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Monosaccharide distribution in particle-size fractions from two ferrallitic soils as determined by capillary GC

Sucres simples des fractions granulométriques de deux sols ferrallitiques. Détermination par CPG

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In order to determine their sugar composition, two ferrallitic soils and their particle-size fractions were subjected to a series of acidic hydrolyses prior to analysis of the extracted monomer sugars by capillary gas chromatography. Amongst the sugars that we were able to identify in the whole soils (12 according to the method of derivatization applied), glucose was the most abundant, followed by mannose. The same observation holds for the particle-size fractions of these soils with the exception of the coarse sand-sized fraction (> 200 µm), where xylose - sugar essentially of plant origin - was the next most abundant sugar after glucose ; this was in accordance with the particulate plant character of this fraction. The ratio galactose+mannose/arabinose+xylose, indicator of the decomposition of plant residues and the accumulation of microbial metabolites, increased 4 to 7-fold from coarse to finer fractions. The value of this ratio and its variations observed in the whole soils or their particle-size fractions were close to those reported in the literature for temperate soils and other tropical soils.

Keywords : organic matter, soil carbohydrates, particle-size fractions, tropical soils, gas chromatography
Mots-clés : matière organique, polysaccharides, fractions granulométriques, sols tropicaux, chromatographie en phase gazeuse.

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INTRODUCTION

Carbohydrate materials in soils, a complex mixture of mono- and polysaccharides, represent 5 to 25 % of the organic matter (Stevenson, 1982). They have been extensively studied for the past 30 years because of their suggested role in soil fertility in three areas : biological nutrition, soil structure, and soil-water relationships. Their determination nowadays, is rapid and sensitive thanks to recent advances in high performance liquid chromatography (HPLC ; Angers *et al.*, 1988) and ion-exchange chromatography (HPAEC-PAD ; Jørgensen and Jensen, 1994). Yet, gas chromatography (GC) remains a widely used technique for detection of saccharides in soils ; it can be even improved by means of an alternative method of derivatization (silylation) which proved to be simpler and more rapid than the classical one (Larré-Larrouy and Feller, 1997).

We tested this method of silylation prior to analysis by GC on two ferrallitic soils of contrasting vegetation and organic matter content ; these soils had been firstly subjected to a sequential acidic hydrolysis which released the monomer sugars contributing to the soil carbohydrate content. We also examined the constitutive saccharides of the soil particle-size fractions, in order to reveal whether the differences in the nature of organic matter associated with the particle-size fractions were reflected in the distribution of the sugars, including the origin of these sugars. Results were finally evaluated in comparison with data provided in the literature for temperate soils.

MATERIALS AND METHODS

Soil samples

The soil samples (0-10 cm) employed here were the two ferrallitic soils from the Congo used in the study described by Larré-Larrouy and Feller (1997) : NS, a clay loam, was obtained from a savannah site (C = 4.3 % - C/N = 17.7), and NCo, a clayey soil (C = 1.8 % - C/N = 13.8) was collected from a field under continuous agricultural

production (cassava) for at least 17 years prior to sample collection. Before fractionation into particle-size classes, the samples were air-dried and gently crushed to pass a 2-mm screen (2 mm for soil analysis, and < 0.2 mm for organic C analysis). Further information on soils, sampling procedures, has been given by Djondo (1994).

Particle-size fractionation of organic matter

20-g subsamples of NS and NCo soils were separated into particle-size fractions by sieving (200-2000 µm, 50-200 µm, 20-50 µm) and by sedimentation (2-20 µm, 0-2 µm), as outlined by Gavinelli *et al.* (1995). Fractions were dried at 60°C, weighed and finely ground. Yields and carbon contents of the various particle-size fractions are presented in Table 1. All results were expressed on oven-dry (105°C) soil weight basis.

Chemical analyses

Carbon and nitrogen determinations. Total C and N of the soils and particle-size fractions were determined using a LECO CHN-600 Elemental Analyzer.

Carbohydrate determinations. Monosaccharides were released by a two-step hydrolysis of the soils and their particle-size fractions using the procedures described by Oades *et al*. (1970) and determined by capillary gas chromatography of the trimethylsilyl derivatives (Larré-Larrouy and Feller, 1997). Saccharides released upon the first hydrolysis with 2.5 M H_2SO_4 refluxing for 20 min may be designated as hemicellulosic saccharides, and saccharides released with 0.5 M H_2SO_4 refluxing for 5 h after soaking with 12 M H_2SO_4 on the residue of the first hydrolysis may be designated as cellulosic saccharides (Cheshire, 1979).

