.

Sample	OC (%)			Particle size distribution[a] (%)		
	Photo-oxidized	Control	Biodegraded	Sand	Silt	Clay
Corn leaves	41.8	45.0	40.0	na	na	na
Pine needles	48.1	50.5	48.5	na	na	na
Soil M	2.1	2.1	na	70	14	16
Soil L	2.1	2.2	na	47	33	20
Peat	45.6	46.2	na	na	na	na

na = not applicable due to the absence of minerals in organic samples.
[a] From Feng and Simpson (2007) and Whalen et al. (2008).

2.2. Design of biodegradation and photo-oxidation experiments

Biodegradation of corn leaves and pine needles was promoted by incubation with soil inoculum for 3 months at the room temperature (23 °C) in the dark (methods modified after Gehrke et al., 1995; Cleveland et al., 2004). Briefly, 100 g of field-moist Soil M was amended with 0.5 g of corn leaf powder and incubated for 5 days at room temperature to stimulate the growth of microbial degraders. The soil was then mixed with 190 ml of deionized water, and 2 ml of the soil–water mixture was taken and mixed with 198 ml of deionized water to prepare the diluted soil inoculum. Then 4.0 g of air dried corn leaves or pine needles was placed in a 450 ml glass jar, wetted with 4 ml of soil inoculum and 4 ml of deionized water, mixed and the jar was capped with an air tight lid. A negligible amount of dissolved organic carbon (OC; <1 μg OC) was added to the litter with soil inoculum. A total of eight replicate jars were prepared for each litter sample, of which two jars served as backup replicates. Jars were open for 15 min and about 1 ml of deionized water was sprayed onto the sample every week to maintain humidity. Biodegradation was assessed by the cumulative carbon respired by microbial community (mg CO_2-C/g), which was measured every week in triplicate using the alkali absorption method (Winkler et al., 1996; Feng and Simpson, 2008). Two replicate jars of each litter type were removed from the experiment on days 30, 60 and 90 of the experiment, freeze dried, ground and homogenized before chemical analysis.

Photo-oxidation was carried out in the laboratory using two ultraviolet (UV-B) lamps (302 nm wavelength; Cole-Parmer, Montréal, Canada). Approximately 1.0 g of air dried litter, 20.0 g of air dried soil, or 2.0 g of Peat was placed in an open top glass petri dish (6 cm in diameter; sample depth <5 mm). In total, 10 replicate dishes of Peat and 32 replicate dishes each of corn leaves, pine needles, Soil M and Soil L were prepared. Samples were lined up under the UV-B lamps, randomly rotated in position every week and the contents of each dish were mixed carefully with a spatula every week. Radiation intensity under the UV-B lamps was measured by a UVX digital ultraviolet intensity meter (302 nm; Cole-Parmer, Montréal, Canada) and averaged about 80 μW/cm^2, comparable to the peak summer solar UV-B irradiance in the mid-latitudes (Lubin et al., 1998; Roeder, 2002). Samples were exposed to 24 h UV-B radiation every day for 3 months to enhance photo-oxidation

without mimicking natural radiation cycles. Peat was sampled on day 90 of the experiment only because preliminary analysis showed minimal changes in the OC content or OM composition of Peat under UV radiation in the first month of the experiment (data not shown), indicating slow photochemical transformation of the Peat OM. Six replicate dishes of each litter (corn leaves, pine needles) or mineral soil (Soil M, Soil L) were removed on days 30, 60 and 90. The contents of the replicate dishes were mixed and homogenized before chemical analysis. Preliminary analysis of phospholipid fatty acids indicated minimal microbial growth in the air dried litter or soil samples under UV-B radiation (data not shown). Hence chemical alteration of OM can be attributed to photo-oxidation alone.

2.3. Extractions and analyses of WEOM and WERs

The OC content of biodegraded, control and photo-oxidized samples was determined at the end of the experiment (day 90) by dry combustion at the University of Guelph Laboratory Services (Guelph, Ontario, Canada). A scheme of chemical analyses is shown in Fig. 1 and Table 1 with details described below.

To assess changes in OM solubility, approximately 500 mg of litter, 20 g of soil, or 4 g of Peat were weighed into 45 ml Nalgene® polyethylene centrifuge tubes and extracted with 30.0 ml of deionized water on a shaker for 24 h at room temperature in the dark. The samples were centrifuged (4500 rpm for 30 min) and the supernatant was filtered through a 0.22 μm Millipore Durapore PVDF membrane to obtain WEOM. Aliquots of the WEOM were quantitatively transferred into pre-cleaned glass vials, diluted with a measured amount of deionized water, acidified with 6 M HCl, and analyzed for water extractable organic carbon (WEOC) content on a Shimadzu TOC 5000 total organic carbon analyzer (Shimadzu Scientific Instruments, Columbia, MD, USA). Another 15.0 ml of the WEOM was quantitatively transferred into Teflon lined bombs for CuO oxidation to isolate lignin derived phenols (Otto et al., 2005). Briefly, the WEOM was extracted with 0.5 g CuO, 100 mg ammonium iron (II) sulfate hexahydrate [$Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$] and 3 ml of 12 M NaOH (a final NaOH concentration of 2 M) under N_2 at 170 °C for 2.5 h. After the reaction, the water phase was acidified to pH 1 with 6 M HCl and kept for 1 h at room temperature in the dark to prevent reactions of cinnamic acids. After centrifugation (2500 rpm for 30 min), the supernatants were liquid–liquid extracted with diethyl ether. The ether extracts were concentrated by rotary evaporation, spiked with a known amount of internal standard (ethyl vanillin), transferred to 2 ml glass vials and dried under N_2 for GC–MS analysis. The whole procedure was carried out in triplicate and finished within 3 days for each sample.

The WERs were rinsed with deionized water and freeze dried. Solvent-extractable compounds were removed from the WERs of litter samples (100 mg) with 30 ml of dichloromethane, dichloromethane:methanol (1:1; v:v) and methanol, respectively. Lignin derived phenols were extracted and analyzed from the air dried residues by the CuO oxidation method as described above. Extractions were conducted in triplicate for both litter samples. Preliminary tests showed that photo-oxidation did not result in any compositional changes in the solvent extractable compounds or lignin derived phenols from the WER of Soil M (data not shown). Hence, the WERs of Peat or soil samples were not analyzed further.

2.4. GC–MS analysis

Aliquots of the CuO oxidation extracts were converted to trimethylsilyl (TMS) derivatives by reaction with 100 μl N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) and 10 μl pyridine for 3 h at 70 °C and diluted with hexane if necessary. GC–MS analysis was performed on an Agilent model 6890N GC coupled to an Agilent model 5973 quadrupole mass selective detector. Separation was achieved on a HP5-MS fused silica capillary column (30 m × 0.25 mm i.d.; 0.25 μm film thickness). The GC operating conditions were as follows: temperature increased from 60 to 200 °C at a rate of 10 °C/min, and then increased to 300 °C at a rate of 4 °C/min with a final isothermal hold at 300 °C for 15 min. Helium was used as the carrier gas. The sample was injected in splitless mode with an Agilent 7683 autosampler and the injector temperature was set at 280 °C. The mass spectrometer was operated in the electron impact mode at 70 eV ionization energy and scanned from 50 to 650 Da. Data were acquired and processed with the Chemstation G1701DA software. Individual compounds were identified by comparison of mass spectra with literature, NIST and Wiley MS library data, authentic standards and interpretation of mass spectrometric fragmentation patterns. Compounds were quantified with internal standards (a measured amount of ethyl vanillin added into samples before derivatization) in the total ion current and normalized to the sample weight. The recovery of lignin derived phenols was assumed to be the same during extraction, which was confirmed by the analysis of control samples on different days (day 0, 30, 60 and 90; see Sections 3.1.2 and 3.2.2). Carbon loss through direct mineralization in biodegradation or photo-oxidation was considered to be small and hence not accounted for in the calculation of compounds' concentrations.

