Communications in Soil Science and Plant Analysis
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lcss20

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Available online: 05 Feb 2007

To cite this article: Kasia Debosz, Lene Vognsen & Rodrigo Labouriau (2002): Carbohydrates in hot water extracts of soil aggregates as influenced by long-term management, Communications in Soil Science and Plant Analysis, 33:3-4, 623-634

To link to this article: http://dx.doi.org/10.1081/CSS-120002768

COMMUN. SOIL SCI. PLANT ANAL., 33(3&4), 623–634 (2002)

CARBOHYDRATES IN HOT WATER EXTRACTS OF SOIL AGGREGATES AS INFLUENCED BY LONG-TERM MANAGEMENT

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ABSTRACT

Microbial carbohydrates are immediate by-products of microbial metabolism and play an important role in the formation and stabilization of soil structure. The effect of long-term management on soil carbohydrate content and monosaccharide composition was investigated in five Danish sandy loams under organic and conventional management with animal manure and mineral fertilizers. Hot-water (80°C) extraction was used to measure the distribution and composition of carbohydrates in aggregate size. Carbohydrates released to hot water were determined after hydrolysis as reducing sugars equivalent to glucose. The monosaccharide composition in hot-water extracts was analyzed as the corresponding alditol acetates. Sites with a history of

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long-term continuos management practices were used. Three treatments from the >100 year Askov long-term field experiment were used to show results of contrasting fertilization on soil carbohydrate content. These were all grown to a four-course crop rotation. Total carbohydrate content was significantly influenced by long-term management practices, with a significantly higher carbohydrate content in soils fertilized with either mineral fertilizers or animal manure (1200 to 800 mg C kg^{-1} DM aggregate) than in an unfertilized soil (600 to 500 mg C kg^{-1} DM aggregate). These results were as true for micro-aggregates (<0.25 mm) as for the 0.5–1.0 mm and 4.0–8.0 mm fractions. The organically managed soil (>40 years) was sited at a commercial farm with forage crop rotations, organic manure and no use of crop protection chemicals. These results showed significantly higher levels of carbohydrate both in micro-aggregate and macro-aggregates (1200 to 900 mg C kg^{-1} DM aggregate) than an adjacent conventionally managed soil with annual cash crop, mineral fertilizers and use of crop protection chemicals (960 to 760 mg C kg^{-1} DM aggregate). Carbohydrate C content generally increased as aggregate size decreased in both soils. Monosaccharide distribution was generally similar among three aggregate size classes studied. In all soils the content of monosaccharide was highest in microaggregates and lowest in macro-aggregates. Mannose and galactose were normally the most common monosaccharides in the hot-water extracts of aggregate fractions, indicating a predominantly microbial origin.

INTRODUCTION

Carbohydrates, which may be of both plant and microbial origin, constitute an important pool of labile carbon (C). Carbohydrates are important as bonding agents for soil aggregates and as energy source for soil microorganisms (1). Various authors have reported that noticeable changes in soil physical properties, such as aggregate distribution and aggregate stability, occur in response to changed land management (2,3). It is likely therefore that such changes in aggregation are related to labile organic bonding compounds. These bonding compounds contain a combination of transient and temporary organic binding agent (4). Carbohydrates are thought to play a major role in this respect and many attempts have been made to characterize the carbohydrates involved (e.g., (1,5)).

There are no techniques for directly quantifying the amount of labile organic bonding compounds in soil. Non-specific extraction methods, such as strong acids or bases do not differentiate between total carbohydrate content and

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the more specific carbohydrate sub-pool that is involved in aggregate stability (1). Haynes and Swift (6) suggested that hot-water extractable carbohydrates might represent such a pool. Hot water is considered not to solubilize or hydrolyze plant structural carbohydrates (1,7) and should, therefore, be relatively enriched in non-structural plant carbohydrates and extracellular microbial carbohydrate (8). Some workers have observed that the hot-water-extractable carbohydrate fraction is more closely correlated with aggregation and aggregate stability than total carbohydrate or organic C content in soil (6,8,9–12). In a review on the subject, Degens (5) found that carbohydrates extractable by hot water could be correlated with aggregation across a range of soil types.

The objective of this study was to determine if different management systems relate to changes in the distribution and composition of soil carbohydrates. This was achieved by characterizing the carbohydrate fraction of various aggregate size fractions in hot-water extracts. To accomplish this goal, we studied soils from a long-term fertilizer experiment and soils under organic and conventional management with animal manure and mineral fertilizers.

