Exploration Geomicrobiology – Developing bio-indicator technology for mineral exploration

Rebecca Pohrib

Department of Geology and Geophysics

School of Earth and Environmental Sciences

University of Adelaide, South Australia

rebecca.pohrib@student.adelaide.edu.au

25 October, 2010

Supervised by Dr. Frank Reith

Abstract

Geomicrobiology is a relatively new approach for mineral exploration research; it shows promise as a means of enabling researchers to cheaply and quickly categorising microbes based on specific factors (geochemistry, underlying geology, regolith landforms, land-use, sample depth, geophysics (magnetic survey) and mineralisation). The research site is located at the Hillside IOCG-style deposit, Yorke Peninsula in South Australia. Above the zone of mineralisation and from background areas, DNA was extracted from the surface (0.03 m) and sub-surface (0.03 – 0.5 m) soils. Terminal restriction fragment length polymorphism (tRFLP) and multivariate statistical methods (nmMDS, CAP, Permanova, RELATE) were employed to analyse the relative similarities between soil communities of bacteria, fungi and archaea. The results of the experiment demonstrate that microbial community composition of the Hillside site can be linked to site relevant factors such as geochemistry, underlying geology, regolith landforms, land-use, sample depth, geophysics and mineralisation. Primarily, land-use and depth stand out as being the major factors driving microbial communities of bacteria and fungi ($P < 0.05$), with archaea showing no significant effect. Genetic richness was highest in bacteria and fungi surface soil samples. Significant differences ($P < 0.05$) were found in microbial communities between the different factors. Geochemistry and biological data sets can be linked together (RELATE). Non-metric multidimensional scaling was not sufficient to elucidate difference in factors between populations. However, using constrained canonical analysis of principal co-ordinates differences become evident. Geophysics, mineralisation and geology displayed some promising results but further research is

needed to gain a better understanding of the interaction of these factors with microbes.

Contents

1. Introduction

Australia has been a tectonically stable continent for a long period of time, allowing millions of years of transported overburden to accumulate. This presents a problem to mineral exploration as rock outcrops are becoming harder to find (Corbett, 1973; Butt *et al*., 2005). At the Hillside Copper mine site, South Australia, this is the exact case with overburden reaching down 5 to 70 (m) (Rex Minerals, 2008) and given the constraints with initial drilling, new and innovating techniques are being looked into to help with the discovery of mineral deposits.

Attention to the use of microbes in mineral exploration has been gaining momentum in the last decade or so with Frank Reith, Henry Ehrlich, Steven Wakelin, Stephen L. Rogers, amongst many others leading the field in this area (Reith and Rogers, 2007; Ehrlich, 1998; Wakelin *et al*., inprep). Geomicrobiological observations were made in the 1800s, with Ehrenberg, Winogradsky and Harder being the first to contribute to the field (Ehrlich and Newman, 2009) and noting how through laboratory studies specific organisms were significant for geomicrobiological transformations. In the last few years, there has been an increased emphasis on the extent of microbial activity out in the field.

Micro-organisms are the dominant life forms on Earth, with bacteria constituting 50% of the living biomass (Reith *et al.*, 2009). Microbial processes are known to drive processes involved in mobilization, distribution and speciation of many trace metals under a wide range of environmental conditions (Reith *et al.*, 2009). Micro-organisms are highly diverse and abundant in soils with vast population sizes. This

can be seen in soils and surface waters containing up to '10^{12} cells/g, sediments and shallow regolith materials up to 10^6 cells/g whereas in the deep sub-surface (between 50 – 10 000 m) 10^4 to 10^5 cells/g have been detected' (Reith and Rogers, 2007). Besides being genetically and ecologically extremely versatile micro-organisms, and in particular bacteria, have developed a wide spectrum of metabolic capabilities including the ability to utilize inorganic compounds in the production of energy. Elements that bacteria are known to reduce or oxidise in order to gain metabolic energy include H, C, P, V, Mn, Fe, Co, Cu, As, Se, Br, Mo, Sn, Sb, Te, Hg, W and U (Ehrlich, 1998).

Microbial processes are known to drive processes involved in the mobilization, distribution and speciation of many trace metals under a wide range of environmental conditions (Reith and Rogers, 2007) and this may be of great importance to mineral exploration. Some bacteria use metal ions as their energy source by converting or chemically reducing the dissolved metal ions from one electrical state to another. This reduction releases energy for the bacteria's use and thus concentrates the metals into what ultimately / possibly can become an ore deposit. Through research certain iron, uranium and even gold ores are thought to have formed as a result of microbe action (Reith *et al.*, 2008; Dexter-Dyer *et al*., 1984).

The particular importance of micro-organisms in processes relevant to mineral exploration lies in their ability to promote mineral dissolution and diagenesis as well as control major and trace metal mobilisation, transport and precipitation that may lead to the formation of secondary mineralisation and anomalies in and around

mineralised zones (Ehrlich, 1998). All microbes employ metal species for structural functions and / or catalytic functions and depending on the quantity of metals microbial interactions can exert a major impact on metal or metalloid distribution in the environment (Ehrlich, 1997). This can be seen at the Teutonic Bore in Western Australia where high S and Fe content of the material provides a rich resource to drive microbial oxidation, thereby supporting bacterial growth to some point (Wakelin *et al*., inprep). It is increasingly recognised that many mineral transport and transformation processes, previously considered to be chemically driven, are in fact controlled by microbes (Ehrlich, 1998).

The Hillside Copper Mine site, South Australia, is an excellent site where the influence of geochemical and environmental factors on microbial communities can be explored. The mine site has known mineralisation and different landscape settings in the area and it provides an excellent environment to work in. To study these relationships, recently developed molecular microbiology techniques were used to study gene expression and environmental processes. Culture independent techniques such as terminal restriction fragment length polymorphism (tRFLP) were used which is a molecular biology technique for the profiling of microbial communities based on the position of a restriction site closest to a labelled end of an amplified gene. The method is based on digesting a mixture of PCR amplified variants of a single gene using one or more restriction enzymes and detecting the size of each of the individual resulting terminal fragments using a DNA sequencer. This then generates a 'fingerprint' of an unknown microbial community (Marsh, 1999). These new and innovating molecular techniques are becoming important

because samples that were taken required growing cultures but now a soil sample can be analysed without the need for culture dependent techniques.

Furthermore, recent research suggests that by identifying biogeochemical processes that lead to the dispersion and accumulation of trace elements in soil, and quantifying the reaction kinetics of these processes in different materials, this new field of research may help mineral explorers to predict dispersion, transport and concentration of trace elements in and around mineralised zones (Reith and Rogers, 2007). Identification of the microbiota and microbial processes that control the solubilisation, transport and precipitation of trace metals in the soil should lead to the discovery of indicator micro-organisms and the development of microbial biosensors for detecting concealed mineralisation (Reith *et al.*, 2007).

This project intends to bring together a geological based perspective and biological based perspective. This will be achieved by using a pre-existing geochemistry data set by Byron Dietman (unpub) and the biological dataset collected. It is innovative in that a new approach is being used in the research area, which has not previously been attempted and of which could revolutionise finding deposits in a much easier and more environmentally friendly manner, without the initial need of drilling.

The hypothesis of this work is:

Microbial community of the Hillside Mine site can be linked to site relevant factors such as geochemistry, underlying geology, regolith landforms, land-use, sample depth, geophysics (magnetic survey) and mineralisation.

To test this hypothesis the aims of the study are;

- To assess microbial community as indicators of mineralisation by looking at the differences in microbial profiles expressed in areas overlying mineralisation compared to background regions;
- To assess what extent environmental factors such as land-use, regolith material, underlying geology, sample depth and geophysics are linked to or influence microbial community structures; and,
- Develop a statistical approach that allows the comparison of geomicrobial and geochemical datasets.

2. Materials and Methods

2.1 Study Location

The research site is located at the Hillside Copper (Cu) mine, north-west of Adelaide close to the township of Ardrossan in South Australia (Figure 1) coordinates; 34^{o}32'04.32" S 137^{o}52'41.81" E. Easy access is provided to the tenement by major sealed roads including National Highway 1 between Adelaide and Port Augusta, its alternative coastal route from Port Augusta to Whyalla, and the Port Wakefield to Yorketown-Warooka roads on Yorke Peninsula. A large network of unsealed roads and tracks provides good access within the tenement. Hillside is located within the geological terrane called the Gawler Craton which is host to the large Olympic Dam iron oxide Cu-Au-U-REE deposit as well as world-class resources

of Cu and Au. Mineralisation at the mine is common within the fault zones, known as the Pine Point Fault zone, and seems typical of an Iron Oxide Copper Gold (IOCG) deposit. The elements which were chosen for analysis were based on their local abundance and expression of known mineralisation. These include; mineralisation commodity elements (Au, Cu, U), secondary mineral trace element host elements (Al, Fe, Ca), as well as pathfinder and mineralisation accessory elements (Ce, Co, Dy, Li, Tl and V) (Dietman, unpub). Research will go into the Hillside Mine site to see if there is a difference in mineralisation and background samples. Recent research by Wakelin *et al* inprep at the Teutonic Bore massive sulphide deposit in the Yilgarn Craton region has found significant differences in microbiology of soil collected from between mineralised and background samples. Most of the rocks of the Gawler Craton are covered by a sequence of younger rocks that have hidden many large copper deposits but in recent times, exploration underneath these cover rocks has led to the discovery of Olympic Dam which is Australia's largest copper deposit. Hillside is an agricultural area based on a rotation of cereals and annual pastures with livestock.

