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Irrigation, fertilization and initial substrate quality effects on decomposing Loblolly pine litter chemistry

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Abstract

Changes in carbon chemistry (i.e., carbon compound classes such as aromatics, phenolics, etc.) of loblolly pine (*Pinus taeda* L.) litter were examined during three years of decomposition under factorial combinations of irrigation and fertilization treatments. Cross polarization magic angle spinning ^{13}C nuclear magnetic resonance revealed that total carbon and nutrient concentrations correlated strongly with carbohydrate and O-alkyl carbon concentrations but did not relate well with concentrations of lignin, aromatic and phenolic carbon, or with lignin-related decomposition indices. The best correlations to carbon and nutrient concentrations occurred with the C/N ($R^2 = 0.86$, $P > 0.0001$) and alkyl/O-alkyl ($R^2 = 0.75$, $P > 0.0001$) decomposition indices. In all situations, the carbon chemistry of the decomposing litter followed the general pattern of accumulation of alkyl and carbonyl carbon with a loss of O-alkyl and methoxy carbon. Only small variations in the aromatic and phenolic carbon concentrations were detected. Since lignin is composed primarily of aromatic and phenolic carbons, the observation that there were only small changes in the aromatic and phenolic carbons of the litter is consistent with the general stability of lignin in these ecosystems. Trends in carbon chemistry during decomposition suggested that fertilization accelerated the decomposition process by about 100% as compared with the control plots. Irrigation also accelerated the decomposition process but to a lower extent (about 62% greater than control plots). Initial litter quality, as defined by the litter C/N, did not have a significant effect on the carbon chemistry of the decomposing litter. This study demonstrated that the decomposition mechanisms were not altered by the treatments but there were important changes in the relative chemistry of the decomposing litter which impacted the rate of decomposition.

Introduction

Decomposing litter can be a major nutrient source for forest soils (Raison et al., 1987; Stump and Binkley, 1993), and external factors that increase nutrient release from decomposing litter may accelerate stand growth (Allen, 1987). In the southern United States, litter decomposition is influenced by the warm climate, annual wet-dry cycle, active decomposer communities, and infertile soils. Many factors including nutrient availability (exogenous and endogenous), climate, and substrate quality affect the rate and mechanisms of forest litter decomposition (Melillo et al., 1982). Part of the challenge in understanding the effects of substrate quality on decomposition processes is quantifying the changes in the carbon (C) chemistry over time. The litter C chemistry includes phenolic, aromatic, alkyl, oxygenated alkyls (O-alkyls) and carbonyl compounds. Various measures of litter and woody debris C quality have been proposed, including initial nutrient concentration (Polglase et al., 1992), C/N ratio (Enriquez et al., 1993), percent aromatics (Baldock and Preston, 1995), lignin concentration (Berg and McClaugherty, 1989), lignin/N ratio (Melillo et al., 1982), carbohydrate/lignin ratio (McColl and Powers, 1998), and alkyl/O-alkyl C ratio (Baldock and Preston, 1995). Several models used to describe and/or predict C dynamics or nutrient cycling

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processes rely on some measure of organic matter quality, with lignin/N and C/N being utilized most often (Powlson et al., 1996). A better understanding of the changes in C chemistry and relationships among C constituents, nutrient levels and mass loss may be instrumental in applying an appropriate measure for litter or woody debris quality.

Cross polarization magic angle spinning ^{13}C nuclear magnetic resonance (CPMAS ^{13}C NMR) is a useful tool to examine the C chemistry of a variety of materials, including forest litter (Haw et al., 1984; Baldock and Preston, 1995; McColl and Powers, 1998). This technique is particularly attractive because homogenous litter samples can be analyzed without chemical preparation but gives information on a wide range of C constituents. In this study, CPMAS ^{13}C NMR was used to monitor the changes in C chemistry in loblolly pine needle litter during decomposition over three years (May 1994–May 1997) in a southern forest ecosystem. Specific objectives were to (1) monitor changes in the C chemistry in decomposing high- and low-quality litter under different irrigation and fertilization treatments, and (2) assess the adequacy of different decomposition indices in describing litter decomposition and nutrient dynamics.

Materials and methods

Site description

The study site is the Southeast Tree Research and Education Site (SETRES) in the Sand Hills region of North Carolina (35° N latitude, 79° W longitude). Mean annual temperature and precipitation at the site are 17 °C and 1210 mm. The Wakulla series soil (sandy, siliceous, thermic Psammentic Hapludult) has a low water holding capacity, and is nutrient deficient for loblolly pine. In 1984, longleaf pine (*Pinus palustris* Mill.) was harvested from the site and in 1985 the site was planted with bare-root loblolly pine. Glyphosate (1.5% by volume) was applied as needed to control competing vegetation.

