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Soil Biology & Biochemistry 34 (2002) 1393–1399

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Improved accounting of carbohydrate carbon from plants and soils

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Received 2 May 2001; received in revised form 12 March 2002; accepted 15 May 2002

Abstract

Soils have a great potential to accumulate carbon from atmospheric sources, but we possess few quantitative tools to predict and understand the conversion of C from plant sources into stable soil organic matter. Evaluation of present methods used for analyzing the major form of carbon present in plants, carbohydrates, found that development of 'total carbohydrate' methods were originally based on colorimetric or gravimetric tests whose accuracy is questionable. Use of ion chromatography found that total carbohydrate extraction and hydrolysis methods based on previously published H_2SO_4 solublization and hydrolysis techniques released from pure cellulose as little as 0.4% and a maximum of 22% of the glucose equivalents due to a failure to solublize the substrate. Optimum solublization conditions with concentrated H_2SO_4 (18 M) for 15–30 min followed by autoclave hydrolysis (1–1.5 M H_2SO_4) resulted in 82–97% recovery of purified cellulose–glucose and accounted for a major portion of the plant glucose that was not released by previously published methods. Application of this methodology to soils resulted in additional release of glucose equivalents if the hemicellulose fraction was first extracted with 1.0 M H_2SO_4 (30 min autoclave digestion). Prolonged hydrolysis times (16 h) recommended by previous methodology resulted in increased formation of carbohydrate degradation products as furan derivatives. Use of improved solublization procedures with autoclave hydrolysis (30 min) accounted for up to 99% of the theoretical carbohydrate content from plant biomass and recovered an additional 2.5–3.5 times of soil carbon content as carbohydrate forms while minimizing formation of furan derivatives.

Keywords: Carbohydrates; Monosaccharides; Cellulose; Ion chromatography; Plant and soil carbon

1. Introduction

Carbohydrates play a major role as structural (e.g. cellulose, hemicellulose and pectin) components of plants and provide a major source of energy for microbial processes in soils. The wide diversity of carbohydrates in nature has led to development of many analytical techniques, though many are not quantitative and are prone to interferences (Martens and Frankenberger, 1990a,b; 1991). Martens (2000a) reported that different plant residues have different decomposition rates in soil and the decomposition rates were related to the chemical composition of the plant residue. Residues with higher contents of carbohydrates and amino acids and lower contents of phenolic acids have faster C mineralization rates than residues with lower carbohydrate contents and higher phenolic acid contents. If carbon sequestration in soils is to be managed, the quantitative composition of different plant residues and the impact of different plant biochemistry on carbon mineralization and cycling must be known.

Little quantitative information is available on the 'total carbohydrate' content as cellulose and hemicellulose of plant material. Weil et al. (1998) reported that popular wood contained 41% cellulose and 19% hemicellulose, and Sommerville et al. (2000) reported that *Arabidopsis* sp. contained 58.7% total carbohydrate by dry weight, although most estimates of cellulose and hemicellulose content have been based on solubility and gravimetric analysis using concentrated acid (Goering and Van Soest, 1970). Work in my laboratory with protocols for total carbohydrate extraction, based on the work of Cheshire and Mundie (1966) from different plant biomass resulted in total values (carbohydrate-C/organic C) ranging from 4.1% for clover biomass to 17% for canola biomass (Martens, 2000a) as determined by ion chromatography (Martens and Frankenberger, 1990a). The poor recovery of carbohydrate-C reported by Martens, (2000a) in light of reported theoretical values (Sommerville et al., 2000) suggests that extraction methods proposed for total carbohydrate extraction may not be quantitative.