The trimethylsilyl derivatives of the monosaccharides were identified with a Delsi Nermag DI 200 equipped with a hydrogen flame ionization detector and a 30-m silica capillary column DB-1 (0.25 mm i.d.) which had He as carrier gas at 1.5 b. Injector and detector temperatures were 265°C and 250°C respectively, and the column temperature programmed from 170°C with a 4°C min^{-1} rise to 230°C.

RESULTS AND DISCUSSION

Monosaccharide concentrations and distribution in the soils

The NS native soil, when compared to the cultivated soil NCo, showed a greater amount of non-cellulosic and total carbohydrates (cf. Larré-Larrouy and Feller, 1997) ; this can be paralleled by a similar increase in the total organic C of the soil. However, the carbohydrate-C, as a percentage of total soil C, was higher in the cultivated soil than in the native soil (10 % for NCo, and 8 % for NS), and mostly released during the first hydrolysis (52 %).

Almost 70 % of the total monosaccharides extracted fron the two soils were hexoses (67 % and 61 % for NS and NCo, respectively). Among them, glucose followed by mannose were the most abundant. The ratios, *r,* of the contents of mannose + galactose / arabinose + xylose in the soils studied, provided an indication of the origin of their polysaccharides (Turchenek and Oades, 1979) ; they were greater than 1, suggesting that NS and NCo contained a higher proportion of carbohydrate material synthesized by microorganisms than plant-derived carbohydrates.

Amounts of monosaccharides in acid hydrolysates of the particle-size fractions

The total carbohydrate content for each particle-size fraction of the NS and NCo soils is presented in Table 2.

Summation of the carbohydrates in the fraction hydrolysates did not give such a good agreement with that obtained by direct hydrolyses of the soils (0.36 % vs 0.84 %, respectively for the NS soil and 0.32 % vs 0.44 %, respectively for the NCo soil ; cf. Larré-Larrouy and Feller, 1997). There are consistent differences as if the size fractions did not have suffered much chemical disruption during sugar extraction. A second reason for the low recovery in the particle-size fractions may be the loss of sugars in water-soluble material during the fractionation process : as much as 4 % of the carbon content (Feller, 199 , unpublished results) is soluble and may represent a higher percentage loss of carbohydrate, since these substances are amongst "the most hydrophilic materials in soil organic matter" (Cheshire *et al.*, 1990). Finally, the differences may also be caused partly by experimental error associated with analyses of very low sugar contents. With the exception of the 50-200 and 200-2000 µm fractions, the majority of carbohydrates were extracted during the first hydrolysis (from 57 % to 95 %), with even higher recoveries from some fractions than from the non fractionated soils.

In the NS soil, the largest concentration (in % of fraction dry weight) of carbohydrates was found in the sand-sized fraction (i.e. the 200-2000 µm fraction - with a concentration of sugars 1.5 times greater than that in the whole soil), followed by the 2-20 and 0-2 µm fractions. The coarse-silt fraction (20-50 µm) was the most depleted. In the same way, the sand-sized separate 200-2000 µm was enriched in carbohydrate-C (6.9 % of fraction C) as compared to any other particle-size fraction. If the concentration of carbohydrates is calculated as a percent of the corresponding whole soil or of the total monosaccharide content of all fractions, the 0-2 µm fraction appeared to contain more total monosaccharides than the other fractions ; this is in accordance with the highest C content of this fraction when expressed as a percent of the soil C content (cf. Table 1). Likewise, the proportions of monosaccharides then decreasing in the order 0-2 µm > 200-2000 µm > 2-20 µm > 50-200 µm > 20-50 µm reflected the distribution of soil C present as carbohydrates in each fraction.

The enrichment of the NS sand-sized fraction (200-2000 µm) in monosaccharides is quite in opposition with the results generally presented in the literature for temperate soils : in these soils indeed, the proportion of carbon present as carbohydrate is usually lowest for the sand fraction (Murayama *et al.*, 1979 ; Cheshire *et al.*, 1990) when compared to organic matter associated with clay and silt. This discrepancy may be explained by the fact that sonication was there applied on the whole soil (0-2 mm), and not on the size-fraction < 50 µm as it was done in the present work (Gavinelli *et al.*, 1995) ; as a matter of fact, under the former conditions, up to 50 % of carbon associated with sand particles can be artificially transferred to the fine soil fractions < 20 µm (Balesdent *et al.*, 1991) and may contribute to a higher proportion of carbohydrate material.