Lignin derived phenols included vanillyls (V; vanillin, acetovanillone and vanillic acid), syringyls (S; syringaldehyde, acetosyringone and syringic acid), and cinnamyls (C; *p*-coumaric acid and ferulic acid). The ratios of vanillic acid/vanillin or $(Ad/Al)_v$ and

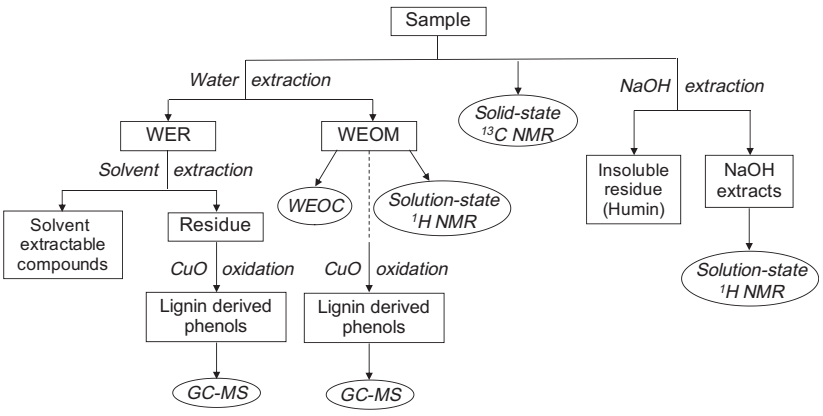

Fig. 1. Scheme of extractions and chemical analyses used to assess terrestrial organic matter transformation. WER: water extracted residue; WEOM: water extractable organic matter; WEOC: water extractable organic carbon.

syringic acid/syringaldehyde or $(Ad/Al)_s$ were used to assess lignin oxidation, which has been observed to increase with increasing degradation (Hedges et al., 1988; Opsahl and Benner, 1995; Otto and Simpson, 2006).

2.5. NMR analysis

The litter samples and Soil M were chosen for NMR analysis to examine compositional changes in the WEOM, NaOH extracts and bulk OM after 90 days of biodegradation and photo-oxidation. To remove paramagnetic materials and to concentrate OC, Soil M was treated repeatedly with 0.3 M hydrofluoric acid (HF), rinsed with deionized water, and freeze dried before NMR analysis. Treatment by 10% HF does not change the bulk chemical composition of soil samples as observed by NMR spectroscopy (Goncalves et al., 2003; Rumpel et al., 2006) and the milder HF treatment (0.3 M) used in this study should preserve the bulk chemical properties of SOM. The HF treated Soil M and litter samples were ground into fine powders for solid-state ^{13}C NMR. The ^{13}C cross polarization-magic angle spinning NMR spectra were acquired on a Bruker BioSpin Avance III 500 MHz NMR spectrometer with a 4 mm probe. The following acquisition parameters were employed: spin rate of 13 kHz, contact time of 1 ms, recycle delay of 1 s, acquisition time of 0.0135 s, line broadening of 25 Hz and with 512 time domain points. Structures in the bulk OM were represented by alkyl (0–45 ppm), *O*-alkyl (45–110 ppm), aromatic and phenolic (110–165 ppm) and carboxylic and carbonyl (165–215 ppm) carbon (Kögel-Knabner, 1997).

Solution-state ^1H NMR was further employed to examine the chemical composition of WEOM and NaOH extracts because it complements solid-state ^{13}C NMR (Olk et al., 1998; Feng et al., 2008) and provides additional structural information on OM composition. WEOM was extracted from a subset of litter samples (~5–10 g; day 90) as described previously. WEOM from Soil M was not analyzed by NMR due to a low yield. NaOH extracts were exhaustively extracted from both litters (1 g) and the HF treated Soil M (40 g of original soil) by NaOH solution (0.1 M) under N_2. Both WEOM and NaOH extracts were filtered through 0.22 μm Millipore Durapore PVDF membrane, ion exchanged with Amberjet 1200(H) ion exchange resin (Sigma–Aldrich), and freeze dried. Approximately 100 mg of WEOM or NaOH extracts were dissolved in DMSO-d_6 (0.75 ml) and transferred into a 5 mm NMR tube for analysis on a Bruker Avance III 500 MHz spectrometer using a 5 mm QXI probe. 1-D solution-state ^1H NMR experiments were performed with 512 scans, a recycle delay of 2 s, 16,384 time domain points and a sample temperature of 298 K. The macromolecular species (i.e., relatively large OM components or stable aggregate species) in the NaOH extracts from litter and soil samples were examined by diffusion edited ^1H NMR (Simpson, 2002; Simpson et al., 2007a). Under the same NMR experimental conditions (including sample size and NMR acquisition parameters), the signal intensity of diffusion edited NMR correlates to the abundance of macromolecular species in the sample (Simpson et al., 2007a,b). Diffusion edited experiments were used with a bipolar pulse longitudinal encode-decode sequence (Wu et al., 1995). Scans (1024) were collected with 16,384 time domain points using 2.5 ms, 53.5 gauss/cm gradient pulses and a 200 ms diffusion time. Spectra were apodized by multiplication with an exponential decay corresponding to 1 Hz line broadening in the transformed spectrum and zero filled with 32,768 spectral points. Chemical shift assignments were made using a range of 2-D experiments and studies on natural OM (Simpson et al., 2003; Kelleher and Simpson, 2006). The 1-D spectra were labeled with general regions that are dominated by the following categories of chemical components: aliphatic (0.6–2.8 ppm), *O*-alkyl from carbohydrates and amino acids (2.8–5.6 ppm), amide and aromatics (6.2–9 ppm; Simpson et al., 2007a).

2.6. Statistical analysis

Two-way ANOVA was used to assess the significance of time and experimental treatments (biodegradation or photo-oxidation) on OM components in litter and mineral soil samples by the General Linear Model in SPSS (v 10.0). A preliminary analysis indicated minimal time effect on OM components in the control samples. Unless mentioned, the interaction between time and experimental treatment was not significant. Linear regression analysis was used to assess the effect of time on OM components in biodegraded and photo-oxidized samples using Origin™ Version 7.0 (Microcal Software, MA, USA) where necessary. Student's *t* test was used to compare the OM components in the control and photo-oxidized Peat samples on day 90. Differences were considered to be significant at a level of $P < 0.05$.

3. Results

3.1. Biodegradation and photo-oxidation of plant litters

3.1.1. Degradation and solubility of bulk OC in litters

The OC content of biodegraded, control and photo-oxidized samples on day 90 is given in Table 2. Approximately 11% and 4% of OC was lost during the biodegradation of corn leaves and pine needles, respectively, consistent with the carbon loss estimated from microbial respiration data (~15% and 3% in corn leaves and pine needles, respectively; Fig. 2). Although the OC loss from pine needles was small due to the slow-to-decay nature of pine needles (Melillo et al., 1989), the OC decrease was significant judging from the respiration data (Fig. 2). The OC content of litter samples decreased by 5–7% with photo-oxidation, which was comparable in magnitude to the biodegradation induced loss in OC content. WEOC accounted for 14.8% and 3.7% of total OC in control corn leaves and pine needles, respectively. WEOC increased considerably in the biodegraded corn leaves on days 60 and 90, and decreased by ~50% in the biodegraded pine needles on all sampling dates in comparison to the control (Fig. 3). By comparison, WEOC significantly increased in pine needles under photo-oxidation ($P = 0.01$) but did not change in corn leaves (Fig. 3).

3.1.2. Lignin degradation and solubility

A considerable fraction of lignin derived phenols were extracted from the WEOM of litter samples (Fig. 4). Approximately 4% of total C phenols (total = WEOM + WERs) were extracted from the WEOM of pine needles while the other phenols in the WEOM accounted for 10–30% of the total in corn leaves or pine needles (Fig. 4). The solubility of V, S and C phenols varied, leading to slightly different C/V (~7 in WEOM and ~5 in WER) and S/V (~1.0 in WEOM and ~1.7 in WER) ratios in corn leaves and a very different C/V ratio in pine needles (~0.3 in WEOM and ~1.8 in WER) between WEOM and WERs (Fig. 5).