This study investigated the efficiency of glucose recovery

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0038-0717/02/$ - see front matter
PII: S0038-0717(02)00082-2

Table 1
Properties of soil and plant biomass used in this study

	Organic C (g kg^{-1} residue)	Total N (g kg^{-1} residue)	C/N ratio
Soil[a]			
Prairie	44.0	3.84	11.4
Soybean	28.8	1.86	15.5
Vegetation			
Clover	464	44.9	10
Prairie[b]	453	3.7	122
Corn	445	4.3	103
Soybean pods[c]	432	8.3	52
Soybean stalks[c]	424	8.8	48
Soybean leaves[c]	423	22.3	19
Oat	433	17.0	25
Canola	410	12.8	32
Soybean[d]	397	10.7	37
Soybean[e]	391	6.5	60
Alfalfa	350	36.0	10

[a] Webster soil with vegetation that was present in 1996 and decomposing in 1997 when sampled.
[b] Unidentified native grass species.
[c] Soybean variety, Great Lakes XP7225.
[d] Unidentified soybean variety collected after over wintering in the field.
[e] Soybean variety. Pioneer 92B71.

from purified cellulose by different extraction methods. Optimized conditions were then employed to evaluate the carbohydrate values (hemicellulose and cellulose plus uronic acids) from different plant biomass and soils.

2. Methods and materials

2.1. Soil and plant samples

Selected properties of the Webster silty–clay loam soil (Typic Haplaquoll) used are reported in Table 1 and a full description of properties was given by Martens (2000b). The soil was collected in April 1997 and stored moist at 4 °C until used. Total C, organic C (total C after acid neutralization) and total N were determined by dry combustion with a Perkin Elmer 2400 C/H/N analyzer (Perkin Elmer, Inc., Fullerton, CA) and inorganic C was determined by the difference between total C and organic C. Properties of the corn (*Zea mays*), soybean (*Glycine max*), an unidentified prairie grass, alfalfa (*Medicago sativa*), oat (*Avena sativa*), clover (*Trifoium pratense*) and canola (*Brassica napus*), harvested from field sites or from glasshouse pots, are listed in Table 1. The plant samples analyzed were a mix of above ground leaf and stem portions (ground through a 1 mm sieve).

2.2. Cellulose glucose extraction

Total carbohydrate extraction efficiency was measured by extracting cellulose with various concentrations of H_2SO_4 and coupled with ion chromatography and pulsed amperometric detection of individual monosaccharides (Martens and Frankenberger, 1990b). Briefly, 20 mg purified cellulose in screw-top test tubes (15 × 125 mm) was treated with 300 μl H_2SO_4, ranging from 6 to 18 M for various contact times (5–60 min) in an attempt to solublize the cellulose. After solublization, the appropriate level of deionised (DI) water was added to the samples to result in 0.25–4 M H_2SO_4 concentrations and the samples were refluxed at 90 °C for 16 h or autoclaved for 30–60 min at 121 °C (104 kPa). Samples were titrated to pH 4–5 with 5 M KOH, centrifuged to remove precipitate and then an aliquot was diluted for analysis.

2.3. Plant carbohydrate extraction

To test the optimized conditions for plant residue carbohydrate analysis, a two-step digestion was used. First, hemicellulose sugars (arabinose, galactose, glucose, xylose with trace levels of rhamnose and mannose) were extracted at room temperature from 20 mg sample of plant biomass in screw-top test tubes (15 × 125 mm) with 800 μl 6 M H_2SO_4 for 30 min, diluted with 4.2 ml DI water to 1 M H_2SO_4, then autoclaved (121 °C, 104 kPa) for 30 min. After centrifugation and collection of the supernatant, the residue was washed with two aliquots of 1 ml DI water and centrifuged between each addition and the three supernatants combined and diluted for hemicellulose analysis. Second, the sample was dried (60 °C) overnight before addition of 300 μl 18 M H_2SO_4 (30 min) for cellulose solublization. After solublization, the samples were diluted with 3.6 ml of DI water for autoclave hydrolysis (1.5 M H_2SO_4, 30 min). The cellulose and monosaccharides standards were obtained from Sigma (Sigma Chemical Company, St Louis, MO).

