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# Bacterial Populations in Soils of a Subantarctic Island

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**Summary.** Bacteria were counted (direct counts using acridine orange) in soil samples from 12 sites on Marion Island (subantarctic). Numbers, cell types and cell volumes all varied widely between sites; numbers from 7 to $151 \times 10^8$ cm$^{-3}$ and from 5 to 1300 g$^{-1}$ oven-dry soil (o.d.s.), volumes from 63 to 825 μm$^3$ cm$^{-3}$ and from 61 to 6570 μm$^3$ g$^{-1}$ o.d.s. Five main cell shapes were distinguished, and each divided into up to 4 size-classes. Numbers were related negatively to climatic severity and positively to soil nutrient concentrations, vertebrate manuring, and availability of organic substrates. Volumes were not markedly related to climate; the main division was simply between edaphically rich and poor sites. Manured sites and high-altitude sites both had characteristic cell-types, and there was a strong altitudinal sequence of cell sizes among fjaeldmarks. Among the manured sites, seal wallows and albatross nest differed from gull- or penguin-manured sites under *Cotula plumosa,* especially in the proportions of different cell types and sizes. One sample, from a high-altitude fjaeldmark, was totally unlike all others. It was excluded from all general comparisons but it is suggested that this site deserves further study. The combination of numbers, volumes, cell types and sizes, and fluorescence characteristics are interpreted as indicators of contrasting strategies for growth and reproduction, especially high or low "standing crop" *vs* high or low turnover, and these strategies related to site conditions.

## Introduction

The soils of Marion Island (46°54′S, 37°45′E) are unusual for deep acid peats and tundra soils in having a preponderance of bacteria over fungi in their microflora (Steyn and Smith 1981; Smith and Steyn 1982), e.g. Holding (1981), gives a general biomass ratio, fungi: bacteria, of about 10:1 in tundras and cold-temperate sites. Previous studies of tundra bacteria, in both northern and southern hemispheres, have suggested positive relationships between numbers of bacteria and climatic mildness, organic matter and nutrient supply (Holding et al. 1974). In southern hemisphere sites, similar relations have been suggested between bacterial populations and vertebrate manuring (Boyd et al. 1970; Smith and Steyn 1982; Ramsay 1983). Here, we present data on numbers, cell volumes and cell types of bacteria in a series of soils on Marion Island, from sites differing in climatic severity, edaphic conditions and degree of vertebrate influence. We assess the applicability to these populations of the climatic and edaphic relationships found in previous studies, and suggest some hypotheses concerning strategies of growth and reproduction of the various bacterial populations.

## Materials and Methods

### *Sites*

Twelve sites were sampled. One was at the edge of the Ice Plateau (Marion Island's relict ice-cap). Four were fjaeldmarks, along a gradient of altitude and hence also of climatic severity. Two were mires, of which one was essentially undisturbed, the other was in an area where wandering albatrosses (*Diomedea exulans*) were nesting, near, but not obviously influenced by, an occupied nest. Four sites were heavily manured by seabirds or seals. The remaining site was a fernbrake under *Blechnum penna-marina.*

The individual sites were as follows:
(Locations defined after the map of Langenegger and Verwoerd 1971; Botanical nomenclature follows Gremmen 1981).

*1 Ice Plateau:* frozen weathered ash deposit (clay-like texture) at the eastern edge of the Ice Plateau near Bob Rand Peak. Completely unvegetated. "Soil" contained no visible organic material.

*2 Fjaeldmark (High-Altitude):* a gravelly ash-plain just above Katedraal Krans. "Wind-desert", with scattered moss cushions (*Ditrichum strictum* and *Andraea acutifolia*) and some epilithic lichens, but total vegetation cover <10%. The soil contained a small amount of organic material, including fragments of dead *Azorella selago* shoots, but there were currently no *Azorella* cushions (live or dead) at the site.

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*3 Fjaeldmark (Medium-Altitude, Between Azorella):* SE of First Red Hill. Fjaeldmark vegetation, i.e. cushions of *A. selago,* various bryophytes (a high proportion of *Ditrichum*) and numerous lichens, on mixed volcanic deposits, mainly black lava rock interspersed with small pebbles, gravel and some ash. Mineral soil (as site 2) but with a higher organic content under *Azorella* cushions. Sampled from an unvegetated area between cushions.

*4 Fjaeldmark (Medium-Altitude, Under Azorella):* as site 3 but sample taken from under an *Azorella* cushion.

*5 Fjaeldmark (Low-Altitude):* a rocky ridge in Nellie Humps, c. 700 m inland. Typical low-altitude fjaeldmark vegetation dominated by *A. selago* cushions with epiphytic *Agrostis magellanica,* and lichens and bryophytes (mainly *Andraea acuminata* and *Racomitrium crispulum*) between the cushions. Soil more organic than sites 3 or 4 but still a "mineral" soil. Sampled from bare areas between cushions.

*6 Mire (Undisturbed):* c. 500 m NW of Meteorological Station (M.S.). Wet mire on deep peat. Vegetation dominated by bryophytes (mainly *Jamesoniella colorata, Clasmatocolea humilis* and *Blepharidophyllum densifolium*) and graminoids (*Agrostis magellanica* and *Uncinia compacta*).