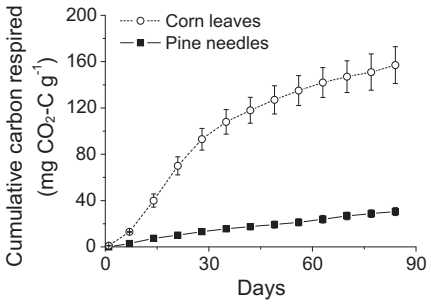

Fig. 2. Cumulative carbon respired during the biodegradation of litter samples. Error bars represent standard errors ($n = 3$).

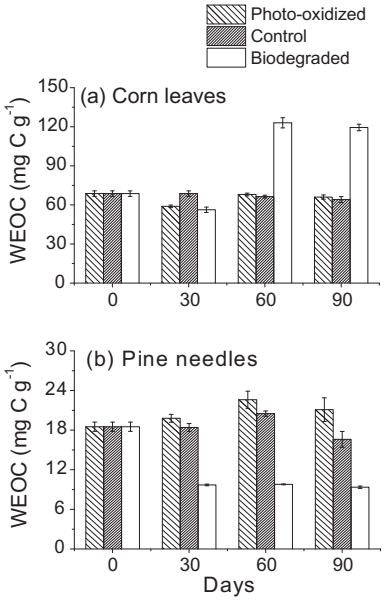

Fig. 3. The water extractable organic carbon (WEOC) content of litter samples during biodegradation and photo-oxidation. Error bars represent standard errors ($n = 3$).

In the WEOM of corn leaves, V and S phenols significantly increased with biodegradation whereas C phenols decreased ($P < 0.05$; Fig. 4a–c). The S/V ratio increased almost fourfold after 90 days of biodegradation and the C/V ratio decreased by half in the WEOM (Fig. 5). As for pine needles, S phenols were not detected. Both V and C phenols significantly decreased in the biodegraded WEOM ($P < 0.05$; Fig. 4g and h) and the C/V ratio decreased by half on day 90 (Fig. 5). The response of lignin derived phenols in WERs varied from those in WEOM to biodegradation. In the WER of corn leaves, V, S and C phenols increased considerably with biodegradation on days 30 and 60 and then decreased on day 90 (Fig. 4d–f). The S/V ratio increased after 90 days of incubation in the WER of corn leaves while the C/V ratio was not significantly different (Fig. 5). In the biodegraded WER of pine needles, V phenols remained unchanged while C phenols decreased over time (Fig. 4i and j), leading to a slightly decreased C/V ratio (Fig. 5). By comparison, photo-oxidation did not induce any significant changes in the abundance of lignin derived phenols in the WEOM of corn leaves (Fig. 4a–c). V and C phenols increased in the WEOM of pine needles under photo-oxidation ($P < 0.05$; Fig. 4g and h) and the C/V ratio slightly decreased in the WEOM of both litter samples (Fig. 5). The abundance of lignin derived phenols remained similar in the WERs of both litters after photo-oxidation (Fig. 4d–f and i and j).

Individual lignin derived phenols also had varied solubility, resulting in a much higher $(Ad/Al)_v$ ratio in the WEOM than in the WER of corn leaves and a lower $(Ad/Al)_v$ ratio in the WEOM than in the WER of control pine needles (Fig. 6a and c). The Ad/Al ratios of lignin derived phenols significantly increased in the WEOM and WERs of both litter samples with biodegradation ($P < 0.05$; Fig. 6), suggesting enhanced side chain oxidation of lignin. In particular, the $(Ad/Al)_s$ ratio in the WEOM of biodegraded corn leaves was more than three times that in the control WEOM or WER. By comparison, the Ad/Al ratios of lignin derived phenols did not increase with photo-oxidation in the WEOM or WERs of litter samples except that $(Ad/Al)_v$ ratio increased in the WEOM of pine needles ($P < 0.05$; Fig. 6c).

Furthermore, biodegradation and photo-oxidation led to varied isomerization of lignin derived phenols in the litter. Both *cis*- and *trans*-isomers of *p*-coumaric acid and ferulic acid (C phenols) were detected in the WEOM and WER of corn leaves. The ratio of *cis*- to *trans*-isomers (*cis/trans*) of C phenols increased with photo-oxidation by day 90 and decreased with biodegradation in the WER of corn leaves ($P < 0.05$; Fig. 7). The *cis/trans* ratios of C phenols were higher in the WEOM than in the WER of corn leaves, which ranged from 0.19–0.30 for *p*-coumaric acid and from 0.02–0.09 for ferulic acid. The ratios were variable in the WEOM of corn leaves and no consistent trend was observed with the experimental treatments (data not shown).

3.1.3. Chemical composition of WEOM and bulk OM in litters

The overall chemical composition of WEOM from corn leaves and pine needles was examined by solution-state ^{1}H NMR, and the spectra were dominated by *O*-alkyl structures with minor contributions from aliphatics (Fig. 8). After 90 days of biodegradation, sharp signals in the *O*-alkyl and aliphatic regions of the WEOM of both litters, probably from small carbohydrates and organic acids, disappeared and were likely utilized by microorganisms. A relatively intense CH_3 peak (Fig. 8e) was observed in the WEOM of biodegraded corn leaves. After photo-oxidation aliphatic components (0.9–1.2 ppm; Fig. 8a and c) were less abundant in the WEOM of corn leaves whereas a sharp peak (~2.4 ppm; Fig. 8b) appeared in the aliphatic region of WEOM of pine needles.

The chemical composition of bulk OM in corn leaves and pine needles was examined by solid-state ^{13}C NMR (Table 3). Biodegradation of plant litters reduced *O*-alkyl structures (mainly from carbohydrates) while alkyl carbon accumulated. By comparison, the solid-state ^{13}C NMR data did not reveal compositional changes to the bulk OM in litters under photo-oxidation. We therefore further examined the solution-state ^{1}H NMR spectra of NaOH extracts from both litters, which can reveal more sensitive environmental changes than bulk OC (Olk et al., 1998; Feng et al., 2008). ^{1}H is also more abundant in OM and is a more sensitive NMR nucleus than ^{13}C, thus, ^{1}H NMR may detect OM transformation products that may not be visible by ^{13}C. In the NaOH extracts of photo-oxidized corn leaves, sharp peaks (notably ~1.9 ppm, 2.4 ppm and 3.9 ppm; Fig. 9a) appeared in the *O*-alkyl and aliphatic regions, most likely representing small carbohydrates and organic acids (possible degradation products from photo-oxidation). Similarly, a sharp peak at the same chemical shift (~1.9 ppm; Fig. 9b and f) was observed in the NaOH extracts of photo-oxidized and biodegraded pine needles. Amide signals increased considerably in the NaOH extracts of biodegraded corn leaves (~8.2 ppm; Fig. 9e). By comparison, the diffusion edited NMR spectra of NaOH extracts from corn leaves showed similar structural distributions in the biodegraded, control and photo-oxidized samples (data not shown). However, aliphatic signals in the region of 1.4–2.1 ppm (Fig. 10a) considerably decreased in the NaOH extracts of photo-oxidized pine needles and aliphatic signals in the region of 0.9–2.1 ppm decreased in the biodegraded pine needles (Fig. 10c), suggesting reduction of aliphatic macromolecular species with both treatments.