2.4. Soil carbohydrate extraction

The carbohydrate composition of soils was compared by analysis of several different extraction and hydrolysis methods. The first extraction was based on the traditional method (Cheshire and Mundie, 1966) using 500 mg soil in screw-top test tube (15 × 125 mm) with an 800 μl 6 M H_2SO_4 treatment for 2 h, diluted with 4.2 ml DI water (1 M H_2SO_4) and refluxed at 90 °C for 16 h (Method 1). A second method involved a 30 min treatment of 100 mg soil in screw-top test tubes (15 × 125 mm) with 800 μl 6 M H_2SO_4, and following addition of 400 μl DI water, digested with 4 M H_2SO_4 for 30 min using the autoclave digestion parameters listed previously (Method 2). The two methods were compared with the method optimized for extraction of plant saccharides describe previously. Briefly, 100 mg soil samples were treated with 800 μl 6 M H_2SO_4 for 30 min in screw-top test tubes (15 × 125 mm) and diluted with 4.2 ml DI water to 1 M H_2SO_4 before autoclave digestion (30 min) to release respective hemicellulose monosaccharides (Method 3, step 1). The sample was then washed with two

aliquots of 1 ml DI water, centrifuged and the three supernatants combined and diluted for hemicellulose analysis. The extracted and washed sample was dried overnight at 60 °C, the soil was treated with 300 μl 18 M H_2SO_4 for 30 min, diluted to with 3.6 ml DI water to 1 M H_2SO_4 and autoclaved to release cellulose (Method 3, step 2).

Caution. Great care must be exercised to quantitatively retain the small fragments of plant residue that may carry over with the supernatant from the hemicellulose fraction for the cellulose extraction step. In addition, the plant and soil material must be thoroughly washed with water (at least 2, 1 ml aliquots) to remove residual H_2SO_4 used to extract the hemicellulose fraction before being dried at 60 °C or poor recovery of cellulose-C will result due to charring. Temperatures higher than 60 °C used to dry the material will also result in reduced cellulose recovery as glucose. Great caution must be exercised to avoid any contact with skin when using concentrated H_2SO_4.

2.5. Plant uronic acid extraction

Uronic acid content (pectin content) was determined as described by Martens and Frankenberger (1990c). Briefly, 200 mg plant sample was digested in 5 ml of 0.25 M H_2SO_4 for 30 min at 121 °C (104 kPa), titrated to pH 4–5 with KOH, centrifuged to remove precipitate and diluted to 10 ml with DI water. A 1 ml aliquot was diluted to 5 ml with DI water, treated with the enzymes pectolyase (3 units ml^{-1}) and *B*-D-glucuronidase (30 units ml^{-1}) at pH 6.8 (phosphate buffer) and the mixture incubated overnight at ambient temperatures (16 h). The enzyme-extract mixture was passed through an activated strong anion (3-quanternary propylammonium, Cl^-) exchange column (Supelco, Bellefonte, PA) and rinsed with 3 ml water. The uronic acids (mainly, galacturonic and glucuronic acids) were eluted with several milliliters 0.1 M NaCl (pH 8.0) and quantified by ion chromatography with pulsed amperometric detection.

2.6. Identification of monosaccharides, uronic acids and furan derivatives

The monosaccharides and furan compounds released as degradation compounds of acid digestion and uronic acids released by enzymatic digestion were separated on a Dionex DX-500 (Dionex Corp., Sunnyvale, CA) ion chromatograph equipped with a CarboPac PAl0 column (2 mm i.d.). Separation was achieved with a NaOH gradient (5–80 mM) for monosaccharides (Martens and Frankenberger, 1990b) and furan derivatives (Bousaid et al., 1999) and a NaOH–Na acetate gradient (50–100 mM NaOH; 125–200 mM Na acetate) for uronic acids (Martens and Frankenberger, 1990c). Furan, furfuryl alcohol, and 5-hydroxymethyl-furfural were obtained from Aldrich (Aldrich Chemical Corp., Milwaukee, WI) and furan derivatives were identified by retention time and coelution with spiked standards.

