New Phytologist

Labile compounds in plant litter reduce the sensitivity of decomposition to warming and altered precipitation

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Received: *6 March 2013*
Accepted: *15 May 2013*

New Phytologist (2013) **200:** 122–133
doi: 10.1111/nph.12376

Key words: ^{13}C NMR, climate change, DRIFT, Japanese knotweed (*Polygonum cuspidatum*), lignin, litter decomposition, recalcitrant.

Summary

- Together, climate and litter quality strongly regulate decomposition rates. Although these two factors and their interaction have been studied across species in continent-scale experiments, few researchers have studied how labile and recalcitrant compounds interact to influence decomposition, or the climate sensitivity of decomposition, within a litter type.
- Over a period of 3 yr, we studied the effects of warming and altered precipitation on mass loss and compound-specific decomposition using two litter types that possessed similar heteropolymer chemistry, but different proportions of labile and recalcitrant compounds.
- Climate treatments immediately affected the mass loss of the more recalcitrant litter, but affected the more labile litter only after 2 yr. After 3 yr, although both litter types had lost similar amounts of mass, warming (*c.* 4°C) and supplemental precipitation (150% of ambient) together accelerated the degradation of alkyl-carbon and lignin only in the more recalcitrant litter, highlighting the role of initial litter quality in determining whether the chemistry of litter residues converges or diverges under different climates. Our finding that labile compounds in litter reduce the climate sensitivity of mass loss and the decomposition of recalcitrant matrix is novel.
- Our results highlight the potential for litter quality to regulate the effect of climatic changes on the sequestration of litter-derived carbon.

Introduction

Litter decomposition sustains ecosystem productivity and can provide a feedback to climate change through changes in the rate of CO_2 return to the atmosphere. Climate change is altering litter decomposition rates through a variety of mechanisms (Dukes & Field, 2000; Fierer *et al.*, 2005; Feng *et al.*, 2008), but little is known about how quickly these rates are changing or the relative importance of the various chemical components of litter driving this response (Cornelissen *et al.*, 2007; Cornwell *et al.*, 2008; Salinas *et al.*, 2011). Understanding the sensitivity of turnover rates and the stability of biochemical components in litter is critical, as these factors influence the amount and chemical composition of organic matter in soil (Crow *et al.*, 2009; Kramer *et al.*, 2012; Wickings *et al.*, 2012).

Biochemical compounds in plant tissues vary in their susceptibility to decomposition, on a spectrum from labile (decaying quickly; e.g. carbohydrates and amino acids) to relatively recalcitrant (decaying slowly; e.g. lignins (Boerjan *et al.*, 2003; Floudas *et al.*, 2012), tannins (Lorenz *et al.*, 2007) and cuticular matrix (Hu *et al.*, 2000)). The recalcitrant compounds are enriched in plant litter as a result of resorption of labile compounds during senescence, and can potentially influence ecosystem functions by modulating the decomposition rates. According to kinetic theory, recalcitrant compounds with high activation energies should be relatively sensitive to temperature (Bosatta & Agren, 1999; Davidson & Janssens, 2006), implying that warming could disproportionately accelerate the decomposition of recalcitrant compounds. This effect of climate on decomposition is not only governed by the kinetics of biochemical reactions, but is also modulated by other abiotic and biotic interactions, many of which have received little attention. For example, climate might have a comparatively small effect on the decomposition of labile compounds, as these substrates can be utilized by a broader spectrum of microorganisms that operate across a wider range of climatic conditions. Further, when labile compounds are abundant, bacterial communities may outcompete fungal communities (McGuire & Treseder, 2010) for non-carbon substrates, thus slowing the decomposition of recalcitrant compounds irrespective of climate. The quality of the litter matrix (as indicated by the proportions of labile and relatively recalcitrant compounds) could thus alter the direct effect of climate on decomposition by affecting microbial community composition (Fig. 1). Apart from the climatic controls, decomposition and associated microbial transformations also depend on how the carbon in the substrates is partitioned

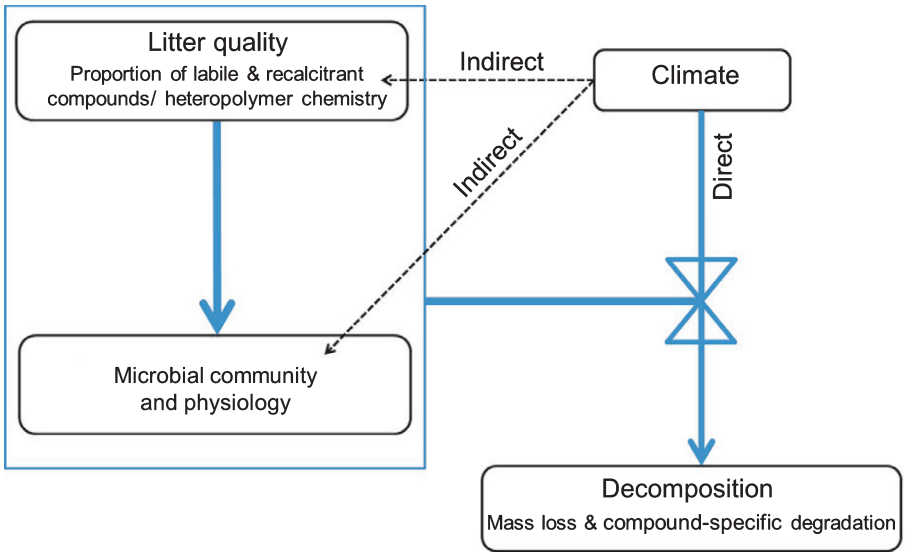

Fig. 1 Conceptual diagram illustrating how litter quality mediates the effect of climate on litter decomposition. Climate may influence litter decomposition indirectly by affecting the chemistry of litter at its formative stage (Tharayil *et al.*, 2011), and by mediating changes in microbial community, physiology and enzyme activity (Allison *et al.*, 2010). The dotted lines represent these indirect effects of climate on decomposition. Climate can also affect the rate of decomposition directly by altering the activation energy of compounds in litter (Davidson & Janssens, 2006). Our study highlights that the effect of climate on decomposition may be modified by litter chemistry (chemistry of heteropolymers and the proportion of labile and recalcitrant compounds) via changes in microbial community composition and physiology.

between microbial biomass production and microbial respiration (carbon use efficiency, CUE; Manzoni *et al.*, 2012), which, in turn, depends on the nutritional quality of the substrates (Keiblinger *et al.*, 2010). Thus, there are several plausible mechanisms by which the chemical composition of litter matrix might modulate the climate sensitivity of recalcitrant compounds. However, this type of litter chemistry-mediated effect of climate on decomposition remains relatively unexplored.

Previous studies have attempted to elucidate the effects of climate on decomposition using litter from different plant species which varies in its composition of heteropolymers (Preston *et al.*, 2009), thus achieving different ratios of labile to relatively recalcitrant compounds. However, the structural identity of plant heteropolymers differs across different species (Ralph *et al.*, 2004; Dixon *et al.*, 2005) and among individuals of the same species as a result of genetic and environmental variation (Vanholme *et al.*, 2010; Tharayil *et al.*, 2011). Most heteropolymers are functionally/operationally defined, their biological reactivity determined by the type and number of monomers, the linkages connecting these monomers (Nierop *et al.*, 2006; Schweitzer *et al.*, 2008; Talbot *et al.*, 2012) and the overall polydisperse matrix, all of which vary significantly within and between species, and could potentially influence processes associated with litter decomposition. For example, the presence of acylated monolignols, by imparting hydrophobicity to lignin (del Rio *et al.*, 2007), could delay its mineralization. Similarly, in tannins, prodelphinidins which contain tri-hydroxy B-rings, have greater enzyme inactivation and protein complexation capacity than procyanidins, which contain di-hydroxy B-rings (Nierop *et al.*, 2006). Thus, studies that use litter from different species to characterize the influence of litter quality on the climate sensitivity of decomposition compare litter types that differ not only in the relative concentrations of compounds (Austin & Ballare, 2010), but also in the molecular structures of their heteropolymers, which also influence decomposition rates. This conflation of the concentration and structure of chemical compounds in litter limits the interpretation of results.

