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# Soluble carbohydrate and organic acid content of soils and associated microbiota from the Windmill Islands, Budd Coast, Antarctica

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**Abstract:** In the cold Antarctic environment labile organic compounds may accumulate in soil due to relatively low utilization rates by heterotrophic microorganisms. Microbial fermentation of these compounds might contribute to the development of strongly acid soils. To test this and assess concentrations, extracts of a range of soils in the Windmill Islands, Budd Coast were analysed by GLC and HPLC for the presence of low molecular weight sugars, polyols and organic acids. Concentrations of sugars and polyols up to 3300 mg $g^{-1}$ were detected in cryptogam dominated soils. Some, such as trehalose, may have principally originated in the soil microflora. Soils from occupied penguin rookeries were found to possess oxalic, acetic, propionic and succinic acids at levels up to 1000 mg $g^{-1}$ soil. Most other soils, however, lacked these acids at detectable levels (1–5 mg $g^{-1}$ soil). No correlation was established between organic acid accumulation and soil pH although those dominated by moss and lichen had been acidified significantly when compared with barren soils. Thus while substantial pools of these readily utilized carbohydrates were probably present in cryptogam dominated soils, there was little accumulation of organic acids which could account for the acidity of mineral soils typical of the Windmill Islands.

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**Key words:** soil, carbohydrates, polyols, organic acids, fermentation, Antarctica

## Introduction

Soils and similar systems, which form at low temperatures or under oxygen limited conditions, are often noted for their accumulation of large quantities of organic matter. This occurs due to an imbalance between the extent of carbon fixation and respirative decomposition, and is encouraged by waterlogging and low turnover rates. Such conditions are characteristic of many Arctic and Antarctic ecosystems (Heal *et al.* 1981). While solid plant residues are the most visible result, other reduced organic compounds may also be produced. Methane commonly accumulates in waterlogged environments (Stevenson 1986, Ch. 1) and its production in peat systems at Signy Island has been reported by Yarrington & Wynn-Williams (1985). Low molecular weight water soluble carbon compounds, which would be utilized in temperate systems in a matter of hours or days (Stevenson 1986, Ch. 1.), may persist in Antarctic terrestrial systems. Tearle (1987) demonstrated the transient accumulation of sugars and polyhydric alcohols (polyols) at levels of up to 2500 mg $g^{-1}$ of wet soil in the fellfield fines of Signy Island during the spring thaw. In the endolithic habitat of the Dry Valleys, ATP can accumulate to greater levels than are present in living microorganisms (Tuovila & La Rock 1987). Bölter (1990a) detected free amino acids (24–136 mg $g^{-1}$ soil) and dissolved 'monocarbohydrates' (12–32 mg $g^{-1}$ soil) in Windmill Islands soils.

Another class of compounds which could accumulate under Antarctic conditions are the short chain alcohols and organic acids which are formed as end-products of microbial fermentation. Under anaerobic conditions they can accumulate in the soil solution at $10^{-3}$ M (equivalent to 60 mg $ml^{-1}$ of acetic acid) and play an important role in controlling the availability of plant micronutrients (Stevenson 1986, Ch.1). To date there have been no searches conducted for organic acids in Antarctic soils and detailed quantification of sugars and polyols in soil has been limited to the studies of Hurst *et al.* (1985) and Tearle (1987). Possible production and persistence of these organic acids was pointed to by the acid pH (4–5) of soils from richly vegetated sites at Signy Island, the Windmill Islands and Cape Hallet (Baker 1970, Ugolini 1970, Christie 1987, Bölter 1990b).

This paper reports on our analyses of Windmill Islands soil from a range of sites for the presence of organic acids typical of fermentation, and the sugars and polyhydric alcohols which could be their precursors in areas where plants are abundant. Its aim was to provide initial survey data on the presence and levels of these compounds in the Windmill Islands and identify soils suitable for further examination.

## Materials and methods

### *Sample selection and treatment*

Soil samples were collected from the Windmill Islands, Wilkes Land, (66°20'S, 110°30'E) during the austral summers of 1989/90 and 1990/91 as indicated in Table I, from a variety of sites