MATERIALS AND METHODS

Soils and Sites

Five arable Danish soils were collected from sites with a history of continuous management practices. All soil types were sandy loams developed on glacial deposits; they were tentatively classified as Hapludalfs. Table 1gives selected information about soils, crops and fertilizers. Soils from the Askov long-term field experiment, which was started in 1893, and is located in the south of Jutland, were used. These included an unfertilized treatment and treatments receiving mineral fertilizers (NPK) or animal manure and use of crop protection chemicals. The main treatments were different levels of nitrogen, phosphorus and potassium (13). For the present study, the fertilized plot received 1.5-fold the amount of NPK normally applied, the manured plot received 1.5-fold the amount of cattle slurry applied and one plot was unfertilized. The experimental site is grown to a four-course crop rotation of winter cereals, root crops, spring cereals and a legume/grass mixture.

Two adjacent sites from Sjaellands Odde, located at a commercial farm in the northwestern part on the island of Zealand were chosen to represent organic and conventional management. On the conventional farm, mineral fertilizers and crop protection chemicals were used, whereas these were excluded on the organic farm. The conventional site had been managed for at least twenty years with no animal manure application and grown with annual crops, generally with removal

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Table 1. Selected Properties of Five Danish Sandy Loams at the Askov Long-Term Experiment (Started 1893–94) and Sjaellands Odde Site (Mineral Fertilizer Site with No Organic Manure Since 1977; Organic Farming Started 1958)

Location	Amendment	pH ($CaCl_2$)	Organic Matter (%)	Clay (%)	Silt (%)
Askov	Unfertilized	5.8	1.7	13	11
	Mineral fertilizer	5.5	2.1	14	11
	Animal manure	5.7	2.5	14	11
Sjællands Odde	Mineral fertilizer	6.1	2.4	19	14
	Organic manure	6.2	3.5	17	17

The three soils at Askov site were on a four-course rotation of spring-grown cereals, legume–grass mixture, autumn-sown cereals, and root crops. The organically farmed soil at the Sjaellands Odde site was on a four-course rotation of spring-grown cereals, barley/peas, legume–grass mixture, and autumn-sown cereals, and the conventionally farmed soil was two-course rotation of annual small grains and rape.

of all aboveground plant residues. The organic site was managed organically since 1958 and had a forage crop rotation based on grass/clover leys and cereals. Nutrients were applied to organic soil as composted farmyard manure and slurry. The site locations and a detailed description of soils, crops, and soil management practices have been reported by Schjønning (14).

Soil Sampling and Sieving

Bulk soil samples were taken in early spring following moldboard plowing in the preceding autumn. In all fields the crop at the time of sampling was winter wheat. None of the soils had received organic inputs (manure or straw) for a period of at least 10 months prior to sampling. Soil samples were collected from the upper 6 to 13 cm of soil using a trowel. In each fertilizer treatment at the Askov long-term field experiment three samples were collected in each of three plots in a randomized design. Soils from organic and conventional sites at Sjaellands Odde were collected at nine sampling points located within each field. Sub-samples of soil were air-dried at 30°C in a drying cabinet with air circulation for 48 h. Dry soil was sieved into three aggregate size fractions, 0.06–0.25, 0.5–1.0, and 4–8, mm, by passing through a nest of sieves. The mass of primary particles (greater than original sieve size) and sand (>0.063 mm) were determined for each aggregate size fraction. The weight of 'true' aggregates was

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calculated after subtracting the weight of particles >0.063 mm in each aggregate size fraction.

The soil material associated with each aggregate size fraction was ground to a fine powder using mortar and pestle and stored in plastic vials until analysis.

Extraction Procedure

An easily extractable carbohydrate fraction was determined in air-dry aggregates. Carbohydrates were extracted according to the methods described by Ball et al. (11), with minor modifications. Soil was shaken with hot-water (80°C) using a 1:6 soil: extractant ratio (wt/vol). The hot-water extract was centrifuged (5800 × g, 10 min) and filtered through a 0.45-μm membrane filter. Before extraction, highly soluble substances and floating debris were removed by shaking the soil with cold water (1:5 soil: extractant ratio, wt:v) for 1 h.