*7 Mire by Albatross Nest:* in Nellie Humps, c. 500 m SW of M.S. Similar to site 6, but in an area in which wandering albatrosses were nesting. Vegetation mainly *Agrostis magellanica, C. humilis* and *B. densifolium*). Some scattered plants of *Acaena magellanica* and stunted tussocks of *Poa cookii* also present. Sample taken under *Agrostis* and bryophytes.

*8 Albatross Nest:* Nellie Humps, c. 350 m SSW of M.S. Luxuriant vegetation (*P. cookii,* with *Cotula plumosa, Callitriche antarctica* and some *Montia fontana*) surrounding an occupied wandering albatross nest. Sampled under vegetation at base of nest.

*9 Gull Cotula:* Paddy Rocks, 600 m S of M.S. Coastal clifftop receiving salt spray, occasionally inundated by waves, and heavily manured by kelp gulls (*Larus dominicanus*) which also deposited substantial quantities of shell material from marine molluscs. A thin dark-brown fibrous peat, covered by *C. plumosa* and *Crassula moschata,* with isolated *Azorella* cushions and *P. cookii* tussocks. Sampled under *Cotula.*

*10 Rockhopper Cotula:* Similar to site 9 but in a spot manured by rockhopper penguins (*Eudyptes chrysocome*) rather than gulls. Vegetation almost entirely *C. plumosa* (100% cover) plus about 5% *P. cookii.* Sampled under *Cotula* as site 9.

*11 Elephant Seal Wallow:* Southern border of Gentoo Lake wallowing area. Elephant seal (*Mirounga leonina*) wallow, recently occupied. Vegetation *C. plumosa* only.

*12 Blechnum Slope:* Slope fernbrake in Nellie Humps c. 450 m N of site 7, 100 m inland from M.S. A complete mat of *Blechnum pennamarina* with very few scattered tussocks of *P. cookii* and no petrel or prion (Procellariidae) burrows. Soil essentially an organic podzol. Sampled under *Blechnum,* beneath the rhizoid zone.

Soil temperatures were not measured at all the sites, but monthly means ranged from 4.5 to between 8 and 10 °C at the low-altitude sites (French and Smith 1985), where the main differences were in the variability of temperatures. The fjaeldmark was subject to fairly wide short-term fluctuations (± 2 – 3 °C within minutes). In contrast, at the *Blechnum* slope, with the same range of monthly means, soil temperatures were maintained almost constant over several days, changing only slowly. The mires and manured sites all had a wider range of monthly means than these two sites, but short-term variation was intermediate between them. The medium- and high-altitude sites were progressively colder, up to the (probably) permanently frozen deposits by the Ice Plateau.

### *Soil Sampling and Analyses*

Soil samples were taken in early May 1982. Single cores (5 cm diameter) were taken from each site to a depth of 3 cm, except at the medium-altitude fjaeldmark and Ice Plateau sites, where a block of the same depth was cut out with a knife, and the high-altitude fjaeldmark, where the soil was too loose to core or cut and a sample to about 3 cm was scooped up with a trowel. Samples from the low altitude sites were brought to the laboratory in the corer, those from the medium-high altitude sites in sterile polythene bags, at field temperature. All samples were counted on the day of sampling, except the sample from the Ice Plateau, which took two days to bring back to the laboratory. Bulk density was determined on a subsample of the core, or on a second sample taken immediately beside the first.

Some physical and chemical attributes of the soils were determined on samples from most sites (Table 1). Wherever possible, material from the same location as the bacterial counts, or from a sample taken with-

**Table 1.** Physical and chemical characteristics of the sample sites

| Sites | Altitude | $H_2O$ | pH | Inorganic | | | | CEC | Organic | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | N | P | K | Ca | | C | N |
| 1. Ice Plateau | 950 | 68 | No chemical data available | | | | | | | |
| Fjaeldmarks: | | | | | | | | | | |
| 2. High-altitude | 750 | c50[a] | | | | | | | | |
| 3. Medium-alt. between *Azorella* cushions | 550 | 47 | 6.2 | <1 | 4 | 0 | 0[b] | 9 | 2 | 0.1 |
| 4. Medium-alt. under *Azorella* | 550 | 116 | 6.2 | 1 | 0 | 0 | 56 | 14 | 4 | 0.2 |
| 5. Low-altitude | 50 | 208 | 5.9 | 2 | 4 | 0 | 107 | 63 | 8 | 0.7 |
| Mires: | | | | | | | | | | |
| 6. Undisturbed | 30 | 1266 | 4.8 | 4 | 70 | 8 | 79 | 80 | 36 | 2.4 |
| 7. By Albatross nest | 30 | 1185 | 4.3 | 34 | 49 | 2 | 137 | 110 | 39 | 2.0 |
| Manured sites: | | | | | | | | | | |
| 8. Albatross nest | 30 | 1000 | 5.4 | 611 | 288 | 1 | 102 | 118 | 40 | 2.0 |
| 9. Gull *Cotula* | 15 | 674 | 5.0 | 101 | 146 | 12 | 181 | 113 | 34 | 2.5 |
| 10. Rockhopper *Cotula* | 15 | 762 | 4.4 | 38 | 675 | 14 | 55 | 95 | 37 | 3.1 |
| 11. Wallow | 5 | 609 | (4.5)[c] | (103) | (41) | n.d. | n.d. | n.d. | (44) | (3.4) |
| 12. *Blechnum* | 30 | 636 | 4.3 | 3 | 289 | 12 | 126 | 162 | 36 | 2.3 |