The study is a 2 × 2 factorial with two levels each of two treatments (irrigation and fertilization), replicated on four blocks. Treatment combinations were: (1) control (no irrigation or fertilization), (2) irrigation, (3) fertilization, and (4) irrigation plus fertilization. Within each treatment plot (50 × 50-m) there was a 30 × 30-m measurement plot. Albaugh et al. (1998) provide a complete description of the site and experimental design.

Table 1. Fertilization history at SETRES (King et al., 1997)

	(kg ha^{-1})						
Date[a]	N	P	K	Ca	Mg	S	B
3/26/92	225	56	112	–	–	–	–
4/12/92	–	–	–	135	56	–	–
6/18/92	–	–	–	–	–	–	1.7
3/30/93	–	28	–	–	–	–	–
4/20/93	26	22	21	–	0.2	–	–
6/10/93	–	–	92	–	56	120	–
8/31/93	56	–	–	–	–	–	–
3/24/94	112	–	–	–	–	–	–
3/2/95	56[b]	28	56	24	34	74	–
5/25/95	–	–	–	–	–	–	1.1
3/25/96	112	12	56	10	–	15	–
4/10/96	–	–	–	–	–	–	1.1
Total	587	146	337	169	146.2	209	3.9

[a]Solid fertilizer was applied to the soil surface except on 4/20/93 which was liquid fertilizer applied to the foliage.
[b]Applied to two plots only.

Irrigated plots were watered with a head sprinkler system beginning in May 1993. Irrigation was provided as needed to maintain available soil moisture in the upper 50 cm of the soil profile between field capacity and 60% available soil moisture. During each irrigation event, 2.5-cm of water was added per plot, and the irrigated plots received, on average, 19 and 61% more water than the non-irrigated plots in 1994 and 1995, respectively (Albaugh et al., 1998). Beginning in March 1992, fertilization was conducted annually with the goal to maintain 1.3 g kg^{-1} leaf N on a dry weight basis (Allen, 1987; Albaugh et al., 1998). The fertilization history at SETRES, through 1996, is shown in Table 1.

Litter qualities

In 1993, loblolly pine needles from SETRES were collected in four 1 × 1-m litter traps randomly placed within each measurement control and fertilized plot in each block during the period of senescence from September through December. Subsamples of the needles from each treatment were analyzed for total C and N on a NA 1500 Carlo Erba C/N/S analyzer[1] (Fisons Instruments, Danvers, MA). Needles from trees on the fertilized plots had a significantly ($P < 0.001$) lower C/N ratio (143) than those obtained from the control

[1]The use of trade or firm names in this publication is for reader information and does not imply endorsement by the U.S. Department of Agriculture of any product or service.

plots (172). In this study, litter quality was defined by the C/N ratios with the litter with the lower C/N ratio representing high-quality litter. Litter from the fertilized plot also had higher concentrations of potassium (K) and N, and lower concentrations of calcium (Ca) and magnesium (Mg). Phosphorus did not significantly differ between the fertilization treatments (Sanchez, 2001). Both high and low quality litters were air-dried and approximately 55 g placed in 30.5 × 20-cm litterbags made from 18 × 16-mm mesh fiberglass screen.

Study design

In May 1994, eight bags containing high quality litter and eight bags containing low quality litter were placed in each of three random locations within each measurement plot of each treatment plot and block resulting in a total of 768 litterbags being placed in the field (4 treatments * 4 blocks * 8 bags * 2 qualities * 3 locations = 768). Litterbags were placed on top of the mineral soil after the forest floor was pushed aside. Time domain reflectometry (TDR) probes were used to monitor soil moisture. One 15-cm probe was installed horizontally in the mineral soil, 2-cm below each set of eight paired litterbags. The TDR measurements were taken twice a month. Barnant 100 model 600-2820 thermocouple thermometers equipped with J-type probes (Barnant Corporation, Barrington, IL) were used to monitor soil temperature. Measurements were taken 2-cm beneath each set of eight paired litterbags when the TDR measurements were taken.

During the first year (May 1994–May 1995), one pair of litterbags (one high and one low quality) was collected from each of the three locations at 3-month intervals. In the second year (May 1995–May 1996), the collections occurred at 6-month intervals. A final collection was done in May 1997. A total of six collections were conducted over the duration of the study. At each interval, 96 litterbags (48 high quality litter and 48 low quality litter) were collected and each were sealed in plastic bags to prevent moisture loss. All litter samples were oven dried at 65 °C overnight and ground in a Wiley mill to pass a 20-mesh screen before laboratory processing.

Composite samples

Because of the long analysis times (8 h per sample) and costs associated with CPMAS ^{13}C NMR analysis, composite samples were used. Composite samples were made by thoroughly mixing 0.25-g ground litter samples collected from each block for each combination of treatment, quality, and sampling time resulting in only one sample for each treatment, quality and sampling time combination. All replication from the blocking was lost at this point. There were a total of 64 composite samples including samples from the study's inception (4 treatments * 2 qualities * 8 sampling times = 64 samples).