**Table I.** Characteristics of soils analysed for organic acids, sugars and polyols.

| Sample | Soil source | pH | Loss on ignition (% w/w) | Organic carbon (% w/w) | |
|---|---|---|---|---|---|
| A1 | occupied penguin colony | 6.8 ± 0.0 | 66 ± 3 | 15 | |
| A2 | " | 7.0 ± 0.0 | 59 ± 6 | 18 | |
| A3 | " | 7.8 ± 0.1 | 40 ± 2 | 13 | |
| | mean ± 1 sd | 7.2 ± 0.5 | 55 ± 13 | 15 ± 3 | |
| X1 | extinct colony | 6.3 ± 0.1 | 17 ± 2 | 9.9 | |
| X2 | " | 5.7 ± 0.0 | 11 ± 1 | 7.6 | |
| X3 | " | 6.4 ± 0.0 | 8.3 ± 2.4 | 5.5 | |
| | mean ± 1 sd | 6.2 ± 0.4 | 12 ± 4 | 7.6 ± 2.2 | |
| P1 | algae rich soil | 3.9 ± 0.0 | 1.1 ± 0.0 | 0.4 | |
| P2[c] | " | 5.7 ± 0.1 | 7.2 ± 1.9 | 4.0 | |
| P3[c] | " | 5.3 ± 0.1 | 9.0 ± 2.9 | - | |
| P4 | " | 4.8 ± 0.0 | 0.9 ± 0.1 | 0.3 | |
| P5[c] | " | 5.1 ± 0.0 | 13 ± 3 | 1.0 | |
| | mean ± 1 sd | 4.9 ± 0.7 | 6.3 ± 5.3 | 1.4 ± 1.8 | |
| L1[a] | lichen rich site | 4.6 ± 0.0 | 1.6 ± 0.2 | 0.1 | |
| L1b[a] | " | 4.3 ± 0.0 | 3.4 ± 0.1 | - | |
| L1c[a,b] | " | 4.8 ± 0.2 | 14.0 ± 3.4 | - | |
| L2 | " | 4.9 ± 0.1 | 1.0 ± 0.1 | 0.4 | |
| L3 | " | 4.5 ± 0.0 | 3.1 ± 0.2 | 1.6 | |
| L4 | " | 4.3 ± 0.1 | 0.9 ± 0.2 | 0.2 | |
| | mean ± 1 sd | 4.5 ± 0.3 | 4.0 ± 5.0 | 0.6 ± 0.7 | |
| M1[a] | moss rich site | 4.4 ± 0.0 | 1.5 ± 0.2 | 3.5 | |
| M1b[a] | " | 4.9 ± 0.0 | 1.5 ± 0.2 | - | |
| M1c[a,b] | " | 4.8 ± 0.1 | 7.0 ± 0.5 | - | |
| M2a | " | 5.4 ± 0.1 | 8.3 ± 4.9 | - | |
| M2b[a] | " | 4.6 ± 0.0 | 1.5 ± 0.1 | - | |
| M2c[a,b] | " | 4.5 ± 0.0 | 38 ± 3 | - | |
| M3 | " | 4.8 ± 0.2 | 0.1 ± 0.0 | 0.3 | |
| M4 | " | 4.8 ± 0.1 | 0.8 ± 0.1 | 1.9 | |
| M5 | " | 5.5 ± 0.2 | 0.5 ± 0.1 | 0.2 | |
| | mean ± 1 sd | 4.9 ± 0.4 | 6.6 ± 12 | 1.5 ± 1.6 | |
| B1 | barren (creek silt) | 6.2 ± 0.1 | 0.1 ± 0.0 | 0.2 | |
| B2 | " (saline sand) | 6.1 ± 0.0 | 3.5 ± 0.7 | 1.3 | |
| B3 | " (moraine sand) | 6.1 ± 0.0 | 0.1 ± .0.0 | <0.1 | |
| | mean ± 1 sd | 6.1 ± 0.0 | 1.2 ± 2.0 | 0.5 ± 0.7 | |
| C1 | cement polluted (site 1) | 7.1 ± 0.1 | 1.5 ± 0.3 | 1.0 | |
| C2 | " | " | 6.8 ± 0.0 | 2.1 ± 0.2 | 0.9 |
| C3 | " | " | 6.9 ± 0.1 | 2.6 ± 0.4 | 0.6 |
| C4 | cement polluted (site 2) | 6.3 ± 0.0 | 1.5 ± .0.2 | 0.7 | |
| C5 | " | " | 5.9 ± 0.0 | 1.2 ± 0.2 | 0.7 |
| C6 | " | " | 6.3 ± 0.0 | 0.7 ± 0.1 | 0.3 |
| | mean ± 1 sd | 6.8 ± 0.5 | 1.6 ± 0.7 | 0.7 ± 0.2 | |

Notes:[1] Samples marked [a] were collected during in January 1991. All other samples were collected during the Austral 1989/90 summer.
[2] Samples marked [b] were from the top 0–1 cm layer and were noticeably richer in decaying vegetation. Samples from algal rich soil marked [c] were from the top 0-1 cm and were noticeably rich in algal cells. The remaining sample (P1 and P4) were sampled after removal of the upper algal rich layer.
[3] Figures given are the average of three replicates ± 1 standard deviation.
[4] '-'= not determined.
[5] Cement polluted 'sites 1'were 10 m downwind of concrete mixing site. Sites '2'were 90 m downwind of concrete mixing site.

