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Ryegrass straw component decomposition during mesophilic and thermophilic incubations

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Abstract The decomposition of perennial ryegrass straw was examined under mesophilic and thermophilic temperatures. Thermophilic conditions were used to define the composting process. The change in lipids, sugars, soluble polysaccharides, cellulose, and lignin was determined during a 45-day incubation. C, H, O, and N steadily decreased in both temperature treatments. The lignin content, as measured by the Klason or 72% H_2SO_4 method, decreased by 10% under mesophilic and 29% under thermophilic conditions. The Klason lignin C loss was 25 and 39% under mesophilic and thermophilic incubations, respectively. The changes in element (C, N, H, and O) ratios indicated that 94% of the lignin fraction was altered during both low- and high-temperature incubations. The changes in the lignin-like fraction as shown by elemental ratios were more extensive than those indicated by the Klason method, showing that this lignin determination has limited value in describing plant residue decomposition. The decomposition of the straw components and the concomitant degradation of the lignin fraction represent an important decomposition process that facilitates the composting of ryegrass straw with a high C:N ratio.

Key words Composting · Lignin · Mesophilic conditions · Ryegrass · *Lolium perenne* L. · Thermophilic conditions · Klason lignin

Introduction

Lignolytic wastes, such as plant residues, from agricultural production systems are difficult to manage and dispose of because of their bulk and low value (Biddlestone et al. 1987). The use of agricultural wastes, such as straw, is becoming a priority issue because disposal practices such as open-field burning are no longer environmentally acceptable. Without field burning, straw residue can affect herbicide efficacy and can negate established herbicide weed control practices (Neate and Rovira 1993). Composting of grass seed straw is an on-farm management strategy that can reduce problems associated with plant residues and will aid in the development of sustainable cropping systems. Benefits of compost additions to cropping systems include fertilizer and soil conditioning value and control of soil-borne pests (Bangar et al. 1989; Nelson and Craft 1992). However, the feasibility of composting lignolytic material with a high C:N ratio, such as grass straw, without intensive co-composting management (lowering of the C:N ratio) has not been assessed.

Composting is a microbially mediated exothermic process that occurs in an aerobic thermophilic environment. Successful grass straw composting appears to require a combined substrate C:N ratio of 30:1 or less to proceed (Biddlestone et al. 1987). The combined C:N ratio of grass straw can exceed 50 and the grass stem often has a C:N ratio of over 100 (Horwath et al. 1994). Under the correct conditions of temperature, moisture, and bulk density, the composting of high-C:N ryegrass straw proceeds rapidly in the field with low-input management (Churchill et al. 1993). The residue undergoes chemical and physical transformation to yield an end-product consisting of microbial products, secondary residue decomposition products, and humic materials (Hammouda and Adams 1987). The product is a potential resource with value as an organic amendment that can improve soil quality (Thomsen 1993; Zaccheo et al. 1993).

The critical processes controlling decomposition of plant residues in the field and during composting must be understood in order to develop methodology that will facilitate the production of a desirable end-product with low-input management. As a first step, the microbial and biochemical aspects of decomposition occurring in high-C:N grass straw must be elucidated. Assessment of lignin de-

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gradation and of the alterations in the lignin macromolecular structure is required to understand the critical factors that control plant residue decomposition, the effect of lignin on the fate of other residue components, and end-product formation. Therefore laboratory experiments were conducted to determine the effect of temperature on the degradation of straw components. Specifically, we examined the changes in available substrates and evaluated the alterations in the lignin fraction in relation to the successful composting of high-C:N residue.

Materials and methods

Plant material

Mature perennial ryegrass straw (7% moisture by dry weight) was obtained locally after grass seed harvest. The straw was chopped, sorted into 1- to 2-cm lengths on a commercial seed cleaner (Kamas/Westrup-Gustafson, Dallas, Tex.), and stored at 4°C until used.