Subsamples of the composites were placed in a 450 °C muffle furnace for eight hours to determine loss-on-ignition. Other subsamples were ashed for 6 h and then digested with 0.3 M HNO_3. The digests were analyzed for P, K, Ca, and Mg by inductively coupled plasma (ICP) spectrometry on a Jobin Yvon 2000 ICP (Instruments S.A. Inc., Edison, N.J.). All subsamples were analyzed for total C and N on a NA 1500 Carlo Erba C/N/S analyzer. All results were reported on an ash free basis.

CPMAS ^{13}C NMR

The CPMAS ^{13}C NMR spectra were obtained at ambient conditions on a Chemagnetics 200S NMR spectrometer (Varian Analytical Instruments, San Fernando, CA) equipped with a CPMAS probe. Relevant parameters were a 300 ppm sweep width, 2.0 ms contact time, 3.0 s pulse delay, 30 Hz line broadening, 5.5 μs pulse length, and magic angle spinning at 4.3 kHz. For each sample, 10,000 scans were obtained and chemical shifts (expressed as ppm) were referenced relative to para-di-tert-butyl benzene that was used as an external standard.

Carbon chemistry fractions were designated based on the chemical shift region in the CPMAS ^{13}C NMR spectrum and were calculated as percentages of the total C from the computerized integrals of the signal intensity within each chemical shift region (Preston et al., 1990). Carbon chemistry fraction designations were based on the signal intensities in the following regions: alkyl (0–50 ppm); methoxy (50–60 ppm); O-alkyl (60–96 ppm); aromatic (96–141 ppm); phenolic (141–159 ppm); and carbonyl (159–185 ppm). The computerized integrals were also used to calculate the percentages of lignin C, carbohydrate C (i.e., cellulose and hemicellulose), and the ratio of carbohydrate to lignin monomers (Cm/Lm) as described by Preston et al. (1990) and deMontigny et al. (1993). The CPMAS ^{13}C NMR instrumental conditions used in this experiment are described as optimal for analyzing wood and, presumably, litter (Haw et al., 1984). However, all calculations obtained from the CPMAS ^{13}C

NMR spectra should be considered semi-quantitative due to signal overlap and because the contact and relaxation delay times may not be optimal for all C types.

Although there is signal overlap in CPMAS ^{13}C NMR spectra, certain compounds dominate sections of the spectra (Preston et al., 2000). Surface waxes and cutin are the major contributors to the intensity in the alkyl C region whereas carbohydrates dominate the O-alkyl region. Lignin and tannins dominate the aromatic and phenolic regions. Preston et al. (2000) do not consider the methoxy region as diagnostically important because it is poorly resolved from the O-alkyl region. Nevertheless, the signal intensity in the methoxy region is primarily from proteins, O-methylated sugars, and methoxy units on lignin.

Condensed tannins

Condensed tannin concentration in the composite samples was determined by proanthocyanidin assay as described by Tiarks et al. (1992). A 20 mg litter subsample was sequentially extracted with methanol and a 1:1 acetone:water solution. The extracts were filtered and the solvent evaporated off. The residue was placed in a test tube and 0.5 mL of methanol and 5 mL of freshly prepared sample reagent were added. The sample reagent was 5% (v/v) concentrated HCl in n-butanol with $FeSO_4 \cdot 7H_20$ added to create a concentration of 200 mg L^{-1} of Fe^{2+}. The samples were stirred in a vortex mixer and heated to 95 °C for 1 h. The test tubes were cooled to room temperature and the solutions were transferred to clean test tubes. The absorbance at 550 nm was measured on a Milton Roy Spectronic 21D spectrophotometer (Milton Roy, Rochester, NY). Condensed tannin concentrations were determined from comparisons with the absorbance of purified tannin standards originating from loblolly pine needles (Fitz Booker, personal communication).