3.2. Photo-oxidation of soils

3.2.1. Degradation and solubility of bulk OC in soils

Due to the slow microbial decomposition rate of SOM (Feng and Simpson, 2008), biodegradation of SOM was not included in this study and we focus on the alteration of SOM under photo-oxidation. The OC content of soil samples decreased by ~1% after 90 days of photo-oxidation (Table 2). This decrease in bulk OC was statistically not significant. However, photo-oxidation significantly increased OC solubility in that WEOC increased in mineral soils as well as Peat under photo-oxidation ($P < 0.05$; Fig. 11). The WEOC increase was linear with time in the photo-oxidized mineral soils ($P < 0.05$) such that WEOC almost doubled in the

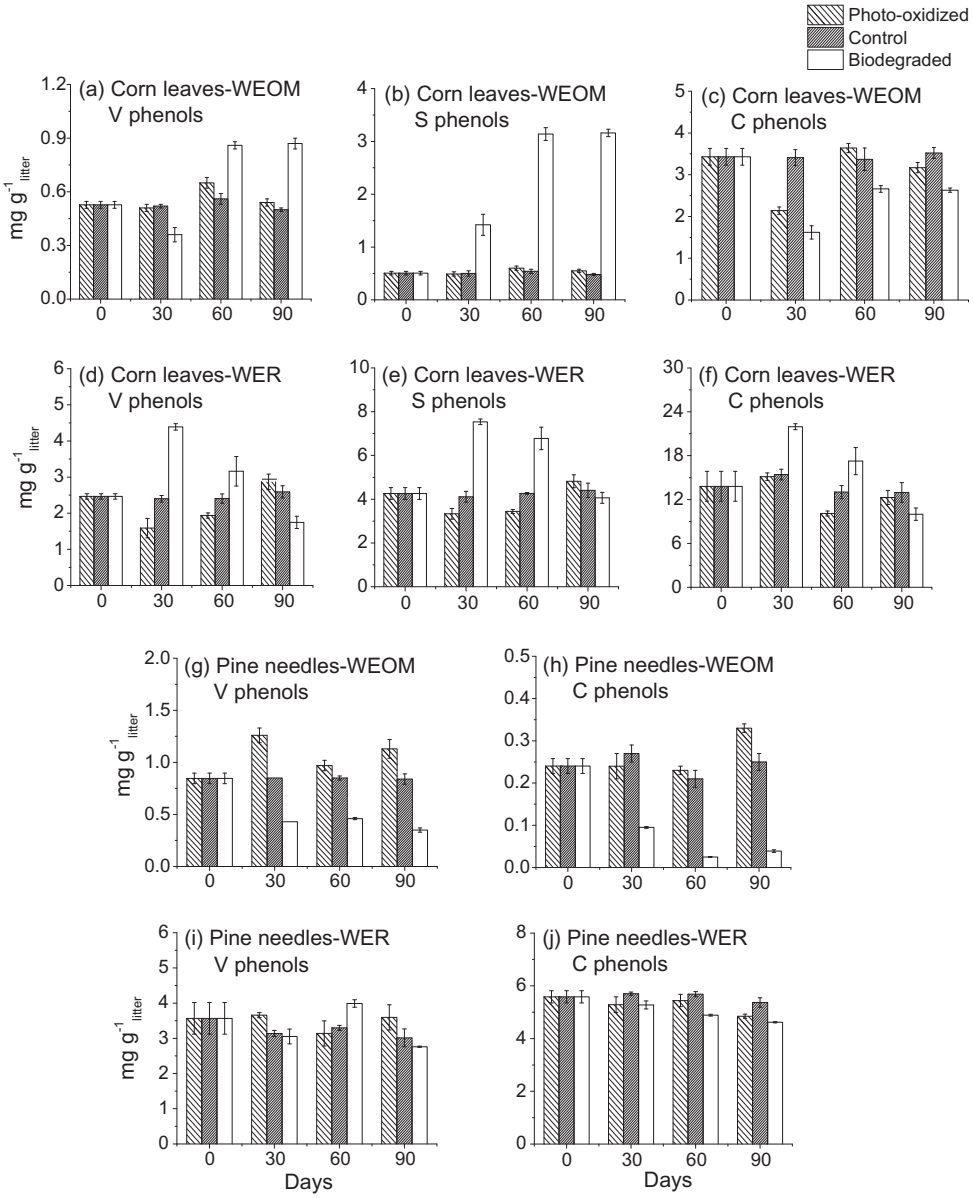

Fig. 4. Lignin derived phenols extracted from the water extractable organic matter (WEOM) and water extracted residues (WERs) of litter samples. Error bars represent standard errors ($n = 3$). V: vanillyl phenols; S: syringyl phenols; C: cinnamyl phenols.

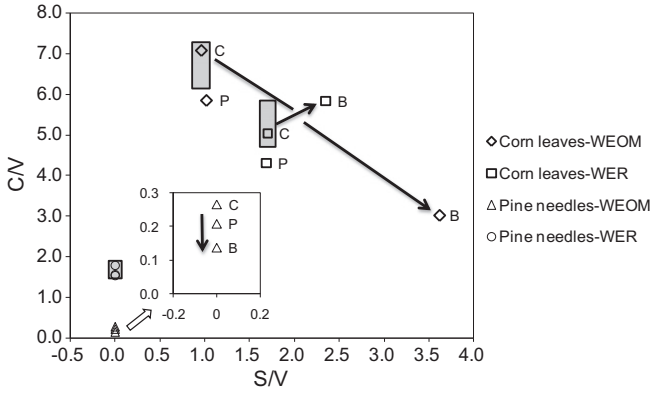

Fig. 5. Changes in the S/V and C/V ratios after 90 days of biodegradation and photo-oxidation of litter samples. V: vanillyl phenols; S: syringyl phenols; C: cinnamyl phenols; P: photo-oxidation; C: control; B: biodegradation. Shaded areas represent the range of ratios observed in control samples on days 0, 30, 60 and 90.

mineral soils compared to the control on day 90 (Fig. 11), accounting for 0.5% and 1.8% of total OC in Soils M and L, respectively.

3.2.2. Degradation of lignin in WEOM

Compared with litter samples, a smaller fraction of lignin derived phenols were extracted from the WEOM of mineral soil samples: approximately 0.1–0.3% and 1–4% of the total V, S and C phenols (total = WEOM + WERs) were extracted from the WEOM of Soil M and Soil L, respectively (data not shown). Lignin derived phenols significantly increased with photo-oxidation in the WEOM of mineral soil samples, and the increase was linear over time ($P < 0.05$; Fig. 12a–f). V and S phenols significantly increased in the WEOM of photo-oxidized Peat ($P < 0.05$) whereas the increase of C phenols was not statistically significant (Fig. 12g–i). The C/V ratio decreased from 0.34 to 0.23 in the WEOM of Soil M under photo-oxidation, but the ratios of C/V or S/V remained similar in all other soil samples. In the WEOM of Soil M, two *iso*-branched fatty acids (C_{16} and C_{17}) were detected and their concentration