Treatments

Two grams (fresh weight) of grass residue were weighed into 140-ml plastic specimen containers (Oxford Labware, St. Louis, Mo.) and placed in sealed mason jars containing 1 ml water to prevent the straw drying out. The straw moisture was adjusted to 80% gravimetric water content (–4.1 MPa). One set of straw residues was incubated at 25°C for 45 days as a low-temperature treatment. A second set of straw residues was incubated at both 25°C and 50°C for 45 days as a high-temperature treatment. The staggered high-temperature treatment was imposed to simulate field composting. It was maintained at 50°C between days 6–25 and 31–40, for a total of 30 days. The samples were aerated daily.

Total C and N

Straw from both the low- and high-temperature treatments was sampled throughout the 45-day incubations. The straw samples were dried at 60°C, weighed, and ground in a Wiley mill to pass a 40-mesh screen. Straw C, H, and N were analyzed on an elemental analyzer (Perkin Elmer, Danbury, Conn.). Straw O was determined by difference. All data were calculated on an ash-free basis.

Lipids and sugars

A modified lipid-extraction method (Bligh and Dyer 1959) was used to remove labile non-polar and polar components of the grass straw. Grass residue (100 mg) was placed in 50-ml centrifuge tubes (Nalgene Co., Rochester, N.Y.) with 4 ml methanol:chloroform:water (12:5:3, v:v). The sample was sonicated for 60 s in an ultrasonic water bath (Branson Ultrasonics Corp., Danbury, Conn.). After standing for 10 min, the sample was centrifuged (1300 *g*, 4°C) for 10 min. The procedure was repeated four times and the supernatants were composited in a separate centrifuge tube.

The supernatant was combined with 5 ml $CHCl_3$ and 4 ml water, vortexed, and centrifuged (1300 *g*, 4°C) for 10 min. The chloroform and methanol-water phases (top) were separated and analyzed separately. The chloroform phase was transferred to a dried (24 h at 50°C) pretared glass vial, allowed to evaporate for 24 h under a fume hood, dried overnight at 50°C, and reweighed to determine the lipid content of the straw. Total sugars in the methanol-water phase were measured colormetrically using the phenol-sulfuric acid technique (Dubois et al. 1956). A standard series was determined from a 1 mg l^{-1} glucose (dried at 80°C for 24 h) stock solution.

Protein extraction

The pellet was placed in a fumehood to evaporate residual chloroform for 1–2 h. The pellet was incubated (30°C for 48 h, with occasional swirling) with 2 ml 0.4% Pronase (Calbiochem, La Jolla, Calif.) in 0.05 *M* TRIS (adjusted to pH 7.4 with HCl). To prevent microbial contamination, 0.1 ml 0.01 *M* N_3Na was added to each sample. The pellet-Pronase mixture was centrifuged (1300 *g*, 4°C) for 10 min and extracted with 2 ml water for a total of four extractions. The supernatants were discarded and the pellet saved for soluble polysaccharide analysis.

Soluble polysaccharides

Following the Pronase digest, the pellet was combined with 10 ml water and placed in a boiling water bath for 1 h to extract soluble polysaccharides. The sample was centrifuged (1300 g, 4°C) for 10 min and the supernatant drawn off with a Pasteur pipette. The pellet was re-extracted with 4 ml water for a total of four extractions and the supernatants composited. Soluble polysaccharides were determined by the phenol-sulfuric acid method (Dubois et al. 1956).

Cellulose and Lignin

The cellulose and lignin fractions were determined by a modified Klason lignin method (Kirk and Obst 1988). One milliliter of 24 N H_2SO_4 was added to the pellet and the mixture was digested for 1 h in a 30°C water bath with occasional mixing with a glass rod. The digestion was terminated by the addition of 28 ml water. The digested samples were autoclaved (1.05 kg cm^{-2} at 121°C) for 1 h and cooled to room temperature. The samples were filtered with a vacuum side-arm flask through a preweighed (dried at 105°C for 24 h) glass fiber filter (Whatman GF/A). The filter was dried at 105°C for 72 h and reweighed to determine the lignin fraction. The acid hydrolysate was analyzed for sugars using the phenol-sulfuric acid method (Dubois et al. 1956) to determine cellulose (included all cell-wall polysaccharides). Separate lignin samples were used to determine the ash content. Lignin samples were analyzed for C, N, and H on an elemental analyzer (Perkin Elmer, Danbury, Conn.). Lignin O was determined by difference, after correction for the ash content, assuming the majority of lignin is composed of C, N, H, and O.