The same observation holds for the cultivated soil, NCo ; the greatest concentrations of carbohydrates (in % of fraction dry weight) were found indeed in the 200-2000 µm fraction and in the clay fraction. As in the NS soil, the 20-50 µm fraction was the most depleted, which is in accordance with the low organic matter content of this fraction (cf. Table 1). Moreover, the sand-sized fraction 200-2000 µm and the clay-sized fraction were enriched in carbohydrate-C as compared to the other fractions (the proportion declined from 8.7 % in the 200-2000 µm fraction to 2.2 % in the 2-20 µm fraction). The proportions of whole soil C present as carbohydrates decreased in the order clay > sand > silt, reflecting the distribution of monosaccharides amongst all the

fractions with a higher proportion in the 0-2 µm fraction and in the 200-2000 µm fraction (5.1 % and 0.8 %, respectively). As for the whole soil, the total carbohydrate-C (sum of the monosaccharide carbon in all the fractions) as a percentage of total soil C, was higher in this cultivated soil (in the 0-2 µm fraction, especially) than in the native soil NS.

Monosaccharide composition of particle-size fractions of NS and NCo soils

The relative concentrations of each monomer sugar in the particle-size fractions of NS and NCo soils are shown in Table 3. Glucose was the predominant sugar in all the fractions, and represented between 35 % and 80 % of the sugars depending on the fraction. It was mainly released during the first hydrolysis, except for the sand-sized fractions (> 50 µm) of NS as well as NCo where it is more highly concentrated and probably present in plant fragments (cellulose - like form of glucose). The second most abundant sugar was mannose, except in the 200-2000 µm fraction of NS and NCo where xylose, indicator of the persistence of plant polysaccharide, was in concentration twice that of mannose ; the proportions of these two sugars varied indeed, in opposite direction and significantly, with increasing particle-size whatever the soils. With the only exception of ribose which represented 14 % of the 0-2 µm fraction monosaccharides in NCo, each of the other identified sugars represented less than 10 % of the total sugars without any sharp differences across the different fractions of the two soils. Surprisingly, ribose appeared here in concentrations consistently greater than what is usually found in the literature concerning the occurrence of this sugar in the soils and their particle-size fractions (Cheshire, 1979). More work must be undergone to confirm this ; but we could already assume that the derivation method (silylation) used in this study before analysis by GC is to be incriminated, when compared to the classical derivation in alditol acetates, by preserving specifically the structure of ribose. In both soils, galactose was above all concentrated in the clay-sized fraction. The deoxysugars, fucose and rhamnose were present in lesser amounts in fraction 50-200 µm (NS and NCo), being relatively constant in the other fractions of NS as well as NCo.

As the soils non fractionated, all the fractions were characterized by greater amounts of hexoses (synthesized with deoxysugars and minor sugars by microbial populations) than pentoses (characteristic of plant polysaccharides). However, *r* values (mannose + galactose / arabinose + xylose) were generally lowest in the 200-2000 µm fractions of NS and NCo soils (Table 3), indicating a high proportion of plant-derived sugars (Oades, 1984). In contrast, higher ratios in clay and silt-sized fractions indicated greater inputs of carbohydrates of microbial origin. Moreover, the proportions of microbial sugars were greater in the savannah soil NS, and decreased with cultivation in the NCo soil. A decrease in the *r* values (or an increase in the xylose / mannose ratio, other indicator for the decomposition of plant residues and soil organic matter, Murayama, 1984), from the finer fractions to the coarser ones is typical in temperate soils and reported as such in the literature (Murayama *et al.*, 1979 ; Turchenek and Oades, 1979 ; Cheshire and Mundie, 1981 ; Angers and Mehuys, 1990). Preliminary results for several other tropical soils (Feller, 1994) showed similar trends.

A question arises about the plant-derived sugars recovered in the sand-size fractions of the soils : do they remain in the same form as the one in the vegetation covering the soil? As a matter of fact, information was lacking in the present study about the sugar composition of the organic materials returned to the soil. The dominance of certain carbohydrates in the parent plant material could explain the persistence of specific

monomer sugars in the coarser separates of the soils according to, or despite of, the different cropping systems applied. More attention should be devoted to this in future works on carbohydrate studies in tropical soils ; especially if we keep in mind that carbohydrates could be considered as good quality indicators of the behavior of soils under cultivation.