2.2 Geology

The geological history of Yorke Peninsula (Figure 2) is varied and includes repeated transgressions and regressions by the sea as faulting and land movements uplifted some areas and allowed others to sink below sea level (Corbett, 1973). The Hillside Cu-Au deposit is situated along the eastern margin of the Gawler Craton, a

large stable block consisting of late Archaean to Mesoproterozoic crystalline basement rocks that have undergone no significant deformation since 1500 Ma (Webb *et al*., 1975; Hand *et al.,* 2007). The deposit is hosted by the metasedimentary and metavolcanic Wallaroo Formation, and the highly brecciated Arthurton Granite; a Hiltaba Suite. These breccias are expressions of the Pine Point fault Zone, which is the dominant structural control on mineralisation. The Hiltaba Suite event is thought to be responsible for the formation of the IOCGU deposits within the Gawler Craton and Hillside. The crystalline basement is assigned to the Moonta Subdomain based on it structural metamorphic and stratigraphic character (Webb *et al*., 1975).

The faulted eastern margin of the Gawler Craton is clearly defined by a deep-seated crustal structure. Basement units in general proximity to the eastern margin of the Gawler Craton often display pervasive hydrothermal alteration and major mineralised districts occur to the south around Moonta-Wallaroo and to the north around Olympic Dam. At 1590 Ma mantel upwelling beneath the thick lithospheric plate of the central and eastern Gawler Craton resulted in intrusion of mafic plugs, extensive anorogenic partial melting of the lower crust, subaerial felsic and minor mafic volcanism (Gawler Range Volcanics), emplacement of granite batholiths and plutons (Hiltaba Suite) and formation of the huge Cu-U-Au-Ag-REE Olympic Dam deposit (Hand *et al.*, 2007). Basement rocks of the Gawler Range Volcanics are only exposed in the very southwest of the project area, though the Gawler Range Volcanics along with Hiltaba Suite granites. Basement units are overlain by undeformed strata that varies considerably in age and thickness, and includes

Mesoproterozoic (Pandurra Formation), Neoproterozoic sediments of the Stuart Shelf and Cainozoic surficial sediments (Webb *et al*., 1975).

2.3 Regolith

The regolith profile is deeply weathered and is seen throughout the Yorke Peninsula. The weathered profile is covered by aeolian sediments and regolith carbonates are widespread within the upper parts of the transported cover (Dietman, unpub). The transported overburden consists of well-sorted, rounded spherical quartzose and calcareous sands with:

- Highly indurated nodular and hardpan regolith carbonate horizon; and,
- Friable, nodular pedogenic carbonates

Coastal sediments show in-situ bedrock altered to saprock and saprolite. Saprolite is found in low relief zones and drainage depressions that are covered by transported regolith (Dietman, unpub). Ferruginous regolith is recognised as ferruginised beach sediments located along the coastline with inter-tidal sediments (Dietman, unpub). Maghemite is also found in palaeodrainage channels occurring along sections of the exposed coastline (Dietman, unpub).

2.4 Climate

Temperatures are usually mild, particularly in the south, where summer maxima are lower and winter minima are higher than in most South Australian

mallee areas (Corbett, 1973). Rainfall is moderate with the wettest months from May to September. The average annual rainfall around the coast is quite low with some places experiencing rainfall of 12 inches (Corbett, 1973). The rate of evaporation is quite high, with dew making a significant contribution to the plants survival (Corbett, 1973).

2.5 Vegetation

Most of the Hillside area has been cleared for agriculture which consists of a rotation between cereals and pastures with livestock. There are some areas of natural vegetation around the main roads, coastal cliff tops and some major drainage depressions. The vegetation found is mostly mallee woodland dominated by red mallee (*Eucalyptus socialis*) and yorrell (*Eucalyptus gracilis*) (Corbett, 1973; Dietman, unpub).

2.6 Soil sampling

Soil samples for 16S rDNA PCR-gel electrophoresis were taken from four major transects corresponding to sampling lines used by Dietman (*unpub*). All areas of mineralisation were covered in the transect these include;

Transect 1 – surface and to depth (GDA)

Transect 2 – to depth (GDA)

Transect 3 – to depth (GDA)

Transect 4 – surface and to depth (GDA)

Background – 50cm (AUS GEO 88)

Natural vegetation – surface and to depth (GDA)

In total 212 samples were taken, with two soil samples taken at each spot (Surface meaning that samples were taken at 0.03 (m) and depth means samples were taken down to 0.5 (m)). The mineralised area lies within Transect 1, 2 and 3. Not all the samples were further analysed thus, a specific subset was used that best corresponds to the factors specified. Refer to Figure 3 for sample number co-ordinates. A total of 93 samples were chosen for further analyses.

The geochemical data set was collected along major fault orientated transects using a hand-auger and shovel by Byron Dietman in 2009. Soil samples were collected at 10 cm intervals and sieved through a 200 µm nylon mesh. All elements were analysed with an emphasis on Au, Fe, Cu and U for the purpose of geochemical exploration.

Samples were collected from the 5th to the 14th of April 2010 using a pick axe, shovel and hand auger, then placed in sterile pre-labelled Falcon tubes. At each sample site all equipment was cleaned with methylated spirits to eliminate contamination. The site location was first found using GPS coordinates already placed into the GPS from Dietman's previous studies. The exact GPS position was reached, followed by a brief description of the soil, land, regolith, depth of sample taken and photograph for future reference. Gloves were worn at all times when cleaning the equipment and taking the samples to reduce contamination.

At each location two samples were taken. One from the near surface where the first 1 cm of soil was brushed away using a plastic hand shovel and then a soil sample of the first 3 cm was taken with an already labelled sterile Falcon tube. The second soil sample was taken at differing depths depending on the depth it took to hit calcrete. From preliminary studies on the geochemical data, using Permanova, it was found there was no major change in geochemistry from 50 cm down, so samples were taken to a depth of 50 cm even though in some instances Dietman took samples down to 1 metre. A small hole was dug out using a pick axe, the depth was measured using a ruler and a photo was taken of this hole with the ruler in it then the sample was taken by 'jamming' the Falcon tube into the bottom side of the hole.

The natural vegetation transect was taken along the roadside and 20 samples (surface and at 50 cm) were collected at random intervals along the section using GPS coordinates AUST GEOD 84. All samples once taken were placed in an esky full of ice to ensure that degradation of the microbes didn't occur. Care was taken when finding the sample location seeing as in the time since Dietman took his samples new roads and drill pads had been made. Wherever this occurred samples were taken as close to the location but with enough distance to avoid any environmental contamination. At the time of sampling, the site had been dry with very light rain in the mornings.

2.7 TRFLP procedure

2.7.1 DNA extraction

Terminal restriction fragment length polymorphism analysis was based on extraction of total community DNA. DNA was extracted from soil samples using the Powersoil™ DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA). Extractions were according to the manufacturer's protocol with the exception that tubes were inverted several times before being placed in the FastPrep.

To ensure sufficient DNA quantities Quant-it Picogreen dsDNA reagent (Invitrogen) and mx3000P (Stratagene) realtime PCR instrument were used. The unknown samples were compared against a standard curve derived from known concentrations of lambda DNA. The assay procedure was made up by pipetting 2 µl of DNA standards and samples to each well of the 96 plate containing 23 µl of Quant-it Picogreen working solution. The plate was then shaken gently and spun down in a plate centrifuge for 10 seconds. Then the plate was placed into mx3000P (Stratagene) realtime PCR instrument. To run the plate a new experiment was selected with quantitative plate read. Then all the wells on the software that contained samples were selected and were set as either standard, unknown or NTC. The analysis run time was 30 seconds for each plate, and once completed the software went to the analysis screen where the results were analysed. This technique ensured sufficient quantities of DNA were present to be able to continue on with the next step.

2.7.2 PCR (Polymerase chain reaction)

Six primers with different gene sequences 63F, 1087R HEX, Ar3F, Ar927R TET, ITS1F FAM and ITS4 were used for amplification. Primer sets and concentrations used are listed in Table 32. Three of these primers have a fluorescent label VIC HEX (1087R), NED TET (Ar927R) and FAM (ITS 1F). These were diluted down with water by the amount of nmol per tube and then vortexed for 20 seconds and refrigerated for 5 minutes at -4^{o}C. These were then further diluted with water to a 1:10 ratio and vortexed for 10 seconds.

A master mix was then produced by adding a certain volume of each primer into one tube with water and 2X buffer and vortexed. 20 µl of this master mix was then pipetted into 0.2 ml PCR tubes and 5 µl of DNA from the field samples was added. There was also two positive controls; E.coli and Thickoderma, and one negative control; water.

The Eppendorf Gradient thermal cycler was then used for the amplification of DNA using the polymerase chain reaction method. The PCR amplification was performed using the following program: 10 min hot start at 95^{o}C followed by 30 cycles of 95^{o}C for 30 seconds, 55^{o}C for 30 seconds and 72^{o}C for 1 minute; and a final extension of 10 minute at 72^{o}C. To assess size and quality of the amplified DNA, 5 µl of the product was run on a 2% (w:v) agarose gel with a molecular size marker (100 bp-DNA-ladder, Promega, USA) and two positive controls (E.coli, tricoderm) and one

negative control (water). All sample products were run with 1/2 µl of dye (Gel Pilot loading gel), for optimum results when photographed.

