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3.5 Television Image Analysis of Microbial Communities in Antarctic Fellfields

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Summary: Epifluorescence microscopy has been combined with television image analysis to quantify the microbial colonization and potential stabilization of Antarctic fellfield substrata. The area of microbial coverage in micro-habitats, together with cell dimensions was measured in undisturbed samples of soil microbial crusts. The selective use of optical filters permitted quantitative differentiation between eukaryotic algae, cyanobacteria and fluorochrome-stained heterotrophic microorganisms via their distinctive wavelengths of fluorescence. Specified exclusion conditions and interactive editing of the image permitted the elimination of electronic "noise" and debris from the measurements. Selected microbial morphotypes, defined by individual cell area and elongation, were quantified independently. User-defined frames facilitate the enumeration of cells on the surface of irregular rock particles. The system was used to quantify the heterogeneity of cyanobacterial colonization of soil fines and to show its correlation with total filament length. The results indicated the occurrence of cyanobacterial "rafts" in soil crusts.

Zusammenfassung: Zur Charakterisierung der mikrobiellen Besiedlung und potentiellen Stabilisierung von antarktischen Böden wurde die Epifluoreszenzmikroskopie in Kombination mit einer Fernsehbildanalyse angewendet. Der mikrobielle Deckungsgrad in Mikrohabitaten und die Zellgrößen wurden in ungestörten Proben von Bodenkrusten gemessen. Mit Hilfe von optischen Filtern konnte quantitativ zwischen eukaryotischen Algen, Cyanobakterien und fluorochromgefärbten heterotrophen Mikroorganismen (auf Grund ihre Fluoreszenz bei bestimmten Wellenlängen) unterschieden werden. Elektronisches "Rauschen" und Schmutz konnten durch spezielle Bedingungen und interaktives Filtern des Bildes ausgeschlossen werden. Ausgewählte mikrobielle Morphotypen, definiert als Einzelzelle nach Flächeninhalt und Ausdehnung, wurden unabhängig gezählt. Spezielle Schablonen, je nach Bedarf, erleichterten die Zählung der Zellen auf der Oberfläche von unregelmäßigen Felspartikeln. Das System wurde benutzt, um die Heterogenität der Cyanobakterien-Besiedelung auf Böden abzuschätzen und um deren Korrelation mit der gesamten Filamentlänge aufzuzeigen. Die Ergebnisse zeigen, daß sich "Flöße" (wohl "Gebinde") von Cyanobakterien in Bodenkrusten bilden.

1. INTRODUCTION

Maritime Antarctic fellfield soils are disrupted by a variety of physical stresses during fluctuations in the prevailing micro-climate. These include major disruption by ice-crystals during frost-heave (CHAMBERS 1967), cracking of the surface during desiccation, abrasion by wind when in the dried condition, and the turbulence of melt-water. These factors impinge directly on the microbial crust which forms on the soil surface. The community is dominated by primary producers including cyanobacteria and eukaryotic algae which may, under conditions of prolonged water-availability, culminate in a visible felt. It is a mixed community which also supports heterotrophic bacteria and, to a lesser degree, yeasts, other microfungi and protozoa.

Filamentous cyanobacteria and algae are common in maritime Antarctic soil crusts, and the production of mucilagenous sheaths is evident on autotrophs and heterotrophs alike. The combination of these structural and adhesive features enables the microbial community to form "rafts" which may be able to withstand the disruptive effects of the climate (WYNN-WILLIAMS 1985). These may then coalesce to form a crust which prevents erosion and provides a stable substratum for subsequent colonization by cryptogams such as lichens and mosses (SMITH 1985) and by invertebrates such as protozoa (SMITH & TEARLE 1985) nematodes and micro-arthropods (BLOCK 1984).

It is therefore hypothesised that the microbial colonizers of Antarctic fellfield soils have characteristics, reviewed in WYNN-WILLIAMS (in 1986), which help them to resist adverse eco-physiological conditions and make an important contribution to the stabilisation of the mineral fines. These morphological and physiological characteristics may be lost or exaggerated in artificial cultures, and the essence of the stabilisation is the interaction with the natural inorganic soil particles. The importance of the soil matrix in influencing the availability to micro-organisms of moisture and nutrients under various conditions of physico-chemical stress has been reviewed by NEDWELL & GRAY (1987). To determine the extent of microbial colonization and stabilising activity, the communities must be examined in situ in relatively undisturbed crusts. This requires epifluorescence microscopy to utilize the autofluorescence of primary producers (SCHREIBER 1980, SIERACKI et al. 1985, WOOD et al. 1985, ESTEP et al. 1986) and to detect the presence of heterotrophs by means of fluorochromes such as fluorescein

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isothiocyanate (BABIUK & PAUL 1970), fluorescein di-acetate (SÖDERSTRÖM 1977, LUNDGREN 1981, CHRZANOWSKI et al. 1984) and Acridine Orange (AO) (TROLLDENIER 1973, WYNN-WILLIAMS 1985). The technique makes cells visible relative to their opaque substratum. However, the heterogeneity and complexity of even relatively species-poor fellfield microbial communities makes quantification of colonization and changes in response to physical and nutritional changes in their micro-environment difficult.