(Fig. 1) characterized by 1) high levels of past and present penguin excretion (i.e. ornithogenic soils), 2) high algal productivity in areas subject to nutrient rich run-off water dominated by *Prasiola crispa* (Lightf. Meneghini), 3) dense stands of moss and/or lichen growth, 4) soil subject to intense pollution, 5) soils with no visible evidence of plant growth or organic matter. Our aim was to examine soils from a wide variety of locations in the Windmill Islands and provide data that was partially complementary to Bölter's (1990*a*, 1990*b*) studies which focused on a single locality in the vicinity of New Casey Station. Some analyses have been reported for half of the samples elsewhere (Roser *et al.* 1993) although different sample numbering was employed. The parent material for most soils not derived from penguin guano (i.e. all samples starting with the letter P,L,M, B or C), was Windmill Metamorphics (Blight & Oliver 1977). The exceptions were B2, M3, and M5 which were derived from Ardery Charnockite and P5 which was rich in penguin guano (Windmill Metamorphics were also the parent material for soil at sites examined by Bölter 1990*a*, 1990b).

Soils underlying moss beds were taken from sites typical of the region, excluding heavily waterlogged areas underlying intermittent stream beds. Samples of polluted soil were collected from points downwind of a concrete mixing site (Roser *et al.* 1992a). Where no vegetation or algal crust was visible, grab samples (100–500 g) were taken from the top 0–5 cm. Where vegetation was present, it was moved aside to expose the underlying material soils except where indicated in Table I. All soils were then sieved through a 2 mm mesh, mixed thoroughly and stored at -20 °C until analysed.

Soil organic matter content was measured as the loss of material on ignition at 400–450 °C. Soil pH was measured at 20 °C in a 20% (w/v) $CaCl_2$ (0.01 M) slurry at the same time. Soil organic carbon content was measured using the sulphuric acid/potassium dichromate method (Rand *et al.* 1975) in December 1991. Levels of polyols and sugars in soils colonized by cryptogams were measured as their silyl derivatives by GLC during February 1991. Levels of selected organic acids were initially determined using gas-liquid chromatography (GLC) during December 1990 in Antarctica. A second set of analyses was made using high-pressure-liquid chromatography (HPLC) during December 1991 at the Australian Government Analytical Laboratories, Hobart. Results are reported as mean ± 1 standard deviation where possible.

### *GLC of sugars and polyols*

Triplicate soil samples for carbohydrate analysis (1 g) were extracted with 2 ml of 70% (v/v) ethanol/water mix on a Chiltern orbital shaker (10 min, 20°C, 90 rpm). Half a millilitre was evaporated to dryness, silylated and analysed using the procedure described previously for cryptogam extracts (Roser *et al.* 1992*a*) based on the method employed by Tearle (1987).

### *GLC of organic acids*

Gas-liquid chromatography assays of organic acids were undertaken using the following adaptation of Holdeman *et al.* (1977). Triplicate 0.5–1.0 g samples of soil were mixed with 2 ml of distilled water, vortexed, heated (50°C, 15 min) and centrifuged (500 G, 15 min) to sediment heavy suspended

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material. Supernatants were divided into two parts. To test for volatile fatty acids (acetic, propionic, butyric), 0.02 ml of 50% (v/v) $H_2SO_4$ and 1 ml of diethyl ether were added to 1 ml of supernatant. This mixture was vortexed (15 sec) and incubated at -20°C overnight to allow the aqueous phase to freeze out. The ether phase was decanted and dried over 0.1 g of anhydrous $MgSO_4$ prior to chromatography. Non-volatile organic acid species (lactic, oxalic, succinic) were methylated prior to analysis. To 0.5 ml of supernatant, 1.0 ml of 100% methanol and 0.2 ml of 50% (v/v) $H_2SO_4$ were added. The mixture was vortexed and then held overnight at 20°C. Finally 0.5 ml of distilled water and 0.25 ml of 100% $CHCl_3$ were added and the mixture was vortexed and allowed to settle.