The gel was placed into the ethidium bromide solution and left for 20 minutes. After 20 minutes, the gel was taken out using a spatula, placed in a tub and immersed in water. This was left for 5 to 10 minutes. Once done the Transilluminator UV source was used and an image was rendered and printed off / saved. Once done the gel was thrown away and the machine cleaned with ethanol and switched off. This step ensured that the sample had sufficient DNA and could be used for the next step.

2.7.3 Restriction digest

PCR clean up of PCR amplifications was done using the Wizard® SV Gel and PCR Clean-up System (Promega, USA) system according to the manufacturers protocol. Restriction enzyme digest procedure was prepared by adding approximately 100 ng of product which was digested with 20U of MspI, HaeIII and TaqI for 3 hours at 37^{o}C or 65^{o}C as per the manufacturer's instructions. Three types of restriction enzymes were used to screen all readily available restriction endonucleases. The plates were cleaned using SigmaSpinTM Sequencing Reaction Clean-up (Promega, USA), 96-well post-reaction clean-up plates according to manufacturer's protocol.

The samples were then processed by the Australian Genome Research Facility (AGRF) on an ABI 3730 Genetic Analyser. 5 µl aliquots of each digest were mixed with 4 µl of formamide and 1 µl of the internal size standard (GeneScan-500 LIZ,

ABI). The samples were denatured at 94^{o}C for 5 minutes then placed on ice prior to capillary electrophoresis.

2.8 Quality Assurance and Quality Control

In order to have quality assurance and quality control in the procedures certain steps were taken to ensure reliable results. During field work contamination was avoided by pre-labelling the sample tubes, wearing gloves at all times, using methylated spirits to clean equipment and placing samples immediately on ice. In the laboratory after each step in the DNA analyses, tests were run to check that DNA was present in the samples and all standards and samples were performed in duplicate for more reliable results. During the gel electrophoresis there were two positive controls (*E.coli* and *tricoderm*) and one negative control (water) which were placed at the end of the gel in the plate and provided a means to tell if the samples were contaminated. A test run was also done using BSA (Bovine Serum Albumin) to see if more DNA would show up in some samples which didn't show any DNA. Before taking the finished samples to the AGRF a test plate (tRFLP) and gel electrophoresis was run on random DNA samples to check that DNA was present and then a cleanup was done using the Wizard SV Gel and PCR Clean-Up system. Random samples were checked to make sure DNA was in the samples.

2.9 Soil physical measurements

Soil pH and EC are factors that have been shown to be important in influencing microbial communities in soils (Wakelin *et al.*, inprep). This was determined by adding 5 g of soil and 25 ml of sterile MilliQ water into a Faclon tube. Before the soil measurements were taken, the samples were mixed for one hour in Southern Cross Science PTY LTD, WA Scientific Instrument at about 1 rpm and then left to sit for 20 minutes. When taking the measurements care was taken to minimise the amount of movement within the tube. The EC was measured first for all the samples, then the pH. The Smartchem – Lab, Multi-Parameter Laboratory Analyser was used to measure the pH and EC values of the samples. The pH meter was calibrated using specific solutions with known pH and EC values with a precision of 98%. For pH and EC values please refer to Table 30.

2.10 Data and statistical analysis

Bacterial, fungal and archaea terminal restriction fragments (T-RF's) were analysed using GeneMarker AFLP/Genotyping software program (SoftGenetics LLC Version1.8) at a detection limit of 200 fluorescent units (FU). Terminal restriction fragments that deviated by less than 1 base pair (bp) in length were considered to be within the same bin set. Peak heights were automatically calculated by the software

and used as a measure of abundance while richness was based on the number of individual peaks obtained (Operational Taxonomic Units). The analysis was performed between 50-500 bp (which is in the linear range of the size standard which is from 35-500 bp). The database of T-RFs for each restriction endonuclease was used to evaluate individual restriction endonucleases.

Differences and similarities of genetic and functional fingerprints as well as geochemical properties were assessed using Primer version 6.1.13 & Permanova+ version 1.0.3 (Primer-E Ltd, Plymouth, U.K.). Permanova+ is an "add-on package which extends the resemblance-based methods of Primer to allow the analysis of multivariate (or univariate) data in the context of more complex sampling structures, experimental designs and models" (Anderson, 2001). Permanova was used as a probabilistic hypothesis test to assess difference in microbial community for: (1) land-use, where land-use was categorized into natural vegetation and agriculture, (2) mineralised zones (where mineralisation was categorized into mineralisation and non-mineralised (background) depending on the elements chosen) and also mineralised for specific elements (such as Au, Cu, U), (3) regolith landforms (agriculture or natural vegetation), (4) underlying geology (5) agriculture (6) land-use (7) sample depth and (8) geophysics.

To assess the difference in microbial community the data was moved into three Excel spreadsheets (bacteria, fungi and archaea). Factors were then added to the spreadsheets (land-use, regolith, samples depth (m), regional geology, basement geology, geophysics and mineralisation) for statistical analyses and opened in Primer to see which factors are driving microbial activity. Land-use, regolith, sample depth,

underlying geology, geophysics were chosen as site important based on geochemistry, maps and what was seen in the field. Land-use was further analysed into agriculture and natural vegetation. The same was done for regolith which was divided into depositional, erosional and transported seeing as these were the regolith landforms present. Sample depth was divided into a compressed factor of surface / deep, and depth length (0.03 (m) to 0.5 (m) and up to 1 (m) in the geochemistry data). The underlying geology was based off of the Maitland 1:250 000 geological map of the area and divided into coastal aeoliante, associated sand spreads and alluvium and low angle slope deposits. Geophysics was based off of magnetic survey data by Rex Minerals, 2009 and divided into groups of high, medium and low. Mineralisation was chosen based on the mineralisation at the site (C, Au, Fe and U) and the environment it is found in. The mineralisation was further divided into groups based around the Goldschmidt element classification and other element associations found in regolith, these were; Calcophiles, uranium associated, Fe and Al, REE and Mn associated (mineralisation_5x_).

The geochemistry dataset provided by Dietman was placed into Primer and was treated by firstly running a 'Draftsman Plot' which showed whether the data was skewed or normal. The data was right skewed, meaning it was not evenly distributed and thus needed to be transformed to get a normal distribution. A 'transform' analysis was run on the data using a Log-expression (for environmental data) and then another draftsman plot was run on the Log data and most of the skewness was gone. The effect of Log-transformation in geochemistry is usually to enhance the 'background' trace element associations (Howarth and Earle, 1979).

For all microbial data sets the same procedures were used. All the bacteria, fungi and archaea datasets were 'overall transformed' to the fourth root (for biological data). Transformations are applied as a method of changing the relative emphasis of the analysis on rare versus more abundant species. Fourth root reduces the contribution of highly abundant species in relation to less abundant ones in the calculation of the Bray-Curtis measure; rare species will contribute more, the more severe the transformation (Anderson, 2001).

The next analyses were run on both the geochemical and biological data sets;

A resemblance analysis (resemblance between every pair of samples based on whether the suite of recorded variables take similar or dis-similar values) was then run on a selection of samples using Euclidean Distance for geochemistry and Bray Curtis similarity for biological assemblage data (Bray Curtis similarity views the space as a grid similar to the city block distance).

A non metric Multi Dimensional Scaling (nmMDS) plot was produced. This constructs a 'map' or configuration of the samples, in a specified number or dimensions, which attempt to satisfy all the conditions imposed by the rank (dis)similarity matrix.

Permanova analysis was run on certain factors to test the simultaneous response of one or more variables to one or more factors in an ANOVA experimental design on the basis of any distance measure, using permutation methods. The 'main test' was left as the default for all tests, and depending on the data either 'Type I Sums of Squares' (Type I SS) or 'Type III Sums of Squares' (Type III SS) was used. Type I SS is order dependent (sequential in this case) and each effect is adjusted for all other

effects that appear earlier in the model, but not for any effects that appear later in the model. This is appropriate for balanced analyses of variance in which the effects are specified in proper order and for trend analysis where the powers for the quantitative factor are ordered from lowest to highest in the model statement for example A, B, AB and B, A, AB etc. Type III SS (partial) is used most often and is corrected for as many other factors in the model as possible. It is calculated by comparing the full model without the variability of interest. So it is considered to be the additional variability explained by adding the variable of interest. Here everything is adjusted for everything else. In a study, the variables of interest are identified. One or more of these variables are referred to as the factors of the study are controlled so that data may be obtained about how the factors influence another variable. Overall, factors can influence each other but can also be co-independent.

The Permutation method chosen was either 'permutation of residuals under the full model' or 'unrestricted permutation of raw data'. Permutations under a full model (all samples) obtain the residuals of the full model by subtracting from each replicate the mean corresponding to its particular cell (combination of factor levels) (Anderson, 2001). An unrestricted permutation was chosen for one factor analysis. This is a good approximate test proposed for complex ANOVA designs where lower sample sizes are used.