Units: altitude m above sea level; moisture ($H_2O$), organic C, N % dry wt.; inorganic nutrients ppm dry wt.; cation exchange capacity (CEC) meq/100 g. Chemical data for sites 3, 4, 6, 8, 10 and 12, and CEC, organic C, N for sites 5 and 9 are from similar locations at the same site. Data for albatross nest are mean of two samples to match bacteria samples. All other data are from the actual sample locations for bacteria

[a] Value approx. only as "soil" was too loose for accurate determination; [b] 0 = not detectable; [c] Values in brackets for Wallow are from under a *Poa* tussock but counts were from open wallow

in a few cm under the same vegetation, was used. Otherwise, we used material from similar locations at the same site. Moisture was measured gravimetrically by drying at 105 °C. pH was measured using a combination electrode in a slurry of 1 part fresh soil: 2 parts distilled water. Inorganic N was determined from 0.5 m NaCl extracts of fresh soils (ammonium-N by indophenol blue, Solorzano 1969, and nitrate + nitrite-N by cadmium reduction, Mackereth et al. 1978). All other analyses were of air-dried subsamples. Organic C was determined as "dichromate-oxidizable" (Allison 1965) with a 0.33 M dichromate solution to enable use of a larger subsample in the analysis; organic N by steam-distillation of the ammonium in Kjeldahl digests; inorganic P as "available" P by a resin-extraction procedure (Smith 1979); CEC by ammonium acetate extraction followed by washing out of excess ammonium acetate with isopropanol, displacement of absorbed ammonium by sodium (as NaCl) and the displaced ammonium determined by steam distillation.

*Bacterial Counts*

Subsamples of the soils (usually c. 50 – 100 g) were slurried by stirring with a known volume of sterile water. (All water used for dilutions was distilled, sterilized and filtered.) Actual soil: water ratios were adjusted for each sample to give a clearly countable sample after filtering and staining. Final dilutions ranged from ×200 to ×1000, with most between ×400 and ×700. Duplicate aliquots of 0.5 – 1.0 ml were taken from each slurry. These were made up to 2 ml with water, stained with 0.5 ml of 0.1% acridine orange (aqueous solution), and filtered through 0.22 µm Nuclepore polycarbonate filters (filter diameter c. 16 mm) previously stained with irgalan black (Hobbie et al. 1977). The bacteria on the filters were counted under oil immersion (×1000) using a Zeiss Standard 14 microscope fitted with a IV FL epifluorescence condenser, HBO 50 mercury lamp, FT510 dichromatic splitter, LP 520 barrier filter and BP 450 – 490 band-pass filter. Counting was done immediately after staining and filtering.

The bacteria were counted in 10 fields selected randomly from each filter, along a randomly selected transect 10 × 100 µm within each field. Except for the two samples from medium-altitude fjaeldmark, at least 200 cells per filter were counted by this method. (Only a single filter was counted from the Ice Plateau sample because of lack of time, but the sample was very homogeneous).

Cells on opaque particles were counted as double, on the assumption that, on average, there would be an equal probability of a cell being on the upper or lower surface (this was evident in the case of transparent or translucent particles).

Cells fluorescing green or yellow-green were assumed to be live. Cells fluorescing yellow-orange or orange (identical colours to soil particles, xylem skeletons and other known non-living items) were assumed to be dead. Red fluorescence, indicating a preponderance of RNA over DNA, (Rigler 1966) could indicate either a dead cell (DNA breaking down) or a very active one (Hobbie et al. 1977). We used the criteria that if a cell had a diffuse outline and was fluorescing only dimly, it was dead. If it was bright and clearly defined it was live and active. Size of cell was also used as a confirmatory criterion, but brightness and definition were given most weight, so that small cells which were also bright and sharply defined were taken to be live and, conversely, large but dim and diffuse cells were presumed dead. Only cells presumed to be live by the above criteria were counted, those fluorescing green or red being counted separately.

The interpretation of colours as indicators of live or dead cells when using acridine orange is notoriously difficult. D. Roser (personal communication) concludes that a "simple live versus dead interpretation of fluorescence metachromasia is not justifiable for in vivo studies". While this is undoubtedly true for any simple distinction applied indiscriminately over all in vivo situations, we feel we have sufficient indirect support for the criteria we have used for our particular samples.

Firstly, it has been established that DNA will fluoresce green and RNA red (see references above). Algae, and other active nucleate cells, in our samples fluoresced red in the cytoplasm (though this could be partly natural fluorescence of chlorophyll in the case of algae) but yellow or yellow-green in the nucleus. Similarly, fragments of higher plants expected to be live but not very active tended to fluoresce green, especially in the nuclear region, while "mini-cells" (of a size likely to lack DNA), when encountered, were uniformly red (cf. Roser 1980). Secondly, all known non-living items fluoresced yellow-orange or orange. Thirdly, orange or yellow-orange bacteria, as well as non-living particles, all tended to be both dimly fluorescent and relatively ill-defined (one notable exception to this being xylem skeletons of higher plants).