Here, we used two litter types that differed in the relative proportions of labile and recalcitrant compounds, but had heteropolymers with similar molecular structure, to characterize the overall mass loss and compound-specific response of plant litter decomposition to the combined effects of warming and altered precipitation. We hypothesized that the influences of warming and altered precipitation on the decomposition of litter would depend on the relative abundance of labile compounds in the litter. We predicted that labile compounds would limit the sensitivity of decomposition to climate, such that the decomposition of litter with a higher proportion of labile compounds (as indicated by a high carbohydrate carbon : methoxyl carbon (CC : MC) ratio; Mathers *et al.*, 2007) would be less responsive to warming and precipitation changes. Further, we predicted that warmer and wetter climates would accelerate the decomposition of recalcitrant compounds only in litter with a lower proportion of labile compounds (low CC : MC ratio).

To hold the structural identity of heteropolymers relatively constant across litter types, we used stem litter of *Polygonum cuspidatum* (Japanese knotweed; syn. *Fallopia japonica*, *Reynoutria japonica*) from a single clonal (genetically identical) population. We achieved a relative difference in proportions of labile and recalcitrant compounds in this litter by collecting samples at two stages after senescence. We collected newly senesced litter (referred to hereafter as *NL*) and old litter (referred to

hereafter as *OL*; this comprised 1-yr-old senesced stems that had been decomposing upright for a year in *P. cuspidatum* stands with no direct contact with soil), which we expected to have a lower proportion of labile compounds than *NL*.

Materials and Methods

Site description

We conducted the litter decomposition study at the Boston-Area Climate Experiment (BACE; Suseela *et al.*, 2012) in Waltham, MA, USA (42°23.1′N, 71°12.9′W). Boston has an annual mean precipitation of 1063 mm and an annual mean temperature of 10.3°C. The topsoil at the study site, a Mesic Typic Dystrudept, had a loamy texture (45% sand, 46% silt, 9% clay; gravel content, 7%). The study site was an old field with grasses and forbs, together with planted seedlings of tree species such as *Acer rubrum*, *Quercus rubra*, *Pinus strobus* and *Belula lenta* (Hoeppner & Dukes, 2012). The BACE had a split-plot, randomized block design, with precipitation treatments applied to *c.* 8-m by *c.* 14-m areas and warming treatments applied to 2-m by 2-m plots within those areas. The plots at BACE were subjected to four levels of warming (referred to as unwarmed, low (+ *c.* 1.0°C), medium (+ *c.* 2.7°C) and high (+ *c.* 4.0°C) warming) using infrared heaters, and three levels of precipitation (ambient (A), wet (W; 150% of ambient rainfall during the growing season) and dry (D; 50% of ambient precipitation)). Within each precipitation area, the four plots were arranged linearly. Each plot had four infrared heaters mounted 1 m above its corners that warmed the canopy and soil. The low, medium and high warming treatments were carried out using 200-, 600- and 1000-W heaters, respectively. Plant canopy temperature in the ambient and high warming plots within each group of four subplots was measured every 10 s using infrared radiometers. These readings were used to achieve feedback control, which limited warming to a maximum of 4°C in the high warming plots. The three precipitation treatments were applied to each precipitation event using rainout shelters and a sprinkler system. The experiment had a factorial design, with each of the 12 climate treatments represented within each of the three blocks (Suseela & Dukes, 2013).

Litter decomposition and chemical analyses

Polygonum cuspidatum Sieb. & Zucc. is a rhizomatous perennial plant that produces upright jointed stems, which reach over 2 m in height (Barney *et al.*, 2006; Tharayil *et al.*, 2013). The aboveground stems senesce each fall. The senesced stem litter for this study was collected from a single clonal population of *P. cuspidatum* stands in Amherst, MA, USA. The stems produced during the growing season of 2006 were tagged at the time of senescence (October 2006) and were left upright in the stands until their harvest in November 2007 (*OL*). New stems produced during 2007 were harvested shortly after their senescence in November 2007 (*NL*). The site experienced mean annual temperatures of 9.8 and 8.6°C and mean annual precipitation of 94 and 86 cm during 2006 and 2007, respectively (NOAA National Climate Data center station ID 190120, Amherst, MA, USA). An analysis of initial litter quality using ^{13}C nuclear magnetic resonance (NMR) spectroscopy showed that *OL* contained a greater proportion of recalcitrant carbon compounds (Table 1). The ^{13}C NMR spectral analysis (described below) provided CC : MC ratios (Almendros *et al.*, 2000; Blumfield *et al.*, 2004) of 17 and 11 for *NL* and *OL*, respectively, indicating a higher proportion of recalcitrant carbon in *OL*. Initial elemental analysis also showed C : N ratios of 123 and 165 for *NL* and *OL*, respectively, also indicating the lower quality of 1-yr-old stems. This approach of depleting labile carbon to understand the response of labile and recalcitrant fractions to temperature has been adopted previously in soils under laboratory settings (Conant *et al.*, 2008).

We placed 3 ± 0.03 g of air-dried *OL* or *NL* in $10 \times 10\ cm^2$ litter bags made of 2-mm mesh screening material. In July 2008, we placed a total of 576 bags of *OL* and *NL* around the edges of the BACE plots, in evenly spaced locations just within the plot borders (36 plots × 2 litter types × 8 replicates per plot per litter type). We retrieved a subset of the bags on each of four dates (after 124, 409, 805 and 1140 d of exposure). The litter remaining in each bag was carefully brushed to remove any soil contamination. The litter mass was corrected for contamination with mineral soil using the carbon content of the topsoil of each plot, with the assumption that any reduction in the carbon content of the remaining litter was a result of contamination with mineral soil. The fraction of litter remaining was calculated using the following equation (Janzen *et al.*, 2002)

$$M = (C_t - C_s)/(C_l - C_s)$$

(M, fraction of mass of litter remaining in the sample; C_t, measured carbon concentration of the sample; C_s, measured carbon concentration of the topsoil of each plot; C_l, measured carbon concentration of the undecomposed initial litter). This method allowed for the quantification of mass loss without washing the litter samples, which could have led to a loss of fragments and leaching of nutrients (Janzen *et al.*, 2002). Previous studies have found that this method gives similar results to ash correction

Table 1 Ratios of the relative peak intensities of chemical shift regions from ^{13}C NMR of *Polygonum cuspidatum* litter after 1140 d of field decomposition

Litter type (treatment)	CC : MC*	Alkyl C : *O*-alkyl C	(Aryl + *O*-aryl C) : *O*-alkyl C
NL (initial litter)	17.26	0.06	0.18
OL (initial litter)	11.51	0.08	0.17
OL (DHt)	7.16	0.08	0.27
OL (A0)	6.24	0.13	0.33
OL (WHt)	2.12	0.14	1.01

These ratios can be used as indicators of plant litter decomposition. DHt, dry + high warming; A0, ambient + no warming; WHt, wet + high warming; CC : MC, carbohydrate carbon : methoxyl carbon. *NL*, newly senesced litter; *OL*, old litter.
*The CC : MC ratio is a more robust indicator of decomposition of woody residues than the alkyl C : *O*-alkyl C ratio (Baldock *et al.*, 1997).