Results and discussion

The initial stages of plant residue decomposition are characterized by the mineralization of labile components, leaving refractory components intact (Kögel-Knabner 1993). In the later stages of decomposition recalcitrant components, such as lignin, are mineralized. The biodegradation of refractory substances is intrinsically limited by chemical structure (i.e., conformational limitations of degradative enzymes) or through association with refractory substances, such as lignin (i.e., lignocellulose) or melanins (Haider 1986; Kögel-Knabner 1993). Describing the alteration and degradation of plant components during decomposition is difficult, due to limitations in methodology that cannot distinguish among components of plant origin, microbial production, and decomposition products (Paul and Van Veen 1978).

The concentration of C, H, and O in the decomposed straw remained similar throughout the incubation in both low- and high-temperature treatments (Table 1). The content of N in the degraded straw decreased from 11.2 to 8.6

and 7.5 g kg^{-1} straw in the low- and high-temperature treatments, respectively. The loss of straw C was 175.7 g kg^{-1} from the low-temperature treatment and 237.1 g kg^{-1} straw from the high-temperature treatment. The loss of H was similar to the C loss. Compared to undecomposed straw, the loss of 0 was 215.8 g kg^{-1} in the low-temperature treatment and 271.6 g kg^{-1} straw in the high-temperature treatment. The C:N ratio decreased from 40:1 to 32:1 in the low-temperature treatment and 28:1 in the high-temperature treatment. According to Biddlestone et al. (1987) mature compost C:N ratios vary from 15 to 20:1, indicating that the decomposed straw in the present study was immature.

The change in straw chemical fractions during decomposition reflects the mineralization of straw components and the increases in microbial products (Paul and Clark 1989; Kögel-Knabner 1993). All the straw components declined during the low-temperature treatment. Cellulose declined from 562.1 to 297.8 g kg^{-1} straw and represented the largest loss of all the chemical fractions measured (Table 2). Lipids increased to 38.3 from 33.1 g kg^{-1} straw on day 7 and then declined to 23.5 g kg^{-1} straw after 45 days of incubation at the low-temperature. Similarly, soluble polysaccharides increased to 21.8 from 17.1 g kg^{-1} straw on day 3 and then declined to 10.1 g kg^{-1} straw. Soluble sugars declined from 33.0 to 6.9 g kg^{-1} straw. Klason lignin steadily declined from 121.5 to 113.0 g kg^{-1} straw in the low-temperature treatment. The initial increase in chloroform soluble material and soluble polysaccharides indicates microbial production of membranes and extracellular polysaccharides (Paul and Clark 1989).

In the high-temperature treatment, the straw chemical fractions were mineralized more rapidly and completely than in the low-temperature treatment (Table 2). Cellulose declined from 562.1 to 213.8 g kg^{-1} straw. Lipids increased from 33.1 to 43.7 g kg^{-1} straw on day 6 and then declined to 18.7 g kg^{-1} straw. Soluble sugars declined from 33.0 to 9.7 g kg^{-1} straw in the high-temperature treatment. Soluble polysaccharides declined to 5.6 from 17.1 g kg^{-1} straw. Klason lignin declined to 89.0 from 121.5 g kg^{-1} straw.

The decomposition of lignin has been reported to be the slowest of all plant components (Minderman 1968; Aber and Melillo 1991; Kögel-Knabner 1993). The Klason lignin method has been used extensively to determine lignocellulose loss in plant decomposition studies (Kirk and Obst 1988), and many ecological and agricultural field studies have used this method to determine lignin loss because of its simplicity (Kirk and Obst 1988; Aber and Melillo 1991). The Klason lignin method has also been used extensively to determine changes in substrate during composting and mushroom culture (Chang 1967; Flaig 1969; Haider 1969; Tsang et al. 1987).