Carbohydrate Determination

The carbohydrates extracted by hot water were hydrolyzed with 12 M H_2SO_4 and 1% thymol (3-hydroxy-4-isopropyl toluene) dissolved in ethanol. The mixture was shaken well, covered with aluminum foil, and placed in a boiling water bath for 35 minutes. During that time samples were shaken two or three times. After cooling and transfer to colorimeter tubes, the absorbance was read at 490 nm. As equal amounts of sample and glucose standard were hydrolyzed and diluted in the same manner, the amount of carbohydrate present in the sample could be calculated by directly comparing the absorbance of the unknown samples with the absorbance of the glucose standards using the relationship:

sample glucose wt. equivalent =

(sample absorbance)(wt glucose standard)/(glucose absorbance)

The calculation of total carbohydrate carbon was based on the assumption that carbohydrates contain 40% (wt/wt) carbon. Coefficient of variation for carbohydrate content of repeated soil samples (n = 10) ranged from 1.4–3.4%.

Monosaccharide Determination

The content of arabinose, xylose, mannose and galactose in hot-water extract was analyzed as the corresponding alditol acetates (15). For

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monosaccharide determination, the repetitions of hot-water extract for each soil were pooled for one sample. The solution was divided into two sub-samples and freeze-dried under vacuum at 23°C. The freeze-dried samples and a standard solution containing arabinose, xylose, mannose and galactose were treated with 2 M H_2SO_4 and heated (100°C) for 1 h to hydrolyze the carbohydrates. Monosaccharides were analyzed as their alditol acetates using Hewlett–Packard 6890 GC with a 30-m DB 225 capillary column and FID detector. Helium was the carrier gas. Monosaccharides were quantified with a standard solution of the four sugars and corrected for loss of a water molecule when monosaccharides are joined to polysaccharides.

The validation of monosaccharide determinations included recovery and repeatability tests. Recovery experiments of the monosaccharide determination were conducted as follows. The amount of arabinose, xylose, mannose, and galactose in soil extracts was determined. Adequate amounts of the four sugars were added to the soil extract and concentrations in the spiked samples determined. The difference between the first and the second determination was divided by the added amount to give the recovery rate. The recovery of individual monosaccharides was between 94 and 100%. Coefficient of variation for monosaccharide content of repeated soil samples (n = 10) ranged from 1.6–9%.

All glassware used in both methods was acid-washed (2 *N* HCl), rinsed with distilled water, covered with aluminum foil, and combusted at 550°C for 6 h.

The total carbohydrate and monosaccharide contents were expressed in milligrams of sugar-C per kg weight of oven-dry aggregates. These values were calculated after subtracting the weight of primary and sand particles >0.063 mm contained in each aggregate size fraction.

Statistical Analysis

A generalized linear mixed model defined with a gamma distribution error and normal random components was used to analyze the results. The random components of this model were specifically designed to take into account the extra variability and correlation generated by the way samples were taken. A detailed study of the residual for each analysis performed (not presented here) showed that the used models were adequate. The statistical inference used is based on hierarchical likelihood technique (16), implemented in SAS macro GLIMIX.

RESULTS AND DISCUSSION

The content of carbohydrate C was significantly higher in the mineral fertilizer and animal manure long-term treatments than in the unfertilized soil at

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the Askov long-term field experiment, while differences between the mineral fertilizer and animal manure treatments were not significant (Fig. 1a). These results were as true for micro-aggregates (<0.25 mm) as for the 0.5–1.0 mm and 4.0–8.0 mm fractions. Lower carbohydrate C content was observed in the 4–8 mm aggregate fractions than in 0.5–1.0-mm and 0.063–0.25-mm fractions. These differences may be because of differences in soil OM (Table 1). The soil OM content decreased in the order animal manure > mineral fertilizer > unfertilized. As reported by Christensen et al. (17) the higher soil OM level in fertilized than in unfertilized plots was ascribed to a greater crop productivity and hence a greater return of crop residues (root deposits and stubble) following fertilization. Crop yields have been generally two to four times higher on mineral fertilizer and animal manure treatments than on unfertilized ones. Cheshire (1) reported that management practices that increase soil OM in the long term also increase soil carbohydrate concentrations.