These difficulties can be overcome by using a combination of epifluorescence microscopy and television image analysis (TVIA). The application of this technique to microbial ecology has hitherto focussed mainly on aquatic populations (SIERACKI et al. 1985, WOOD et al. 1985, BJØRNSEN 1986, ESTEP et al. 1986). Aspects of the theory and practice of the technique relevant to environmental micro-organisms have been sumarized by ESTEP et al. (1986). The technique, as applied for the first time to Antarctic fellfield soils (WYNN-WILLIAMS 1986), quantifies the dimensions of cells, the total area of their coverage, such as cocci, bacilli and filaments. Not only can morphotypes be differentiated in terms of their size and shape, but they can also be grouped according to the wavelength of their fluorescence by the use of selective optical filters. Photo-autotrophs can be imaged using the auto-fluorescence at 685 nm of their primary photosynthetic pigment, chlorophyll$_\alpha$ (Chl.$_\alpha$). The cyanobacteria can be separated from the algae by selectively transmitting the fluorescence of phycobilin accessory pigments (COHEN-BAZIRE & BRYANT 1982). All cyanobacteria contain the accessory pigment phycocyanin (PC) with a fluorescence peak at 655 nm (SCHREIBER 1980). An important filamentous cyanobacterium in soil fines at Signy Island, *Phormidium autumnale* (WYNN-WILLIAMS 1985, 1986), produces the additional, gold-fluorescing, accessory pigment phycoerythrin (PE) with a peak at 585 nm which can be imaged independently of PC (WOOD et al. 1985).

The wavelengths of fluorochromes responsive to the chemical and metabolic status of both autotrophs and heterotrophs can be used both to reveal the cells and, under certain conditions, indicate their viability. Auramine O appears to selectively stain viable algal cells better than non-viable ones (HAWES & DAVEY in press). Under standardized low concentrations of stain, the majority of AO-stained fellfield bacterial cells fluoresce apple green which has been interpreted as the normal dormant or slowly metabolising state of soil bacteria, although the status of orange cells is ambiguous (JONES & SIMON 1975, WYNN-WILLIAMS 1985). Fluorescein diacetate has been recommended as a vital stain for soil bacteria (LUNDGREN 1981) and would be suitable for TVIA.

Even in the naturally-sorted mineral fines of frost polygons, particle size heterogeneity and the presence of debris requires interactive editing of the television display to enhance the microbial images. However, the use of pseudocolour not only facilitates this process but also permits the quantification of fluorescence of defined intensity. This further aids the interpretation of grouping and viability.

The aim of the present study was to apply selective optical filtration and television image analysis to epifluorescing preparations of microbial communities in Antarctic fellfield soils as part of a programme to determine their role in soil colonization and stabilisation.

2. METHODS

The British Antarctic Survey Fellfield Ecology Research Programme (FERP) study site at Jane Col on Signy Island (60° 43' S, 45° 35' W), South Orkney Islands is a primitive fellfield at 150 m altitude with frost-sorted stone polygons and negligible macrovegetation. The studies reported here concentrated on mineral fines of mean grain side-length 45 µm (range 13—132 µm). The mean water-holding capacity of the soil was *c.* > 75% and it was saturated for much of the spring when meltwater was abundant.

Cores (15 mm diameter x *c.* 5 mm deep) were taken at 5 cm intervals on a 25 cm x 25 cm grid in the centre of a frost polygon. They were transported to the BAS research station within 2 h. in Repli dishes (Sterilin Ltd.) at temperatures <15° C and either held at 4° C, usually for <3 h. before treatment, or frozen and stored in the dark at –20° C for subsequent examination. The cores were examined either directly to detect natural aggregations or they were homogenized for enumeration as described fully in WYNN-WILLIAMS (1985).

The undisturbed surface microbial crust was examined directly by mounting in water-soluble Citifluor AF2

photofading retardant (Citifluor Ltd., London) under a cover slip, rehydrating if necessary. This retardant was effective for autofluorescence as well as for Acridine Orange (WYNN-WILLIAMS 1985). Observations were made with a Leitz Laborlux 12 microscope with a Ploemopak epifluorescence illuminator with an HBO-200 50 w mercury arc lamp and either an N2.1 filter block, primarily for cyanobacteria and algae, or an I2 filter block, primarily for fluorochromed bacteria. The combinations of filters used for exciting and selecting specific wavelenghts of fluorescence representative of different pigments and taxonomic groups are given in Table 1. Leitz Fluotar objectives of large numerical aperture were used to optimise fluorescence. Magnifications of x100 and x250 were used for general surveys of algae and larger cyanobacteria, and x500 magnification was used for more detailed studies of pigmentation. Water immersion objectives were preferred to oil for flexibility of examination at various magnifications and to decrease drag and lift on the cover-slip.