(Dukes & Field, 2000). At each retrieval date, subsamples were dried at 50°C for 48 h, weighed and analyzed for carbon and nitrogen content (with an ECS 4010 Elemental Combustion System, Costech Analytical Technologies, Valencia, CA, USA). The decomposition rate constants of both litter types within each individual treatment were calculated by fitting the percentage of mass remaining at each date of collection to the exponential function (Austin & Vitousek, 2000)

$$M_t = M_0 \times e^{-kt}$$

(M_0, percentage of initial mass; M_t, percentage of initial mass remaining at time t; k, decomposition rate constant). The curve was fitted with SigmaPlot version 12 (Systat Software Inc., San Jose, CA, USA). The carbon quality of air-dried subsamples was determined using diffuse reflectance infra-red Fourier transform (DRIFT) and ^{13}C NMR spectroscopy.

DRIFT spectroscopy

Three replicate litter samples from the unwarmed (0) and high warming (Ht) treatments in the dry (DHt and D0), ambient (AHt and A0) and wet (WHt and W0) precipitation treatments were analyzed using DRIFT spectroscopy. The infrared spectra of the samples were collected in transmission mode using a Spectrum One DRIFT spectrometer (Perkin-Elmer, Waltham, MA, USA) equipped with a deuterated triglycine sulfate detector. In short, finely ball-milled litter samples and spectral grade KBr were mixed at a ratio of 1 : 50 and further ground using an agate mortar and pestle. The finely ground (< 10 μm) mixture was gently packed into a macrocup sampling accessory and DRIFT spectra were recorded from 4000 to 650 cm^{-1} at 4 cm^{-1} resolution. Forty interferograms per sample were recorded, co-added and averaged, and corrected against the spectrum of pure KBr (Tharayil *et al.*, 2011). The spectra were Kubelka–Munk transformed and baseline corrected using ACD Spec Manager (Advanced Chemistry Development Inc., Toronto, ON, Canada). A typical mid-infrared spectrum (4000–400 cm^{-1}) can be divided into four distinct regions: the X–H stretching region (4000–2500 cm^{-1}), the triple bond region (2500–2000 cm^{-1}), the double bond region (2000–1500 cm^{-1}) and the fingerprint region (1500–600 cm^{-1}). The peaks between 3000 and 2800 cm^{-1} correspond to C–H stretching and, within this region the dominant peak at 2926 cm^{-1} corresponds to the asymmetric stretching of the methylene (CH_2) group. The shoulder at 2850 cm^{-1} corresponds to symmetric stretching of the methylene group (Smith, 2011). In decomposing plant litter, these vibrations can be associated with the presence of aliphatic compounds, such as cuticular waxes, which are abundant in methylene groups (> 96%) and low in methyl groups (< 2%, peaks at 2940 and 2870 cm^{-1}; Filley *et al.*, 2008). The bending vibration of CH_2 (scissoring) may also contribute to the peak at 1465 cm^{-1}. Based on sample properties, such as N content, spectra of purified standards and peak assignments in the literature, peaks in the remainder of the spectrum were identified as follows: 1510 cm^{-1}, C=C aromatic skeletal vibration (attributed to lignin); 1430 cm^{-1}, C–O–H in-plane bending (attributed to carboxylic acids); 1375–1370 cm^{-1}, C–H symmetrical bending (attributed to phenols and aliphatic compounds); 1320 cm^{-1}, CH deformation and C–O stretch; 1235 cm^{-1}, C–O–C stretch (attributed to aliphatic esters; acetyl side chain cleavage of hemicellulose). The broad peak from 1200 to 900 cm^{-1} corresponds to a combination of C–O stretching and O–H deformation, representing carbohydrates. Nine identifiable peaks corresponding to major functional groups were chosen for statistical analyses. For spectral overlay, within a litter type (*OL* and *NL*), the baseline-corrected spectra were normalized at 1060 cm^{-1}, corresponding to C–O and O–H vibrations of mixed carbohydrates. As the initial chemical composition within each litter type was similar, overlaying the spectra after normalization at 1060 cm^{-1} identified the relative differences in the decomposition ratio of carbohydrates to non-carbohydrates under different climatic treatments within *OL* and *NL*. The relative peak heights of functional groups in each sample, calculated as the ratio of the intensity of each of the individual peaks to the sum of the intensities of all of the peaks (Haberhauer & Gerzabek, 1999), was used to compare across samples within each litter type exposed to different climates.

^{13}C cross-polarization magic angle spinning (CPMAS) NMR spectroscopy

To further refine our understanding of the results obtained from DRIFT analysis, subsamples of *OL* from the climatic treatments that differed according to principal component analysis (PCA) of DRIFT peaks (samples retrieved after 1140 d from A0, D0, DHt, W0 and WHt) were subjected to ^{13}C NMR analysis. We combined three replicate litter samples into one composite sample for each treatment (Preston *et al.*, 2009), and analyzed that sample using ^{13}C NMR. To understand the initial litter quality, ^{13}C NMR analysis was also performed on initial litter of *NL* and *OL* not subjected to decomposition. Spectroscopic techniques, such as ^{13}C NMR, have proven to be non-destructive methods that provide suitable indices to successfully identify the qualitative changes that occur during litter decomposition (Mathers *et al.*, 2007; Preston *et al.*, 2009). To assess litter carbon chemistry (by carbon functional group), we obtained solid-state ^{13}C NMR spectra using a Chemagnetics CMX400 spectrometer (Varian Inc., Palo Alto, CA, USA) operating at a frequency of 100.6 MHz. Ball-milled samples were dried, packed in a 4-mm zirconium rotor and spun at 5 kHz. We used a standard cross-polarization pulse sequence (Mathers *et al.*, 2007). Spectra were acquired with a contact time of 3.5 ms, an acquisition time of 20 ms and a recycle delay of 3 s, optimized using preliminary experiments. Approximately 16 000 transients were collected for each sample; these were processed using a line broadening of 22 Hz, and were baseline corrected (Mathers *et al.*, 2007; Preston *et al.*, 2009). Chemical shift values were referenced externally to glycine at 176 ppm. Relative intensities for the chemical shift regions were integrated using an ACD/Spectrus processor (version 2012, Advanced Chemistry Development Inc.). The regions of chemical shift in a ^{13}C NMR spectrum provide information about the different carbon fractions, and the ratios of

these fractions can be used to describe changes in carbon quality. For instance, the CC : MC ratio can be determined from ^{13}C NMR, where a larger ratio indicates the potential for faster decomposition and a smaller ratio indicates that the material is more resistant to decomposition (Almendros *et al.*, 2000; Blumfield *et al.*, 2004; Mathers *et al.*, 2007). Spectra were analyzed within seven standard chemical shift regions: alkyl C (0–50 ppm); methoxyl and N-alkyl C (50–60 ppm); *O*-alkyl C (60–93 ppm); di-*O*-alkyl and some aromatic C (93–112 ppm); aromatic C (112–140 ppm); phenolic C (140–165 ppm); and carboxyl/carbonyl C (165–190 ppm; Preston *et al.*, 2009). We used the area of these chemical shift regions, expressed as a percentage of total intensity, to calculate three ratios: alkyl/(alkyl + methoxyl + di-*O*-alkyl), referred to as alkyl C : *O*-alkyl C (Preston *et al.*, 2009); *O*-alkyl C : methoxyl and N-alkyl C, referred to as CC : MC (carbohydrate C : methoxyl C; Blumfield *et al.*, 2004; Mathers *et al.*, 2007); and aryl + *O*-aryl C : *O*-alkyl C (Mathers *et al.*, 2007).