The organically managed soil at Sjaellands Odde showed significantly higher levels of carbohydrate C in micro-aggregate (<0.25 mm) and 4.0–8.0 mm fractions than the adjacent conventionally managed soil with mineral fertilizers (Figure 1b). However, no significant differences were found for the 0.5–1.0 mm

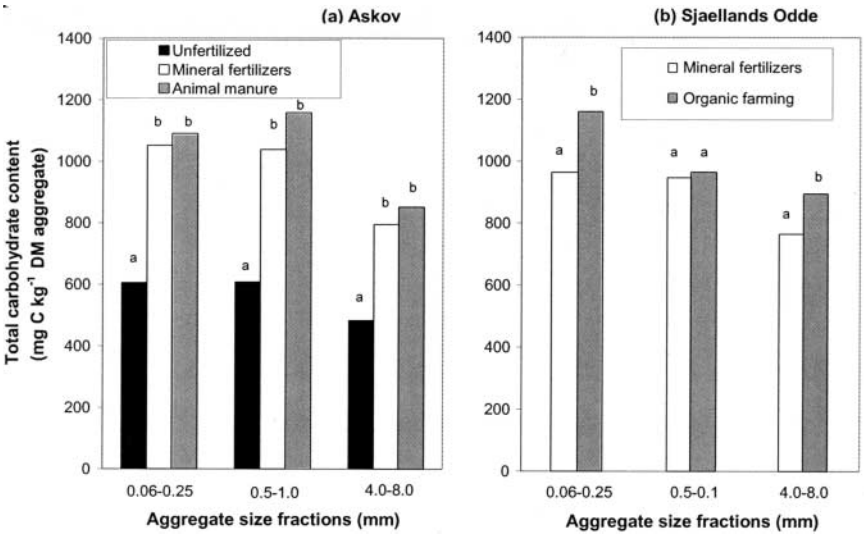

Figure 1. Total carbohydrate C content in three aggregate size fraction a) at Askov long-term field experiment in the unfertilized treatment and treatments receiving either mineral fertilizer or animal manure and b) at Sjaellands Odde in organically cultivated soil and an adjacent conventionally cultivated soil. Values within each location and aggregate size fraction followed by different letters are significantly different at 0.05 level.

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fraction. This difference correlates well with the management history outlined in Table 1. Organically managed soil had a long history of clover and grass in the crop rotation and a nutrient input in the form of composted farmyard manure and slurry, and the organic C content in the soil was 2.06%. Conventionally managed soil had not received animal manure for a minimum of twenty years and had been grown continuously with small grain cereals and rape, generally without mulching of plant, and the soil organic C content was only 1.41%. In both soils the carbohydrate C content was lowest in the 4.0–8.0 mm fraction. Several long-term studies have shown that rotation with legumes can increase the amount of carbohydrates in soil (6,10,18). Soil carbohydrates were strongly influenced by soil management systems, including amendments with bark compost or leaf litter (19). The increase in the carbohydrate C content with decreasing aggregate size shown in Fig. 1 is in marked contrast to data presented by Dormaar (20) and Puget et al. (21). Both authors observed a reduction in carbohydrate content with decreasing aggregate size when carbohydrate content was expressed per unit weight of the total soil material (i.e., aggregates plus gravel and sand grains). However in the study by Baldock et al. (3), an increase in the carbohydrate content similar to the present study was observed as aggregate size decreased. Both in the present study and in the study by Baldock et al. (3), the weight of the carbohydrate fraction was adjusted for primary particles content in contrast to the previously mentioned studies. Results from the present study (data not shown) indicate that the proportion of the primary particles ($>$0.063 mm) contained in each size fraction varied from 27 to 51%.

Monosaccharides distribution was generally similar among three aggregate size classes studied and increased in the order arabinose $<$ xylose $<$ mannose $<$ galactose (Table 2). The management system influenced the amounts of monosaccharides in aggregate size fractions. Monosaccharides were more abundant in mineral and animal manure fertilized soils than in the unfertilized soil, and more abundant in organic soil than in conventionally managed soil at Sjaellands Odde (Table 2). In all soils the content of monosaccharide was highest in microaggregates and lowest in macro-aggregates. However, the sum of the four monosaccharides studied always represented a similar proportion ($\approx$11.5%) of the total hot-water soluble carbohydrate content, regardless of the aggregate size. The hierarchical model of soil aggregation, presented by Hadas (22) and Dexter (23), assumes that a range of different mechanisms will combine primary particles and organic matter into flocculated micro-aggregates (0.063–0.250 mm) and gradually larger macro-aggregates $>$0.250 mm. According to theories on soil aggregation mechanism (4), the carbohydrates are transient binding agents, which glue together primary soil particles (clay, silt, and sand) into micro-aggregates. They consist primarily of extracellular carbohydrates and are produced by bacteria, fungi, and plants. Many studies showed that bonding of carbohydrates were the main aggregating agent (10,24).