For direct enumeration of the microflora in soil smears, cores were homogenized in 1/10 strength Ringers solution containing 0.2% sodium hexametaphosphate to prepare a ten-fold dilution series. For direct counts of algae and bacteria, after settling the 10^{-1}, 10^{-2} and 10^{-3} dilutions respectively for 30 s., 0.5 µl surface aliquots were pipetted on to PTFE-coated 3 mm-aperture Multispot slides (C. A. Hendley (Essex) Ltd.). After air-drying, the preparations for algae and cyanobacteria were mounted in Citifluor AF 2, a glycerol/phosphate-buffered-saline based photofading retardant, under a cover slip. Bacteria and other heterotrophs were stained with a sterile-filtered 1:15,000 dilution of Acridine Orange (AO) for 5 min. The AO stain resulted in excessive background fluorescence when the smears were mounted directly in Citifluor AF2. The smears were therefore rinsed in distilled water, immersed in a 1:1 dilution of Citifluor AF2 for 3 min and rinsed for a further 10 s in water. The smears were then air-dried and observed without a cover slip in low-fluorescence immersion oil or Citifluor AF87, an oil-based

Microbial group	Pigment selected	Excitation filters (nm)	Fluorescence peak (nm)	Suppression filters (nm)
Cyanobacteria	Phycoerythrin (PE)	515–560 Leitz N2.1 block 450–490 Leitz I2 block	565	LP* 580 (orange) Leitz N2.1 LP 515 (green) Leitz I2
				BP** 520 (green) Schott VG6
Cyanobacteria	Phycocyanin (PC)	515–560 Leitz N2.1	655	LP 580 Leitz N2.1
				IF*** 640 Oriel 53940
	PC + Chl_a			LP 610 (orange) Schott RG 610
Algae and Cyanobacteria	Chlorophyll$_a$ (Chl_a)	450–490 Leitz I2	680	LP 515 Leitz I2
		515–560 Leitz N2.1	655	LP 580 Leitz N2.1
				LP 665 (red) Schott RG665
Bacteria and microfungi	Acridine Orange	450–490 Leitz I2	530–650	LP 515 (green) Leitz I2
		515–560 Leitz N2.1	600–650	LP 580 Leitz N2.1 BP 520 (green)
				Schott VG6 RG 610 (orange)
	Fluorescein diacetate/ isothiocyanate	450–490 Leitz I2	525	LP 515 Leitz I2
Algae	Auramine O	450–490 Leitz I2	550	LP 515 Leitz I2
				BP 520 Schott VG6

Tab. 1: Optical filters used for selective observation and imageing of autofluorescing and fluorochromed soil microbial colonizers. * Long pass filter: ** Band pass filter: *** Narrow band interference filter

photofading retardant, both of which give less background fluorescence. At least 10 fields (totalling >400 cells) per sample were routinely counted at magnifications ranging from x100 to x1000 as appropriate.

The samples were quantified using a Panasonic WV1850 Extended-red Newvicon camera with automatic gain control, coupled to a Seescan I3000 image analyser (Seescan Devices, Cambridge, U.K.). The system comprised a Solitaire Plus processing module with 256 x 256 x 8 bit resolution, combined with a high resolution monitor displaying monochrome images with pseudocolour superimposed. Of the image processing facilities available, the optimal combination for the present samples consisted of a high pass filter and contrast enhancement (requiring the setting of the respective black to white limits of the 0—128 grey-scale range for optimal contrast between cell and background). The thresholds were set on the same scale and the images were edited in original or zoomed mode as necessary. Exclusion conditions based on area and elongation were used to select specific cell morphologies and to eliminate electronic "noise", debris and cell clumps. The results consisted of total and mean (± SD) object data for counts, length (l), breadth (b), elongation (l/b), area (a), perimeter (p), and shape factor ($4\pi a/p^2$). Using any combination of up to 8-band pseudocolour, it was possible to quantify allocated bands of grey-shades (on the 0—128 shade scale) corresponding under certain conditions to the fluorescence of different cell pigments in the same field of view. Using a software routine, the analyser was calibrated with a stage micrometer slide observed with transmitted light. A resulting table giving the full height of the monitor display in microns at the required magnification provided a quick reference for calibration thereafter. The true dimensions of *Phormidium autumnale* cells were determined by calibration with an eyepiece graticule. These data were used for internal calibration of the thresholds for TV imageing, using the 'Measure object' menu (Fig. 3b), by adjusting the image breadth to the measured breadth of the cells. Filters were selected to avoid overloading or underexposing the camera, and the sensitivity of the detector was adjusted to ensure a calibrated image with accurate dimensions. The intensity of fluorescence varied but the dimensions of a bright or faint object were adjusted to be the same.