Statistical analyses

We used two model structures to gain detailed insight into the treatment effects on the percentage mass remaining (Finzi *et al.*, 2001; Wickings *et al.*, 2012; Mueller *et al.*, 2013). The data for the percentage mass remaining were analyzed by both time and decay stage. First, to identify broad patterns of mass loss, we used mixed-model restricted maximum likelihood (REML) analysis with warming, precipitation, time of litter collection and type of litter as main effects and percentage mass remaining as the response variable. Second, we examined how the percentage mass loss and decomposition rate constants of each litter type responded to warming and precipitation treatments (main effects) at each individual time of collection using mixed-model REML. The above analyses of percentage mass remaining for each litter type at consistent decay stages would provide more explanatory power for the influence of climate on decomposition, as litter types vary in their chemical quality over time (Wickings *et al.*, 2012). In all of these analyses, Tukey's honestly significant difference (HSD) multicomparison test was used to detect differences among individual treatments. Interpretations of DRIFT spectra were based on PCA, and on ANOVA of PCA coordinate scores, followed by Tukey's HSD multicomparison test. The relative heights of individual peaks of normalized DRIFT spectra of *OL* in A0, WHt and DHt treatments were also analyzed using one-way ANOVA, and differences among groups were tested using Tukey's HSD multicomparison test. All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

Mass loss

Analyses of mass loss with warming, precipitation, time of collection and litter type as main effects revealed that mass loss varied with precipitation ($P = 0.013$), time ($P < 0.001$) and litter type ($P = 0.001$; Supporting Information Table S1). In general, litter exposed to ambient ($P = 0.032$; 50% mass loss) and supplemental ($P = 0.013$; 52% mass loss) precipitation treatments lost more mass than litter in the dry treatment (43% mass loss). Averaged across all climate treatments, mass loss after 124, 409, 805 and 1140 d of field incubation was 21%, 40%, 58% and 74%, respectively. After 1140 d of decomposition, *OL* had lost more mass (50%) than *NL* (47%), on average. However, the effect of litter type on mass loss also varied with time (litter type × time interaction; $P = 0.004$; Fig. 2a); *OL* had lost more mass than *NL* after 409 d ($P = 0.001$) and 805 d ($P = 0.05$) of field incubation, but mass loss did not differ between *OL* and *NL* after 124 and 1140 d of decomposition.

Analyses of mass loss at individual times of collection revealed that the effects of the climate treatments varied with the initial quality of the litter (Fig. 2b,c; Tables S2, S3). During the early phase of decomposition (the first 124 d of field incubation) *c.* 20% of the litter mass was lost from both *NL* and *OL*, and neither warming nor altered precipitation treatments affected mass loss rates of *NL* (Fig. 2b). However, mass loss from *OL* varied with precipitation treatments after 124 d of decomposition (Fig. 2c; $P = 0.004$), with more mass lost from litter exposed to ambient ($P = 0.016$) and wet ($P = 0.007$) treatments than the dry treatment. Similarly, after 409 d of decomposition, litter in the

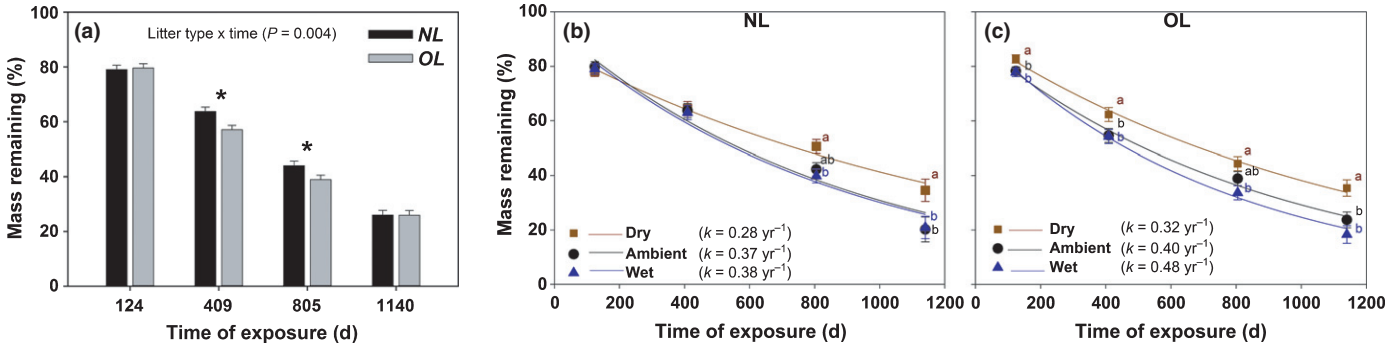

Fig. 2 Percentage initial mass remaining in (a) newly senesced litter (*NL*; $n = 36$) and 1-yr-old litter (*OL*; $n = 36$) of *Polygonum cuspidatum* across 1140 d of decomposition, and in (b) *NL* ($n = 12$) and (c) *OL* ($n = 12$) at each time of collection in the different precipitation treatments. Values represent means ± SE. (a) Asterisks indicate significant treatment effects ($P < 0.05$). In (b, c), different letters identify differences in means (Tukey's honestly significant difference (HSD)) among the precipitation treatments.

wet ($P = 0.018$; 46% mass loss) and ambient ($P = 0.025$; 46% mass loss) precipitation treatments had lost more mass than litter in the dry treatment (38% mass loss). Climate treatments did not affect mass loss of *NL* after 409 d, but, by 805 d and later, the dry treatment slowed mass loss of both litter types ($P < 0.05$; Fig. 2b,c). At the final time point (1140 d), *OL* had lost more mass in the ambient ($P = 0.05$; 76% mass loss) and wet ($P < 0.014$; 82% mass loss) treatments than in the dry treatment (63% mass loss), and *NL* had similarly decomposed more rapidly in ambient (80% mass loss; $P = 0.021$) and wet ($P = 0.015$; 79% mass loss) treatments than in the dry treatment (65% mass loss). Decomposition rate constants varied with precipitation ($P < 0.05$) treatments in both *NL* and *OL*, such that the dry treatment lowered the decay constant relative to ambient and wet treatments (Fig. 2b,c).

Litter quality

The progress of decomposition can be visualized in DRIFT spectra as a decrease (> 75%) in the intensity of bands representing the carbonyl stretching of esters and carboxylic acids at 1750 cm^{-1} and C_1–H vibration of β-glycosidic linkages in cellulose at 896 cm^{-1} (Fig. 3a,b; Hatakeyama *et al.*, 1976; Kataoka & Kondo, 1998; Artz *et al.*, 2008), which were transformed to less distinct shoulders after 1140 d of decomposition. At the same time, bands representing the C–H stretching of aliphatic compounds at 3000–2800 cm^{-1} and phenolic C=C and C=O stretching at 1660–1590 cm^{-1} increased in intensity, with greater increases in *OL* (63% and 72%, respectively) than *NL* (45% and 66%, respectively) after 1140 d.