3. Results and discussion

The poor recovery of the total carbohydrate fraction from the plant samples as reported by Martens (2000a) suggests that current methodology for carbohydrate extraction and hydrolysis is not quantitative. Plant or soil carbohydrate recovery is based on two factors, first, the carbohydrate fractions must be quantitatively solublized and second, the solublized carbohydrate polymers must be hydrolyzed to their respective monosaccharides for colorimetric or ion chromatographic analyses. The much copied original method published by Cheshire and Mundie (1966) used 12 M H_2SO_4 (shaken at ambient temperatures) to solublize the carbohydrate fraction present in soil including plant residues and 0.5 M H_2SO_4 with heat (100 °C) to hydrolyze the solublized carbohydrates to the monomeric forms. The Cheshire and Mundie (1966) method was based on the work presented by Walksman and Stevens (1930), who first proposed using 12 M H_2SO_4 to solublize humus polysaccharides for gravimetric analyses. The H_2SO_4 method and its variations have been the standard for releasing microbial and plant monosaccharides present in soil for the past 30 years (Swincer et al., 1969; Martens and Frankenberger, 1990b; Puget et al., 1999). Accurate accounting of plant carbohydrate-C is vital if C balances are to answer the questions related to plant-C cycling and soil C sequestration, since carbohydrates compose the majority of plant C cycling through terrestrial systems. The properties of the plant biomass used are shown in Table 1.

Table 2 shows the recovery of glucose from purified cellulose influenced by acid concentration used for solublization and the concentration of acid used for digestion. Acid or bases have traditionally been used to solublize carbohydrates with acid hydrolysis preferred due to the added hydrolysis action by the acid for the release of respective monosaccharides from carbohydrate polymers, which are more suitable for analysis. The recovery of glucose from cellulose extractions was directly related to the solublization of the cellulose by the acid before hydrolysis (Table 2; Fig. 1). Cellulose that is not first solublized, cannot be hydrolyzed. Use of H_2SO_4 concentrations less than 18 M (concentrated H_2SO_4) failed to completely solublize the cellulose (Fig. 1) and subsequent hydrolysis (0.5 M H_2SO_4) resulted in low glucose recoveries due to poor solublization (Table 2). The concentration of the acid for the hydrolysis step was less critical than acid concentrations used for solublization (Table 2). The ratio of sample (mg) to concentrated acid (μl) is also important as >0.07 mg sample μl^{-1} acid (20 mg cellulose and 300 μl 18 M acid) failed to completely solublize the cellulose. The autoclave method gave comparable or a better glucose recovery compared with the 90 °C for 16 h method and is

Table 2
Recovery of glucose from purified cellulose (20 mg, 7.98 mg C) as solubilized and hydrolyzed with various H_2SO_4 concentrations

Solublization time (6 M)	Hydrolysis concentration (M)	Heating time (h)	Cellulose-C recovered (mg)	Recovery (%)
2 h	0.25	16, 90 °C	0.03 ± 0.01	0.4
2 h	0.50	16, 90 °C	0.06 ± 0.04	0.8
2 h	1.00	16, 90 °C	0.18 ± 0.04	2.3
2 h	2.00	16, 90 °C	0.32 ± 0.01	4.1
2 h	4.00	16, 90 °C	0.37 ± 0.02	4.6
2 h	0.25	0.5[a]	0.05 ± 0.03	0.6
2 h	0.50	0.5[a]	0.07 ± 0.05	0.8
2 h	1.00	0.5[a]	0.36 ± 0.05	4.6
2 h	2.00	0.5[a]	0.56 ± 0.06	7.0
2 h	4.00	0.5[a]	0.86 ± 0.06	11.0
0.5 h (9 M)	0.50	0.5[a]	0.43 ± 0.04	5.0
0.5 h (12 M)	0.50	0.5[a]	1.74 ± 0.23	22
0.5 h (18 M)	0.50	0.5[a]	5.20 ± 0.31	65