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Table 2. Monosaccharide-C Content and Their Molar Ratios at Three Aggregate Size Fractions (A, B, C) in Unfertilized, Mineral Fertilized, and Animal Manure Fertilized Soils at Askov Long-Term Experiment and Mineral Fertilized and Organic Manure Fertilized Soils at Sjaellands Odde (mg C kg^{-1} DM Aggregate)

Location	Treatment	Arabinose	Xylose	Mannose	Galactose	Ratio M + G/A + X
A. 0.06–0.25 mm aggregate size fraction						
Askov	Unfertilized	14.4a[a]	14.4a	21.8a	22.4a	1.5
	Mineral fertilizer	23.6b	22.7b	34.9b	36.4b	1.5
	Animal manure	24.9b	28.2b	35.7b	39.6b	1.4
Sjaellands Odde	Mineral fertilizer	20.5a	25.3a	36.2a	35.2a	1.6
	Organic manure	24.1b	30.7b	43.3b	43.9b	1.6
B. 0.5–1.0 mm aggregate size fraction						
Askov	Unfertilized	14.7a	15.3a	20.4a	20.4a	1.4
	Mineral fertilizer	25.4b	26.3b	36.3b	37.8b	1.4
	Animal manure	27.6c	31.3c	38.9c	44.4c	1.4
Sjaellands Odde	Mineral fertilizer	18.6a	22.8a	33.0a	32.1a	1.6
	Organic manure	19.5b	24.6b	35.3b	35.7b	1.6
C. 4.0–8.0 mm aggregate size fraction						
Askov	Unfertilized	13.1 a	11.6 a	16.5 a	15.4 a	1.3
	Mineral fertilizer	17.9 b	18.4 b	26.2 b	25.9 b	1.4
	Animal manure	18.9 b	16.6 b	25.2 b	28.1 b	1.5
Sjaellands Odde	Mineral fertilizer	14.9 a	20.2 a	27.9 a	26.4 a	1.6
	Organic manure	17.4 b	23.3 b	34.7 b	34.9 b	1.7

[a] Within each location and aggregate size fraction, column values followed by different letters are significantly different at 0.05 level.

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The hot-water extractable pool is probably dominated by mucigel of microbial origin (6,25). The monosaccharide composition of microbial carbohydrates clearly differs from that of plant material; microorganisms produce predominantly galactose (G) and mannose (M), while plant tissue and root mucigels are the major sources of arabinose (A) and xylose (X) (26,27). Based on the observation that hot-water extracts showed sugar ratios $(G + M)/(A + X) > 2$ (8), assume that these were mostly composed of extracellular microbial carbohydrates, since this ratio is low (<0.5) for plant carbohydrates (27). In the present study the ratio $(G + M)/(A + X)$ in aggregate size fractions was in the order 1.7–1.3, indicating a contribution from plant residues and root mucigels (Table 2). Root mucigels are soluble in hot water and may contribute to this fraction. Ball et al. (11) and Puget et al. (21) also found ratios within this range which they attributed to the presence of both plant and microbial components.

CONCLUSIONS

Results suggest that the carbohydrate C content be significantly influenced by different management practices. Long-term fertilization (>100 years) with mineral fertilizers and animal manure increased the content of carbohydrate C in aggregates when compared with unfertilized soil. However no significant differences between the carbohydrate C contents of soils subject to mineral and animal manure fertilization were detected. This seems to be a result of similar crop yield and hence a similar return of crop residues in both fertilized treatments. Organic cultivation with a long history (>40 years) of clover and grass in the crop rotation, animal manure input, and no use of crop protection chemicals had higher carbohydrate C content than conventionally cultivated soil with an annual cereals, no input of animal manure, and use of crop protection chemicals. Monosaccharide analysis indicated that a significant proportion of carbohydrate in the soil and aggregate size fraction was of microbial origin [$(G + M)/(A + X)$ ratios > 1.3]. The sum of monosaccharides always represented the same proportion ($=11.5\%$) of the total hot-water extractable carbohydrates, regardless of the aggregate size.

ACKNOWLEDGMENTS

The technical assistance of Anette Clausen, Palle Jørgensen, and Lis Sidelmann is gratefully acknowledged. Per Schjønning, Danish Institute of Agricultural Sciences, is kindly acknowledged for valuable discussions on the manuscript. This work was partly financed by the Danish Environmental Research Program (Research Centre for Organic Farming).

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