We used DRIFT spectroscopy to assess the response of compound-specific degradation to the climate treatments (DHt, D0, AHt, A0, WHt and W0) in litter remaining after 1140 d of decomposition (Fig. 4; spectra normalized at carbohydrate band 1060 cm^{-1}). Alkyl carbon, derived from cutin, waxes and lipids, decreased in abundance in *OL* exposed to the warmed, wet treatment relative to *OL* from warmed treatments experiencing dry conditions (Fig. 4a; lower intensity of peaks at 2926 cm^{-1}, $P = 0.005$ and 1461 cm^{-1}, $P = 0.013$; Filley *et al.*, 2008; Lammers *et al.*, 2009) or ambient precipitation (at 2926 cm^{-1}, $P = 0.029$). Similarly, *OL* exposed to a warmer, wetter climate also had lower intensities of DRIFT peaks in the fingerprint region of 1500–1200 cm^{-1} ($P < 0.05$; Fig. 4a), indicating the greater decomposition of non-carbohydrate compounds per unit of carbohydrate. We used the Savitsky–Golay second derivative to resolve peaks in the 1700–1596 cm^{-1} region (Smith, 2011). The signals in the original spectrum in this region were identified as the minima of the second derivative and were at 1660, 1625 and 1596 cm^{-1} (Kihara *et al.*, 2002; Fig. 4c). These bands originate from lignin: the band at 1660 cm^{-1} corresponds to ring-conjugated α and β unsaturated bonds in lignin monomers, the band at 1620 cm^{-1} represents stretching of the carbonyl group and the band at 1590 cm^{-1} can be assigned to aromatic C=C stretching (Kihara *et al.*, 2002). Aromatic compounds, such as lignin, decreased more in *OL* exposed to the WHt treatment than to the DHt treatment, as indicated by the reductions in the peaks

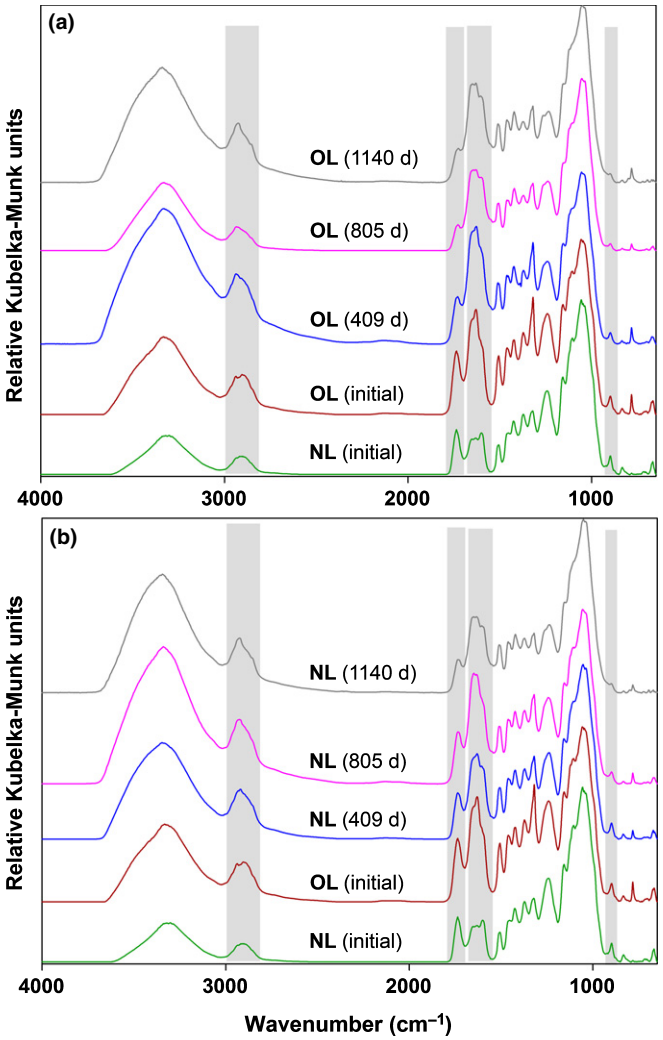

Fig. 3 Mean normalized (carbohydrates, 1060 cm^{-1}) diffuse reflectance infra-red Fourier transform (DRIFT) spectra of (a) 1-yr-old (*OL*; $n = 3$) and (b) newly senesced (*NL*; $n = 3$) *Polygonum cuspidatum* litter, including spectra for initial litter and litter after three stages of decomposition in the control (ambient + unwarmed) treatment, indicating changes in the relative intensity of various functional groups. Shaded regions from left to right indicate peaks: 3000–2800 cm^{-1} (aliphatic compounds), 1750 cm^{-1} (carbonyl stretch of aliphatic esters and carboxylic acids), 1660–1590 cm^{-1} (phenolic C=C and C=O stretching) and 896 cm^{-1} (C1–H vibration of polysaccharides). The progress of decomposition can be visualized as a decrease in the intensity of bands at 1750 and 896 cm^{-1} and as an increase in intensity of bands at 3000–2800 and 1660–1590 cm^{-1}.

in the 1700–1596 cm^{-1} region (Fig. 4a; $P < 0.05$). The lignin to carbohydrate ratio, as computed by the ratios of the peak intensities at 1631 to 1108 cm^{-1}, was 30% higher in the dry than in the wet treatments (Fig. 5). By contrast, in *NL*, although the precipitation treatments influenced the mass loss at 1140 d of decomposition (Fig. 2b), this mass loss was not accompanied by a differential loss of compounds from the litter matrix, as evident from the similarity in the DRIFT spectrum of *NL* decomposed under different climates ($P > 0.05$; Fig. 4b).

PCA of the relative intensities of the DRIFT peaks of *OL* showed sharp contrasts in litter chemistry among the climatic treatments after 1140 d of decomposition (Fig. 6). PC axes 1