Cell shapes and sizes were noted in each sample (sizes were estimated from the 5 µm graduations on the eyepiece graticule) and the proportions of cells in each shape and size class estimated to the nearest 5%, for green and red cells separately. Shapes and sizes were defined in as much detail as possible, as Lundgren (1984) has shown that use of too broad size-classes can give extremely erroneous results. Assumptions such as that of Jenkinson et al. (1976) that all bacteria can be assumed to be more or less spherical, even though of different sizes, are also questionable. We aimed to go as far as was practically possible towards the ideal of measuring individual cells. Shapes, dimensions and

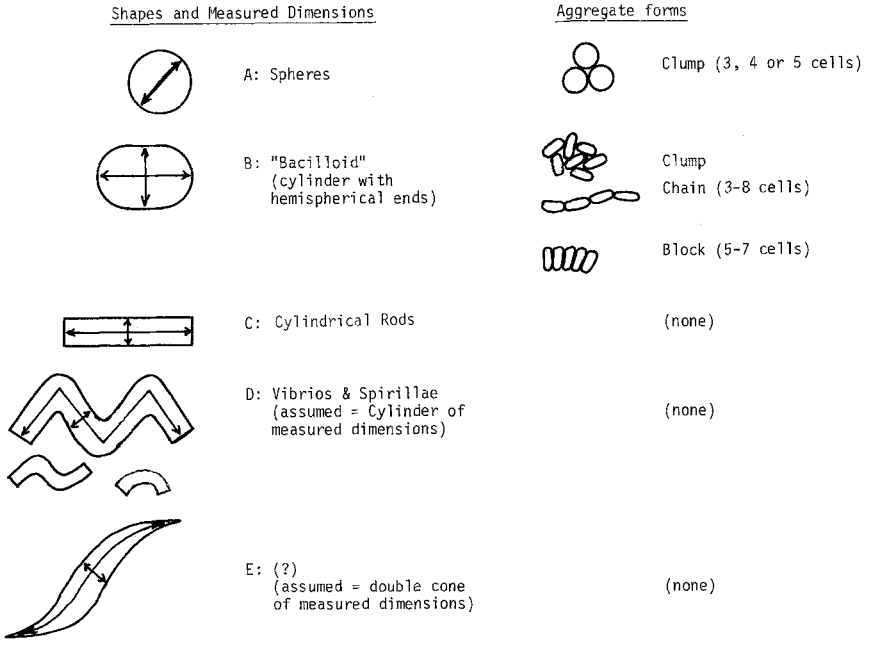


**Fig. 1.** Cell morphology. Criteria for description (shapes recognized, dimensions measured, and list of aggregate forms where present)

**Table 2.** (a) Cell dimensions (μm) and (b) Cell volumes ($\mu m^3$). Cell types as defined in Fig. 1

| | Cell type | Size class | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 |
| (a) | A (spheres) | ⩽0.25 | 0.33 | 0.5 | n.p. |
| | B (bacilloid) | ⩽0.5 ×0.25 | 0.67×0.33 | 1.0 ×0.50 | ⩾1.5×0.75 |
| | C (cylindrical rods) | 0.75×0.25 | 1.5 ×0.25 | 1.75×0.33 | 2.5×0.33 |
| | D (vibrios/spirillae) | 1.0 ×0.25 | 2.0 ×0.25 | 3.0 ×0.25 | 4.0×0.25 |
| | E (?) | n.p. | n.p. | 3.0 ×0.33 | 4.0×0.5 |
| (b) | A | 0.008 | 0.03 | 0.07 | n.p. |
| | B | 0.01 – 0.02[a] | 0.05 | 0.16 | 0.5 |
| | C | 0.04 | 0.07 | 0.15 | 0.25 |
| | D | 0.05 | 0.1 | 0.15 | 0.35 |
| | E | n.p. | n.p. | 0.1 | 0.25 |

[a] In albatross nest and *Cotula* sites type B1 cells were nearly all very small so a volume of 0.01 was used. In all others except *Blechnum* B1 cells were larger and volume = 0.02. In *Blechnum* B1 cells were a mixture of both types so we assumed an average volume of 0.015
n.p. = not present

cell volumes are listed in Fig. 1 and Table 2. The degree of clustering of cells in each sample was also noted, together with the approximate portion of cells on solid particles or in solution.

*Statistical Analysis*

Because some of the soil data were not from the actual sample locations, detailed statistical comparisons of bacterial counts with soil physical and chemical characteristics were not possible. However, the data were sufficient to enable us to group the sites as e.g. wet/dry or high/low-nutrient, and we could test for differences between these groups.

Specific a priori groupings of sites were tested by analysis of variance, using $Log_{10}$ counts or volumes as a variance-stabilizing transform.