The Klason lignin method is a gravimetric approach that measures acid-insoluble material broadly defined as lignin. Precipitation and condensation of other plant com-

Table 1 Mean (SD) concentrations (g kg^{-1}) of C, H, O, and N in straw on day 0 and after 45 days of incubation at low (LT) and high (HT) temperatures

Day	Treatment	C	H	O	N	C:N
Undecomposed straw						
0		450.3 (0.7)	68.2 (0.5)	470.3 (0.8)	11.2 (0.4)	40:1
Decomposed straw						
45	LT	468.6 (10.8)	82.6 (2.2)	434.2 (13.3)	14.7 (0.5)	32:1
45	HT	467.3 (17.6)	80.6 (2.5)	435.6 (20.8)	16.5 (0.7)	28:1
Original content remaining						
45	LT	274.6 (6.2)	48.4 (1.3)	254.5 (9.1)	8.6 (0.3)	
45	HT	213.2 (8.0)	36.8 (1.2)	198.7 (9.5)	7.5 (0.3)	

Table 2 Mean (SD) change (g kg^{-1}) in chemical fractions during low- and high-temperature incubations. Chloroform soluble, soluble sugar, soluble polysaccharide, cell-wall polysaccharide, and lignin fractions do not equal 100% because unknown fraction not included

Day	Lipids	Sugar	Soluble polysaccharide	Cellulose	Lignin
Low temperature					
1	33.1 (1.8)	33.0 (2.5)	17.1 (1.7)	562.1 (26.2)	121.5 (1.8)
3	33.7 (0.1)	13.3 (0.3)	21.8 (0.2)	542.0 (35.7)	127.9 (6.9)
7	38.3 (1.4)	9.8 (0.6)	19.0 (3.6)	481.9 (7.5)	125.6 (7.6)
12	31.5 (1.4)	8.2 (0.4)	11.4 (1.5)	450.8 (13.6)	125.1 (3.8)
20	26.9 (1.5)	7.3 (1.0)	11.1 (0.8)	381.8 (3.8)	122.2 (1.0)
30	26.4 (1.4)	7.0 (0.9)	8.9 (2.6)	312.9 (11.8)	114.1 (1.8)
45	23.5 (3.5)	6.9 (0.6)	10.1 (1.6)	297.8 (12.7)	113.0 (2.1)
High temperature					
6	43.7 (1.5)	12.6 (1.0)	13.7 (1.2)	513.8 (23.1)	119.1 (2.3)
8	38.0 (2.5)	7.5 (0.7)	12.3 (0.7)	522.2 (35.8)	119.5 (6.0)
12	35.0 (1.5)	17.9 (3.8)	9.0 (1.1)	315.8 (29.7)	109.1 (2.1)
17	30.4 (2.9)	16.7 (0.8)	8.9 (2.5)	289.0 (40.7)	106.0 (5.6)
25	26.0 (4.1)	11.6 (1.2)	6.2 (0.8)	219.4 (24.0)	102.8 (6.2)
30	25.6 (1.9)	6.1 (1.2)	6.1 (1.2)	185.5 (18.4)	102.4 (8.5)
45	18.7 (1.1)	9.7 (0.9)	5.6 (0.3)	213.8 (9.4)	89.0 (1.7)

ponents, such as proteins and suberins, can interfere with the Klason method (Kirk and Obst 1988). Lignin can be released or altered during the degradation of cell walls during plant residue decomposition (Crawford 1981; Crawford and Pometto 1988). Oxidation of lignin phenolic and branch chain structures increases the O content of the lignin fraction (Kirk 1971; Chang et al. 1980; Kögel 1986). In addition, the biosynthesis of microbial aromatic compounds during decomposition can interfere with the gravimetric analysis (Flaig et al. 1975; Martin and Haider 1980; Haider 1991). For these reasons the Klason lignin method may have limited value in plant residue decomposition studies (Crawford 1981). The approach used in the present study eliminated many of these interfering substances through the extensive use of chemical and enzymatic extraction procedures.