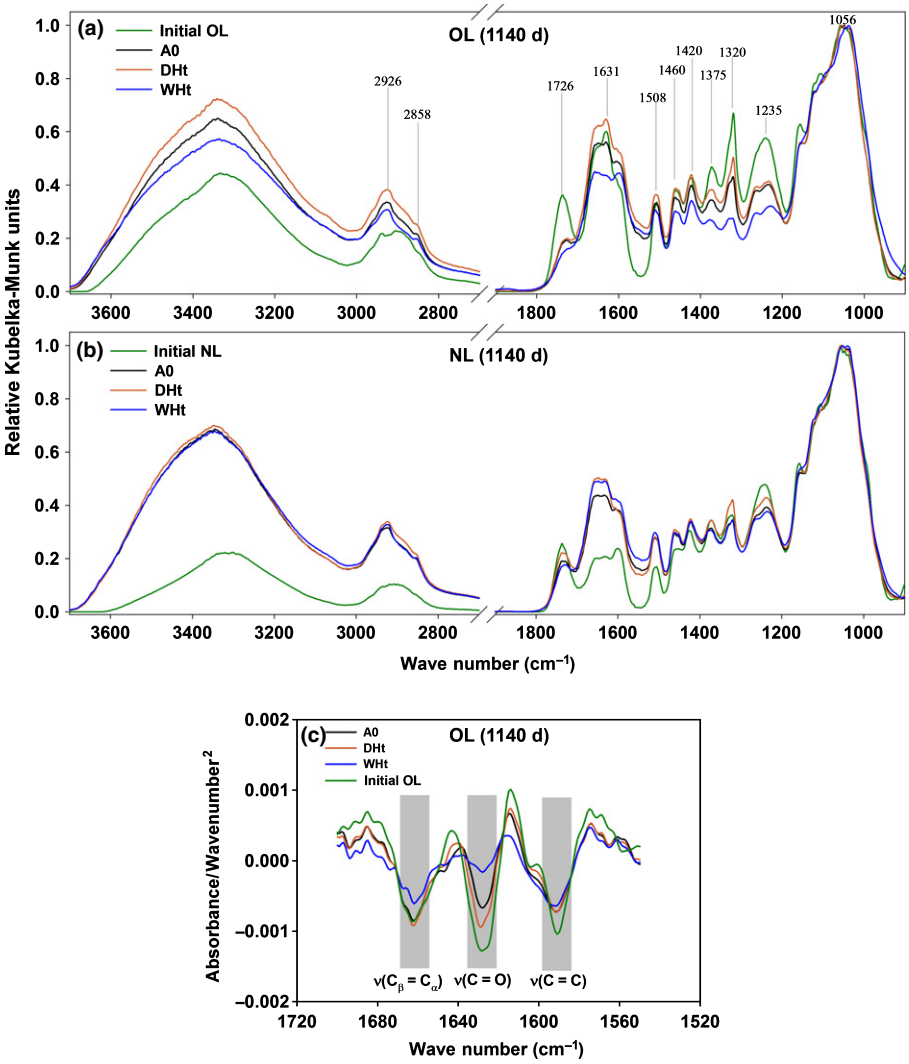

Fig. 4 Mean normalized (carbohydrates, 1060 cm^{-1}) diffuse reflectance infra-red Fourier transform (DRIFT) spectra of (a) 1-yr-old litter (*OL*; $n = 3$) and (b) newly senesced litter (*NL*; $n = 3$) of *Polygonum cuspidatum*, including spectra for initial litter and litter after 1140 d of decomposition in the different treatments, and (c) the second derivative of the peak between 1700 and 1590 cm^{-1} from the spectra of *OL* exposed to different treatments for 1140 d, showing the bands at 1660 cm^{-1} (ring-conjugated α and β unsaturated bonds in lignin monomers), 1620 cm^{-1} (stretching of the carbonyl group) and 1590 cm^{-1} (aromatic C=C stretching), which originate from lignin monomers. The normalized DRIFT spectra of *OL* indicate a significant reduction in the intensity of lignin (1700–1590 cm^{-1}) in WHt relative to DHt. A0, ambient + no warming; DHt, dry + high warming; WHt, wet + high warming.

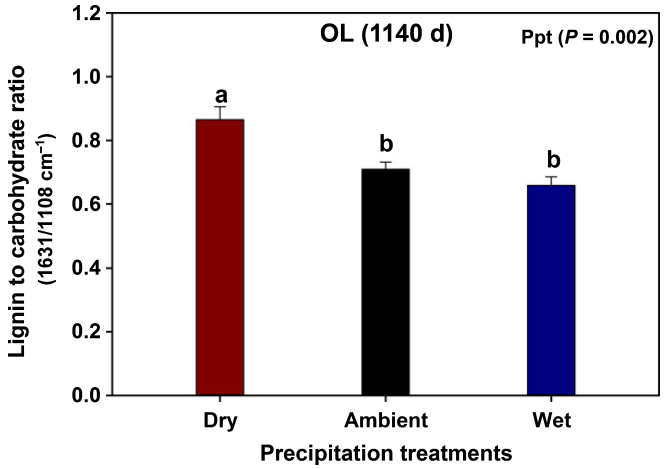

Fig. 5 Intensity ratio of diffuse reflectance infra-red Fourier transform (DRIFT) peaks between 1631 and 1108 cm^{-1} (lignin to carbohydrate ratio) of 1-yr-old *Polygonum cuspidatum* litter (*OL*) exposed to different climatic treatments after 1140 d of decomposition. Values represent means ($n = 3$) ± SE. Different letters identify differences in means (Tukey's honestly significant difference (HSD)) among the precipitation treatments.

and 2 explained 57% and 25% of the variance in the dataset, respectively. PC axis 1 corresponds to the effect of precipitation treatments, as determined by the analysis of variance of the coordinate scores ($P = 0.007$; Table 2). The chemistry of *OL* in the dry, high warming (DHt) treatment differed from that of all other treatments (Table 2), with higher intensities of peaks at 1320 cm^{-1} (CH deformation and C–O stretch corresponding to the outer surface of cutin) and 1631 cm^{-1} (aromatic skeletal vibrations of lignin; Fig. 6; PC axis 1). The chemistry of litter in the W0 and WHt treatments differed from that in the litter in the ambient and dry treatments along PC axis 1 (Tukey's *post-hoc* comparison; Table 2), with higher relative intensities of peaks at 1461 cm^{-1} (aliphatic compounds) and 1420 cm^{-1} (O–H deformations of phenolic and aliphatic groups). PC axis 2 identified litter chemistry differences in the warming treatments ($P = 0.017$), as *OL* from the WHt treatment differed from that of all other treatments (Fig. 6; Table 2), and had a higher peak intensity at 2926 cm^{-1} (asymmetric stretch of methylene (CH_2) group). Although the PCA of the relative intensities of the DRIFT peaks of *OL* after 1140 d of decomposition identified significant treatment

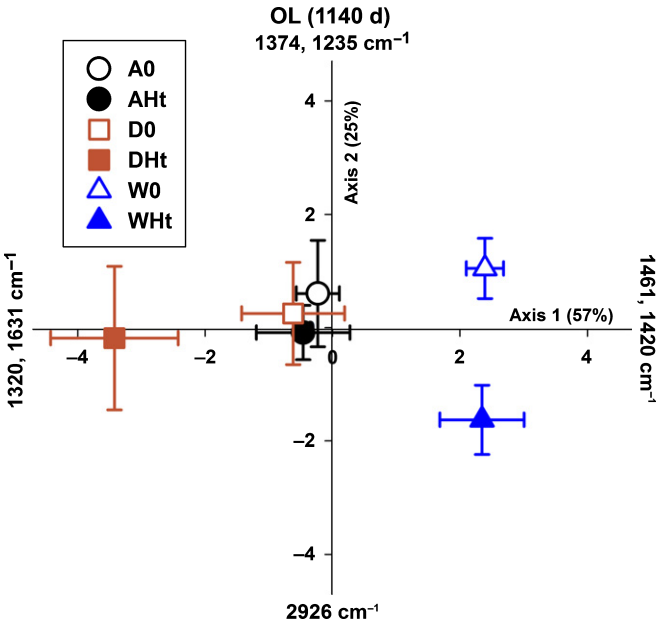

Fig. 6 Principal component analysis (PCA) of the relative intensities of dominant diffuse reflectance infra-red Fourier transform (DRIFT) peaks of 1-yr-old *Polygonum cuspidatum* litter (*OL*) from different climatic treatments after 1140 d of decomposition. The wave numbers with the highest eigenvector loading are listed on each principal component axis. Values represent means ($n = 3$) ± SE. A0, ambient + no warming; AHt, ambient + high warming; D0, dry + no warming; DHt, dry + high warming; W0, wet + no warming; WHt, wet + high warming.

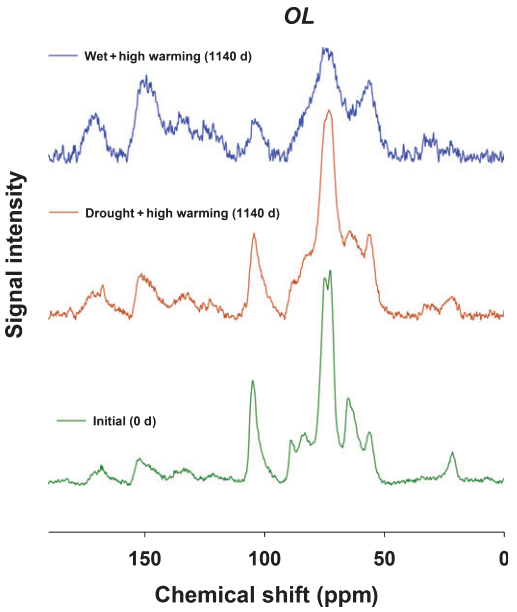

Fig. 7 ^{13}C NMR spectra of 1-yr-old (*OL*) *Polygonum cuspidatum* litter exposed to different climatic treatments after 1140 d of decomposition.

differences, PCA of *NL* data did not reveal any treatment effects on litter chemistry (Fig. S1; Table S4).