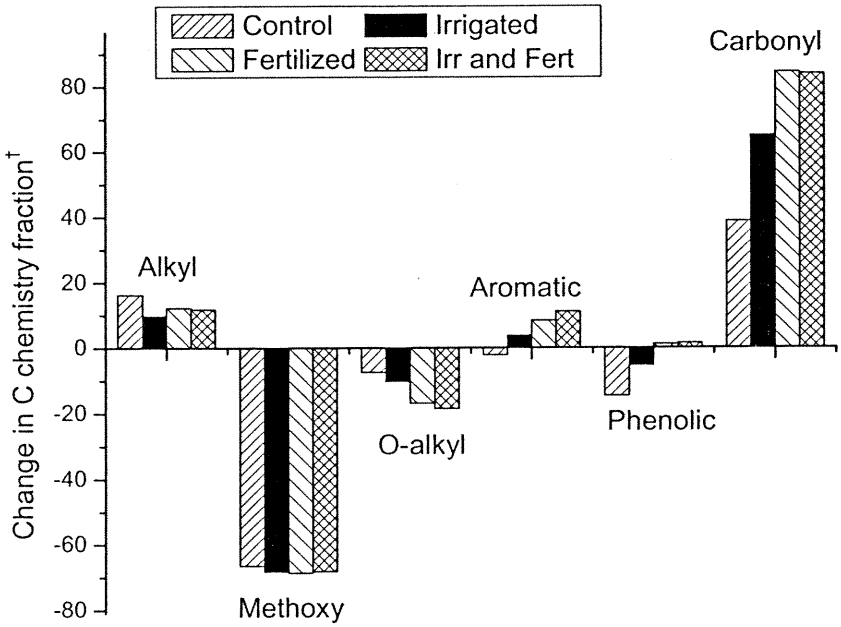
It is possible that the concentration of tannins could be altered by the oven drying of the litter samples. However, any degradation of heat sensitive compounds would probably not alter the results of the CPMAS ^{13}C NMR analysis. If tannins are degraded to lower monomer phenols and carbohydrates, we would anticipate changes in the details of the aromatic, phenolic and carbohydrate regions of the NMR spectra but would not alter the overall area of these regions and thus the corresponding C chemistry fraction calculations.

Statistical analyses

Lack of replication, resulting from the compositing of samples across blocks, precluded direct testing of treatment means for C chemistry fractions, nutrients or decomposition indices measurements, at any given collection time. Instead, decomposition curves were plotted for each C chemistry fraction and decomposition index and the parameters and regression curves were tested for treatment effects. Measured values for C chemistry fractions and decomposition indices were plotted and linear or quadratic models were fitted as appropriate. The linear model was $y = a + b*X$ and the quadratic model was $y = a + b_1*X + b_2*X^2$ where y = C chemistry fraction (or decomposition index value) and X = months in the field. Parameter estimates and 95% confidence intervals were calculated using the PROC REG procedure in the SAS® 8.01 statistical software package (SAS Corporation, Cary, NC). Significant differences in the parameter estimates between the treatments were determined by lack of overlap in the confidence intervals of each parameter. In addition, significance of pairs of treatments for the entire model was determined by comparing the full and reduced general linear models of the treatment effects on the C chemistry fractions. This consisted of fitting a treatment specific model and a pooled general model and then using the F statistic to determine if the models were significantly different. There were four treatments that resulted in six pairwise tests for each model in a C chemistry fraction (or decomposition index). Thus, to ensure an experimentwise type I error rate of 0.05, the Bonferroni approach (Rawlings et al., 1998) was used where each of the six pairwise tests was determined significant if it resulted in a *P*-value of less than $0.05/6 = 0.0083$, reducing the chance of a false positive to 0.05 for all six tests.

Results and discussion

At the end of the study and for each composite sample, there was an accumulation of alkyl and carbonyl C atoms with a concurrent loss of methoxy and O-alkyl C atoms and a small change in the aromatic and phenolic C atoms (Figure 1). This observation is in agreement with the results of previous studies on changes in litter C chemistry with decomposition (Baldock and Preston, 1995). Decomposition of organic matter results in the oxidation of different C fractions and thus the increase in the highly oxidized C compounds (i.e., carbonyls). Baldock and Preston (1995)



†Change in C chemistry fraction= [(C chemistry fraction at yr = 3) - (C chemistry fraction at yr = 0)]

Figure 1. Change in the carbon chemistry fractions of loblolly pine litter, after 3 years of decomposition, for litter samples placed on control, irrigated, fertilized and irrigated + fertilized plots on a sandy soil.

attributed the large increase in carbonyl C with the synthesis of carbonyl structures during the decomposition process. The large decrease in methoxy C atoms probably resulted from the loss of proteins and O-methylated sugars. The trends in Figure 1 suggest that fertilization increased the decomposition of the litter as evidenced by the higher production of carbonyl compounds in the litter decomposing on the fertilized plots. The increase in carbonyl C came primarily at the expense of methoxy and O-alkyl C, and not from the oxidation of lignin. Irrigation also seemed to accelerate decomposition but its effect is less than that observed for fertilization. The implication that fertilization has a stronger effect than irrigation on the C chemistry of the decomposing litter is consistent with the observations that fertilization (but not irrigation) increased litter decomposition rates ($k = 0.36\ yr^{-1}$ for the fertilized and irrigated + fertilized plots, $k = 0.27\ yr^{-1}$ for the control and irrigated plots) and nutrient dynamics in the replicated study (Sanchez, 2001).