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Keywords : organic matter, soil carbohydrates, particle-size fractions, tropical soils, gas chromatography

Mots-clés : matière organique, polysaccharides, fractions granulométriques, sols tropicaux, chromatographie en phase gazeuse.

Table 1. Yields and carbon contents of particle-size fractions* isolated from two soils of Congo

Fraction	Fraction weight	C		C/N
(µm)	*(% of soil dry weight)*	*(% of fraction)*	*(% of total C)*	
NS				
0-2	41,6	3,0	30,8	11
2-20	21,6	4,6	24,7	17
20-50	6,4	3,5	5,6	24
50-200	19,6	4,1	20,1	20
200-2000	9,3	7,2	16,5	32
Recovery**	98,5		97,7	
NCo				
0-2	61,2	1,8	59,8	11
2-20	15,9	2,1	18,1	19
20-50	6,5	0,9	3,2	18
50-200	11,4	1,6	9,9	16
200-2000	3,2	5,1	8,9	36
Recovery**	98,1		99,9	

*The particle-size fractionations were carried out in triplicate, the variations of the fraction weights did not exceed 5%.
**Sum of fractions as % of whole soil.

Table 2. Concentrations of monosaccharides* released by successive sulphuric hydrolyses from particle-size fractions of NS and NCo soils

Fraction	Monosaccharides released by first hydrolysis			Monosaccharides released by second & third hydrolyses			Monosaccharides released by all hydrolyses				
(µm)	*(percentage fraction d.w.)*	*(percentage total)*	*C (percentage fraction C)*	*(percentage fraction d.w.)*	*(percentage total)*	*C (percentage fraction C)*	*(percentage fraction d.w.)*	*(percentage soil d.w.)*	*(percentage in each fraction)*	*C (percentage fraction C)*	*C (percentage soil C)*
NS soil											
0-2	0,3	81,6	3,8	0,1	18,4	0,9	0,4	0,2	38,7	4,7	1,4
2-20	0,3	81,8	2,8	0,1	18,2	0,6	0,4	0,1	20,1	3,4	0,8
20-50	0,1	56,5	0,9	0,1	43,5	0,7	0,1	0,0	1,5	1,7	0,1
50-200	0,1	49,6	1,3	0,1	50,4	1,3	0,3	0,1	13,7	2,6	0,5
200-2000	0,3	23,7	1,6	0,9	76,3	5,2	1,2	0,1	26,0	6,9	1,1
Total								0,4			4,0
NCo soil											
0-2	0,4	93,4	8,0	0,0	6,6	0,6	0,4	0,2	75,3	8,6	5,1
2-20	0,1	87,3	1,9	0,0	12,7	0,3	0,1	0,0	4,9	2,2	0,4
20-50	0,1	83,9	3,9	0,0	16,1	0,7	0,1	0,0	2,0	4,6	0,1

50-200	0,1	34,2	2,1		0,1	65,8	4,0		0,2	0,0	7,0	6,1	0,6				
200-2000	0,5	49,0	4,2		0,6	51,0	4,4		1,1	0,0	10,8	8,7	0,8				
Total										0,3			7,0				

C (percentage soil C), soil C present as carbohydrates.

Table 3. Monosaccharide composition of hydrolysates of the particle-size fractions of NS and NCo soils