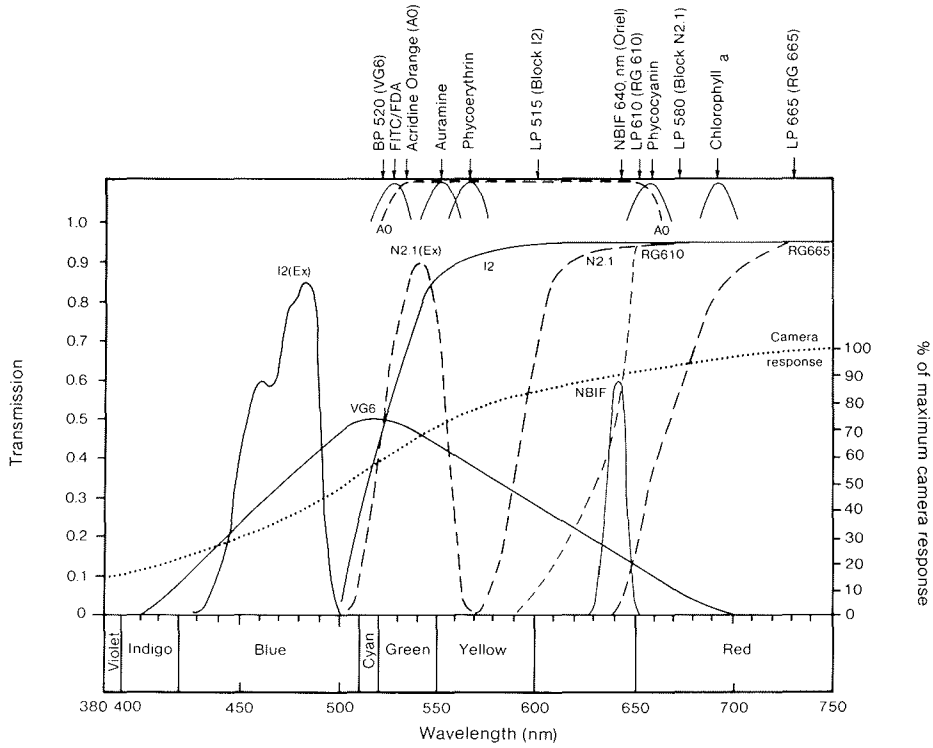


Fig. 1: Transmission curves of selected excitation (Ex) and suppression filters together with stylized fluorescence peaks of fluorochromes and photosynthetic pigments, relative to the TV camera response curve. BP = Band pass filter (peak wavelength), LP = Long pass filter (wavelength at 50% transmission. NBIF = Narrow band interference filter (peak wavelength). Codes are Schott catalog numbers. FITC = fluorescein iso-thiocyanate, FDA = fluorescein di-acetate.

The excitation and transmission spectra in Figure 1 show that the choice of excitation and suppression filters for the selective transmission and image analysis of a particular pigment is necessarily a compromise. Separation of PC fluorescence from Chl_{α} emissions requires a narrow band filter at 640 nm despite the PC peak being at 655 nm. Moreover, the TV camera was highly sensitive to red light (maximal at *c.* 750 nm and >90% maximal at the fluorescence peaks of PC and Chl_{α}) but with lower sensitivity to green light (c. 40% maximal at the fluorescence peaks for FITC, FDA and green Acridine Orange).

For photomicrography, an Olympus OM2 camera mounted on a Micro Instruments Ltd. adaptor with a x3.2 eyepiece was used on automatic setting. Exposure times (ranging from <1 sec to 25 sec at ISO 400 setting) were recorded as a measure of the intensity of fluorescence resulting from various combinations of filters. These times were converted into percentage of maximal fluorescence emission relative to the shortest exposure time. Colour slides were obtained using Fujichrome 400 film (ISO 400). Exposures in excess of 20 sec showed evidence of reciprocity failure, and also drained the camera battery. An external power source has since been devised. Photographs were taken from the image analyser monitor using the same camera and film.

3. RESULTS

3.1 *Selective Detection of Photopigments and Fluorochromes*

Initial studies of the microflora of undisturbed fellfield soil crusts at Jane Col (WYNN-WILLIAMS 1985, 1986) showed the very large cyanobacterial filaments of *Phormidium autumnale* (BROADY 1979, BROADY et al. 1984) to be dominant colonizers and valuable biological markers (Fig. 2a). As they contain all three photo-pigments, Chl_{α}, PC and PE, they were used to test the use of filter blocks and accessory suppression filters for discrimination between the pigments both optically and by TVIA.

Accessory suppression filter[1]	Wavelength transmitted (nm)	Dominant fluorescing pigment	Percentage fluorescence
Green excitation (N2.1 filter block):			
None	>580	PE, PC, Chl_a	100
VG6	520±35	PE	33
RG 610	>610	PC, Chl_a	50
NBIF 640	640±10	PC	29
RG 665	>665	Chl_a	26
Blue excitation (I2 filter block)			
None	>515	PE, PC, Chl_a	100
VG6	520±35	PE	53
RG 610	>610	PC, Chl_a	62
NBIF 640	640±10	PC	36
RG 665	>665	Chl_a	53

Tab. 2: Diminution of the intensity of auto-fluorescence from *Phormidium* cells transmitted by selective suppression filters relative to the fluorescence evoked by green and blue excitation.