In this study, estimates of lignin are included in three measurements (lignin, lignin/N and Cm/Lm ratio). However, estimates of lignin concentrations from NMR data are difficult because of the interference of soluble phenolics, especially tannins. Tannins occupy the same signal range in the NMR spectra as do lignin and carbohydrate compounds. Even traditional measures of lignin, such as Klason lignin, are often contaminated by soluble phenolics and cutin (Zech et al., 1987). Soluble phenolics interfere less in carbohydrate C calculations because carbohydrates typically make up the majority of the C signal in fresh litter (Zech et al., 1987; Preston et al., 1990; Baldock and Preston, 1995), and soluble phenolics are only present in small amounts (deMontigny et al. 1993). Unlike lignin, soluble phenolics do not remain long in woody debris or litter (Schlesinger, 1977; Lorenz et al., 2000). Therefore, the error in calculating lignin concentrations and relating these measures to decomposition processes depends on the proportion of soluble phenolics in the sample, and the length of time that the decomposition has progressed. Litter tannin concentrations were determined in a separate procedure (proanthocyanidin assay) independent of the CPMAS ^{13}C NMR analysis. The initial concentrations of condensed tannins was essentially the same for the high quality litter ($32.6\ mg\ g^{-1}$) as compared with the low quality litter ($30.9\ mg\ g^{-1}$) and were quickly lost; 65% of the initial concentration was lost in the first year for

all litter qualities and treatments. The concentration of condensed tannins after 3 years of decomposition was about 1.7 mg g^{-1} for the high quality litter and 1.5 mg g^{-1} for the low quality litter. Since the rate of decay was very rapid and consistent between samples, we infer that tannins did not alter the observed trends in lignin and carbohydrate C. It is hypothesized that this would also be the case for any tannin degradation products potentially produced during the oven drying of the litter.

To examine how the different metrics changed in relation to each other, the Pearson's correlation coefficients for all C chemistry fractions, nutrients, and decomposition indices were calculated (Table 2). The aromatic, phenolic and lignin C chemistry fractions did not significantly correlate, at the $P < 0.05$ level, to C or any nutrient with the exception of lignin which had a weak correlation with Ca ($r = -0.30$). Potassium did not correlate significantly to any C chemistry fraction or decomposition index. Carbon correlated with the relative concentrations of alkyl ($r = -0.53$), methoxyl ($r = -0.32$), O-alkyl ($r = 0.67$), carbonyl ($r = -0.28$), and carbohydrate ($r = 0.67$) C chemistry fractions. Generally, the O-alkyl and carbohydrate C concentrations showed the best correlation with all the nutrient concentrations except Mg and K. All decomposition indices significantly correlated with C (and most nutrients) concentration, with the C/N and alkyl/O-alkyl C ratios having the best fit ($r = 0.86$ and 0.75, respectively). The lignin/N ratio was modestly correlated ($r = 0.58$) to the C concentration and the Cm/Lm ratio had the poorest correlation ($r = 0.31$) with C concentration. Nitrogen showed significant correlations with all the decomposition indices, especially the alkyl/O-alkyl C ratio ($r = 0.80$). Phosphorus and Mg correlated with all the decomposition indices except the Cm/Lm ratio. Calcium correlated significantly with all the indices except the lignin/N ratio. In general, the strongest correlations between nutrients and decomposition indices were with the C/N and alkyl/O-alkyl ratios.

An examination of the treatment effects on decomposing litter was made by examining the C chemistry changes in the litter as decomposition proceeded. Using the pooled data set, the R^2 and P-values ranged greatly, with the O-alkyl C ($r^2 = 0.75$), carbohydrate C ($R^2 = 0.75$), alkyl/O-alkyl C ($R^2 = 0.64$), C/N ($R^2 = 0.87$), and lignin/N ($R^2 = 0.70$) measures giving the best fit (Table 3). Better relationships were obtained when the data was separated by quality and treatment. Examples of this are shown in Table 4 and Figures 2A–2C where the R^2 and p values for the pooled data set are compared with the data set separated by quality and treatment. Although the p values were higher, largely due to smaller n values, the R^2 values using data from each treatment separately (Figure 2B) were generally higher than those obtained utilizing the pooled data set (Figure 2A). However, even a small deviation from the general trend could result in low R^2 values (Figure 2C).

In the replicated study, fertilization significantly ($P < 0.05$) affected decomposition rates and nutrient release or immobilization while initial litter quality only affected nutrient release or immobilization (Sanchez, 2001). This was not reflected in this study in which no significant ($P < 0.05$) main treatment or quality effect was detected in trends for nutrients or C chemistry fractions in decomposing litter. Parameter values for the regression equations for C chemistry fractions and nutrients that exhibited predictable trends ($R^2 > 0.5$, $P < 0.0001$) in the regression analysis of the pooled data set are shown in Table 5. The slope (b-values) suggests that irrigation, compared with the control treatments, impacted the rate of loss of these measures except for N which was not affected by initial quality or treatments. Losses of aromatic, O-alkyl and carbohydrate, and overall C were greater in litter decomposing on the irrigated plots. However, these losses were not statistically significant at the $P < 0.05$ level.