Fraction	Ara	Rha	Rib	Fuc	Xyl	Man	Gal-NH2	Gal	Glc-NH2	Glc	Gal-AU	Glc-AU	Total	Pentoses	Hexoses	Deoxyhexoses	r	Xyl / Man
(µm)	*(µg/g fraction)*												*(mg/g fraction)*					
NS soil																		
0-2																		
first hydrolysis	116	100	249	92	167	674	40	251	15	1159	n.i.	n.i.	2,9					
second & third hydrolyses	21	15	33	n.i.	9	121	n.i.	32	n.i.	412	n.i.	n.i.	0,6					
Total hydrolyses	137	115	282	92	176	795	40	283	15	1571	n.i.	n.i.	3,5	0,6	2,9	0,2	3,4	0,2
2-20																		
first hydrolysis	60	89	163	n.i.	248	694	47	227	n.i.	1679	n.i.	22	3,2					
second & third hydrolyses	n.i.	n.i.	13	n.i.	n.i.	41	n.i.	12	n.i.	654	n.i.	n.i.	0,7					
Total hydrolyses	60	89	176	n.i.	248	735	47	239	n.i.	2333	n.i.	22	3,9	0,5	3,4	0,09	3,2	0,3
20-50																		
first hydrolysis	18	12	29	n.i.	55	77	18	33	n.i.	581	n.i.	14	0,8					
second & third hydrolyses	n.i.	n.i.	10	n.i.	n.i.	23	n.i.	n.i.	n.i.	596	n.i.	n.i.	0,6					
Total hydrolyses	18	12	39	n.i.	55	100	18	33	n.i.	1 177	n.i.	14	1,5	0,1	1,3	0,01	1,8	0,6
50-200																		
first hydrolysis	31	20	77	n.i.	90	265	26	54	n.i.	779	n.i.	n.i.	1,3					
second & third hydrolyses	13	n.i.	19	n.i.	7	50	n.i.	10	n.i.	1268	n.i.	n.i.	1,4					
Total hydrolyses	44	20	96	n.i.	97	315	26	64	n.i.	2047	n.i.	n.i.	2,7	0,2	2,4	0,02	2,7	0,3
200-2000																		
first hydrolysis	78	47	168	34	990	362	n.i.	171	n.i.	1089	n.i.	n.i.	2,9					
second & third hydrolyses	59	99	26	16	303	178	141	61	17	8525	n.i.	19	9,4					
Total hydrolyses	137	146	194	50	1293	540	141	232	17	9614	n.i.	19	12,4	1,6	10,6	0,2	0,5	2,4
NCo soil																		
0-2																		
first hydrolysis	243	136	511	126	229	758	33	321	n.i.	1214	n.i.	10	3,6					
second & third hydrolyses	20	n.i.	42	n.i.	n.i.	42	n.i.	12	n.i.	137	n.i.	n.i.	0,3					
Total hydrolyses	263	136	553	126	229	800	33	333	n.i.	1351	n.i.	10	3,8	1,0	2,7	0,3	2,3	0,3
2-20																		
first hydrolysis	32	44	55	20	88	232	12	79	n.i.	436	n.i.	9	1,0					
second & third hydrolyses	n.i.	n.i.	n.i.	n.i.	n.i.	9	n.i.	n.i.	n.i.	137	n.i.	n.i.	0,1					
Total hydrolyses	32	44	55	20	88	241	12	79	n.i.	573	n.i.	9	1,1	0,2	1,0	0,1	2,7	0,4

20-50																		
first hydrolysis	46	24	79	12	75	183	18	61	n.i.	376	n.i.	n.i.	0,9					
second & third hydrolyses	n.i.	n.i.	n.i.	n.i.	n.i.	8	n.i.	n.i.	8	151	n.i.	n.i.	0,2					
Total hydrolyses	46	24	79	12	75	191	18	61	8	527	n.i.	n.i.	1,0	0,2	0,8	0,04	2,1	0,4
50-200																		
first hydrolysis	44	30	86	12	124	74	16	62	n.i.	367	n.i.	10	0,8					
second & third hydrolyses	10	9	24	n.i.	16	59	n.i.	12	n.i.	1462	n.i.	n.i.	1,6					
Total hydrolyses	54	39	110	12	140	133	16	74	n.i.	1829	n.i.	10	2,4	0,3	2,1	0,1	1,1	1,1
200-2000																		
first hydrolysis	280	177	579	73	1289	538	n.i.	295	n.i.	2157	n.i.	n.i.	5,4					
second & third hydrolyses	28	38	59	n.i.	102	187	n.i.	42	n.i.	5152	n.i.	n.i.	5,6					
Total hydrolyses	308	215	638	73	1391	725	n.i.	337	n.i.	7309	n.i.	n.i.	11,0	2,3	8,7	0,3	0,6	1,9

n.i., non- identified.

Ara, arabinose ; Rha, rhamnose ; Rib, ribose ; Fuc, fucose ; Xyl, xylose ; Man, mannose ; Gal-NH2, galactosamine ; Gal, galactose ; Glc-NH2, glucosamine ; Gal-AU, galacturonic ac. ; Glc, glucose ; Glc-AU, glucuronic ac.