[a] Autoclave hydrolysis at 121 °C, 104 kPa for 0.5 h.

more efficient to employ. Thus, further studies focused on use of the autoclave digestion step (Table 2). Cheshire and Mundie (1966) reported that under the conditions outlined by their method, release of nonglucose saccharides was complete in 8 h at 100 °C, but recovery of glucose continued to increase with increase in extraction time up to 40 h suggesting that the method was slowly releasing glucose, possibly from the less soluble cellulose fraction.

Table 3 shows the efficiency of 18 M H_2SO_4 for recovery of cellulose–glucose as affected by solublization and hydrolysis times and hydrolysis acid concentration. A solublization time of 30 min and a hydrolysis time of 30 min in 1.0–1.5 M H_2SO_4 was the most effective method tested and resulted in greater than 95% recovery of the cellulose-C as glucose-C. Timing of the solublization was very important since the glucose equivalents not recovered was due to poor solublization (5 min treatment) or contact with the acid for longer than 30 min, which resulted in a brownish solution, suggesting charring had started. Also the substrate must be as dry as possible as the heat generated by the reaction of H_2SO_4 with moisture will increase the rate of charring.

To determine if the optimized method will increase

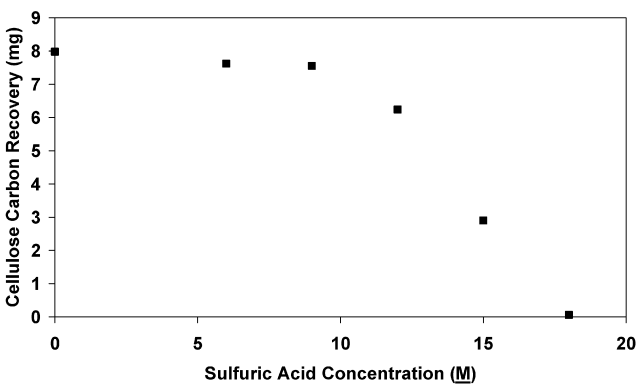

Fig. 1. Recovery of cellulose-C (mg) following solublization with different concentrations of H_2SO_4 and digestion with 0.5 M H_2SO_4.

recovery of carbohydrate-C from plant biomass, a range of samples were digested first for release of hemicellulose sugars and second for quantification of the insoluble cellulose fraction. The use of the autoclave hydrolysis with a reduced sample size (20 mg compared to 100 mg) substantially increased the recovery of the hemicellulose fraction from 9.7 ± 4.5% (average of seven residues) reported by Martens (2000a) to 30.7 ± 9.0% for the same seven residues (Table 4). Additional tests showed that the sample size digested with the autoclave method is important as a 100 mg plant sample used in previous work (Martens, 2000a) with 300 μl acid was less effective for monosaccharides recovery than the 20 mg sample. Addition of the cellulose digestion increased release of plant carbohydrate-C (Table 4, Fig. 2) an additional 29 mg C (canola) to 101 mg C (corn), nearly doubling the C accounted for with the hemicellulose analysis. Plant pectins composed of uronic acids (mainly galacturonic and glucuronic acids), can account for a large portion of plant C in certain plant species and with the agronomic plants tested here, up to an additional 7.5 mg C (canola) was recovered. With the exception of the clover, soybean leaves and the alfalfa biomass, the recovery of total plant carbohydrate-C content was close to the 59% total carbohydrate content reported for an *Arabidopsis* sp. (Sommerville et al., 2000). The residues with the low carbohydrate recovery had the lowest C/N ratio (highest N content) suggesting that more plant C is present as N-containing compounds such as amino acids.