Since litter chemistry is not a static measure during the decomposition process, examination of the trends in the decomposition indices of the litter during decomposition may give an indication of the relative change in litter recalcitrance during decomposition. Parameter values for the regression equations for three decomposition indices for each treatment and quality combination are shown in Table 6. Initial litter quality was statistically different among treatments ($P < 0.05$) for the C/N and lignin/N measures but not for the alkyl/O-alkyl C measure. The low quality litter had higher initial C/N values (a-value) and exhibited a more rapid drop (b-value) during decomposition. The low quality litter had higher initial lignin/N values but did not differ in rate of lignin/N drop. Neither fertilization nor irrigation had a significant effect on any parameter value for any of these measurements. From the C/N and lignin/N trends, it could be hypothesized that there was a significant difference in the initial litter qualities and thus a difference in recalcitrance. Since a litter quality difference was not detected in the alkyl/O-alkyl measure, its value as a decomposi-

Table 2. Pearson's correlation coefficients between nutrients and carbon chemistry fractions and decomposition indices

	C	N	P	Mg	Ca	K
Alkyl	−0.53***	0.55***	0.30*	−0.40***	−0.38**	ns
Methoxyl	−0.32**	0.30*	ns	−0.33**	0.63***	ns
O-Alkyl	0.67***	−0.70***	−0.47***	ns	0.42***	ns
Aromatic	ns	ns	ns	ns	ns	ns
Phenolic	ns	ns	ns	ns	ns	ns
Carbonyl	−0.28*	0.34**	0.42***	ns	ns	ns
Lignin	ns	ns	ns	ns	−0.30*	ns
Carbohydrate	0.67***	−0.70***	−0.47***	ns	0.42***	ns
C/N	0.86***	−0.91***	−0.64***	0.43***	0.29*	ns
Alkyl/O-alkyl	0.75***	0.80***	0.49***	−0.37**	−0.49***	ns
Cm/Lm	0.31*	−0.26*	ns	ns	0.29*	ns
Lignin/N	0.58*	−0.76***	−0.57***	0.34**	ns	ns

*, **, *** indicate relationship is significant at $P < 0.05$, 0.01, and 0.001 levels, respectively; ns indicates relationship is not significant at $P < 0.05$ level.

Table 3. Coefficient of determination (R^2) and probabilities (P values) for the regression analyses for carbon types and decomposition indices for pooled data set

Measure	Curve type[a]	R^2	P	N
	Carbon types			
Alkyl	linear	0.15	0.002	64
Methoxyl	quadratic	0.18	0.002	64
O-Alkyl	linear	0.75	<0.0001	64
Aromatic	linear	0.53	<0.0001	64
Phenolic	quadratic	0.17	0.004	64
Carbonyl	quadratic	0.08	0.09	64
Lignin	quadratic	0.21	0.0007	64
Carbohydrate	linear	0.75	<0.0001	64
	Decomposition indices			
Alkyl/O-Alkyl	linear	0.64	<0.0001	64
C/N	quadratic	0.87	<0.0001	64
Cm/Lm	quadratic	0.08	0.07	64
Lignin/N	quadratic	0.70	<0.0001	64

[a]Curves were described by linear or quadratic equations. Linear equations followed the general form, $Y = a + bX$. Quadratic equations followed the general form, $Y = a + b_1X + b_2X^2$.

tion index might be assumed to be less sensitive than C/N and lignin/N measures. However, this may be an erroneous assumption since no significant effect of initial litter quality (i.e., C/N ratio) on decomposition rates was detected in the replicated study (Sanchez, 2001). Furthermore, the predictability of changes in alkyl and O-alkyl C observed in this study is one of the reasons this index was initially recommended

Table 4. Coefficient of determination (R^2) and probabilities (P values) for the regression analyses for O-Alkyl carbon (linear) and lignin/N (quadratic) values for pooled data set and all treatments and litter quality combinations

Treatment	Quality	R^2	P	N
	O-Alkyl carbon			
All	All	0.75	<0.0001	64
Control	High	0.91	0.0002	8
Irrigated (I)	High	0.91	0.0002	8
Fertilized (F)	High	0.76	0.005	8
I + F	High	0.76	0.005	8
Control	Low	0.74	0.006	8
I	Low	0.92	0.0002	8
F	Low	0.93	0.0001	8
I + F	Low	0.89	0.0005	8
	Lignin/N			
All	All	0.70	<0.0001	64
Control	High	0.94	0.0008	8
I	High	0.85	0.009	8
F	High	0.42	0.25	8
I + F	High	0.63	0.08	8
Control	Low	0.52	0.16	8
I	Low	0.97	0.0001	8
F	Low	0.99	<0.0001	8
I + F	Low	0.97	0.0001	8