## Results

### *Numbers*

Numbers $cm^{-3}$ fresh soil ranged from $7\times10^8$ in the medium- and high-altitude sites to more than $100\times10^8$ in most of the sites subject to vertebrate manuring (Table 3). The high-altitude fjaeldmark had surprisingly high counts but was also exceptional in other ways (see below). Counts from this site were therefore omitted from all statistical comparisons. Excluding them, there was a clear altitudinal trend among the fjaeldmark sites from low numbers in the highest sites to higher numbers at low altitudes, together with higher numbers under *Azorella selago* cushions than in unvegetated soil between them. Numbers in the mire sites and, more surprisingly, also in the seal wallow were similar to those in the low-altitude fjaeldmark. The other manured sites (all by birds) had much higher counts, as did the *Blechnum* site which, while not subject to any great amount of manuring and by no means high in inorganic N, was still rich in "available" P and "exchangeable" cations.

The two counts from each site were generally similar, indicating homogeneous conditions within samples. The

**Table 3.** Numbers of fluorescing bacteria per $cm^3$ fresh soil and per g oven-dry soil (o.d.s.) in Marion Island sites

| Site | Nos $\times10^8$ $cm^{-3}$ | | | Nos $\times10^8$ $g^{-1}$ o.d.s. | | |
|---|---|---|---|---|---|---|
| | Green | Red | Total | Green | Red | Total |
| H-a Fjaeldmark[a] | 95±2 | none | 95±2 | 113±3 | none | 113±3 |
| Ice Plateau | 7 | none | 7 | 5 | none | 5 |
| M-a Fjaeldmark between *Azorella* | 7±1 | none | 7±1 | 7 | none | 7 |
| M-a Fjaeldmark under *Azorella* | 25±2 | none | 25±2 | 53±4 | none | 53±4 |
| L-a Fjaeldmark | 52±1 | neg.[b] | 52±1 | 161±4 | neg. | 161±4 |
| Mire by Albatross nest | 44±1 | neg. | 44±1 | 569±9 | neg. | 569±9 |
| Mire (undisturbed) | 59±1 | neg. | 59±1 | 810±12 | neg. | 810±12 |
| Wallow | 47±5 | 16±1 | 63±6 | 334±36 | 114±8 | 447±44 |
| Albatross nest | 87±20 | 13±7 | 100±33 | 961±440 | 140±75 | 1102±368 |
| *Blechnum* | 65±4 | 41±4 | 106±7 | 481±25 | 303±27 | 784±52 |
| Gull *Cotula* | 56±12 | 68±15 | 124±26 | 433±95 | 531±112 | 965±207 |
| Rockhopper *Cotula* | 32±5 | 119±1 | 151±4 | 273±39 | 1025±5 | 1300±30 |

Counts given are means ± maximum deviation
[a] H-a, M-a, L-a = high, medium, low-altitude; [b] neg. = present but "negligible" (<15 cells per counted filter)
Brackets in Totals columns indicate significant groupings for analysis of variance

**Table 4.** Percentages of the 17 cell types recognized in samples of bacteria from Marion Island sites

| Site | Spheres | | | Bacilloids | | | | Cylinders | | | | Vibrios etc. | | | | (?) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A1 | A2 | A3 | B1 | B2 | B3 | B4 | C1 | C2 | C3 | C4 | D1 | D2 | D3 | D4 | E3 | E4 |
| Ice Plateau | | | 31 | | | 25 | 25 | | | | 45 | | | | | 2 | |
| Fjaeldmarks: | | | | | | | | | | | | | | | | | |
| High-altitude | | 8 | 7 | | 5 | 5 | 5 | | | | 20 | | | | | 35 | 15 |
| Medium-altitude between *Azorella* cushions | | 12 | 8 | | 30[c] | 20[c] | | 16 | 18 16 | 12 | | | | | | | |
| Medium-altitude under *Azorella* | 13 | 13 | 7 | 14 | 14 | 7 | | | | | | | | | | | |
| Low altitude | 40 | | | 60 | | | | | | | | | | | | | |
| Mires: | | | | | | | | | | | | | | | | | |
| Undisturbed | 32 | 8 | | 48 | 6 | 6 | | | | | | | | | | | |
| By Albatross nest | 30 | 12[a] | 8[a] | 27[d] | 11[d] | 7 | | 3 | 2 | | | | | | | | |
| Manured sites: | | | | | | | | | | | | | | | | | |
| Albatross nest Filter I | 40[a] | 8[a] | 2 | 40[b] | | 8[b] | 2 | | | | | | | | | | |
| Filter II | 18[a] | 18[a] | 9 | 18[b,c,d] | 18[b,c,d] | 2 | 7[c] | 2 | 2 | + | 1 | 2 | 2 | + | 1 | | |
| Gull *Cotula* Green | 19 | 19 | | 19 | 19 | | | 6 | 6 | | | 6 | 6 | | | | |
| Red | 15 | 11 | 11 | 15 | 8 | 7 | 7 | 5 | 4 | 4 | | 5 | 3 | 3 | 2 | | |
| Rockhopper *Cotula* | | | | | | | | | | | | | | | | | |
| Green | 32 | 8 | | 32 | 8 | | | 10 | 10 | | | | | | | | |
| Red | 40 | 8 | 2 | 36 | 5 | | 5 | 2 | 2 | | | | | | | | |
| Wallow | 12 | 12[a] | 36[a] | 7 | 7[b] | 7[b] | 14[c] | | | | | | 5 | | | | |
| *Blechnum* Green | 18 | 14 | 14 | 18 | 14 | 11 | 2 | 2 | 2 | 1 | + | 2 | 2 | | | | |
| Red | | 45 | | 9 | 18 | 18 | 10 | | | | | | | | | | |