Recovery of monosaccharides by the extraction and hydrolysis method of Cheshire and Mundie (1966) from microbial extracellular polymers purified from pure culture studies was nearly quantitative as reported by Martens and Frankenberger (1991), but purified microbial carbohydrate polymers from pure culture growth may not be as structurally complex as cellulose–carbohydrate polymers found in plant tissue or microbial products in soil. Quantification of carbohydrate-C from soils with the method outlined by Cheshire and Mundie (1966) rarely

Table 3
Recovery of glucose from purified cellulose (20 mg; 7.98 mg C) as solubilized with 18 M H_2SO_4 and hydrolyzed with various H_2SO_4 concentrations (121 °C, 104 kPa)

Solublization time (min)	Hydrolysis concentration (M)	Heating time (min)	Cellulose-C	
			Recovered (mg)	Recovery (%)
5.0	0.5	30	3.91 ± 0.13	49
5.0	1.0	30	6.86 ± 0.28	86
5.0	1.5	30	6.86 ± 0.25	86
5.0	2.0	30	4.23 ± 0.30	53
15	0.5	30	4.07 ± 0.27	51
15	1.0	30	6.54 ± 0.32	82
15	1.5	30	7.36 ± 0.33	92
15	2.0	30	4.15 ± 0.23	52
30	0.5	30	5.52 ± 0.23	69
30	1.0	30	7.74 ± 0.32	97
30	1.5	30	7.74 ± 0.32	97
30	2.0	30	3.19 ± 0.32	40
30	0.5	60	6.30 ± 0.23	79
30	1.0	60	6.30 ± 0.21	79
30	1.5	60	5.52 ± 0.23	69
30	2.0	60	3.59 ± 0.23	45

extracted more than 5% of the total C content as carbohydrate (Martens and Frankenberger, 1992; Martens, 2000a). Since the new methodology resulted in a substantial increase in recovery of plant biomass carbohydrates, the new method was applied to soil samples with contrasting management. Tables 5 and 6 show that the recovery of the soil carbohydrates from an agricultural managed soil or a native prairie was increased 3.5 or 2.5 times, respectively, by application of the autoclave digestion step to release hemicellulose sugars (Method 3, step 1) and low levels of glucose were recovered with a second solublization with 18 M H_2SO_4 and autoclave digestion step (Method 3, step