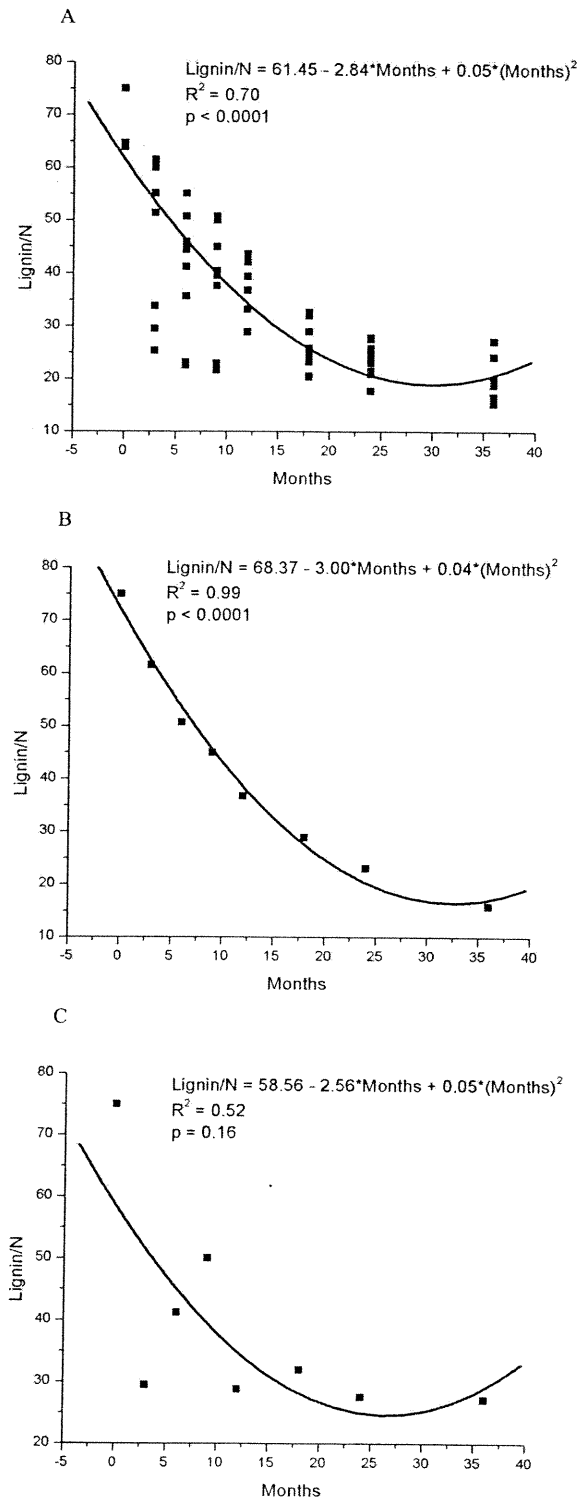


Figure 2. Changes in litter lignin/N ratio with time for (A) all treatments and qualities, (B) low quality litter on control plots, and (C) low quality litter on fertilized plots.

for use in decomposition studies. Baldock and Preston (1995) proposed using the alkyl/O-alkyl C ratio as a sensitive index of decomposition because they observed that alkyl C increased with concurrent decreases in O-alkyl C in forest litter and peats. This ratio was much more reliable in estimating decomposition than measurements of aromaticity (Baldock and Preston, 1995), which comprises the majority of lignin (Manders, 1987; Zech et al., 1987; Preston et al., 1990). No attempt was made by these authors to compare the alkyl/O-alkyl C measure with C/N as a decomposition index. In this study, metrics utilizing lignin (i.e., lignin/N and Cm/Lm ratio), as compared with C/N and alkyl/O-alkyl measures, had the lowest correlation to C and nutrients and exhibited the least predictive trends during the decomposition process. These results are consistent with the work of Enriquez et al. (1993) who utilized a data set of 256 published reports containing decomposition rates and C and nutrients contents to conclude that C:N:P ratios explained 89% of the variance of plant decomposition rates for a variety of detritus from diverse origins. When lignin was substituted for C, the lignin:nutrient ratios explained only 37% of the variance in decomposition rates. As such, caution must be used when using aromatic (i.e., lignin) measures in modeling and decomposition (mechanistic or quantitative) studies.

Conclusions

This study has demonstrated that there are significant correlations between C chemistry and nutrient concentrations during litter decomposition. Although not verified statistically, at the $P < 0.05$ level, trends in the litter C chemistry data suggest that fertilization accelerated the oxidative decomposition of litter resulting in a greater synthesis of highly oxidative C compounds (i.e., carbonyls). Irrigation and initial litter quality had a much smaller effect on decomposition. These conclusions are consistent with the results of the replicated analysis where only fertilization significantly affected decomposition (Sanchez, 2001).