The ratios of the intensities of different carbon functional groups from ^{13}C NMR also identified differences in litter chemistry at different stages of decomposition (Fig. 7; Table 1). Initial, undecomposed *NL* had the highest CC : MC ratio and *OL* exposed to WHt had the lowest ratio. The ratios of alkyl C : *O*-alkyl C and (aryl + *O*-aryl C) : *O*-alkyl C increased during decomposition; the highest value was for *OL* exposed to the WHt treatment.

Discussion

Overall, our results suggest that the effect of climate on litter decomposition depends on the proportion of labile compounds in the litter. The higher proportion of labile compounds in *NL* prevented climate from affecting the mass loss rates for 2 yr. After 3 yr of decomposition, although the mass loss varied similarly with climate in both litter types, warmer, wetter conditions accelerated the degradation of recalcitrant matrices only in litter that was initially low in labile compounds (*OL*). Thus, the proportion of labile compounds regulated both the overall mass loss dynamics and the decomposition dynamics of the recalcitrant compounds. Our results also emphasize that, after 3 yr of decomposition, although mass loss was similar in *OL* and *NL*, the chemical composition of the remaining litter in *OL* diverged with climatic treatments. We also found that precipitation treatments affected mass loss more strongly than warming treatments, and this effect persisted even when *c.* 80% of the mass had been lost from both litter types.

Effects of warming and altered precipitation on rates of litter decomposition

Although the climate treatments significantly affected the mass loss of *OL* within the first 124 d of field incubation, their effects on *NL* could only be detected later – after 805 d of field

Table 2 Results of *post-hoc* mean separation test (Tukey's honestly significant difference (HSD)) from ANOVA of principal component axis coordinate scores of diffuse reflectance infra-red Fourier transform (DRIFT) peaks from old litter (*OL*) after 1140 d of decomposition

Precipitation ($P = 0.007$)	Warming ($P = 0.116$)	Scores	Tukey group*	Precipitation ($P = 0.881$)	Warming ($P = 0.017$)	Scores	Tukey group
PC axis 1 (57%)				PC axis 2 (25%)			
Wet	Unwarmed	2.3928	A	Wet	Unwarmed	1.0448	A
Wet	High warming	2.3455	A	Dry	Unwarmed	0.2513	AB
Ambient	Unwarmed	−0.2318	B	Dry	High warming	−0.1850	AB
Ambient	High warming	−0.4632	B	Ambient	Unwarmed	0.6016	AB
Dry	Unwarmed	−0.6202	B	Ambient	High warming	−0.08606	AB
Dry	High warming	−3.4231	C	Wet	High warming	−1.6267	C

*Treatments that do not share letters differ significantly ($\alpha = 0.05$).

incubation (Fig. 2b,c). Thus, in the earlier stages of decomposition, litter with a smaller proportion of labile compounds (*OL*) was more sensitive to climate than litter with more labile compounds (*NL*). As all the heteropolymers in both litters were structurally similar, we attribute this delay in the appearance of climatic effects on decomposition of *NL* to its greater initial abundance of labile compounds. During the early stages of decomposition, microbes target unprotected and high-energy-yielding substrates, such as cellulose and hemicellulose (Berg & McClaugherty, 2008). As most of these labile compounds can be utilized by a wide range of microbial communities (Jones *et al.*, 2009; McGuire & Treseder, 2010), having different climatic and other environmental tolerances (Pett-Ridge & Firestone, 2005; Cruz-Martinez *et al.*, 2009), the potential for climate to affect the utilization of these compounds may be limited. Irrespective of climate, in the presence of labile carbon, bacteria are superior competitors for non-carbon compounds and may competitively exclude the slower growing fungi that specialize in the decomposition of relatively recalcitrant compounds (Fontaine *et al.*, 2003; Moore *et al.*, 2003; Waldrop & Firestone, 2004; McGuire & Treseder, 2010). In addition, fungal communities may have more specific climatic requirements (McGuire *et al.*, 2010; Hawkes *et al.*, 2011). This difference in response of bacterial and fungal communities to climate and labile carbon could explain the initial confinement of climatic influence on decomposition to *OL*. Recently, it has been shown that, in soil, the microbial utilization of labile substrates that do not require enzymatic breakdown is insensitive to warming (Frey *et al.*, 2013). In addition, as most of the initial depolymerization of heteropolymers is facilitated by microbial exo-enzymes, greater moisture should accelerate the mineralization of heteropolymers by increasing diffusion rates, facilitating enzyme–substrate interactions. This could explain the greater mass loss from *OL* exposed to ambient and wet precipitation (relative to dry) in the initial stages of decomposition. In *NL*, a similar response to precipitation appeared only during the later stages of decomposition (at 805 d), probably after the advanced degradation of labile compounds. Previous studies have shown that the decomposition of recalcitrant compounds, such as lignin, starts only after low-molecular-weight labile compounds have been degraded (Berg & McClaugherty, 2008; Adair *et al.*, 2008; Schneider *et al.*, 2012). Our study demonstrates that this initial decomposition of labile compounds is relatively insensitive to climate, and hence the effect of climate on tissue decomposition should be more pronounced only after the relative depletion of labile substrates. Our finding that litter quality determines the climate sensitivity of litter decomposition suggests that the traditional conceptual model for decomposition, in which climate and litter quality act as two separate factors affecting litter decomposition, should be modified to include an interaction (Fig. 1).

Climate has been considered to have a negligible influence on decay rates during the later stages of litter decomposition (Berg & Meentemeyer, 2002), as the influence of nutritional constraints increases (Prescott, 2010). However, we found a contrasting pattern: the decomposition of *NL* initially did not respond to the climate treatments and, in later stages, ambient and wet treatments accelerated the mass loss of both *NL* and *OL* relative to the dry treatment, even after 1140 d, when *c.* 80% of the initial mass had been lost. Our results thus suggest that sufficient moisture can remove some of the constraints on decomposition in the later stages of decay, resulting in greater mass loss.

In this study, precipitation treatments regulated litter mass loss and decay rates more strongly than did warming treatments (Fig. 2b,c). As moisture availability is normally much higher in mesic systems such as ours than in arid and semiarid systems, one might expect only a modest response of ecosystem processes to moisture relative to the response to temperature. The Boston area's climate has strong seasonality to temperatures, and the magnitude of the BACE warming treatments was relatively small relative to seasonal temperature swings (Fig. S2a). Although the region experiences relatively constant average monthly precipitation, there was considerable variability among months and years during this experiment (Fig. S2b). Soils can go through pronounced drying and wetting cycles during the growing season, and the BACE treatments strongly affected soil moisture (e.g. Hoeppner & Dukes, 2012; Suseela *et al.*, 2012). We would expect the litter wetting and drying cycles to be even more rapid and more dramatic. Our results suggest that the precipitation treatments altered the degree to which moisture levels constrained the degradation of compounds in litter during the warmer seasons, when temperatures were optimal. At the same study site (BACE), we found that precipitation treatments had a stronger effect on soil microbial respiration than did warming (Suseela *et al.*, 2012).