Space indicates absent; + indicates $<0.5\%$. Where green and red cells are not distinguished, cell types were approximately the same in both. In the albatross nest green and red cells were similar within each filter but the two filters were very different so are given separately. All other values are lumped over both filters

[a,b,c,d] indicate aggregate forms present: type A,a = "clump"; type B,b,c,d = "clump", "chain" and "block", respectively (see Fig. 1). With parentheses indicates $<1/3$ of the class was aggregate form, without indicates $>1/3$

one exception was the albatross nest, where one filter was similar to the mires, the other with high counts similar to the remaining manured sites. Partly because of the within-site homogeneity, grouping the sites as: medium-high altitude (unvegetated); medium-altitude fjaeldmark under *Azorella*; low-altitude fjaeldmark and mires; and all "enriched" sites (Table 3) accounted for 93% of the total variance (analysis of variance, $\log_{10}$ counts).

Numbers $g^{-1}$ oven-dry soil (o.d.s) ranged from $5\times10^8$ to $1300\times10^8$. As with numbers $cm^{-3}$, there was a clear altitudinal and vegetation-related trend in the fjaeldmarks and related sites (Table 3) but the order of counts in the milder low-altitude sites was completely different. The counts from the two mire sites were more widely separated, the wallow had lower counts than either of them, and all three sites had much higher bacterial numbers than the fjaeldmark. Of the edaphically rich sites, only the two *Cotula* sites had significantly higher counts than the undisturbed mire, though all (except the wallow) had higher counts than the mire by the albatross nest. Again, the high-altitude fjaeldmark was exceptional.

A group order of: high-medium altitude (unvegetated) < medium-altitude fjaeldmark under *Azorella* < low-altitude fjaeldmark < mires < edaphically rich sites (Table 3) accounted for 97% of the variation in $\log_{10}$ counts.

Only the edaphically richer sites (manured sites and *Blechnum*, see Table 1) had a substantial proportion of red cells (Tables 3 and 4), with the two *Cotula* sites having the highest. A similar increase in the proportion of red cells in enriched sites was found by Smith and Hilmer (1984) in counts of bacteria from water bodies on Marion Island.

### *Cell Types and Sizes*

Numbers alone can give a misleading estimate of actual bacterial populations, as a total count may cover a wide variety of cell types and sizes. Among the Marion Island sites, there was considerable variation in composition of the population as well as in total numbers (Table 4).

Type E cells were recorded only at the two high-altitude sites, especially the fjaeldmark, while only edaphically rich sites contained type D (vibrios and spirillae). At the opposite extreme, the undisturbed mire and low-altitude fjaeldmark had the simplest population composition, with only type A (spheres) and B (bacilloid) cells, and those nearly all in a single size-class.

In the fjaeldmark sites, the percentage of larger cells increased with altitude. The general trend of decreasing population size with increasing climatic severity implied

**Table 5.** Volume ($\mu m^3$) of fluorescing bacteria, per $cm^3$ and per g.o.d.s., in Marion Island sites

| Site | $\mu m^3 \times 10^6\ cm^{-3}$ | | | $\mu m^3 \times 10^6\ g^{-1}$ o.d.s. | | |
|---|---|---|---|---|---|---|
| | Green | Red | Total | Green | Red | Total |
| H-a Fjaeldmark[a] | 1560 | none | 1560 | 1800 | none | 1800 |
| M-a Fjaeldmark between *Azorella* | 63 | none | 63 | 61 | none | 61 |
| L-a Fjaeldmark between *Azorella* | 79 | neg.[b] | 79 | 243 | neg.[b] | 243 |
| M-a Fjaeldmark under *Azorella* | 122 | none | 122 | 257 | none | 257 |
| Ice Plateau | 196 | none | 196 | 148 | none | 148 |
| Mire by albatross nest | 159 | neg. | 159 | 2040 | neg. | 2040 |
| Mire (undisturbed) | 161 | neg. | 161 | 2200 | neg. | 2200 |
| Rockhopper (*Cotula*) | 73 | 455 | 528 | 525 | 327 | 3800 |
| Gull *Cotula* | 193 | 580 | 773 | 1490 | 4500 | 5990 |
| Wallow | 169 570 | 69 193 | 238 763 | 1860 4040 | 759 1370 | 2620 5410 |
| Albatross nest | 914 542 | 42 55 | 957 597 | 10050 5960 | 462 605 | 10530 6570 |
| *Blechnum* | 403 | 422 | 825 | 2970 | 3110 | 6080 |

Volumes calculated from mean nos in Table 3, cell-type distributions in Table 4 and cell volumes in Table 2 (b) (see text) – also separately for the two filters from the albatross nest (values to left of main columns) because of the extreme variability of this sample
[a] H-a, M-a, L-a = high, medium, low-altitude; [b] neg. = present but negligible
Brackets in Totals columns indicate significant groupings for analysis of variance

by numbers may not, therefore, be maintained when cell type composition is taken into account.