In this study, all three indices (alkyl/O-alkyl, C/N, and lignin/N) correlated very well to most nutrients and followed a predictable pattern during the decomposition processes. Litter C/N measurements had the best correlation to nutrient concentrations and exhibited the most predictable decomposition trends as compared with the lignin/N and alkyl/O-alkyl C

Table 5. Parameter values for linear[a] regression for different carbon types, nutrients and C/nutrient ratios

Measure	Parameter	Control	Irrigated (I)	Fertilized (F)	I + F
		Treatment			
Aromatic	a	11.16 (0.38)[b]	12.00 (0.30)	10.77 (0.34)	10.74 (0.37)
	b	−0.08 (0.02)	−0.15 (0.02)	−0.05 (0.02)	−0.08 (0.02)
O-Alkyl	a	23.01 (0.61)	22.41 (0.47)	22.67 (0.68)	22.61 (0.84)
	b	−0.22 (0.03)	−0.30 (0.03)	−0.28 (0.04)	−0.32 (0.05)
Carbohydrate	a	27.61 (0.74)	26.89 (0.56)	27.20 (0.82)	27.14 (1.01)
	b	−0.26 (0.04)	−0.36 (0.03)	−0.34 (0.05)	−0.39 (0.06)
C	a	53.95 (0.39)	55.27 (1.03)	53.67 (0.62)	53.91 (1.22)
	b	−0.35 (0.02)	−0.60 (0.06)	−0.37 (0.04)	−0.51 (0.07)
N	a	0.38 (0.02)	0.38 (0.03)	0.33 (0.02)	0.35 (0.02)
	b	0.01 (<0.01)	0.01 (<0.01)	0.02 (<0.01)	0.03 (<0.01)

[a]Linear regression equation used are in the general form of $Y = a + bX$.
[b]Standard Errors are shown in parenthesis.

Table 6. Parameter values for linear[a] and quadratic[b] regression equations for different decomposition indices for high and low quality litter

Measure	Parameter	Control	Irrigated (I)	Fertilized (F)	I + F
		Treatment			
Alkyl/O-Alkyl[c]	a	0.44 (0.02)[d]	0.45 (0.03)	0.47 (0.02)	0.49 (0.02)
	b	0.01 (<0.01)	0.01 (<0.01)	0.01 (<0.01)	0.01 (<0.01)
		High quality			
C/N	a	137.1 (8.9)	137.5 (4.5)	138.2 (4.4)	140.9 (4.7)
	b_1	−4.29 (1.29)	−4.05 (0.65)	−5.75 (0.63)	−6.68 (0.67)
	b_2	0.06 (0.03)	0.04 (0.02)	0.08 (0.02)	0.11 (0.02)
Lignin/N	a	114.0 (5.5)	113.4 (9.3)	84.71 (20.3)	90.36 (16.9)
	b_1	−4.17 (0.79)	−4.33 (1.35)	−3.11 (2.94)	−3.95 (2.45)
	b_2	0.07 (0.02)	0.09 (0.04)	0.05 (0.08)	0.08 (0.07)
		Low quality			
C/N	a	164.4 (8.5)	167.0 (8.5)	164.5 (4.8)	166.0 (6.0)
	b_1	−5.55 (1.23)	−6.86 (1.23)	−7.44 (0.70)	−8.62 (0.87)
	b_2	0.08 (0.03)	0.10 (0.03)	0.11 (0.02)	0.14 (0.02)
Lignin/N	a	109.1 (19.3)	137.6 (5.0)	133.1 (4.3)	132.4 (5.6)
	b_1	−4.41 (2.80)	−4.33 (0.73)	−3.11 (2.94)	−3.95 (2.45)
	b_2	0.09 (0.08)	0.10 (0.02)	0.09 (0.02)	0.11 (0.02)

[a]Linear regression equation used are in the general form of $Y = a + bX$.
[b]Quadratic equations used are in the general form of $Y = a + b_1X + b_2X^2$.
[c]Pooled data from low and high quality litter since no significant difference, at $P < 0.05$ level, in this metric was detected between the litter qualities
[d]Standard Errors are shown in parenthesis.

measures. The relative ease in taking C/N measures as compared with taking lignin/N and alkyl/O-alkyl makes this the metric of choice in most decomposition studies. Although C/N measures do not provide molecular level information, it is still a simple and important diagnostic for modeling C and nutrient dynamics on a variety of soils. However, for mechanistic studies of the decomposition process, more detailed information can be gained through CPMAS ^{13}C NMR analyses, especially in the alkyl, O-alkyl, and carbonyl spectral regions.

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