Analyses of the ether and chloroform phases were performed on a Varian 3400 Gas Chromatograph using a stainless steel AT-1000 column (Alltech; length 1.8 m ; internal diameter 3 mm) for both the ether and chloroform extracts. Conditions were as follows: sample size = 4 ml; injector temperature = 160°C; detector oven temperature = 300°C; detector type = flame ionization detector; sensitivity = $10^{-11}$; helium carrier gas flow rate = 30 ml $min^{-1}$. Column oven temperature was increased isothermally from 60°C to 200°C at 10°C $min^{-1}$ during the course of each run. Peak sizes were measured as area units by integration. Acids in samples were identified and quantified by comparison with peak retention times and areas of standards. Where feasible, constituent identity was confirmed by co-chromatography. Sensitivity was of the order of 1–5 mg fatty acid $g^{-1}$ dry soil depending on the species being measured for all species other than formic acid. Formic acid was assayed as its methyl ester because the retention time of the free acid on the packed columns tested was too close to that of ether. Sensitivity was of the order of 100 mg $g^{-1}$ soil. Aqueous solutions containing fatty acids at known concentrations in the range of 100–1000 mg $ml^{-1}$ were treated in an identical manner to the soil extracts to act as standards. These were run after every ten samples and at the beginning of each day's measurements.

Recovery rates were estimated as follows. A mixture of seven known fatty acids (c. 200–800 g $g^{-1}$ soil) was added to triplicate samples, representing four soil types (A1, X2, P1, L1a). These samples were then extracted as described, their fatty acid content quantified and the percentage recovery estimated from the difference in content between the amended and the original sample.

### *HPLC of organics acids*

All samples (0.5 g) were extracted (5 ml $H_2O$, 5 min, 20°C), centrifuged (3500 G, 5 min, 20°C) and filtered (Dynagard 0.2 mm). Samples of soils A1, A2 and A3 were also extracted with 0.1 M HCl. Analysis of aqueous and neutralized acid extracts was performed by ion exclusion chromatography on a 300mm x 7.8 mm Biorad Aminex HPX-87H column; eluant 5 mM $H_2SO_4$; flow rate 0.8 ml min-1. A Shimadzu variable wavelength UV-Vis SPA-10 AU detector, set at 210 nm, was used for detection. Peak retention times and area were calculated

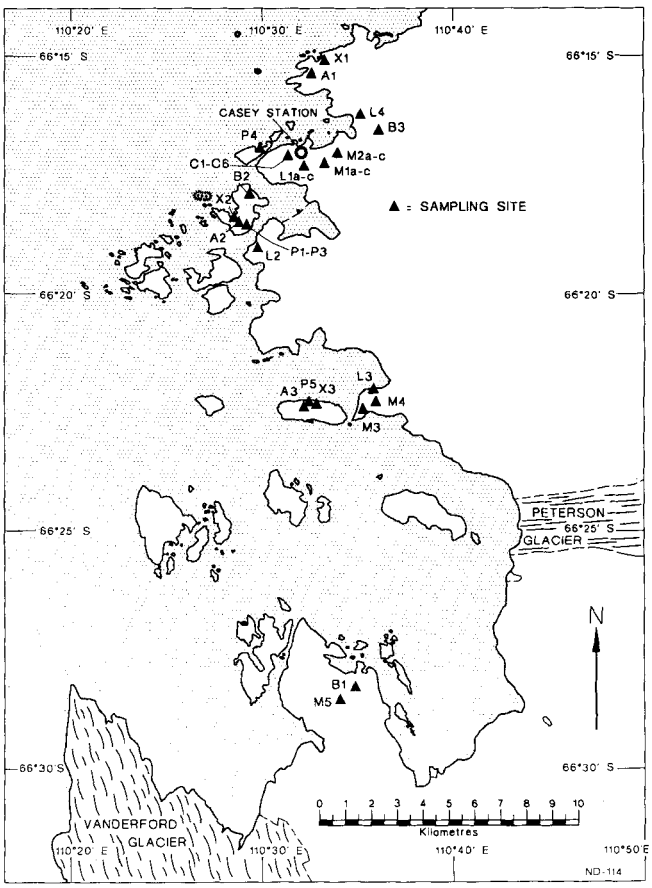


**Fig. 1.** Map of the Windmill Islands and sampling site localities.

using an Hewlett Packard 3394 Integrator and standard solutions of organic acids prepared at concentrations of 10, 100 and 1000 mg g-1. Possible contamination from sample bags was appraised by extraction with $H_2O$ and 0.1 M HCl (20 h, 20°C). This system was used to test for acrylic acid, expected in ornithogenic soils (Sieburth 1960) in addition to the acids tested for using the GLC system. Sensitivity was of the order of 1 mg $g^{-1}$.