The percentage transmission of fluorescence calculated from photographic exposure times (Tab. 2) shows that the 33% transmission by the VG6 filter of green light from the N2.1 block coincides with only 40% maximal sensitivity of the TV camera (Fig. 1), greatly restricting the amount of green fluorescence transmitted. Despite a lower total fluorescence emission than that evoked by green excitation (8 s exposure and 6 s respectively), the excitation by blue light of a greater proportion of green fluorescence is therefore be more suitable for FDA, Acridine Orange (green cells) and PE fluorescence in the presence of PC. Conversely, the relatively low percentage transmissions of red light filters were more than compensated for by the red-sensitivity of the camera, necessitating a neutral density filter to prevent overloading of the automatic gain control (Tab. 3).

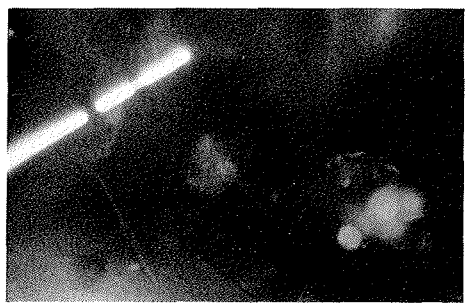
3.2 *Quantification of Microbial Cells by TV Image Analysis*

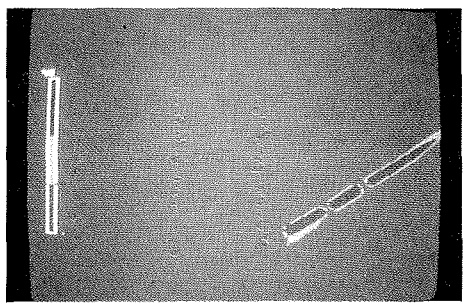
The reproducibility of object dimensions at various wavelengths is shown in Table 3. The errors of over- and underexposure, and the different total emissions from N2.1 and I2 blocks are also conspicuous. The variation in intensity of fluorescence, whether due to emissions from different pigments or differences due to the metabolic state or viability of the cells, was quantified using bands of pseudocolour. Subtle differences in shades of grey in the original image become conspicuous when converted into colours (Fig. 2b). Usually, less than the eight colours

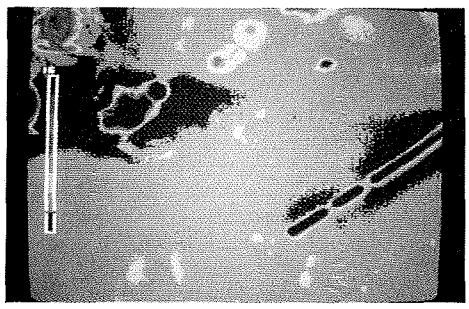
Filter block	Accessory filters*	Dominant pigments	Area of cell image** (μm^2)
I2	None	PE, PC, Chl_a	145.2
N2.1	None	PE, PC, Chl_a	Overloaded
N2.1	9% neutral density (ND)	PE, PC, Chl_a	159.4
N2.1	VG6	PE	159.4
N2.1	9% ND + RG 610	PC, Chl_a	146.6
N2.1	9% ND + RG 630	PC, Chl_a	144.5
N2.1	NBIF 640	PC	161.6
N2.1	9% ND + RG 665	Chl_a	127.6
N2.1	RG 665	Chl_a	359.7 (overloaded)
I2	RG 665	Chl_a	67.2 (under-compensated)

Tab. 3: Relative constancy at selected wavelengths of the area of epifluorescence TV images of a *Phormidium* cell containing phycoerythrin (PE), phycocyanin (PC) and chlorophyll$_a$ (Chl_a). * See Table 1 for details; ** Measured at x500, see Fig. 3.

were needed. Green, red and dark blue were found to give good visual contrast, and such displays ably demonstrated the selective power of accessory filters for specific pigments, such as NBIF 640 for PC in cyanobacteria with the total exclusion of algae containing Chl_a alone (compare Figs. 2a, 2b and 2c).







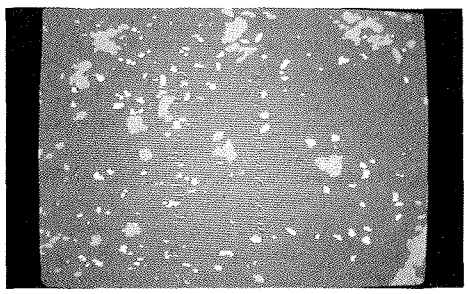


Fig. 2a: Group of auto-fluorescing algae (red, chlorophyll$_a$) and cyanobacterial *Phormidium* cells (yellow, phycoerythrin) in an undisturbed fellfield soil crust. Mountant: Citifluor AF2; excitation and suppression by I2 block alone. Magnification x500. Breadth of *Phormidium* cells = 6—7 µm.