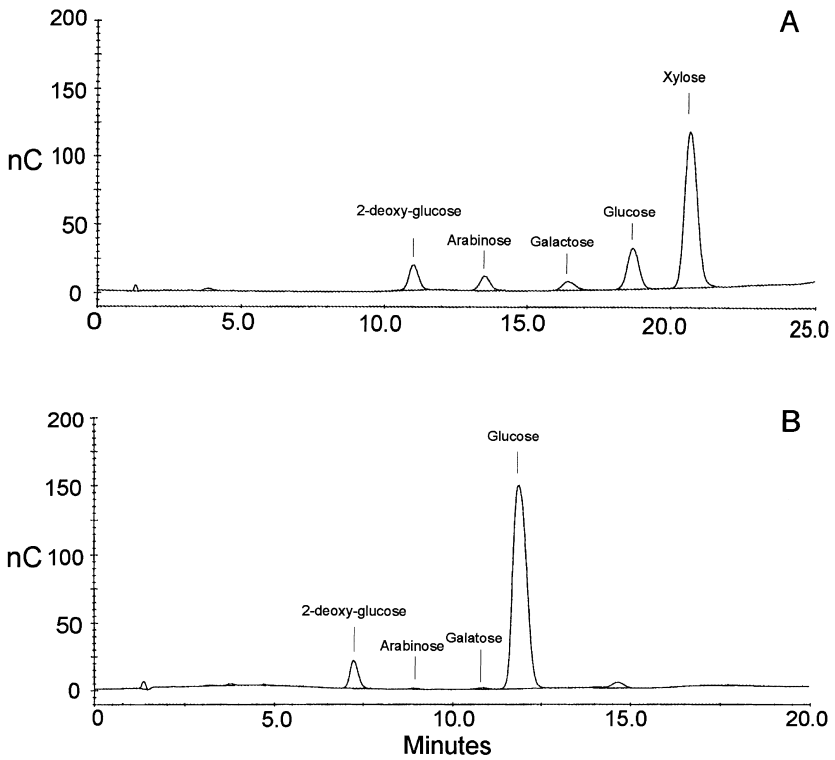

Fig. 2. Chromatograms of the corn residue (A) hemicellulose carbohydrate fraction extracted with 1.5 M H_2SO_4 digestion (autoclave 0.5 h) and (B) cellulose fraction extracted with 18 M H_2SO_4 pretreatment and 1.5 M H_2SO_4 digestion (autoclave 0.5 h) following the hemicellulose digestion 2-deoxy-glucose was added as an internal standard. The shifts in retention time from A to B are due to a stronger elution gradient used for the cellulose analysis. Response was measured as nanocoulomes (nC). 2-deoxy-glucose was added as an internal standard.

Table 4
Recovery of hemicellulose, cellulose and uronic acids by the methods described and percentage of total plant C recovered as carbohydrate-C from different plant biomass tested

Vegetation	Hemicellulose (mg g^{-1})	Cellulose (mg g^{-1})	Uronic acids (mg g^{-1})	Total C[a] (mg g^{-1})	Recovery (%)
Clover	171.5	75.5	17.0	105.1 ± 8.7	22.6
Prairie[b]	394.7	221.7	5.3	248.6 ± 10.2	54.9
Corn	339.8	253.7	0.9	238.3 ± 8.9	53.5
Soybean pods[c]	332.1	168.5	16.9	206.6 ± 7.6	47.8
Soybean stalks[c]	295.2	253.7	10.9	223.6 ± 10.5	52.7
Soybean leaves[c]	337.1	90.1	12.5	175.5 ± 12.5	41.5
Oat	357.8	186.8	4.3	219.4 ± 7.8	50.6
Canola	449.2	72.6	20.3	216.3 ± 9.6	52.8
Soybean[d]	327.7	165.7	6.1	199.7 ± 12.0	50.2
Soybean[e]	342.3	227.9	14.2	246.5 ± 6.5	59.0
Alfalfa	217.5	174.9	8.0	157.0 ± 8.2	44.8

[a] Calculated for hemicellulose and cellulose by multiplying the mg sugar by 39.99% and uronic acid by multiplying the mg uronic acid by 37.12% for a by C basis. Recovery is calculated by total carbohydrate C/total plant C.
[b] Unidentified native grass species.
[c] Soybean variety, mature Great Lakes XP7225.
[d] Unidentified soybean variety collected after over wintering in the field.
[e] Soybean variety, mature Pioneer 92B71.

Table 5
Carbohydrate content of the Webster soybean soil extracted by different acid hydrolysis concentrations and autoclave vs. reflux methods

Monosaccharide	Method 1[a] (μg g^{-1} soil)	Method 2[b] (μg g^{-1} soil)	Method 3[c] (μg g^{-1} soil)	
			Step 1	Step 2
Fucose	136.8 ± 25.3	109.9 ± 4.7	189.9 ± 0.3	0.0
Arabinose	864.3 ± 17.0	1652 ± 162	2169 ± 46	0.0
Rhamnose	423.9 ± 85.0	151.4 ± 104	356.2 ± 5.8	0.0
Galactose	796.7 ± 27.3	2036 ± 83	2983 ± 41.3	0.0
Glucose	276.9 ± 32.3	1781 ± 34	3165 ± 743	542.4 ± 10
Xylose	903.3 ± 53.6	313.5 ± 6.5	658.0 ± 171	0.0
Mannose	349.3 ± 47.8	1322 ± 71	1782 ± 87	0.0
Furan	8250 ± 358	5326 ± 48	1583 ± 80	0.0
Total[d]	3751 (12,001.2)	7365 (12,691.08)	11,845 (13,428.7)	542.4