Statistical analyses was carried out using the subroutine available within the Microsoft Excel 4 spreadsheet system.

## Results

### *Soil acidity and organic matter content*

Many of the soils from the Windmill Islands region were strongly acidic (pH ) (Table I), particularly in the case of soils from areas rich in moss and lichen. Soil organic matter content was highly variable, ranging between 0.1 and 10% of dry soils (other than those from occupied penguin colonies) (Table I). Ignition loss and soil carbon content corresponded well with one another (organic carbon = 0.48 ignition loss; $r^2$= 0.62, df = 22 for soils other than those from actively occupied colonies). The proportion of carbon comprising the ignitable matter of three occupied colony soils (27±4 %) was consistent with *high levels*

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**Table II.** Sugars and polyols detected in Windmill Islands soils.

| Sample | Sugars and polyols detected | mg carbohydrate[1] $g^{-1}$ soil | carbohydrate as % of ignition loss | carbohydrate as % of organic carbon |
|---|---|---|---|---|
| P2 | sorbitol[2] (44)[3], sucrose (26), trehalose (23), glucose (2), inositol | 1300 ± 100 | 1.8 | 6.9 |
| P3 | sorbitol[2] (43), sucrose (32), trehalose (15), glucose (3), arabitol?, inositol | 3300 ± 900 | 3.7 | ND |
| P5 | sorbitol[2] (24), sucrose (48), trehalose (28) | 1100 ± 200 | 0.9 | 3.9 |
| L1a | arabitol/ribitol(26), trehalose (74) | 23 ± 6 | 0.1 | 5.5 |
| L2 | arabitol (70), mannitol[2] (30) | 56 ± 5 | 0.53 | 3.3 |
| L3 | trehalose | 25 ± 2 | 0.1 | 0.4 |
| L4 | arabitol/ribitol (22), trehalose (77) | 22 ± 4 | 0.2 | 2.7 |
| M1a | arabitol (11), ribitol (10), mannitol/sorbitol[2] (31) sucrose (21), trehalose (27) | 100 ± 20 | 0.7 | 0.7 |
| M2a[4] | arabitol (21), ribitol (7), mannitol/ sorbitol[2] (17), glucose (15), sucrose (25), trehalose (6) | 800 ± 700 | 0.9 | ND |
| M3 | ND | <2[6] | <0.1 | <0.1 |
| M4 | ND | <2 | <0.1 | <0.02 |
| M5 | ND | <3 | <0.1 | <0.3 |
| C1 | trehalose | 22 ± 5 | 0.1 | 0.5 |
| C2 | sucrose (53), trehalose (47) | 40 ± 1 | 0.2 | 1.0 |
| C3 | trehalose | 26 ± 5 | 0.1 | 1.0 |
| C4 | sucrose (51), trehalose (49) | 21 ± 4 | 0.1 | 0.7 |
| C5 | ND | <2 | <0.1 | <0.1 |
| C6 | ND | <3 | <0.1 | <0.2 |

Notes:
[1] Total carbohydrate quantities are given as mean ± 1 standard deviation. Where carbohydrates were identified, their % contribution to the total carbohydrate is given in brackets.
[2] Differentiations of mannitol and sorbitol are tentative and based on slight differences consistently observed in retention time of peaks in the three replicates (P2, P3 and P5).
[3] Percentage of total carbon calculated to be present as sugars and polyols was estimated using the assumption of molecules containing 40% carbon by weight (i.e. glucose).
[4] Sample M2a containing unknowns comprising >2% of the total carbohydrate.
[5] Despite the high standard deviation, > 100 mg $g^{-1}$ of carbohydrate was detected in all three replicates of soil M2a.
[6] The minimum amount of carbohydrate that was detectable in soil was *c.* 3 mg $g^{-1}$.