Fig. 2b: Pseudo-colour TV display of the algae and *Phormidium* in Fig. 2a showing the 8 colour segments of 16 shades of grey.

Fig. 2c: Pseudocolour TV display of the cells in Fig. 2a after filtering the fluorescent image generated by a N2.1 block through an NBIF 640 filter to exclude all pigments but phycocyanin. Only three colour bands have been selected.

Fig. 2d: The use of exclusion conditions to eliminate "noise", debris and clumps (all pink) from a field of bacteria (yellow) for enumeration of an enrichment culture of fellfield soil from Jane Col. Bacteria stained with Acridine Orange, mounted in Citifluor AF87. Illumination: I2 block. Magnification x1000.

Exclusion conditions based on area and elongation were especially valuable for quantifying fields of fellfield bacteria. Acridine Orange fluorochromed dilution slides of enrichment cultures of bacteria from a fellfield soil crust from Jane Col were dominated by a relatively homogeneous field of coccobacilli but also contained aggregates and small particles of debris. Both these unwanted components of the image were eliminated in terms of maximum and minimum area, and a "window" of elongation was defined so that only the required objects were quantified (Fig. 2d). To ensure internal standardisation, these exclusion conditions were based on data obtained from the objects themselves, using the "Measure object" menu (Fig. 3). Finally, histograms of frequency distribution of cell dimensions were displayed before printing of storing. Exclusion conditions could also be used to differentiate morphotypes in field-fresh samples.

3.3 *Microbial Colonization of Soil Crusts*

When reproducible thresholds have been established and calibrated, TVIA can be used for micro-ecological surveys of communities too complex for visual analysis. A magnification of x100 permitted enumeration of the large cyanobacterial filaments.

Figure 4 illustrates the heterogeneity of phototrophic microbial colonization of fellfield fines in a 25 cm^2 quadrat at Jane Col. The apparent patchiness was confirmed statistically by the variance (s^2) exceeding the mean for both the total area of cells and their total length per unit area (Tab. 4). There was a tenfold variation in the extent of colonization, greatly exceeding the range of the 95% confidence limits. Nevertheless, the total percentage cover was still very low (maximum 6%) on the micro-ecological scale of a field of view measuring 866 x 1262 µm.

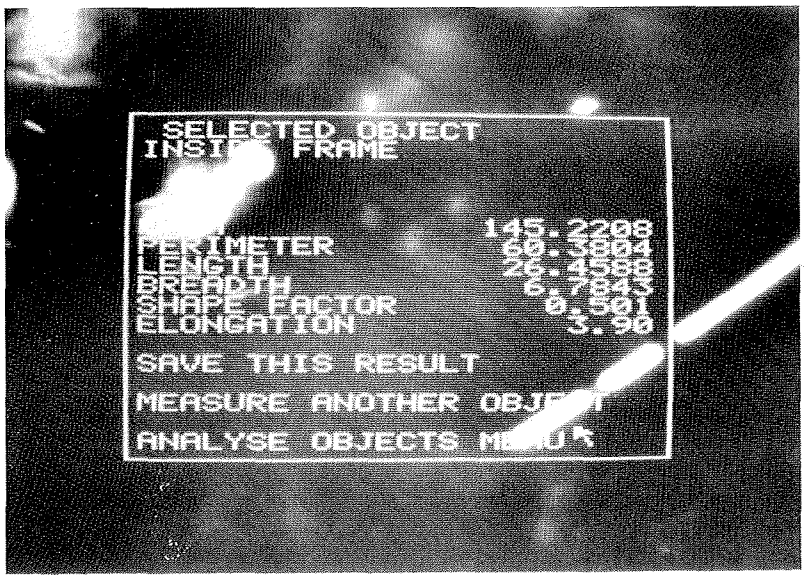


Fig. 3: The "Measure object" display of cells in Fig. 2a showing the dimensions of the *Phormidium* cell arrowed.

The apparent relationship between total cell area and length (Fig. 4) was confirmed by a correlation coefficient of 0.968 ($P < 0.001$,) so that 93.7% of the variation in area could be explained by variation in total length of cells which were predominantly filamentous.

At the thresholds required to ensure continuity of the images of filaments, there was a tendency to exaggerate their breadth by a factor of up to two (Tab. 4). However, the breadth calculated from the brighter pseudocolour band (grey shades 80—128) gave a measurement close to the true width. It was therefore possible to obtain reliable dimensions by a combination of object measurements and grey-shade band data to compensate for the difficulties of imageing cells of variable fluorescence and delineation. The exceptions to this correlation at coordinates x2, y2 and x3, y2 indicated a disproportionately large amount of filaments at these locations.