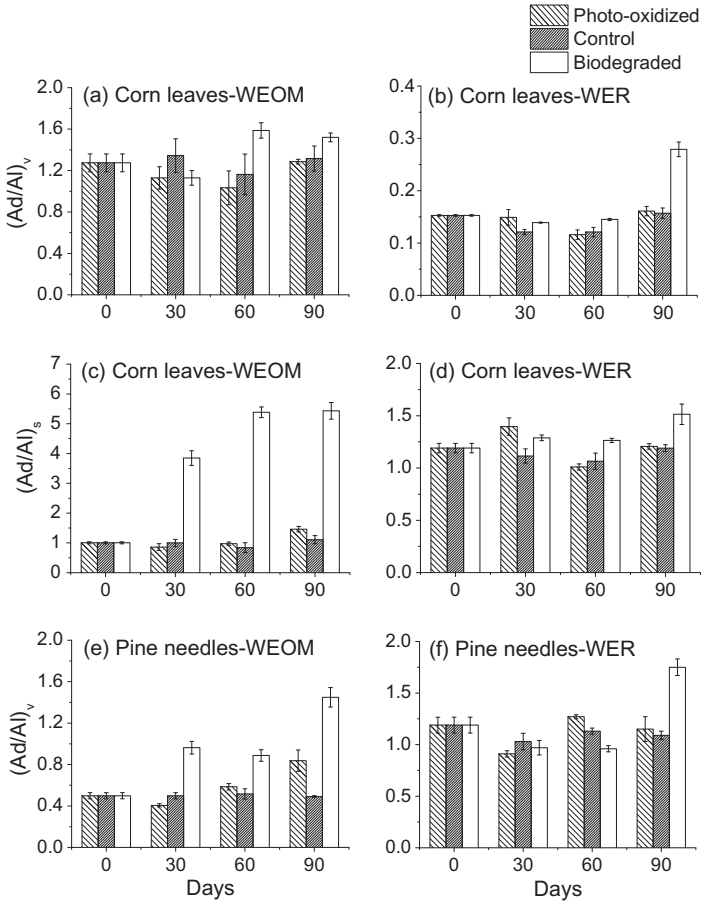

Fig. 6. The Ad/Al ratios of lignin derived phenols in litter samples. Error bars represent standard errors ($n = 3$). WEOM: water extractable organic matter; WER: water extracted residue. Subscripts 'V' and 'S' indicate vanillyl and syringyl phenols, respectively.

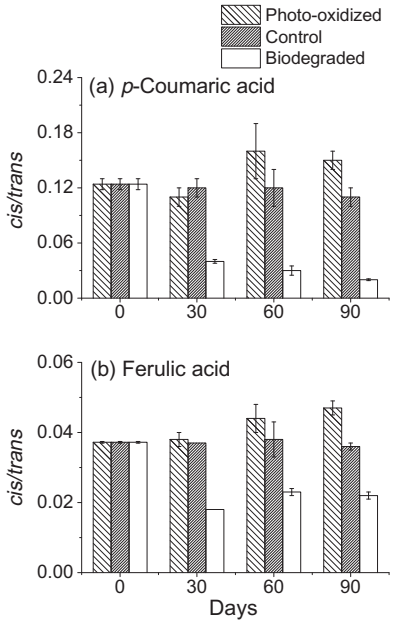

Fig. 7. Ratios of *cis*- to *trans*-isomers (*cis*/*trans*) of cinnamyl (C) phenols in the water extracted residue (WER) of corn leaves. Error bars represent standard errors ($n = 3$).

remained fairly constant in both control and photo-oxidized samples (0.22–0.27 μg/g). Furthermore, the $(Ad/Al)_v$ ratio increased in the WEOM of both mineral soil samples ($P < 0.05$) but was not significantly different in Peat (Fig. 13a–c). The $(Ad/Al)_s$ ratio increased significantly in the WEOM of Soil M ($P < 0.05$) and remained constant in the WEOM of Soil L and Peat (Fig. 13d–f).

3.2.3. Chemical composition of bulk OM in soil

Bulk OM in Soil M was examined by solid-state ^{13}C NMR (Table 3), which did not reveal any compositional changes under photo-oxidation. We further examined the solution-state ^{1}H NMR spectra of NaOH extracts from Soil M, which had a similar structural distribution under photo-oxidation and control conditions (data not shown). However, the signal intensity of diffusion edited NMR for the photo-oxidized soil was only half of that for the control soil under the exactly same NMR conditions (data not shown), suggesting a considerable reduction of macromolecular species in SOM after exposure to UV radiation.

4. Discussion

4.1. Transformation of terrigenous OM under biodegradation and photo-oxidation

Biodegradation of litter samples by soil inoculum was optimized in this study, where the magnitude of carbon mineralization in 3 months was comparable to that of carbon loss through mineralization and leaching during the decomposition of fresh corn leaves (~12%) and pine needles (~3.2%) in the field (Melillo et al., 1989; Burgess et al., 2002). The carbon loss from corn leaves was much higher than that from pine needles mainly due to its higher content of soluble OM (Fig. 3), which controls OM

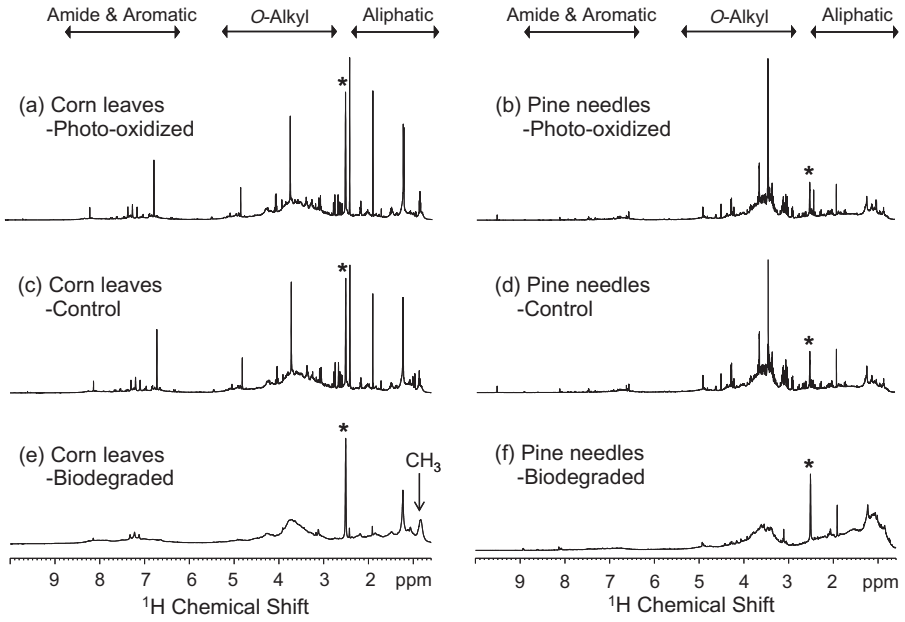

Fig. 8. Solution-state ^{1}H NMR spectra of water extractable organic matter (WEOM) from litter samples on day 90. *DMSO-d_6 solvent.

Table 3
Relative percentages (normalized to the total signal of ^{13}C) of carbon structures in the solid-state ^{13}C NMR spectra of bulk OM on Day 90.

	Corn leaves			Pine needles			Soil M	
	Photo-oxidized	Control	Biodegraded	Photo-oxidized	Control	Biodegraded	Photo-oxidized	Control
Alkyl (0–45 ppm)	15	17	29	21	22	27	46	45
O-Alkyl (45–110 ppm)	70	68	53	57	55	51	33	33
Aromatic and phenolic (110–165 ppm)	9	9	11	19	20	18	14	14
Carboxylic and carbonyl (165–215 ppm)	6	6	7	3	3	4	7	8

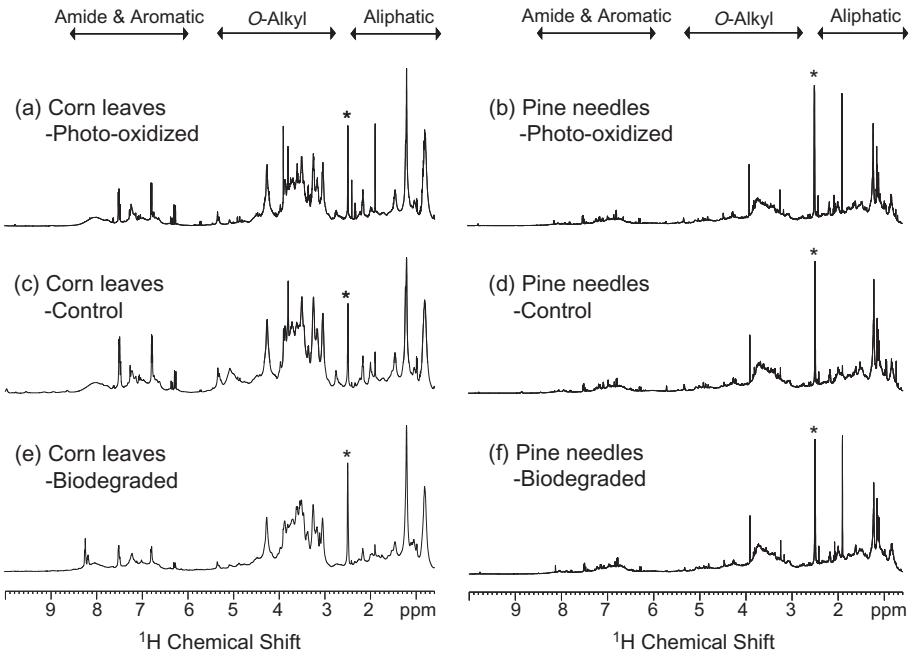

Fig. 9. Solution-state ^{1}H NMR spectra of NaOH extracts from litter samples on day 90. *DMSO-d_6 solvent.