**Table III.** Organic acids detected in Windmill Islands soils.

| Sample | Organic acid detected mg $g^{-1}$ soil | (mean ± 1 standard deviation) GLC |
|---|---|---|
| HPLC | | |
| A1 | acetic (330 ± 260) | -[a] |
| " | oxalic (2100 ± 400) | |
| " | succinic (48 ± 7) | |
| A2 | acetic (700 ± 500)acetic (110 ± 10) | |
| " | propionic (320 ± 310) | propionic (500 ± 1000) |
| " | oxalic (2200 ± 400) | oxalic (4600 ± 2000) |
| " | succinic (29 ± 7) | succinic (60 ± 6) |
| " | formic (52 ± 5) | |
| A3 | acetic (700 ± 340) | -[a] |
| " | propionic (110 ± 160) | |
| " | oxalic (250 ± 10) | |
| " | succinic (6.5 ± 0.6) | |
| X1 | ND | ND |
| X2 | ND | acrylic (trace)b |
| X3 | ND | acrylic (4) |
| P1 | ND | acrylic (trace) |
| P2 | ND | acetic (trace) |
| " | acrylic (4) | |
| P3 | acetic (250 ± 80) | - |
| P4 | - | ND |
| P5 | - | acetic (10)acrylic (trace) |
| L1a | ND | succinic (11)acrylic (3) |
| L1b | ND | - |
| L1c | succinic (1.2 ± 0.7) | - |
| L3 | ND | acrylic (2) |
| L2, L4 | ND | ND |
| M1c | succinic (1.3 ± 1.2) | - |
| M1b, M2a, M2b, M2c | ND | - |
| M1a, M3a, M3b, M4 | ND | ND |
| M5 | - | ND |
| B1, B2, B3 | - | ND |
| C1, C2 | - | ND |
| C3, C4, C5, C6 | - | acrylic (1.5–2) |

Notes: 'ND'= no organic acid detected; '-'= sample not assayed using this method. Figures given are the average of three replicates ± 1 standard deviation where possible. a. assay results conducted on these penguin colony soil samples marked[a] were unreliable due to saturation of the HPLC column by other organics. b. Identification of acrylic acid[b] in several samples was based on retention compared to a standard solution. The reliability of this result is uncertain as it was detected in several samples (e.g. C3-C6) for which no likely source could be ascribed.

of nitrogenous material reported for guano e.g. uric acid, ammonia.

One way analysis of variance showed that there were significant differences between the soil classes for all three parameters at significance levels of < 0.00001. The lichen and moss dominated soil samples were found to have mean pHs significantly less ($P$ = 0.00002 and 0.0003 respectively) than those of soils from barren sites. The soils from the active colony sites were found to have significantly higher pH, organic matter and organic carbon ($P$ = 0.02 to 0.00002) than for any other samples with the exception of the contaminated and extinct colony soils (pH only).

### *Sugars and polyol concentration*

Low molecular weight sugars and polyols (mono- and disaccharides) were found in 72% of the samples analysed (Table II). By far the highest levels (1100–3300 mg $g^{-1}$) were found in soils located near penguin colonies and characterized by some visible algal growth. The principal carbohydrates detected were sorbitol, sucrose and trehalose. Lichen dominated

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soils contained sugars and polyols typical of those present in lichen thalli, particularly arabitol, ribitol and trehalose. Of the soils colonized by moss, three contained no detectable levels of sugars or polyols while two, M1a and M2a, contained sucrose, trehalose, glucose and a number of polyols, at levels totalling 100 and 800 mg $g^{-1}$ respectively. In those soils in which they were detected, these low molecular weight carbohydrates accounted for 0.1–7% of the total soil organic matter measured as loss on ignition or calculated from organic carbon content (Table II). Of the soils from sites contaminated by cement dust, four out of six were found to have detectable levels of sucrose and trehalose.

As had been found previously with our examination of cryptogam samples (Roser *et al.* 1992*b*) there were some difficulties in separating and identifying all polyols, particularly sorbitol and mannitol. Of more concern was our observation that the ethanol extracts of the P2, P3 and P5 soils were green, indicating the extraction of chlorophyll from live cells.

*Organic acids (GLC)*

There appeared to be no clear relationship between soil pH and the levels of organic acids as determined by GLC. The highest quantities of organic acids, of the order of 1000 mg $g^{-1}$ soil, were detected in ornithogenic soils from active penguin colonies (A1, A2, A3) (Table III) which were neutral to alkaline. All three samples of this class were dominated by acetic and oxalic acid and contained smaller quantities of succinic. Propionic acid was also detected in two of the three samples (Table III). No formic, lactic or butyric acid was detected.

Concentrations of organic acids comparable to those from occupied colony soils were only detected in one of the acidic soils (P3). This was obtained from an area characterized by heavy algal growth and located near an active colony. The only other organic acid detected was succinic, at a concentration of »1 mg $g^{-1}$ soil in samples M1c and L1c. All three soils were notable for containing higher percentages of organic matter (9, 14 and 7% respectively) than other similar samples.

For the four soils tested, oxalic acid was found to have the poorest recovery (58 ± 15 %). The recovery rates estimated for the other six acids ranged on average from 87–152%. Though there was clearly some variation in recovery achieved it was comparable to the variation observed between replicates. Overall it seemed unlikely that significant quantities of organic acids were going undetected in the samples.