In the *Blechnum* and *Cotula* sites, the distribution of red cell types were very different from the green, both in the proportions of different cell types and sizes, and in the red cells being more associated with solid particles in the *Cotula* sites. This was not so in the albatross nest and wallow. These two sites, together with the mire near the albatross nest, were also the only low-altitude sites with a significant proportion of aggregate forms. The two samples from the albatross nest had totally different compositions. Filter I resembled the mire samples but Filter II showed a typical "enriched site" pattern, matching the difference in counts between the two filters from this site.

The general spatial distribution of cells on the filters was roughly classified as "even" (no marked gaps or clusters), "patchy" (cells tending towards local groupings with distinct empty areas but no actual clustering and "clustered" (distinct, fairly tight clusters of cells, other than aggregate forms). Clusters were rare and confined in all sites to type A and B cells, as were aggregate forms. Aggregate forms (Fig. 1, Table 4) except for the "chains" in the medium-altitude fjaeldmark, were all associated with a "patchy" distribution.

We also noted the approximate proportion of cells on solid particles or in the soil solution. In the fjaeldmark sites there was a gradation from nearly all cells adhering to particles in samples from low altitude to nearly all being in the soil solution in samples from the Ice Plateau. The milder low-altitude sites all had between 50% and 75% of the cells on particles, with the highest proportion on particles in the wallow and among red cells in the *Cotula* sites.

### *Biovolumes*

From the mean numbers in Table 3, the cell-type distributions in Table 4, and the cell volumes in Table 2(b), we calculated the total volume of (assumed live) bacterial cells per $cm^3$ and per g.o.d.s. In each site, by:

$$V = \sum (N \times p_i \times v_i)$$

where
$N$ = total count (mean of 2 filters),
$p_i$ = proportion of cell type i (separately for red and green i = A1 to E4),
$v_i$ = volume of cell type i.

We also calculated volumes for the two filters from the albatross nest separately, as they were so different.

We did not attempt to convert counts or biovolumes to biomass estimates. Any such estimate must depend on essentially arbitrary assumptions about cell density and wet: dry weight ratios, the latter of which, especially, are likely to bear little relation to reality.

Considering volumes $cm^{-3}$ (Table 5), the effect of the contrasting trends with altitude in numbers and sizes among the fjaeldmark sites, and of a similar tendency in the milder sites for the most numerous cells to be in the smaller size-classes, was to reduce the overall variation in bacterial populations to just two groups corresponding to edaphically poor and edaphically rich sites. Analysis of variance (as for numbers) shows that this simple division (Table 5) accounts for 92% of the variance in total biovolumes (red + green). The overall variation, too, was reduced especially if the high-altitude fjaeldmark (which by this measure is more exceptional than ever) is ignored.

The order of sites within the two main groups was also altered by conversion of numbers to volumes. In the edaphically poor group, the medium-altitude fjaeldmark under *Azorella* had greater biovolume than the low-altitude fjaeldmark; and the Ice Plateau, with lowest numbers, had the highest volume. Similarly, bacterial num-

bers in the wallow were the lowest of all the enriched sites, but biovolume among the highest, while the rockhopper-enriched *Cotula* soil, with highest mean numbers, had lowest mean biovolume. The two filters from the albatross nest were, however, still widely different, spanning the entire range from minimum to maximum of the edaphically rich sites.

When volumes were expressed per g.o.d.s. (Table 5), the mires separated from the fjaeldmarks, giving a three-way division of the sites into fjaeldmarks and Ice Plateau, unenriched mires, and edaphically rich sites (Table 5). This division accounted for 95% of the variance (analysis of variance, as before). The high-altitude fjaeldmark and Ice Plateau still had higher volumes than would be expected, but the volume at the Ice Plateau, at least, was not higher than the two mildest fjaeldmark sites. The two mires were closer than by any other measure, as were all the edaphically rich sites.

Generally, volumes showed a simpler pattern than numbers. In particular, the climate-related sequence among the sites in numbers was much reduced or even completely obliterated when populations were expressed as volumes.

## Discussion

The numbers of bacteria counted in our samples were generally much higher than population estimates using plate counts from the same or similar antarctic and sub-antarctic sites (Steyn and Smith 1981), or from a wide range of tundra sites (Holding et al. 1974). This is to be expected when comparing a direct count with a plate count method, but many of our counts were also higher than most direct counts from tundra sites listed by Parinkina (1974), even though her counts may have included some dead cells. Our counts do, however, agree broadly with those of Ramsay (1983) who used acridine orange for direct counts of samples from an antarctic island. If our assumptions concerning the distinction between live and dead cells are valid, then our counts are likely to be a good estimate of the actual bacterial populations in our samples.

The wide range of both numbers and volumes, and the variety of cell types, are typical of tundra sites (e.g. Parinkina 1974; Holding 1981), where there may be as much variation among individual sites as between polar and tropical ecosystems (Swift et al. 1979).