Response of recalcitrant compounds to warming and altered precipitation

Although similar percentages of mass were lost from *NL* and *OL* after 1140 d of decomposition (Fig. 2a), *OL* experienced faster decomposition of the recalcitrant matrix (alkyl carbon and lignin) per unit of degradation of labile matrix (Fig. 4a). In addition, the normalized (1060 cm^{-1}, carbohydrates) DRIFT spectra of *OL* exposed to climatic treatments after 1140 d revealed a significant reduction in the intensity of lignin in WHt relative to DHt (Fig. 4a,c; 1700–1596 cm^{-1}). Similarly, the decrease in the intensity of DRIFT peaks in the fingerprint region (1500–1200 cm^{-1}) of *OL* exposed to a warmer and wetter climate suggested that there had been greater decomposition of non-carbohydrates per unit of carbohydrate (Figs 4a, 6). The lignin degradation in *OL* exposed to WHt was further evident from the relative increase in condensed and cross-linked lignin, as indicated by the ratios of the peak intensities at 1510 and 1631 cm^{-1} (Fig. 8; 42% increase relative to DHt; Mann *et al.*, 2009). Condensed and cross-linked lignin is composed of carbon–carbon linkages, which are more difficult for microbes to break down than less condensed lignin, with its greater proportion of labile β-O-4 linkages (Boerjan *et al.*, 2003; Talbot *et al.*, 2012). The higher relative abundance of condensed and cross-linked lignin in the residue of *OL* exposed to WHt indicates advanced decomposition relative to *OL* in the DHt treatment. This greater

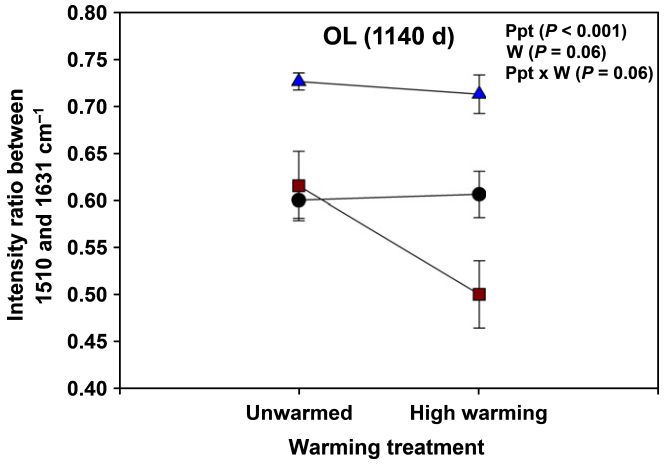

Fig. 8 Intensity ratio of diffuse reflectance infra-red Fourier transform (DRIFT) peaks between 1510 and 1631 cm^{-1} of 1-yr-old (*OL*) *Polygonum cuspidatum* litter after 1140 d, showing the relative enrichment of condensed and cross-linked lignin in the wet treatment (triangles) relative to the dry (squares) and ambient (circles) treatments (higher ratios signify greater proportions of condensed and cross-linked lignin). The higher relative abundance of condensed and cross-linked lignin in the residue of *OL* exposed to WHt (wet + high warming) indicates advanced decomposition relative to *OL* in the DHt (dry + high warming) treatment. Values represent means ($n = 3$) ± SE. Ppt, precipitation; W, warming.

decomposition of recalcitrant compounds in WHt is further supported by the ^{13}C NMR analysis (Fig. 7), where *OL* from the WHt treatment had the lowest CC : MC ratio, indicating that decomposition had advanced further than in DHt litter. Previous studies have shown steady declines in the CC : MC ratio with advancing decomposition (Almendros *et al.*, 2000; Blumfield *et al.*, 2004; Mathers *et al.*, 2007). The CC : MC ratio is a more robust indicator of the decomposition of woody residues than the alkyl C : *O*-alkyl C ratio (Baldock *et al.*, 1997; Mathers *et al.*, 2007). After both litter types had decomposed for 3 yr, this effect of the climate treatments on the decomposition of the recalcitrant matrix was visible only in *OL*, suggesting that the warmer, wetter conditions accelerated the decomposition of recalcitrant compounds only after substantial degradation of a labile structural matrix. As decomposition progresses, the cellulose and hemicellulose matrices that are less protected are more easily degraded, increasing the relative abundance of lignocellulosic material (Berg & McClaugherty, 2008). Thus, in the advanced stages, during the decomposition of lignocellulose, we would expect the degradation of cellulose to be tightly associated with the degradation of lignin, which would result in an increase in the decomposition of lignin per unit degradation of carbohydrate, as observed in *OL* at 1140 d. We did not observe the above compound-specific degradation pattern in *OL* exposed to ambient precipitation treatments (Fig. 4a), underscoring the importance of greater moisture availability in the facilitation of the decomposition of recalcitrant compounds. The climatic treatments did not instigate a preferential decomposition of recalcitrant compounds from *NL*. The greater abundance of relatively labile compounds could have altered the sequence of compounds decomposed in *NL*, thus nullifying the effect of climate. Our finding that labile compounds in litter decrease the climate sensitivity of decomposition of recalcitrant matrix is novel and suggests that, within a species, indices of litter quality (the proportion of labile to recalcitrant compounds) may signal the degree to which climatic factors can affect soil carbon sequestration. After 3 yr of decomposition, both litter types had lost similar amounts of mass (Fig. 2a), but the chemistry of the residual *OL* varied with climatic treatments (Fig. 4a), whereas the chemical composition of *NL* exposed to different climatic treatments was similar (Fig. 4b). These results indicate that initial litter quality can influence whether the chemistries of residual litter converge (Wallenstein *et al.*, 2013) or diverge (Wickings *et al.*, 2012) over time under different climates.

The results from this study indicate that the effect of climate on litter decomposition depends on the quality of litter; litter with a greater initial proportion of labile compounds was less sensitive to warming and altered precipitation. Similarly, the warmer and wetter climate treatment preferentially accelerated the decomposition of recalcitrant compounds in the more recalcitrant litter. The significant effects of precipitation on mass loss and chemical composition, even in the late stages of litter decomposition, reveal the potential of climate to alter the amount and quality of carbon in plant litter available for sequestration. These results emphasize that litter chemical composition has an overriding effect on the climate sensitivity of decomposition; thus, litter quality may regulate litter-derived carbon sequestration under future climates.

Acknowledgements

We thank Carol Goranson and Hollie Emery for help with fieldwork. We also thank the field assistants who helped build and maintain the infrastructure of BACE. We are grateful to two anonymous reviewers for their constructive and thoughtful comments that improved the quality of the manuscript. This study received financial support from the National Science Foundation (NSF) (DEB-0546670 to J.S.D.; DEB-1145993 to N.T.) and the US Department of Energy's Office of Science (BER, to J.S.D.), through the Northeastern Regional Center of the National Institute for Climatic Change Research. This is publication No. 1331 of the Purdue Climate Change Research Center.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Principal component analysis of the relative intensities of dominant diffuse reflectance infra-red Fourier transform (DRIFT) peaks of newly senesced litter (*NL*) in different climatic treatments after 1140 d of decomposition.

Fig. S2 (a) Average monthly rainfall in the ambient treatment and cumulative rainfall in the dry, ambient and wet treatments during the study period (June 2008 to August 2011) at the Boston-Area Climate Experiment (BACE) and (b) average monthly soil temperature (2 cm depth) in the warming treatments from October 2008 to August 2011.

Table S1 Results of the mixed-model restricted maximum likelihood (REML) analysis of the responses of mass loss to warming, precipitation, litter type and time of sampling

Table S2 Results of the mixed-model restricted maximum likelihood (REML) analysis of the responses of mass loss of new litter (*NL*) to warming and precipitation treatments at each time of sampling

Table S3 Results of the mixed-model restricted maximum likelihood (REML) analysis of the responses of mass loss of old litter (*OL*) to warming and precipitation treatments at each time of sampling

Table S4 Results of principal component analysis (PCA) of the diffuse reflectance infra-red Fourier transform (DRIFT) peaks of newly senesced litter (*NL*) after 1140 d of decomposition

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