The 'Number of permutations' was adjusted to 9999 permutations, it is clearly desirable to perform as many permutations as time and computing power will allow. The pseudo-F statistic shows that as the pseudo-F statistic gets larger, the likelihood of the null hypothesis being true diminishes (Anderson, 2001).

The pseudo–*f* statistic (*f*) is a ratio of the mean sum of squares between groups to the mean sum of squares within group. As the pseudo-*f* statistic gets larger, the likelihood of the null hypothesis being true diminishes. The P-value (*P*) is the probability of obtaining a test statistic at least as extreme as the one that was actually observed, assuming that the null hypothesis is true.

After the Permanova a CAP analysis was run to further analyse specific factors. CAP is a routine for performing canonical analysis of principal coordinates. The purpose of CAP is to find the axes through the multivariate cloud of points that either (i) are the best at discriminating among a priori groups or (ii) have the strongest correlation with some other set of variables (Anderson, 2001). Cap analysis was then employed to discriminate among a priori groups or to predict values along a gradient; also to do distance-based canonical correlation and provide a constrained ordination diagram.

To establish the relation between the similarity matrices for geochemistry and the microbial samples the RELATE procedure was used. RELATE is a measure of how closely related two sets of multivariate data are, for a matching set of samples, by calculating a rank correlation coefficient between all the elements of their respective (dis)similarity matrices (Anderson *et al*., 2006).

2.11 Assumptions

The geophysics data used for this study was based off of a magnetic survey done at the Hillside Mine site by Rex Minerals (Rex Minerals, 2009); where regions of low magnetic activity appear blue, regions of high magnetic activity appear purple and regions of medium magnetic activity appear green / yellow. If the geophysics was high then there was mineralisation, if the geophysics was medium or low this was assumed to mean there was no mineralisation. Mineralisation for the natural vegetation values where there was no data set was based on geophysics. Mineralisation for the surface samples was acquired by assuming that mineralisation at depth reflects the surface as well.

In the initial microbiology data set any columns that had one or more cells with no data or less than 5 bands of DNA were not used. It is very difficult for Permanova to analyse data with zeros because not all marginal and cell means can be estimated and therefore not all main effects and interaction can be tested (Quinn and Keough, 2003). In addition, seeing as the data set is small when looking at the P-values we are also assuming that 0.1 (10%) is significant as well as 0.05 (5%). Geology was based off of the Maitland Geological Survey of South Australia 1:250 000 sheet map. For statistical assumptions please refer to the PERMANOVA A+ for Primer manual by Anderson *et al*., 2006.

Molecular fingerprinting techniques like tRFLP do not measure the original relative abundances of the identified operational taxonomic unit and thus cannot answer

questions about OTU richness or about OTU relative abundances (Ramette, 2009). For this study it is assumed that the sizes and the fluorescence intensities of labelled DNA fragments can be analysed and used as an indicator of OTU abundance where intersample variation in relative peak areas are not affected by PCR conditions as they similarly apply to all OTUs and samples.

3. Results

The soil samples were collected to show microbial community profiles in relation to the environment at Hillside. This was done using field and laboratory based techniques. For the geochemistry, bacteria, fungi and archaea datasets the same procedures were used.

3.1 Geochemistry

Permanova analysis was run on a number of factors using the geochemistry samples. The effects of sampling soil from all the samples when using Type 1 SS (Table 1) shows there is a strong effect (f = 2.1766) of depth and it is significant on a 5% level (P = 0.0005). These effects are apparent in the nmMDS plot (Figure 4) of the sample depth (m) where there is no distinct clustering at depth below 50 cm.

The effect of sampling soil from all the samples (Table 2) shows that when using Type 1 SS regolith has a strong effect (f = 2.2749) and is significant on a 5% level (P = 0.0082). These effects are apparent in the CAP analysis on the regolith, Figure 5 where observations in the same group are clustered together in the plot. There is a distinct separation between the regolith landform units of transported, depositional and erosional with no overlapping between any units. There is also a tendency for the transported samples to occur in the top right-hand area of the plot, the erosional in the top left hand side and depositional at the bottom (spreading towards the middle and right side of the plot). The analysis shows that the three groups of regolith landform units are indeed distinguishable from one another.

When using Type I SS (Table 3) geophysics has an effect (f = 4.6545) and is significant on a 5% level (P = 0.0001), while regional geology has an effect (f = 2.2108) and is also significant on a 5% level (P = 0.0227). Mineralisation has an effect and is significant on a 10% level (f = 1.2163, P = 0.2672). These effects are apparent in the CAP analysis of the geophysics, Figure 6 where there are three distinct clustering of groups. The ‘low’ and ‘medium’ clusters are found in the right of the plot, with ‘medium’ towards the top corner and ‘low’ occurs in the bottom corner with some spreading towards the middle. The ‘high’ cluster is found in the left corner of the plot towards the middle, with a bit of spread.

Figure 7 was obtained by running a cluster analysis on the geochemistry elements and shows the group average linking. The elements on the x-axis can be split into groups by further analysing the dendogram. These are chalcophiles, Fe and Al,

accessories, Uranium accessories, REE, Mn accessories and solution which shows the regolith element associations at the Hillside Mine site.

Figure 8 was obtained by using a RELATE analysis and shows a strong correspondence was observed between the data sets.

3.2 Bacteria communities

Permanova was used to show univariate or multivariate data in response to factors, groups or treatments in an experimental design. Bacteria community profiles were on average more significantly affected by sample depth (compressed or (m)) and land-use.

Genetic diversity of the bacterial communities was recorded between 5 – 57 OPUs in different positions, with a total of 489 BINs for restriction enzyme MspI, 362 BINs for HaeIII and 301 BINS for TaqI. Overall, the genetic richness was larger in the surface samples than the depth samples. There also seemed to be some separation between mineralised and non-mineralised zones with genetic richness higher in areas overlying the mineralisation.

There is a strong effect of sample depth and it is highly significant at 5% level (Table 1; P = 0.0001), with CAP analyses showing a distinct clustering of 0.03 (m) and 0.5 (m) samples, with all the other values in between.

The effect of sampling soil from all the samples (Table 4) when using Type III SS shows that there is a strong effect (f = 8.8167) of depth and it is significant on a 5% level (P = 0.0001). Land-use is weaker (f = 2.0785) and not significant on a 5 % level (P = 0.0563) but significant on a 10% level. This is seen again in Table 5 table where depth is significant and land-use is less significant. These effects are apparent in the CAP analysis of the depth compressed data, Figure 9 where observations in the same group are clustered together in the plot. There is a clear grouping of the 'deep' samples in the right side centre of the plot, and surface samples in the left side centre of the plot with some overlapping within the units. The deep samples do however have a larger spread than the surface samples.

The effect of sampling soil from the surface samples (Table 6) shows that regional geology has an effect (f = 3.7729) and it is significant on a 5% level (P = 0.0107). While mineralisation has less of an effect (f = 1.362) and not significance on a 5% level but significant on a 10% level (P = 0.1921). These effects are apparent in the CAP analysis of the regional geology, Figure 10 where observations in the same group are clustered together in the plot. There is a grouping of the 'alluvium and low angle slope deposits' samples in the left side centre of the plot, and the 'associated sand spreads' samples in the right side centre of the plot. There is some overlapping with the 'associated sand spreads' and 'coastal aeoliante' samples.

The effect of sampling soil from the surface samples shows that (Table 7) the influence of regional geology is strong (f = 3.6099) and is significant on a 5% level (P = 0.0381). While regolith has a strong effect (f = 2.323) and is significant on a 5% level (P = 0.0411). These effects are apparent in the CAP analysis of the regolith, Figure 11

where observations in the same group are clustered together in the plot. The transported and erosional groups are distinctly separated with the transported occurring towards the left centre of the plot and the erosional occurring towards the centre right of the plot with larger spread towards the right. The depositional samples overlap with both the transported and erosional groups.

The effect of sampling from all samples shows that (Table 8) the influence of sample depth has a strong effect (f = 4.6123) and is significant on a 5% level (P = 0.0001). Whereas mineralisation has an effect (f = 1.4208) and is significant on a 10% level (P = 0.1042).

The results from Table 9 Permanova show when using Type I on selected samples geophysics has an effect (f = 3.9407) and it is significant on a 5% level (P = 0.001), regional geology has a strong effect (f = 6.4611) and is significant on a 5% level (P = 0.0002) and mineralisation has an effect and is significant on a 5% level (f = 2.7034, P = 0.0086).

The effect of sampling soil from the surface samples shows that (Table 10) the influence of regional geology is strong (f = 4.2544) but it is significant on a 5% level (P = 0.0383). While regolith has an effect and is significant on a 5% level (f = 2.8909, P = 0.023), and geophysics has a strong effect (f = 3.1738) and is significant on a 5% level (P = 0.0019). These effects are apparent in the CAP analysis of geophysics, Figure 12 where observations in the same group are clustered together. There is a separation between all three geophysics groups, high (left of plot), medium (bottom of plot) and low (middle – top of plot) but there is overlapping between all samples.