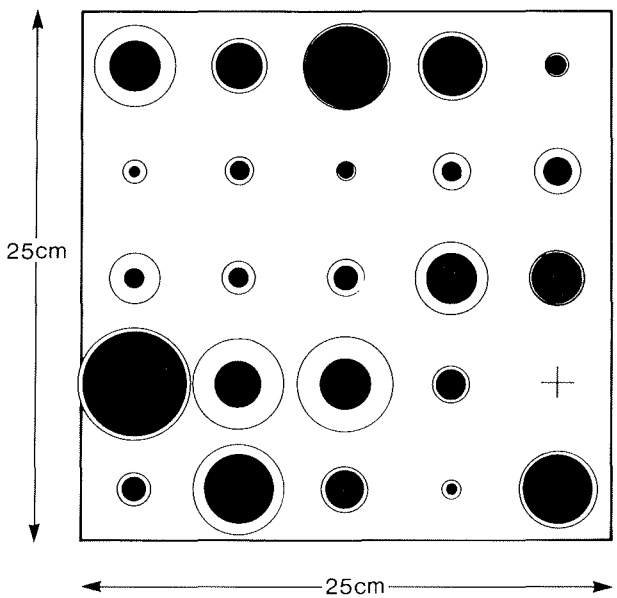


Fig. 4: Heterogeneity of autofluorescent algal/large cyanobacterial cell cover of mineral fines in a quadrat in the centre of a frost polygon at Jane Col. Quantification was by TV image analysis of epifluorescence microscope displays at magnification x100. The diameter of the black inner disc is proportional to total cell area of cover, and the outer to total cell length per unit area (c.f. Tab. 4).

Statistic	Mid-Grey band* (% cover)	Grey-white band** (% cover)	Total cover (%)	Total Area ($\mu m^2\ mm^{-2}$)	Total length ($\mu m^2\ mm^{-2}$)	Object breadth (μm)	Grey-White breadth*** (μm)
Mean (x)	1.03	1.35	2.37	95 646	6 202	15.0	8.2
S.E. (n=24)	±0.13	±0.19	±0.31	±11 195	±660	±0.4	±0.5
Confidence limits (95%)	±0.27	±0.39	±0.64	±23 162	±1 336	±0.8	±1.0
Maximum	2.67	3.60	6.26	223 197	11 989	18.8	12.3
Minimum	0.20	0.26	0.46	23 405	1 724	11.2	3.8

Tab. 4: TV image analyses of the heterogeneity of microbial percentage cover and total microbial cell area and length per unit area of fellfield polygon fines at Jane Col. * Grey shades 60–80 (medium fluorescence) on the TV image grey scale 0–128. ** Grey shades 80–128 (bright fluorescence). *** Bright central portion (grey-shades 80–128) of the pseudocolour image.

4. DISCUSSION

The mineral fellfield soils of patterned ground at Jane Col have a microbial crust which ranges in composition from a scattering of filaments and aggregates to a mixed algal-cyanobacterial felt visible to the naked eye (WYNN-WILLIAMS 1986). The phototrophic colonizers range from large eukaryotic algae such as filaments of *Zygnema*, diatoms and coccoid unicells, through large cyanobacterial filaments such as *Phormidium autumnale*, to small cyanobacteria of similar size to associated heterotrophic fellfield bacteria. The energy-independence of the phototrophs enables them to be primary colonizers, and preliminary studies (WYNN-WILLIAMS 1985) indicate that the filaments form stabilising meshes while their mucilages (DREWS & WECKESSER 1982) provide cement for aggregation of soil particles, resulting in local "rafts" of stable crust. The phototrophs are therefore an essential starting point for a study of colonization processes in Antarctic fellfield soils.

The conspicuous gold-fluorescence of the large filaments of *Phormidium autumnale* in the polygon soil crusts at Jane Col suggested that they were primary structural contributors to the soil crust. The ability to separate them optically from algae and other cyanobacteria devoid of phycoerythrin (PE) is of value for interpreting the relative contributions of various micro-organisms to soil stabilisation. PE yields a fluorescence 19.3x brighter than equimolar fluorescein (ONG et al. 1984), so that seasonal and spatial variation in dominant species such as *P. autumnale* can be readily detected. However, the sensitivity of the camera to the orange-red fluorescence of PC provides even more available light for the optical separation of cyanobacteria from PC-free algae by TVIA. The

camera was more sensitive in the red region than that used by ESTEP et al. (1986) for similar studies, but not as comprehensively sensitive as the silicon-intensifier target (SIT) camera used by BJØRNSEN (1986) which has the additional advantage of high sensitivity at the green end of the response curve. High sensitivity is especially advantageous at the low magnifications required for quantifiying wide fields of large micro-organisms because the epifluorescence objective also acts as the condenser for the excitation beam. This results in a lower intensity of illumination at lower magnifications.