Lignin C declined to 25 and 39% in the low- and high-temperature treatments, respectively, as determined by elemental analysis of the Klason lignin fraction (Table 3). This compared with a decline in the lignin fraction of 10 and 29% in the low- and high-temperature treatments, respectively, as determined by the Klason lignin method. N in the lignin fraction increased by 12% in the low-temperature treatment and by 16% in the high-temperature treatment. The loss of lignin H was similar to that of C in both treatments. The mass of O remained similar to undecomposed straw lignin O in the high-temperature treatment and increased to 127% in the low-temperature treatment. The constant or increased level of O and loss of C and H indicated that the lignin fraction was oxidized during the decomposition process. Reviews of degradative reactions during the decomposition of lignin have indicated that increases in O content occur through the oxidative splitting of side chains and oxidative ring cleavage to form carboxylic acid groups (Kirk 1971; Flaig et al. 1975; Chang et al. 1980; Crawford 1981; Kirk and Farrell 1987; Kögel-Knabner 1993).

The shift in the elemental ratios of the decomposed lignin fraction indicated a greater change than that determined by the Klason method. The increased N and decreased C contents of the lignin fraction resulted in a fall in the C:N ratio, from 52.9:1 to 35.6:1 and 28:1 in the low- and high-temperature treatments, respectively. The C:O ratio declined from 2.4 to 1.4 and 1.5 in the low- and high-temperature treatments, respectively. The C:H ratio changed little, indicating that the loss of C and H was similar in both treatments. It was calculated that approximately 6% of the original lignin was unaltered in both treatments. The percentage of altered lignin was calculated from the change in element ratios between undegraded lignin and degraded lignin. The increased N content of the decomposed lignin suggests that humic substances formed during decay of the straw (Flaig et al. 1975; Kögel-Knabner 1993). Other composting studies of straw residues have shown similar increases of N in the analyzed lignin-like fraction, using the Klason method (Bremner 1954; Flaig 1969; Haider 1969; Hammouda and Adams 1987).

During the composting of wheat straw residues, Chang (1967) found minimal lignin degradation using the Klason method. Similarly, only an 11% lignin loss was reported in wheat straw compost used for the culture of *Pleurotus* sp. (Tsang et al. 1987). In contrast, Durrant et al. (1991) showed 30–40% lignin degradation using specifically ^{14}C-labeled ring and side-chain lignin and uniformly labeled lignin in wheat straw compost used for *Agaricus bisporus* production. These authors found that the Klason lignin method underestimated the amount of lignin lost compared to ^{14}C-lignin mineralization. Similarly, in a study on methods of determining lignin loss in forest litter, Johansson et al. (1986) found that the Klason method produced the highest amount of residual lignin. These studies indicate that the Klason method is a conservative estimate of lignolytic activity. In contrast, Inbar et al. (1990) found little change in the lignin content of composted cattle manure using the acid-detergent method, but the use of decomposed materials (cow manure) for composting may have confounded the interpretation of lignin loss and degradation.

Elemental ratios in the decayed lignin fraction closely resembled those of soil organic matter (Flaig et al. 1975; Volk and Loeppert 1982). These findings were similar to those of other studies in which different methods were used to determine lignin degradation in different plant materials (Table 4). It is doubtful that the majority of the lignin-like fraction was completely contaminated with humic substances. It is more probable that the lignin fraction was both chemically altered and contaminated with microbial products and humic substances.