Permanova analysis was run on a number of factors using the bacteria samples, Tables 11 and 12. The effect of sampling soil from surface samples shows that when using Type 1 SS (Table 11) geophysics has an effect (f =2.6397) and it is significant on a 10% level (P = 0.0775), while regolith has an effect (f = 2.8343) and is significant on a 10% level (P = 0.1019). Mineralisation also has an effect (f = 1.6982) and is significant on a 10% level (P = 0.2444). CAP analysis of mineralisation at the surface, Figure 13 shows there is a good separation between all mineralisation sub-factors. Most samples are clustered in the middle left of the plot with all samples overlapping, except for Au, Cu and U which is located in the top right of the plot.

The effect of sampling soil from all samples excluding Transect 1 shows that when using Type 1 SS (Table 12) regional geology has the strongest effect (f = 3.8801) and is significant on a 5% level (P = 0.0079). Mineralisation has an effect (f = 1.4299) but is significant on a 10% level (P = 0.1481).

3.3 Fungi communities

Genetic diversity of soil fungal communities was recorded between 5 - 22 OPUs in different positions, with a total of 437 BINs for restriction enzyme MspI, 464 for BINs for HaeIII and 301 BINs for TaqI. The genetic richness was larger in the surface samples but there was not that much difference between surface and depth. Permanova results indicate that there is a strong effect of sample depth and that it is significant (P < 0.05). CAP analysis (Figure 14) shows this separation of depth. There

also seemed to be some separation between mineralised and non-mineralised zones with genetic richness higher in areas overlying the mineralisation.

Permanova analysis was run on a number of factors using the Fungi samples. The effect of sampling soil from all samples shows that when using Type III SS (Table 13) shows that that the influence of depth compressed has a very strong effect (*f* = 14.622) and is significant on a 5% level (*P* = 0.0001). Land-use has an influence (*f* = 3.5961) and is significant on a 5% level (*P* = 0.0019).

Table 14 shows that that the influence of regional geology has an effect (f = 2.0004) and is significant on a 5% level (*P* = 0.033).

Table 15 shows that that the influence of sample depth (m) is strong (*f* = 4.3622) and significant on a 5% level (*P* = 0.0001), and land-use also has an effect (*f* = 3.2314) and is significant on a 5% level (*P* = 0.0044). These effects are apparent in the CAP analysis of sample depth (m), Figure 15 where there is a distinct clustering of 0.03 (m) and 0.5 (m) samples towards the right and left of the plot, respectively. The rest of the samples fall within these two major clusters, there is some overlapping between all samples. There seems to be a gradational effect within the plot starting at 0.03 (m) at the right of the plot and goes across to the left with sample depth increasing to 0.5 (m) at the right of the plot.

The effect of sampling soil from the surface samples shows that when using Type III SS (Table 16) the influence of regolith has a very strong effect (*f* = 3.6399) and is significant on a 5% level (*P* = 0.0004). While geology has an effect (*f* = 3.806) and is significant on a 5% level (*P* = 0.0382). These effects are apparent in the CAP analysis

of regolith, Figure 14 where there is a distinct separation of all three groups. Transported samples are located towards the upper left of the plot with a large spread over the middle, the erosional samples are grouped in the upper right of the plot with a small spread over middle and the depositional samples overlap with both transported and erosional groups.

Table 17 shows that that the influence of geology when using Type III SS has an effect (f = 3.3179) and is significant on a 5% level (P = 0.0027), while the influence mineralisation has an effect (Pseudo-f = 1.1614) but is significant on a 10% level (P = 0.3241).

Table 18 sample shows that sample depth (m) has a strong effect (f = 4.1974) and is significant on a 5% level (P = 0.001), mineralisation also has an effect (f = 1.8665) and is significant on a 5% level (P = 0.011) when using Type III SS.

The effect of sampling soil from all samples excluding Transect 1 (Table 19) shows that when using Type 1 SS the influence of mineralisation has a strong effect (f = 2.0892) and is significant on a 5% level (P = 0.0057). Geophysics also has a strong effect (f = 2.269) and is significant on a 5% level (P = 0.0091), whilst the influence of regolith has an effect (f = 1.9478) and is significant on a 5% level (P = 0.0349).

Table 20 samples that regional geology has an effect and is significant (f = 3.445, P = 0.0247), regolith also has an effect and is significant on a 5% level (f = 3.3544, P = 0.001) and mineralisation has no effect (f = 0.84322) and is significant on a 10% level (P = 0.6577).

When using Type I SS on selected samples the Permanova results (Table 21) showed geophysics has an effect (f = 2.6043) and is significant on a 5% level (P = 0.0017). Whereas regional geology has no effect (f = 0.89881) but is significant on a 10% level (P = 0.543) and mineralisation has an effect and is significant on a 5% level (f = 2.0717, P = 0.0078).

3.4 Archaea communities

Genetic diversity of soil archaea communities was recorded between 5 – 21 OPUs in different positions, with a total of 303 BINs for the restriction enzyme MspI, 362 BINs for HaeIII and 147 BINs for TaqI. The genetic richness was slightly larger in the surface samples than at depth, although the difference was very small. All the samples taken from the surface at Transect 1 returned results, whereas Transect 4 had only one depth and 4 surface samples returned significant results. There also seemed to be some separation between mineralised and non-mineralised zones with genetic richness higher in areas overlying the mineralisation.

Permanova analysis was run on a number of factors using the Archaea samples. The effect of sampling soil from all samples when using Type III SS (Table 22) shows that the influence of depth compressed (m) has a strong effect (f = 3.1274) and is significant on a 5% level (P = 0.0136), whist the influence of land-use has an effect (f =2.8105) and is significant on a 5% level (P = 0.0206).

Table 23 shows that the influence of land-use has an effect (f = 2.6242) and is significant on a 5% level (P = 0.0374) and sample depth (m) has an influence (f = 1.6528) but is significant on a 10% level (P = 0.0801). These effects are apparent in the CAP analysis of sample depth (m), Figure 16 where there is a distinct clustering of 0.03 (m) and 0.5 (m) samples towards the left and right of the plot, respectively. The rest of the samples fall within these two major clusters, there is some overlapping between all samples.

The effect of sampling soil from the surface samples shows that when using Type III SS (Table 24) regional geology has a strong effect (f = 4.0678) and is significant on a 5% level (P = 0.0177), although regolith has an influence (f = 2.3935) and is significant on a 5% level (P = 0.0483).

These effects are apparent in the CAP analysis of regional geology, Figure 17 where there is a distinct clustering of the two groups, coastal aeoliante (left of the plot) and associated sand spread (right of the plot) towards the bottom of the plot. There is also some overlapping between the two groups, with the coastal aeoliante having the biggest spread around the bottom right of the plot.

The effect of sampling soil from the surface samples shows that when using Type I SS (Table 25) regolith has the strongest effect (f = 3.3449) and is significant on a 5% level (P = 0.0363), and geophysics also has a strong effect (f = 2.4922) but is significant on a 10% level (P = 0.078). While the mineralisation has an effect and is significant on a 10% level (f = 1.8473, P = 0.1572).

The effect of sampling soil from all samples excluding Transect 1 shows that when using Type 1 SS (Table 26) regional geology has a strong influence (f = 4.0892) and is significant on a 5% level (P = 0.0123), whilst the mineralisation has an effect and is significant on a 10% level (f = 1.4671, P = 0.1808).

The effect of sampling soil from the surface (Table 27) shows that sample depth has no test and mineralisation has an effect and is significant on a 10% level (f = 1.8169, P = 0.0757), when using Type I SS. Although, when using Type III SS on the surface (Table 28), sample depth has an effect and is significant on a 5% level (f = 2.6417, P = 0.0036) and mineralisation has an effect but is significant on a 10% level (f = 1.5845, P = 0.0702).

The effect of sampling surface samples when using Type I SS (Table 29) also showed that regional geology has an effect and is significant on a 5% level (f = 3.9418, P = 0.0211). Furthermore regolith has an effect but is significant on a 10% level (f = 2.4177, P = 0.0627) and mineralisation has an effect but is significant on a 10% level (f = 1.0787, P = 0.4089).

The results from Table 30 Permanova show that when using Type I SS on selected samples geophysics has an effect (f = 2.1515) and is significant on a 10% level (P = 0.1175), regional geology has strong effect (f = 4.5344) and is significant on a 5% level (P = 0.031) and mineralisation has an effect and is significant on a 5% level (f = 1.5405, P = 0.2244). Overall archaea showed the least amount of significance with any tests.

4. Discussion

The hypothesis is that microbial composition of the Hillside site can be linked to site relevant factors such as geochemistry, underlying geology, regolith land-forms, land-use, sample depth, geophysics (gravity and magnetic surveys) and mineralisation. The results show that microbial composition can be linked to these factors to a certain extent.

The Primer procedure was based off of Anderson and Willis, 2003 analysis of multivariate data where; (1) a robust unconstrained ordination (such as a non metric MDS plot); (2) an appropriate constrained analysis (such as a CAP plot) by reference to a specific hypothesis; (3) a rigorous statistical test of the hypothesis; and (4) characterization of species responsible for multivariate patterns or effects were used. From this it was found that the soil samples analysed the levels of microbes were the highest in the bacteria and fungi, with archaea having significantly lower amounts. In most samples, there was an absence of archaea which is indicative of bacteria thriving in harsh environments with high pH and arid climate.

Out of the three restriction enzymes, MspI had the highest frequency of resolving single populations. This observation is supported by a previous study into restriction endonucleases in determining microbial diversity using tRFLP (Engebretson et al., 2003).