The fluorescence of Chl$_\alpha$ at 665 nm is augmented by that of allophycocyanin (APC), another accessory pigment found in all cyanobacteria (COHEN-BAZIRE & BRYANT 1982) Therefore there is no shortage of light for TVIA of total soil photo-autotrophs. SCHREIBER (1980) states that at low excitation intensity, green light at 515—560 nm barely excites Chl$_\alpha$. The resulting red fluorescence transmitted through the RG 665 filter used here must therefore be mainly due to cyanobacterial APC (COHEN-BAZIRE & BRYANT 1982). It is therefore not possible to assume that the light transmitted through the RG 665 filter represents the total Chl$_\alpha$ present in the colonizing cells, unlike the conclusion drawn for PC using the narrow-band 640 nm filter. Combinations of the field diaphragm, trinocular head prism and neutral density filters were used to obtain the optimal image intensity which the results here show to have a relatively broad "window".

The energy transfer from phycobilins to Chl$_\alpha$ is proportional to the attachment of phycobilisomes to the thylakoids, and PC fluorescence increases as the efficiency of the energy transfer decreases. In mesophilic *Anacystis nidulans* the efficiency decreases below 10° C, being especially temperature sensitive in the range +2° to –2° C (frequent in spring and summer at Jane Col), resulting in a maximum increase in PC fluorescence of 300% at 5° C (SCHREIBER 1980). This phenomenon may be a valuable ecological indicator of metabolic activity under controlled conditions in situ which is quantifiable by pseudocolour band TVIA as described here. However, other factors affecting phycobilisome efficiency such as pH, decreasing concentration of divalent cations and increasing monovalent anions must remain constant as they also stimulate cold-induced PC-fluorescence (SCHREIBER 1979).

The gold appearance of the *P. autumnale* with blue excitation indicated a greater stimulation of PE than the orange-red fluorescing PC. However, green excitation was necessary to stimulate enough PE fluorescence for filtration through a green filter (to exclude PC and Chl$_\alpha$ fluorescence) for TV image capture at the less efficient end of the camera response spectrum. Further losses due to photofading, particularly in high-energy blue light, were overcome in two ways: Firstly, the use of Citifluor (WYNN-WILLIAMS 1985) delayed fading during the scanning of samples, and secondly the selected TV image was "frozen" so that the excitation shutter could be closed.

Throughout the examination of the colonizer microflora, the relative position of the cells was maintained with minimal disturbance. This sustains the main advantage of TVIA over bulk determinations of fluorescent pigments and fluorochromes which lies in monitoring the spatial interaction of the colonizers with the substratum and each other. This permits the quantification of area of cover as for macroscopic cryptogams (SMITH 1985), providing ecological continuity in analysis of the colonization process. The complexity of the filamentous meshes and variety of morphology has precluded such analysis without TV assistance. Nevertheless, the TV image analyser does not have the visual acuity and interpretive powers of the human eye and brain. Decisions must be taken on the optimal threshold necessary to maintain the integrity of images of filaments without exaggerating their dimensions or detecting background debris. SIERACKI et al. (1985) and ESTEP et al. (1986) have discussed the digitization errors which occur with cyanobacterial images, using marine strains of *Synechococcus* as their example. The present studies revealed an exaggeration of breadth measurements during the analysis of terrestrial cyanobacteria by pixel summation. The use of selective bands of pseudocolour permitted better estimates of the known breadth of *Phormidium* cells whilst retaining the continuity of filaments. The error was lower at higher magnifications as the percentage of pixels in the peripheral grey zone was porportionately lower. This must be balanced against the shallower depth of focus at higher magnifications which is a major limitation for the examination of undisturbed soil crusts. This limitation is minimized by the use of naturally-sorted fines from frost polygons which frequently have flat, relatively stone-free surfaces. Such material is valuable for investigating fundamental ecological processes even though it represents only one type of fellfield substratum.

Elongation was underestimated because of the exaggeration of breadth so that it is necessary to determine the true breadth of the required cells at high magnification and to relate this to the apparent breadth at the field scanning

magnification. This will permit the generation of appropriate exclusion conditions and correction factors length was proportionally much less. The pseudocolour band area method used here is an alternative method of breadth determination. The measurement of the dimensions of the required objects in the field of view was a valuable internal standard for setting exclusion conditions for whole fields.

The use of TVIA is a novel approach to the micro-ecology of Antarctic soil micro-organisms. CAMERON & DEVANEY (1970) related scanning electron microscope images of soil crusts to their optical appearance but were unable to quantify the micro-organisms. WYNN-WILLIAMS (1986) used a monochrome TV analysis system to detect the response of Antarctic soil microbial autotrophs and heterotrophs to nutritional amendments and to different particle sizes for settlement from meltwater. However, the absence of pseudocolour restricted the interpretation of dimensional errors. The present results are consistent with the hypothesis that the occurrence of "rafts" of filamentous and mucigel-cemented micro-organisms, primarily phototrophs, is an important factor in the colonization of mineral fellfield soils (WYNN-WILLIAMS 1985). The close correlation of heterogeneity of area coverage with total cyanobacterial filament length emphasized the apparent importance of filaments in the colonization process as revealed by TVIA.

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