*Organic acids (HPLC)*

Levels of soil organic acids determined using HPLC were similar to those measured by GLC (Table III). That the occupied colony soils contained total concentrations of organic acids c. 1000 mg $g^{-1}$ soil, was indicated by analysis of sample A2. In addition to acetate, propionate, oxalate and succinate, two other acids were detected. In sample A2 low levels of formate were detected, while in several others soils, peaks corresponding to the acrylic acid standard were detected. The origin and identity of the latter soil constituent was unclear. While it was not derived from the plastic bags used for collection and storage, it was detected in several moss and lichen soils well away from the most likely source of this compound i.e. penguin excreta. Its absence from A2 was conceivably due to interference from high levels of other organic acids which chromatographed very closely. The use of the HPLC with the occupied colony soils was made difficult by the binding of other organic species to the column and prevented reliable analyses of soils A1 and A3.

## Discussion

*Accumulation of sugars and polyols*

In his studies of maritime Antarctic cryptogams, Tearle (1987) showed that there was transient development of high concentrations of polyols in the fellfield soils of Signy Island. Our results showed comparable levels in extracts of algal soils and somewhat lower levels in soils where moss and lichen were predominant. The difference in abundance could have been explained by viable plant material in the algal soils. Chlorophyll *a* levels in samples P2, P3 and P5 ranged from 110 to 1800 of g $g^{-1}$ soil whereas the quantities extracted from samples L1a, L4, M1a, B1, B2 and B3 ranged from 0.2 to 14 g $g^{-1}$ (Roser & Seppelt unpublished data). This highlights both the importance of reporting extensive details on site characteristics when sampling soils for such mobile materials and the variation possible between microsites. Similar observations have been made by Bölter (1990*a*).

The range of sugars and polyols was somewhat different from that detected by Tearle (1987). Whereas arabitol, ribitol and mannitol were most abundant at Signy, our results indicated that the compounds that were present and predominated depended on the dominant cryptogam flora. As might be expected from the known cryptogamic composition for the Windmill Islands (Roser *et al.* 1992*b*), sorbitol, sucrose and trehalose were commonly present in the algae dominated soils. By contrast the lichen dominated soils contained a substantial proportion of arabitol and/or ribitol, compounds entirely confined to this group of plants. The presence of substantial levels of arabitol and ribitol in soil underlying two of the moss samples pointed to a mixture of lichen and moss growing together at the sample site, consistent with the observation of such associations in the area e.g. *Rinodina olivaceobrunnea* Dodge et Baker growing on *Grimmia antarctici* Cardot (Roser *et al.* 1992*b*). The high proportion of trehalose in polluted soil samples was also noteworthy. The absence of arabitol (indicating lichen) or sorbitol (indicating algae) and its high levels in relation to sucrose (predominant in moss) pointed to a fungal origin, traceable to the decomposition of moss and lichen killed by cement dust pollution (Roser *et al.* 1992*a*). That an increase in trehalose could be expected under these conditions was indicated by the observations of Hurst *et al.* (1985) on decomposing grass leaves on South Georgia.

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*Accumulation of organic acids in cryptogam dominated soil systems*

Yarrington & Wynn-Williams (1985) reported that the Signy Island peat moss microflora was capable of producing significant quantities of methane in slurries, implying anoxic conditions at least at microsites. They also noted the production of acidity and suggested that fatty acids (e.g. formic, acetic, succinic, propionic acids) might be responsible. Since both lichen and moss dominated soil in the Windmill Islands were clearly acidified compared to soil from barren sites, we thought that fatty acids might be accumulating, possibly from microbial use of polyols and sugars. Such accumulation of fatty acids has been reported for rice paddy soils (Tsutski & Ponnamperuma 1987, Turtura *et al.* 1989) and landfills (Grainger *et al.* 1984). In these cases, common fermentation acids including acetic, propionic, butyric, succinic and lactic have been detected at concentrations between 1 and 1000 g $g^{-1}$ with total concentrations of 50–100 g $g^{-1}$ soil being typical.

Such substantial accumulation was apparently not taking place in Windmill Islands soils. While there are likely to be low levels of organic acids (< 1 mg $g^{-1}$), as indicated by the detection of these compounds in samples X3, M1c and M2c, they would still comprise < 0.1% of the total organic matter content. Such low concentrations imply either rapid leaching or utilization or the virtual absence of fermentation leading to fatty acid production. Either way their absence points to the soils examined being predominantly aerobic. To settle this question it would be desirable to measure the Eh profile (Yarrow & Wynn-Williams 1985).