4.1 Depth

Differences in soil depth have been found to be important for micro-organisms. Micro-organisms require nutrients from the surroundings and there is a major difference between near surface and subsurface conditions (Ehrlich, 1997). The results from this study show that bacterial, fungal and archaea community structure is influenced by the sampling depth. From Permanova analysis on the data it was found that there was no major change in geochemistry from 50 cm deeper, so samples were taken to a depth of 50 cm as seen by the MDS analysis (Figure 4). Two samples were initially taken into account for the fact that the samples were being taken from 'agricultural' lands which had not been done before for this type of experiment. One sample was taken at the surface and another at depth, to see if agriculture had a difference on microbial community. There was a difference found between the samples taken at the surface and samples taken at depth between bacteria, fungi and archaea. This is to be expected seeing as studies have found that the topsoil layer has significantly higher microbial activity than the subsoil regardless of the management strategy (Kibunja *et al*, 2009; Ge *et al*., 2008).

Permanova and CAP indicated that 0.03 (m) and 0.5 (m) depths, were different at the different sample depths. Although it can be seen from bacteria, fungi and archaea that surface samples are highly significant compared to non-surface samples (Tables 1 and 4, $P < 0.05$) when tested for all factors in the model. This is similar to

the findings by Taylor et al 2002 who found no significant change from mid soil to deep. Furthermore, CAP analysis of depth also shows a separation of the two surface and depth samples. The deep samples are seen to have quite a large spread in the plot compared to the surface samples which are clustered quite close together, suggesting that the surface samples are more reliable.

The significance of surface samples is also seen in the abundance of micro-organisms when compared to the depth samples. The results show that the bacteria depth samples had the least amount of genetic richness, but were still considerably higher compared to fungi and archaea. Bacteria had the largest data set from the tRFLP procedure. Fungi also had the least amount of genetic richness in the depth samples. Archaea had the smallest data set from the tRFLP procedure, implying that archaea would not be a suitable community for profiling.

Furthermore, the microbial results corroborates well with the geochemical data which showed the same separation between surface and depth samples (Figures 9, 13 and 15) and was highly significant from the Permanova Table 12. RELATE testing was used to assess if a link between the microbial data sets exists (Figure 8). A strong correspondence was observed between the data sets, with a high significant p of 0.904 (sample statistic 0.1%).

This has important implications for research in the area. The difference in top soil and depth could indicate an effect of agricultural practices on the microbial activities, increasing the activity closer to the surface where the agricultural chemicals are placed. There is also the fact that the further down the samples are

taken, the microbial community decreases due to lack of nutrients further down. Bacteria have the most genetic richness out of fungi and archaea, with the most peaks for each restriction enzyme. This suggests that bacteria are the most appropriate microbial community to use for analyses.

Implying that it is more significant to take surface samples in this area even though the land has been used for agriculture, and seeing as throughout the Yorke Peninsula agricultural processes are in progress this technique of taking surface samples is viable.

4.2 Land-use

Land-use change can have significant impacts on soil conditions and microbial communities with general community composition differing through different land-uses (Bossio *et al.*, 2005). The conversion of natural vegetation to pasture or cultivated fields is a common occurrence in many landscapes and is one of the dominant factors affecting the biodiversity and functioning of terrestrial ecosystems. The results show that fungal community is influenced by the land-use change from agriculture to natural vegetation, bacterial and archaeal communities showed some influence although they were the least affected by the change in land-use. This coincides with a study done by Kasel *et al* 2008 who found that 'while location effects were weak or uncertain, the data indicated clear, stronger effects of land use on soil fungal community composition.'

Land-use is also significant to microbial communities but only from the Permanova results which can be seen in from Tables 4, 13 and 23. When tested for all the factors in the model land-use was found to be significant on a 10% level for bacteria while land-use was significant on a 5% level for fungi and archaea. This observation suggests that microbial community is influenced to some degree by the change in land-use, which is supported from a previous study by Singh et al (2009) who found that multivariate analysis of the microbial communities suggested that bacterial and fungal communities are influenced by sample location. This suggests that land-use has a distinct effect on fungal and bacterial communities, with archaea showing the least amount of effect at the Hillside Mine. Catriona *et al* 2009 found that land-use was a key factor influencing microbial properties with bacteria communities less responsive to land-use compared to site and fungal communities was more affected by land-use change than by site, although site still had some influence.

The differences in microbial responses to land-use change can be attributed to the fact that agricultural impact on soils affects the chemical properties. The importance of land-use as determinants of soil microbial communities was examined using Permanova and over all this study found that there is a strong separation of the land-use into agriculture and natural vegetation from bacterial and fungal communities. The influence of land-use on microbial profiles was supported in both bacterial and fungal profiles, confirming previous findings that land-use strongly influence microbial community composition (Grayston *et al.*, 1998).

4.3 Regolith

Regolith characteristics are determined by a number of factors including climate, geology, topography, biological activity and time. The regolith can either help mineral exploration by providing broader targets or hinder it by concealing the bedrock targets, preventing easy sampling, mapping and photo interpretation of structure (Craig *et al*., 1999). It's important to understand the different regolith materials found, as this defines whether geochemistry or microbial communities will reflect underlying bedrock or mineralisation. Biological activities can have physical, chemical and other biological effects on the regolith. It has been found that bacteria and fungi promote the formation of silicate, carbonate and sulphide minerals, as well as mediate metallic mineral formation by binding metal cations to negatively charged groups of the cell all or cell envelope (Ehrlich, 1998). In turn, the structure and activity of microbial communities resident in the regolith are strongly influenced by the prevailing geochemical conditions, such as pH, redox condition, organic matter and trace elements (Kizilkaya *et al*., 2004). There haven't been many studies undertaken on the effect of regolith on microbes. The results from this study indicate that regolith has an effect on the microbial community and this is supported by a study which found that the presence of micro-organisms influenced the distribution of metals in the regolith (Wilford *et al*., 2009).

Furthermore, Permanova and CAP analyses showed regolith has a significant influence on microbiology at the Hillside Mine. The regolith found at Hillside consists

of three major landforms; erosional, depositional and transported. The results show that fungi and bacteria are strongly influenced by regolith at the surface (Tables 7 and 16; $P < 0.05$). In addition, CAP analyses of regolith for bacteria and fungi (Figures 11 and 14) show a good separation of the regolith into the three groups. There were no significant findings for archaea which could have arisen from the fact that archaea had some of the lowest OPUs compared to bacteria and archaea at the surface. This suggests that archaea does not thrive in the conditions present at Hillside. It was also found that for all three biological datasets there were no significant results at depth, this means that the microbial communities do not correlate to depth.

In addition, the geochemical data set fits in well with the microbiology at the site for regolith, when tested for all factors in the model (Table 2, 5, 15 and 23; $P < 0.05$). Figure 6 shows a significant separation between transported, depositional and erosional regolith landforms. This implies that there is a difference in microbiology between these three different landform units, and that the interaction of regolith and soil microbial community could be a method of finding geochemical anomalies. This corroborates well with a study done by Wakelin *et al* inprep, who found that the interaction of mulga, the regolith and soil microbial community is responsible for the surficial geochemical anomaly at the Jaguar exploration site.

Overall, the results show that regolith has a significant effect on both microbial and geochemical data sets, implying that bacterial and fungal communities can be used as an indicator in differentiating between regolith landform units.

4.4 Geophysics

Geophysical methods were used at Hillside to analyse the mineral deposit present. These methods include magnetic and gravity surveys used to identify drilling targets within the Pine Point Fault Zone. Detailed high resolution magnetic surveys have redefined a large number of copper targets. The copper discovered to date is associated with magnetite, which in high concentrations will produce magnetic anomalies on detailed survey allowing to 'see through' the cover rocks (Rex Minerals, 2009). Furthermore, drilling has shown a strong relationship between the magnetite and copper. Airborne EM (electromagnetic) survey over Hillside revealed several deep seated fault zones interpreted to be shallow, conductive bodies potentially associated with copper sulphide mineralisation (Rex Minerals, 2009).

Recent attention to the effects of microbiology on geophysics has surfaced with some interesting results. It has been well documented that microbes play a role in altering environmental systems (Singh *et al.,* 2008; Ehrlich, 1997; Crowley, 2008) in various ways. Studies have been undertaken on the effects of microbial processes on geophysical properties of the shallow subsurface (Atekwana *et al*, 2006) which may show potential changes in the bulk physical properties of the subsurface and are potentially measurable by geophysical techniques. These alterations of geologic media may come about when microbes directly colonize sediment surfaces forming biofilms that alter properties such as texture, surface area, pore size and pore

geometry and cementation among others (Atekwana *et al.*, 2006). The physical properties of the shallow subsurface detected by geophysical techniques depend on the mineralogy of the rock or regolith, the type and natural pore spaces which control volume of pore fluids, and the interconnectedness or pores, which control the ability of pore fluids to flow through the material (Atekwana *et al.*, 2006). Laboratory experiments using geophysical techniques such as direct current, resistivity, induced polarization, self potential and seismic were used and suggest that there is a potential for the geophysical monitoring of biological activity and resulting geomicrobiology processes in field settings (Atekwana *et al.*, 2006). Although, field effects of microbial processes on geophysical properties have been isolated to hydrocarbon impacted sites, seeing as these sites are considered natural laboratories where excess organic substrates stimulate microbial activity.