[b] 6 M H_2SO_4 for 2 h with a 1 M reflux for 16 h.
[c] 18 M H_2SO_4 for 0.5 h, autoclave with 4 M for 0.5 h.
[a] Step 1, 12 M H_2SO_4 for 0.5 h, autoclave with 1 M for 0.5 h; Step 2, 18 M H_2SO_4 for 0.5 h, autoclave with 1 M for 0.5 h.
[d] Value in parentheses is the total value of extracted carbohydrates plus furan.

Table 6
Carbohydrate content of the Webster prairie soil extracted by different acid hydrolysis concentrations and autoclave vs. reflux methods

Monosaccharide	Method 1[a] (μg g^{-1} soil)	Method 2[b] (μg g^{-1} soil)	Method 3[c] (μg g^{-1} soil)	
			Step 1	Step 2
Fucose	432.4 ± 23.6	367.2 ± 8.9	493.2 ± 8.9	0.0
Arabinose	1869 ± 98.6	3520 ± 76	3220 ± 126	0.0
Rhamnose	982.9 ± 29.4	1042 ± 157	1342 ± 157	0.0
Galactose	2134 ± 55.2	2036 ± 83	3741 ± 49	0.0
Glucose	1183 ± 110	1781 ± 34	3849 ± 205	773.4 ± 156
Xylose	2043 ± 245	1183.5 ± 6.5	1709 ± 67	0.0
Mannose	1301 ± 58.9	1322 ± 71	2653 ± 187	0.0
Furan	11,645 ± 231	12,169 ± 141	4257 ± 93	0.0
Total[d]	9945 (21,590)	11,251 (23,420)	17,007 (22,037)	773.4

[a] 6 M H_2SO_4 for 2 h with a 1 M reflux for 16 h.
[b] 18 M H_2SO_4 for 0.5 h, autoclave with 4 M for 0.5 h.
[c] Step 1, 12 M H_2SO_4 for 0.5 h, autoclave with 1 M for 0.5 h; Step 2, 18 M H_2SO_4 for 0.5 h, autoclave with 1 M for 0.5 h.
[d] Value in parentheses is the total value of extracted carbohydrates plus furan.

2). The traditional reflux approach (Method 1) that had a longer digestion time (16 h), with a less concentrated digestion acid resulted in a lower total carbohydrate release, but the longer contact time favored formation of furan and trace levels of furfuryl alcohol as compared to the 30 min autoclave digestion (Tables 5 and 6). Increased formation of decomposition products was also noted when the soil samples were autoclave digested in 4 M H_2SO_4 (Method 2). The formation of furan and furfuryl alcohol was not noted with 18 M digestions of purified cellulose or the tested plant residues and formation of furan appears to be only a phenomenon during the digestion of soil samples with H_2SO_4 especially with longer digestion-hydrolysis times (>30 min). If an 18 M H_2SO_4 solublization followed by a 1 M-autoclave digestion step (Method 2) was used to extract hemicellulose and cellulose from the soil samples in one step, an increased furan formation was noted in the chromatograms. Furan formation was limited with the soil hemicellulose extraction procedure outlined (Method 3, step 1). Thus, for a total carbohydrate extraction, soils need to be extracted first to account for the hemicellulose sugars and second with 18 M acid to release the possible cellulose fraction (Method 3, steps 1 and 2, sequentially).

Use of the modified solublization and autoclave digestion with ion chromatography for carbohydrate analysis resulted in quantitative recovery of glucose-equivalents present in purified cellulose and substantially increased the recovery of plant and soil carbohydrates. Ion chromatography can account for the monosaccharides present in the acidic extracts and also detects the possible furan decomposition products. Quantitative accounting of plant and soil carbohydrate C must be included for accurate accounting of C balances during C cycling in soil.

Acknowledgments

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