decomposition rate during the early stages of biodegradation (Berg and Staaf, 1980). Biodegradation reduced the labile *O*-alkyl structures mainly derived from carbohydrates in litters (Table 3). In particular, small carbohydrates and organic acids were efficiently removed from the WEOM of both litter samples with biodegradation (Fig. 8), consistent with the expectation of intense degradation of soluble OM in plant litter (Berg, 2000; Cleveland et al., 2004). In contrast, the chemical composition of NaOH extracts was less altered (Fig. 9) because NaOH extracts contain more macromolecular species that are harder to break down. A decline of

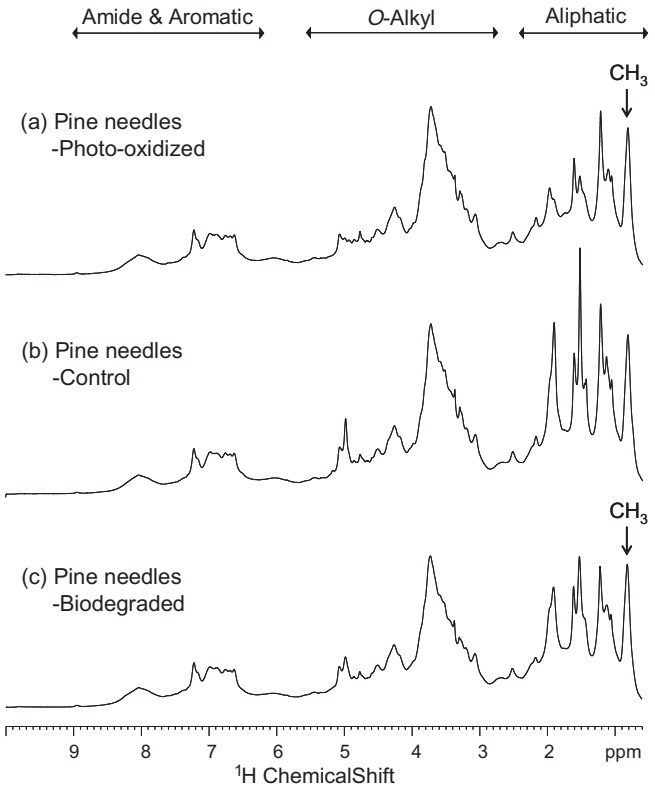

Fig. 10. Diffusion edited solution-state ^{1}H NMR spectra of NaOH extracts from pine needles on day 90.

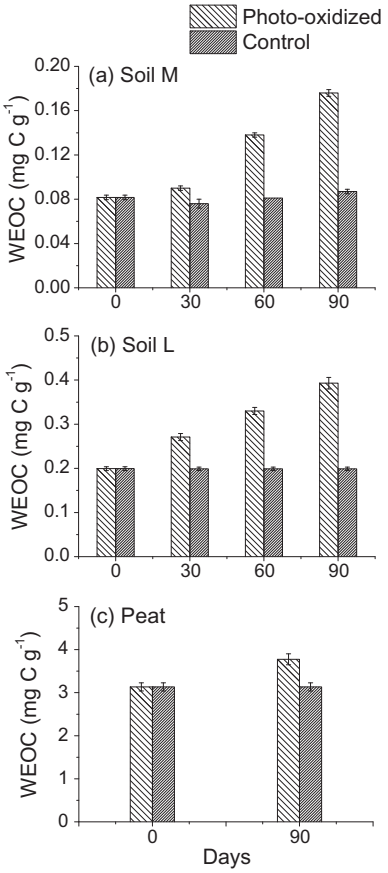

Fig. 11. The water extractable organic carbon (WEOC) content of soil samples during biodegradation and photo-oxidation. Error bars represent standard errors ($n = 3$).

macromolecular species in the aliphatic region was observed during the biodegradation of pine needles (Fig. 10). Waxes, lipids and cutin are believed to the major contributors to this region (1–1.8 ppm; Simpson et al., 2007a,b). Among them, cutin is unlikely to decompose during the early phase of litter degradation due to its recalcitrance (Hu et al., 2000; Gleixner et al., 2001). However, microbial attack of wax lipids coated on the surface of pine needles is known to induce a faster decay of 'free' lipids than cellulose or lignin (Bridson, 1985). This process may explain the observed reduction of aliphatic macromolecular species in pine needles. The biodegraded corn leaves had a faster decomposition rate and more visible microbial colonization than pine needles, as well as an intense CH_3 peak in the WEOM (Fig. 8e). Although lipids (such as plant cuticles) contain CH_3 groups, they are unlikely to substantially contribute to the CH_3 peak in the WEOM due to their low solubility. Alternatively, the CH_3 peak could be derived from microbial peptides, which are also evident from the increased amide signals in the NaOH extracts (Fig. 9e; Simpson et al., 2007a). Aliphatic structures from microbial biomass may also contribute to the accumulation of alkyl carbon in the solid-state ^{13}C NMR spectrum of biodegraded corn leaves (Table 3).

In comparison to biodegradation, litter samples were less transformed by photochemical degradation. Mineralization of DOM to CO_2 and CO has been shown to occur under UV radiation (Kieber et al., 1990; Miller and Zepp, 1995). Photochemical mineralization of terrigenous plant litter has also been documented in arid and semi-arid regions (Austin and Vivanco, 2006). The same mechanism was very likely to contribute to the OC content decreases (1–7%) in litter and soil samples exposed to photo-oxidation in this study. Substantial alterations to the bulk chemistry of WEOM from litter were not observed after photo-oxidation (Fig. 8). However, slight decreases of aliphatic components in the WEOM of corn leaves and NaOH extracts of pine needles were observed under UV radiation (Figs. 8 and 10), likely reflecting photochemical breakdown of cell wall lipids in litter (Day et al., 2007). In addition, small aliphatic molecules, which are likely to be photochemical products (such as organic acids; Allard et al., 1994; Moran and Zepp, 1997; Brinkmann et al., 2003), were detected in the WEOM of pine needles and the NaOH extracts of both litter samples (Figs. 8 and 9), suggesting photochemical alteration of litter OM. More importantly, a considerable reduction of macromolecular SOM species was detected by diffusion edited NMR in the photo-oxidized Soil M NaOH extract. Similar trends have been reported for OM in aqueous solutions (De Haan, 1993; Allard et al., 1994; Osburn et al., 2001) but not in terrigenous environments. Smaller molecules produced from photochemical degradation of macromolecular SOM species are very likely to contribute to the increased WEOC content from soil under UV radiation (Fig. 3). These observations confirmed our hypothesis that photo-oxidation may increase the solubility of terrigenous OM by reducing macromolecular species.