In the case of Hillside, where the area is carbonate rich studies such as these have not been undertaken as such. The results of the Primer analyses showed that geophysics did have an effect on the microbial communities to some degree. Permanova results showed geophysics has an effect and is quite significant for both bacteria and fungi, at surface and all samples excluding Transect 1, respectively. The results for bacteria samples at the surface are quite significant because this means that the microbial community is mostly influenced in the upper surface of the soil and that a sample from the surface could be sufficient data in analysing geophysics in the area. The fungi results excluding Transect 1 (which is over the mineralised zone) implies that there is still a high significance of geophysics throughout the mine area. CAP also showed the presence of three different clusters containing low,

medium and high samples (Figure 11) for bacteria in surface samples, which further corroborates that geophysics is influenced by microbes.

The geochemistry data also showed a high significance of geophysics, with Permanova results indicating geophysics has a strong effect and is highly significant this can be seen from Table 3 (f = 4.6545, P = 0.0001). Which is to be expected seeing as the elements being tested are what make the geophysical anomalies present in the ground to begin with. Areas where geochemical and geophysical anomalies overlap are considered prospective (Henkle *et al.*, 1993).

The results from this study indicate that there could be an effect of microbial processes on geophysical properties in carbonate rich areas with aeolian transported cover and that geochemistry is influenced by geophysics, and maybe a good indicator as to where the mineralisation lies.

4.5 Mineralisation

A geochemical anomaly was present in the surficial soil above the buried ore deposit at the Hillside Mine site. The anomaly consists of elevated concentrations of Cu, Au, Fe and which is very similar to an IOCG (iron-oxide, copper, gold) type-style deposit. Mineralisation commodity elements are Au, Cu and U, secondary mineral trace element host elements are Al, Fe, Ca, and pathfinder and mineralisation accessory elements are Ce, Co, Dy, Li, Tl and V (Dietman, unpub).

Primary geological processes result in the association of particular elements in specific minerals, these associations may persist: the elements may become separated or new associations may develop. Depending on weathering conditions and the types of regolith formed, particular elements may undergo fractionation during weathering or concentrated in particular regolith components to form regolith – related element associations (Scott and Pain 2008). Iron is the fourth most abundant element, and Fe oxides (primarily goethite and hematite) are major end products of near surface chemical weathering. Their ability to take up a wide range of cations means that these minerals exert a significant control over the outcome of many trace elements in the regolith, including target and pathfinder elements. Secondary Mn minerals are geochemically important and a component of parts of the regolith. Manganese is the tenth most abundant element in the crust, and it generally associates with Fe, Mg, Ni and Co. It is readily oxidised under near-surface conditions (Scott and Pain 2008).

At the Hillside Mine site the common element associations in the regolith tested for are chalcophiles, Fe and Al, Uranium associated, Rare Earth Elements and Mn associated. These element associations are based on the Goldschmidt's classification. Goldschmidt's classification is based primarily on the energy of formation of oxides and sulphides. Lithophile elements (such as Na, K, Li, Al, Ti, Mg and Ca) generally concentrate in the rich-forming silicate and oxide minerals of the crust and mantle. Chalcophile elements (such as Cu, Ag, Zn, Pb and S) readily form sulphides (Scott and Pain 2008). Chalcophiles also exhibit a bonding affinity with selenium, tellurium, arsenic and antimony, and therefore also exhibit high levels of

derivatives of these elements. Siderophiles exhibit a weak affinity to both oxygen and sulphur. But show an affinity for iron and a distinguishing characteristic of siderophiles is that they exhibit high solubility in molten iron. Siderophile elements generally have a low reactivity and exhibit an affinity to form metallic bonds. As a result, siderophiles are most often found in their native state. Atmophile elements (O, N, H and the inert gases) are the main components of the atmosphere. Biophile elements (C, N, O, H, P and S) make up the main part of the biosphere (Scott and Pain 2008).

There is a definite element association in the regolith at the Hillside Mine site (Figure 7). The groups in Figure 7 represent the Goldschmidt's classification with more emphasis on regolith related element associations. It is apparent that most elements are grouped according to the element associations, although there are some elements which are found in another group and this can be attributed to the fact that some elements behave differently under conditions of high O_2, S or H_20 and different temperatures (Scott and Pain 2008). The chalcophiles group reflects the elements found at the Hillside Mine site and are clustered together. Fe + Al associations are based on the fact that Al and Fe are two key elements in processes such as weathering and pedogenesis (Van-Hees *et al.,* 2006). Uranium associated elements were based on elements with a common association with uranium and the hierarchical grouping in Figure 7 shows the clustering of elements. The environment is also host to rare earth elements. Rare earth elements (REE) was based on the fact they share many properties in the periodic table (lithophiles) and the rare earth metals occur together in minerals. It can be said that these elements cluster together

in the CLUSTER analysis (Figure 7). Manganese – associated was based on common associations with Mn and it can be said that there is an association of these elements in the regolith to some degree. Accessories group was based on all other elements, there seems to be an association between the elements clustered together (Sb, Ag, Te, W, Se). Mineralisation was also based on 5x mineralisation, which involved taking the five highest and lowest values from certain elements (in this case U, Au, Cu as these are site important), to calculate background and any elements that had 5x background was considered mineralised. This was further compressed to “yes” or “no” which meant that if any of the three elements were present, it was mineralised and if none were present this meant there was no mineralisation (Figure 13). From Figure 13 it can be said that there is an association between the mineralisation groups, although there is quite a bit of overlapping and spread between all points.

Furthermore, the genetic richness of the microbial communities may say a great deal about the mineralisation of the site. The genetic richness of bacteria increased over the mineralisation and natural vegetation transect, with the highest OPUs found (Transect 1, 2 and 3) and decreased in the non-mineralised zones (Transect 4 and background). There is also some distinction between mineralised and non-mineralised areas with a decrease in genetic richness evident over the non-mineralised areas. The genetic richness of the fungal community increased over the mineralisation zones (Transects 1, 2 and 3) and decrease in genetic richness over the non-mineralised area (Transect 4). However, there was an increase in OTUs over the background samples. Archaea showed the least genetic richness over both mineralised and non-mineralised zones. These results show that overall bacterial

communities of mineralised and non-mineralised soils may show a difference in genetic richness over the areas. The same cannot be said for fungi and archaea, which showed no difference and may suggest that overall the fungal and archaeal communities for the mineralised and background soils at the Hillside Mine site are quite similar. Need to put in the data

Moreover, from field analyses and previous studies it has been found that the Yorke Peninsula area around Hillside is a calcrete environment with carbonate associations of Ca, Mg, Au, Ba, Ni, Sr, U, V, Z. Calcrete is the most widespread rock on the Peninsula, and masks a great deal of the underlying geology (Corbett, 1973). Calcrete also plays a major role in the pH of the area. In Figure 7 the Solution refers to the pH and EC taken from the samples at the Hillside Mine site. Chemical interactions between elements are largely controlled by what happens in the electron shells of atoms. The activity of protons (pH) and the activity of electrons (EC) are important controls on chemical reactions and the stability of minerals and ions in solution. Overall, all the samples had a high pH ranging from 8.4 – 9.94, and EC values ranged from 100 – 499 uS, with some samples producing larger amounts. The high pH is to be expected in the Hillside, Yorke Peninsula area where the soils are highly calcareous in the root zone. Generally, microbial activity is higher in calcareous soils than in acidic soils because they provide more favourable conditions to support a highly diverse population of microorganisms (Matinizadeh *et al.,* 2008). The small EC values can also be attributed to the environment and less electrons in the soil.

From this study and many other studies it may be said that mineralisation is known to be affected by microbes (Wakelin et al., inprep; Ehrlich and Newman, 2009; Reith

and Rogers, 2007) and corroborates well to a certain extent by findings from Reith and Rogers (2008) who found the bacterial community of auriferous soils displayed genetic differences when compared to non-auriferous (background) soils. It has also been found that bacterial communities of Zn-contaminated soils were dominantly influenced by the concentration and speciation of the Zn in the soils (Brim *et al* 1999) which may be the case in overlying buried mineralisation. From Permanova, mineralisation has a significant influence when it comes to bacteria surface samples and fungi samples (excluding transect 1). This is favourable for the Yorke Peninsula seeing as calcrete is wide spread in the region and mimics the conditions found at Hillside. This is a good starting point for further exploration down the Yorke Peninsula using this technique.

4.6 Geology

The results show that regional geology has a strong effect on bacteria and archaea, with no noticeable effect on fungi. The Hillside Mine underlying geology is based on the Maitland Map 1:250 000 sheet which shows the regional geology of the Yorke Peninsula. This has then been grouped into coastal aeoliante, associated sand spreads and alluvium and low angle slope deposits. There have been few studies undertaken on the differences of microbial communities over different geological settings. Previous researchers have focused their attention on microbial interactions

with metals present in the environment (which were covered in the section *4.5 mineralisation*).