Differentiation between plant residue chemical fractions and microbial production during decomposition is rarely studied (Paul and van Veen 1978). The obvious lack of analytical methods, especially in field research, has contributed to the dearth of information concerning the processes occurring during plant residue decomposition (Paul and Clark 1989; Kögel-Knabner 1993). This research and other studies that have probed lignin degradation have raised questions concerning the actual processes occurring during plant residue decomposition. The change in the chemical nature of lignin during decomposition has generally been ignored. Furthermore, most studies on plant residue decomposition do not relate changes in substrate qual-

Table 3 Mean (SD) change in elements and elemental ratios in the lignin fraction for low- (LT) and high- (HT) temperature treatments

	C	H	O	N
Content day 0 ($g\ kg^{-1}$)	640.2 (44.5)	81.9 (6.2)	265.8 (51.9)	12.1 (1.4)
Percentage remaining				
LT	75.0 (0.4)	75.1 (0.9)	126.5 (5.5)	111.6 (9.2)
HT	61.3 (0.3)	60.0 (0.8)	98.2 (3.7)	116.1 (6.7)
		C:H	C:O	C:N
Day 0		7.8	2.4	52.9
LT		7.8	1.4	35.6
HT		8.0	1.5	28.0

Table 4 Mean (SD) change (%) in elements during lignin degradation

Lignin	Days of decomposition	C	H	O	N
Perennial ryegrass[a]	0	64.02 (4.45)	8.19 (0.62)	26.58 (5.19)	1.21 (0.14)
	45	53.84 (1.07)	6.90 (0.08)	37.75 (1.06)	1.51 (0.11)
	45	54.78 (0.66)	6.85 (0.14)	36.41 (0.77)	1.96 (0.11)
Ryegrass[b]	0	63.10	5.92	30.67	0.54
Ryegrass[c]	180	61.15	5.42	32.42	1.75
Ryegrass[d]	0	62.73	5.64	30.55	0.53
	180	62.20	5.41	31.30	0.56
Wheat straw[e]	0	63.39	5.41	30.98	0.22
	180	60.40	5.66	32.86	1.08

[a] Horwath and Elliott (This study)
[b] Freundenberg and Harkin 1994 (cited in Flaig et al. 1975)
[c] Maeder (1960) (cited in Flaig et al. 1975)
[d] Flaig (1969)
[e] Flaig et al. (1975)

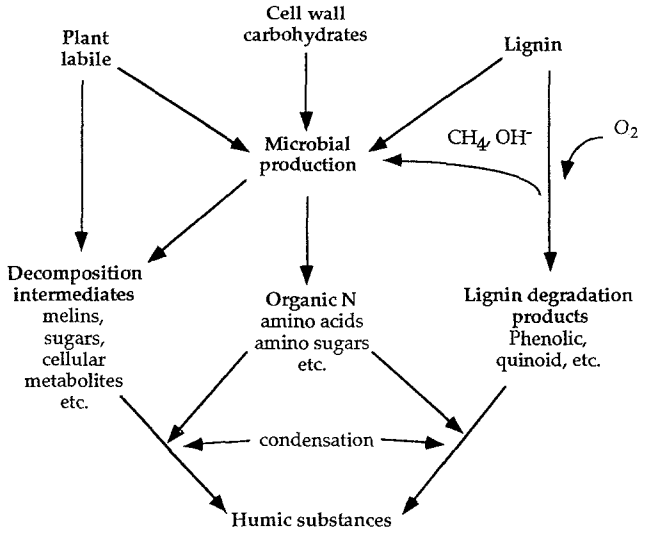

Fig. 1 Proposed pathway for lignin degradation and formation of humic substances in decayed ryegrass straw

ity to the fate of lignin. Future research should delineate between microbial production and plant residue decomposition and alteration to give a more realistic interpretation of decomposition dynamics.

The extensive alteration and decomposition of lignin throughout the 45 days of the present study provide evidence of why grass straw composts successfully in the field without the addition of N to lower the C:N ratio. In this study, the results indicate that lignin was degraded concomitantly with the other straw components measured. Churchill et al. (1993) showed a 80% volume reduction in composted ryegrass after 20 weeks in the field. The breakdown of lignin likely increases the availability of cell-wall polysaccharide and related compounds for microbial use (Fig. 1). The relationships among the formation of humic materials, the alterations in the lignin fraction, and the production of microbial by-products are poorly understood (Kögel-Knabner 1993). Understanding the degradation of the lignin fraction during plant residue decomposition and composting will lead to practices that can tailor the end product to specific uses and provide insights on the nature and origin of humic substances in soil.

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