There remains the question of why the soils underlying the lichen and moss beds were acid. The acidity could be due to humic and/or fulvic acids which control the acid base balance of peatlands (McKnight *et al.* 1985, Urban & Bayley 1986). Lichen acids are another possibility (Jones & Wilson 1985) although there was no evidence of the most common agent, oxalic acid. Similarly if the acids had been released by soil microorganisms (Rozycki & Strzelczyk 1986, Lapeyrie *et al.* 1987) they should have been detected eluting near succinic, lactic and oxalic acids.

*Accumulation of organic acids in ornithogenic soil systems*

The high levels of oxalic acid in penguin colony soils were probably a by-product of uric acid breakdown (Hutchinson 1950). An alternative source may have been the breakdown of acrylic acid originating from *Phaeocystis* algae, the major diet of the euphausids, and excreted in penguin faeces (Sieburth 1960). This would be possible via a cleavage and hydrolysis of the molecule's double bond. Our failure to detect acrylic acid in the fresh ornithogenic soils may not indicate its absence but may be due to the high levels of organic matter in this material masking small peaks and inhibiting binding to the HPLC column. The presence of high levels of oxalic acid may have accounted for the paucity of algal growth close to occupied penguin colonies. The detection of large pools of organic acids typical of those derived from microbial fermentation (Dawes & Sutherland 1976, table 4.2), probably reflected anaerobic/microaerophilic conditions at these sites and contents of the penguins' excreta.

*Methodological considerations*

This initial study of soil carbohydrates and organic acids highlighted some possible limitations in current methodology which it would be desirable to address in any future work. For the quantification of sugars and polyols, the use of other types of capillary column or alternatively HPLC (Sancho *et al.* 1986) needs to be investigated so that the pairs arabitol ribitol, and sorbitol mannitol, can be more readily distinguished. The relatively high levels of carbohydrates which appear to develop in this soil could make the latter technique viable despite its lower sensitivity relative to GLC. It has been reported to us (R. Arnold personal communication 1993) that improved GLC resolution of the common polyols may be achieved with the use of a 25 m x 0.2 mm (internal diameter) Hewlett-Packard Ultra 2 column (25m x 0.2 mm, film thickness 0.3 m, crosslinked with 5% Ph Me Silicone). P. Monteil (personal communication 1993) has noted that Thin Layer Chromatography (TLC) would be an additional useful technique, which could be readily employed in Antarctica. A more powerful technique is $^{13}C$ nuclear magnetic resonance spectroscopy, particularly for tracing the fate of labelled compounds.

The method for extracting sugars and polyols from soils needs to be revised. The standard method used by Tearle (1987), and followed here for consistency, involved the use of 70% ethanol. The cryptogam soil does not appear to interfere with the recovery of polyols and sugars (D.R. Melick personal communication). This extraction reagent, however, is very effective in killing microorganisms and disrupting cell membranes. Indeed its activity is the basis of the Gram stain reaction. It is also a good solvent for chlorophyll and sugars. Though leakage of sugars and polyols into soil can occur naturally (Dudley & Lechowicz 1987, Melick & Seppelt 1992), it is conceivable that a portion of the carbohydrate detected was stored in viable cells and released during the extraction procedure.

The analysis of organic acids by both GLC and HPLC would benefit by both improving sensitivity to give greater resolution and by an improved extraction technique. Although we obtained satisfactory recovery of added standards, the levels at which these were added were substantially higher than the limits of detection of the GLC technique. One approach would be to replace the currently recommended packed columns by capillary column systems. Concentration of aqueous extracts by freeze drying could also increase sensitivity. This would facilitate measurements of extraction efficiencies where low concentrations of organic acids are present.

## Conclusions

Although our study was of limited scope it established that substantial pools of carbohydrates can accumulate in the continental Antarctic environment as demonstrated for the maritime Antarctic at Signy Island (Tearle 1987). It follows that these sugars and polyols, including glucose and related sugars are valid model substrates for studying major nutrient pathways in the Antarctic soil systems where cryptogams are abundant (see Bölter 1990*a*). Levels of organic acids tend to be low in Windmill Islands soils except in nutrient rich areas such as penguin colonies and their surroundings. Though both organic acids and carbohydrates comprise only a small portion of the total soil organic matter, an understanding of their utilization and production should be central to studies of soil microbiology of Antarctic soils. Of particular interest would be differences associated with the three distinct dominant cryptogam groups – algae, lichens and mosses.

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