Both numbers and volumes of bacteria separate the sites into groups related to climate and to soil nutrient conditions, especially enrichment by vertebrate excreta (e.g. compare Table 1 with Tables 3 and 5). The climatic relationship, however, is not discernible when population size is expressed as volumes $cm^{-3}$. The most important nutrients appear to be inorganic N and P, followed by cation supply, especially K and Ca. Additionally, the high volumes in the medium-altitude fjaeldmark under *Azorella* compared to the other fjaeldmark sites, coupled with the differences between populations (as numbers or volumes) expressed per $cm^3$ or per g.o.d.s., suggest that a third significant factor is supply or availability of an organic substrate, as suggested by Holding et al. (1974) for tundra bacteria generally.

Among the edaphically rich sites, there is a distinction between sites without significantly more, but with larger, cells than the mires (e.g. albatross nest and wallow), and those with higher numbers, but not larger cells (e.g. the two *Cotula* sites). The former had relatively few red cells (25% of total) while the latter were typified by the two *Cotula* sites, with a high proportion (>50%) of red cells, of which many were small. In water bodies at the same or similar sites, the difference in proportion of red cells does not appear so pronounced. Smith and Hilmer (1984), for example, state that nearly all cells from a rockhopper penguin-influenced pool fluoresced red, but also that "a high proportion" of cells in a wallow pool were red.

A combination of moderately high numbers with a high proportion of large cells (including many red cells) of all types except E, gives the *Blechnum* sample its high cell volume.

From the combination of numbers. Volumes and cell types, we can suggest some tentative hypotheses regarding the growth and reproductive strategies of the bacteria relative to site conditions. Following from our argument against converting volumes to biomass, we will confine our hypotheses to number and volume relationship. It is, however, likely that distinction between high and low volume populations, or relationships based on rank order of volumes, will also hold for biomass, despite the lack of a simple proportional relation between them.

There are two main divisions: between high and low "standing crop" and between high and low turnover. Turnover was assesed mainly on the proportions of red and green cells in each sample, and the proportions of different cell sizes of each type. A high proportion of red cells, indicating a very active population, meant that turnover was likely to be high. Small cells also suggested relatively high turnover, especially if they were also red, following the suggestion of Jenkinson et al. (1976) that small cells would more easily obtain the necessary resources for division than large cells, hence would "turn over" more rapidly. Additionally, a general knowledge of plant production and litter decomposition rates, where available, was used as a corroborative index, generally more "active" sites being assumed also to have more active microflora, hence faster turnover.

The mires have moderate levels of both standing crop and turnover, so were used as a standard with which to compare the other site groups.

The edaphically rich sites all have high standing crop, but the wallows and albatross nest can be interpreted as low-turnover sites (relative to the others in this group), the *Blechnum* site as medium-turnover, and the two *Cotula* sites as high-turnover.

Among the fjaeldmarks, the converse pattern applies. The low-altitude site has highest volume and turnover (though lower in both than the richer sites). In the medi-

um-altitude site under *Azorella* there is sufficient organic substrate to maintain an equivalent biovolume but turnover is relatively inhibited, possibly by climate. Between *Azorella* cushions climate, substrate and nutrients are all restrictive and both biovolume and turnover are low.

The high volume at the Ice Plateau is due entirely to the extremely large size of nearly all cells at this site. Here, the low temperatures might produce a general slowing of all biological activity, including both reproductive and degenerative processes, leading to a gradual accumulation of large, long-lived cells, producing in turn the observed population characteristics. It is, however, probable that in the two days it took to transport the sample to the laboratory there was a "thaw flush" of growth, since it was not possible to keep it frozen.

We therefore expect the fjaeldmarks all to be low-turnover sites, with standing crop depending mainly on substrate supply, the mires to be medium-biomass, medium-turnover sites, limited mainly by nutrients, and the edaphically rich lowland sites all to have high standing crop and turnover, with no simple limits to either, but both dependent rather on a more complex combination of environmental constraints. In this last group, we can distinguish relatively high and low-turnover sites. These hypotheses concerning relative turnover rates could be tested by measuring microbial activity at the same sites, since the two should be correlated. Widden (1977) derived a similar pattern of turnover and biomass divisions for two high-arctic soils on Devon Island, Canada, including corroborative evidence from decomposition studies. (We did attempt activity measures, using a $^{14}C$-glucose kinetic method, but some of the sample vials were damaged on the return voyage from the island, so we were not able to obtain valid results from these tests.)

There remain the two exceptional sites: the albatross nest with its considerable heterogeneity, and the high-altitude fjaeldmark, which was exceptional in almost every possible way. The former can probably be accounted for by the fact that it was not strictly a soil, but rather a conglomerate of diverse materials including peat, live and dead plant material, and extreme local concentrations of excreta, all of which could be expected to harbour very different microbial populations, both qualitatively and quantitatively.

The high altitude fjaeldmark is less easily explicable. In cell sizes, it fits the altitudinal sequence fairly well, but its extremely high numbers, and the presence of large quantities of type E cells (half the total numbers and nearly half the volume) together with the high degree of clustering, make it a unique sample in this set. Sample contamination might be a partial explanation, but even discounting, for example, the type E cells makes no great difference to the way it stands out from all the other fjaeldmark sites. A second (unlikely) possibility is some form of local enrichment (e.g. by birds) that happened to coincide with the chosen sample spot. It is, however, most likely that this sample is a genuine reflection of the microflora at a high-altitude site. While that can only be postulated, it is clear that such sites warrant further attention.

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