4.2. Response of lignin derived phenols to biodegradation and photo-oxidation

Lignin derived phenols are known to have variable stability to microbial decomposition (Hedges et al., 1988; Opsahl and Benner, 1995; Bahri et al., 2006). C phenols that link carbohydrates and lignin in the ligno-cellulose complex of non-woody vascular plant tissues are more accessible to microbial degraders and hence less stable than V phenols (Lam et al., 2001; Bahri et al., 2006). Consistently, C phenols decreased over time in the WER of biodegraded pine needles while V phenols remained relatively constant (Fig. 4i and j). By contrast, the sum of lignin derived phenols increased in the WER of corn leaves in the first 2 months of

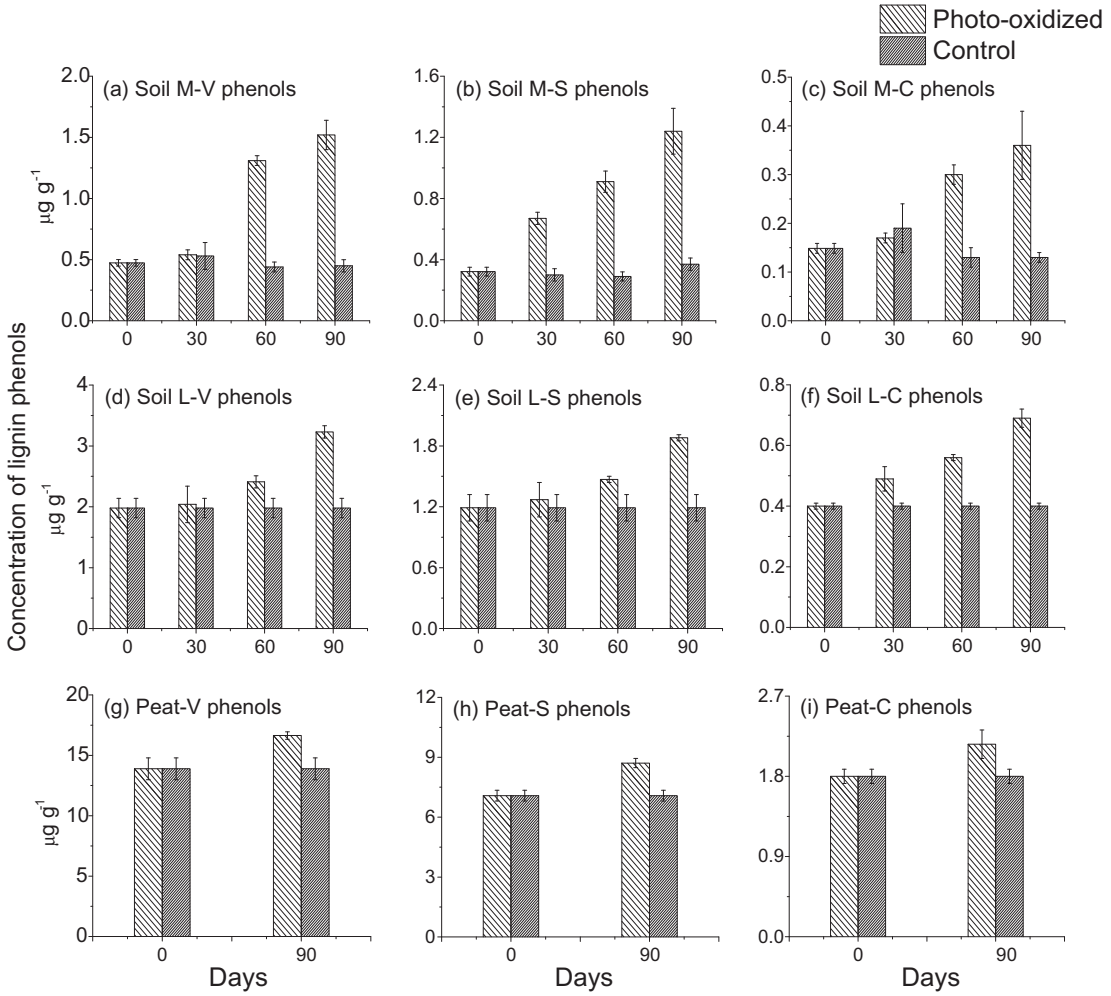

Fig. 12. Lignin derived phenols in the water extractable organic matter (WEOM) of soil samples. Error bars represent standard errors ($n = 3$). V: vanillyl; S: syringyl; C: cinnamyl.

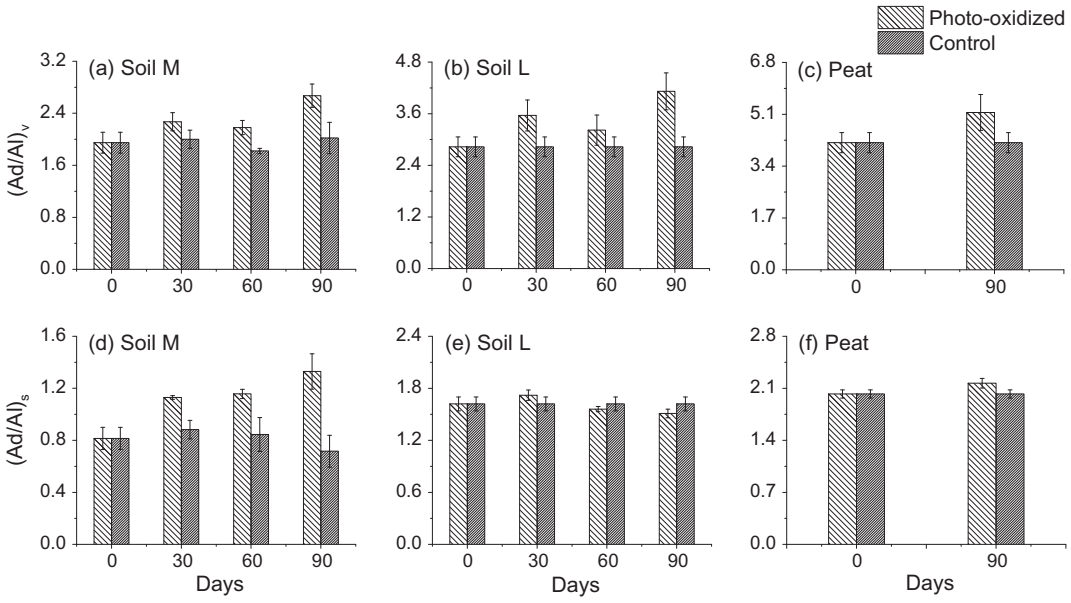

Fig. 13. The Ad/Al ratios of lignin derived phenols in the water extractable organic matter (WEOM) of soil samples. Error bars represent standard errors ($n = 3$). Subscripts 'V' and 'S' indicate vanillyl and syringyl phenols, respectively.

biodegradation together with an increase in V and S phenols in the WEOM (Fig. 4a–f). Microbial transformation of fresh litter at the initial stage of litter decomposition may have cleaved the cross linkages between phenylpropanoid units in lignin and released smaller units from the biopolymer (Said-Pullicino et al., 2007), which contributed to the increase of V and S phenols in the WEOM and the increased extractability of lignin derived phenols in the WER. With more extensive biodegradation, lignin derived phenols were mineralized or transformed (Bahri et al., 2006) and hence the V, S and C phenols decreased in the WER of corn leaves on day 90 (Fig. 4d–f). The decrease of C phenols in the WEOM of biodegraded corn leaves (Fig. 4c) may be attributed to their greater vulnerability to biodegradation than V or S phenols. Similar to C phenols, S phenols are reported to degrade faster than V phenols in the environment (Hedges et al., 1988; Opsahl and Benner, 1995; Otto et al., 2005). However, increases in the S/V ratio have been documented at the early stage of litter decomposition (Kögel, 1986; Miltner and Zech, 1998) and were observed in biodegradation of corn leaves (Fig. 5). This was presumably due to a preferential release of S phenols from the lignin polymer (i.e., higher yield of S phenols from CuO oxidation) or the preferential microbial attack of V phenols. Alternatively, a higher S/V ratio was found in the WEOM of corn leaves during biodegradation (Fig. 5), suggesting that microbial transformation led to a higher solubility of S phenols in corn leaves. Preferential loss of S phenols through leaching during biodegradation may lead to a lower S/V ratio in the remaining litter residue. However, no leaching occurred in this experiment and hence more S phenols were extracted. The counter-intuitive change in the S/V ratio observed during the early stage of litter decomposition suggests caution in reconstructing OM inputs from different plant groups (gymnosperm versus angiosperm) based on data of lignin derived phenols. The yields of V, S and C phenols from diverse OM media deserve further investigation to make the lignin data comparable across various environments.

Consistent with other studies (Hedges et al., 1988; Opsahl and Benner, 1995), biodegradation significantly increased the Ad/Al ratios of V and S phenols in both the WEOM and WER of litter samples due to side chain oxidation of lignin (Fig. 6). However, there was a notable difference in the Ad/Al ratios between WEOM and WER of the same sample. Significant increases in the Ad/Al ratios have been reported in the leachates or DOM from litters due to a preferential leaching of the acid forms of lignin derived phenols (Benner et al., 1990; Hernes et al., 2007). The difference in the Ad/Al ratios between WEOM and WER was especially large in corn leaves, likely due to a higher solubility of OM in corn leaves. This phenomenon warrants caution in comparing the Ad/Al ratios between litters with different solubility and chemistry.

Compared with biodegradation, photo-oxidation did not change the Ad/Al ratios of lignin derived phenols in corn leaves. However, lignin derived phenols increased in the WEOM of photo-oxidized pine needles (Fig. 4g and h) and mineral soil samples (Fig. 12a–f) together with an increase in the $(Ad/Al)_v$ ratio (Figs. 6 and 13a and b). V and S phenols also increased in the WEOM of photo-oxidized Peat (Fig. 12g–i) but the increase of Ad/Al ratios was not significant (Fig. 13c and f), probably due to the slow transformation of Peat OM under photo-oxidation as compared with mineral soils. These observations suggest that while photochemical alteration of lignin may be slow or absent in Peat and litter (corn leaves) OM, lignin transformation is enhanced in mineral soils under UV radiation. Enhanced solubility of lignin may contribute to the increased SOM solubility under photo-oxidation. An elevated $(Ad/Al)_v$ ratio has been previously reported for DOM exposed to photodegradation (Opsahl and Benner, 1998). Collectively, UV radiation is shown to induce transformation of lignin in solid residues as well as in aqueous solutions. Alternatively, the $(Ad/Al)_s$ ratio was reported to be unresponsive to photodegradation in DOM (Opsahl and Benner, 1998). In this study, the $(Ad/Al)_s$ ratio increased in the WEOM of Soil M under photo-oxidation and remained constant in all other samples (Figs. 6 and 13), suggesting that side chain oxidation by UV radiation was less prominent in S than in V phenols. The S/V ratio remained similar in all samples while the C/V ratio slightly decreased in the WEOM of both litter samples (Fig. 5) and Soil M, suggesting a higher susceptibility of C phenols to UV radiation. This observation was in contrast to the findings by Opsahl and Benner (1998) where the S/V ratio decreased while the C/V ratio increased in DOM exposed to UV radiation. As Opsahl and Benner (1998) suggested, selective degradation of S phenols by marine microorganisms as well as their higher susceptibility to photochemical alteration may have contributed to the decrease in S/V ratio observed in their study. In contrast, the litter and soil samples were kept in dry conditions under UV radiation and minimal microbial activity prevented microbial utilization of the S phenols in this study. Alternatively, lignin responses to photo-oxidation varied among different litter and soil types, indicating a control by OM chemistry. DOM, SOM and litter OM have distinct chemical composition and resistance to degradation (Benner et al., 1992; Marschner and Kalbitz, 2003), which may contribute to their different responses to UV radiation.

Finally, isomerization of lignin C phenols leading to an increase of *cis* isomers has been reported under light exposure (Hartley and Jones, 1975; Turner et al., 1993). A similar pattern was observed in the WER of corn leaves exposed to UV radiation (Fig. 7). By contrast, biodegradation selectively removed *cis* isomers and led to a decrease in the *cis*/*trans* ratio of corn leaf WER. The distinct responses of the *cis*/*trans* ratio of lignin C phenols may be utilized to distinguish between the two decay mechanisms of litter. Moreover, similar to the Ad/Al ratio, the *cis*/*trans* ratio of C phenols varied considerably between the WER and WEOM such that *cis* isomers were more enriched in WEOM. Because WEOM extraction was conducted in the dark and lignin derived phenols were analyzed by the same method from WEOM and WER, isomerization during extraction procedures was unlikely. Intuitively, a higher proportion of lignin that was close to the leaf surfaces was exposed to UV radiation, photochemically transformed, and became more soluble in water. Hence, WEOM contained a higher proportion of transformed lignin phenols in the *cis* form.

4.3. Origin and implications of increased SOM solubility under UV radiation

As discussed previously, the radiation induced increase in SOM solubility can be attributed to the photochemical degradation of macromolecular SOM species and the increase of lignin derived phenols in WEOM under photo-oxidation. UV radiation is also known to alter microbial community composition and biomass (Klironomos and Allen, 1995; Lindell et al., 1995; Johnson, 2003). However, microbial activity was minimal in the air-dried soils and the concentration of two *iso*-branched fatty acids that were microbially derived (Boon et al., 1977; Otto et al., 2005) remained similar in the WEOM of Soil M. Hence, microbial contribution to the increased WEOC was unlikely. Higher energy UV radiation is known to disrupt SOM–mineral interactions (Skjemstad et al., 1993). However, it is not known whether this mechanism contributed to the increased SOM solubility at a lower UV level as used in this study.

Photo-oxidation doubled the amount of WEOC in Soils M and L after 3 months of UV radiation, releasing an additional of 0.10–0.19 mg C/g of soil carbon into the aqueous phase. It should be noted that the intensity of UV radiation used in this study is higher than the average natural solar radiation in order to promote photo-oxidation in the laboratory. Nonetheless, the magnitude of WEOC increase is significant given the low level of WEOC content

in agriculture, grassland and forest soils (0.003–0.4 mg C/g; (Boyer and Groffman, 1996; Embacher et al., 2007; Rennert et al., 2007). Increased SOM solubility may lead to an increased soil carbon loss through leaching. Moreover, DOM provides the most readily available substrate for soil microorganisms (Zsolnay, 1996; Marschner and Kalbitz, 2003). Photochemical products are shown to be less resistant to microbial attack than the parent OM in aquatic systems (Lindell et al., 1995; Wetzel et al., 1995; Moran and Zepp, 1997; Judd et al., 2007). Microbial mineralization of photo-oxidized SOM is hence very likely to increase. These processes may play a key part in SOM decomposition and carbon cycling in surface soils that are exposed to solar radiation.

5. Conclusions

Molecular transformation of terrigenous OM and its lignin components was examined and compared during microbial and photochemical decomposition of plant litter and soil samples in this study. As expected, biodegradation dominated in the litter OM decomposition, which increased lignin oxidation, reduced carbohydrate content in the bulk OM and removed soluble carbohydrates and organic acids from the WEOM in both litter types investigated. In contrast, photo-oxidation did not substantially alter the chemical composition of litter OM. However, small aliphatic molecules, probably representing photochemical breakdown products, were detected in WEOM and NaOH extracts from litter by NMR analysis. Most importantly, photo-oxidation significantly increased the solubility of SOM, which was attributed to an enhanced oxidation of lignin derived phenols and a photochemical degradation of macromolecular species as observed by diffusion edited NMR. Our data provide the evidence that UV radiation may play a role in destabilizing SOM and may contribute to soil carbon loss through leaching and oxidation. Photochemical transformation of terrigenous OM, in particular, SOM, merits further investigation to promote soil management practice that prevents photochemical degradation of soil carbon in future.

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