It is well known that soil is formed from bedrock via geological processes such as tectonic movement, which gives rise to sedimentary or consolidated rock. Turbidity current s deposit sedimentary rock, where as igneous rocks (unconsolidated) are formed by volcanic activity. Both tectonic and volcanic geological processes give rise to the parent material, which supplies the soil with most of its minerals. And as such there should be a correlation between the soil microbiology and geology of the area. From this study it can seen that the geology of the area has a significant effect on micro-organisms. Bacterial communities and archaea are highly affected by the regional geology ($p < 0.05$); where as fungi show limited affects ($p > 0.05$). CAP analysis also shows that there is a good separation of the regional geology into clusters for bacteria and archaea. This corroborates to a certain extent with a study done on different karst environments where it was found that there were differences in the number of soil bacteria and the predominant bacterial species between the two different karst areas (Li et al., 2004). This may imply that bacterial communities can be used to differentiate between geological environments.

From the results it may be said that the bacterial communities at the Hillside Mine site can be employed to study regional geology relationships, but this still needs further investigating.

4.7 Implications

The results from this study have major implications for mineral exploration in the Hillside Mine site area. Depth is very significant and from the results implies that it is more significant to take surface samples in the area even though the land has been used for agriculture, and seeing as throughout the Yorke Peninsula agricultural processes are in progress this technique of taking surface samples is viable. This is of great importance in the mining industry as the potential for geomicrobiology in this area was unknown and may be able to save time and money by lessening the need for initial drilling. The clearing of the Hillside area for agricultural practices has also presented the problem for geochemical analyses where there may be no plants present. Geomicrobiology overcomes this problem as a soil or near surface sample can be taken from any location.

5. Conclusion

The project aimed to assess the microbial community as indicators for mineralisation, the influence of environmental factors on microbial community structures and use of highend statistical modelling on geomicrobial and geochemical datasets.

The main findings in this study are:

- Bacteria and fungi communities are the best option for analyses.

- Land-use and depth are the main factors effecting microbiology at the Hillside Mine.

- Geophysics, mineralisation and geology factors are the secondary factors effecting microbiology at the Hillside Mine.

- The geochemistry and geomicrobiology of a site, such as at Hillside, can be linked together.

- tRFLP is a valuable technique in comparative community analysis.

This information can supply new insights into the effects of land management. Depth is very significant in the Hillside area and from the results it may imply that it is more significant to take surface samples in the area even though the land has been used for agriculture, and seeing as throughout the Yorke Peninsula agricultural processes are in progress this technique of taking surface samples is viable. The complex regolith environments both challenges and provides opportunities for mineral exploration. The regolith presents numerous exploration problems, affecting geological, geophysical and geochemical mapping and exploration techniques, and constraining their use (Butt *et al* 2005). There is still the need for more research into geomicrobiology especially the effects of geology on microbiology. This promising field should one day lead to discovery of indicator micro-organisms and the development of microbial biosensors for detecting concealed mineralisation.

6. Acknowledgments

I would like to thank the people at Rex Minerals for allowing me to take samples from the Hillside Mine Site. Thank you to Byron Dietman for his geochemistry dataset. A big thank you to Steven Hill for believing in me and giving me the chance to do Honours in the first place. A special mention to Andreas Schmidt Mumm for all his support and guidance throughout Honours and undergrad, I really appreciate everything you've done. I couldn't have done any of the lab work required without the help from Adrienne Gregg at CSIRO. Thank you to my supervisor Frank Reith for all his help, patience and explanations on everything statistical. Without your continued help throughout the year I could not have made it this far, so thank you very much Frank. Also, thank you to the Honours group of 2010 for some unforgettable memories, in particular Andrew Knight for his uncanny 'nut' throwing ability from the desk behind me and Steph McLennan, Teena Rusak, Vair Pointon and Ashlyn Johnson. To my family (and Milky) thank you for everything. Last but not least, I'd like to make special mention to Thi Van for being my truest friend and Phill Adams for always being there for me, helping me out and never giving up on me.

7. References

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. Austral Ecology 26: 32-46.

Anderson, M. J. Gorley, R. N. and K. R. Clarke (2006). Permanova+ for Primer: Guide to software and statistical methods."The University of Auckland.

Anderson, M.J., Willis, T.J., 2003. Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. Ecology 84, 511–525.

Atekwana, E. A.; Werkema, D. D.; Atekwana, and E. A. Biogeophysics: The effects of microbial processes on geophysical properties of the shallow subsurface, Chapter 6. In *Applied Hydrogeophysics*; Vereecken, H., Binley, A., Cassiani, G., Revil, A., Titov, K. Eds.; Springer: The Netherlands, 2006; pp 161–193.

Bossio, D.A., Girvan, M.S., Verchot, L., Bullimore, J., Borelli, T., Albrecht, A., Scow, K.M., Ball, A.S., Pretty, J.N., and A.M. Osborn, 2005. Soil Microbial Community Response to Land Use Change in an Agricultural Landscape of Western Kenya. Microbial Ecology.

Brim, H., E. Heuer, et al. (1999). Characterization of the bacterial community of a zinc-polluted soil. Canadian Journal of Microbiology 45: 326-338.

Butt, C.R.M., Robertson, K.M., Scott and M. Cornelius (2005). Regolith expression of Australian ore systems – A compilation of exploration case histories with conceptual dispersion, process and exploration models. Corporate Research Centre for Landscape Environments and Mineral Exploration.

Corbett, D. (1973). Yorke Peninsula – a natural history, Department of Adult Education, The University of Adelaide, Adelaide.

Crawford, A. R. (1960). Maitland Geological Survey of South Australia. Department of Mines.

Crowley, D. (2008). Impacts of metals and metalloids on soil microbial diversity and ecosystem function. International Symposium ISMOM 2008.

Craig, M.A., Wilford, J.R., and I.J, Tapley, I.J (1999). Regolith-landform mapping in the Gawler Craton - an alternative approach. Cooperative Research Centre for Landscape Evolution and Mineral Exploration. MESA Journal 12.

Dexter-Dyer, B., M. Kretzschmar, et al. (1984). Possible microbial pathways in the formation of Precambrian ore deposits. 141: 251-262.

Dietman, B. J. (2009). Regolith and associated geochemical and biogeochemical expression of buried copper-gold mineralisation at the Hillside prospect, Yorke Peninsula. Adelaide, University of Adelaide. [Unpublished Honours Thesis].

Ehrlich, H. L. (1998). Geomicrobiology: its significance for geology. Earth-Science Reviews 45: 45-60.

Ehrlich, H. L. (1997). Microbes and metals. Appl Microbiol Biotechnol 48: 687-692.

Ehrlich, H. L. and D. K. Newman (2009). Geomicrobiology. Taylor & Francis

Engebretson, J.J., and C.L. Moyer (2003). Fidelity of select restriction endonucleases in determining microbial diversity by terminal-restriction fragment length polymorphism. Applied and Environmental Microbiology 69: 4823-4829.

Grayston, S.J., Wang, S., Campbell, C.D., and A.C. Edwards (1998). Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biology and Biochemistry 30: 369-378.

Ge, Y., He, J.Z., Zhu, Y.G., Zhang, J.B., Xu, Z., Zhang, L.M., and Y.M. Zheng (2008). Differences in soil bacterial diversity: driven by contemporary disturbances or historical contingencies. The ISME Journal 2: 254-264.

Henkly, W.R., Thomas, J.W., and C.C. Hyde (1993). Geochemical and geophysical signatures at three precious metals exploration targets in Nevada. Journal of Geochemical Exploration 47: 29-43.

Howarth, R.J., and S.A.M. Earle (1979). Application of a generalized power transformation to geochemical data. Mathematical Geology.

Howchin, W. (1929). The geology of South Australia.

Kasel, S., Bennett, L.T., and J, Tibbits (2008). Land use influences soil fungal community composition across central Victoria, south-eastern Australia. Soil Biology and Biochemistry 40: 1724-1732.

Kibunja, C. N., Mwaura, F.B., and D.N, Mugendi (2009). "Long-term management effects on soil properties and microbial populations in a maize-bean rotation at Kabete, Kenya." African Journal of Agricultural Research Vol. 5 (2), 108-113.

Kizilkaya, R., Askin, T., Bayrakli, B., and M, Saglam 2004. Microbial characteristics of soil contaminated with heavy metals. *Europ. J. Soil Biol.* 40, 95-102.

Li, W., Yu, L.J., Yuan, D.X., Xu, H.B., and Y. Yang (2003). Bacteria biomass and carbonic anhydrase activity in some karst areas of Southwest China. Journal of Asian Earth Sciences. 24: 145-152

Macdonald, C.A., Thomas, N., Robinson, L., Tate, K.R., Ross, D.J., Dando, J., and B.K, Singh 2009. Physiological, biochemical and molecular responses of the soil microbial community after afforestation of pastures with *Pinus radiata.* Soil and Biochemistry 41: 1642-1651.

Marsh, T.L (1993). Terminal restriction fragment length polymorphism (T-RFLP): an emerging method for characterising diversity among homologous populations of amplification products. Current Opinion in Microbiology. 2: 323-327.

Matinizadeh, M., Korori, S.A.A., Teimouri, M., and W. Praznik 2008. Enzyme activities in undisturbed and disturbed forest soils under oak (*Quercus brantii var*. persica) as affected by soil depth and seasonal variation. Asian J. Plant Sci., 7: 368-374.

Parduhn, N. L. (1991). A microbial method of mineral exploration: a case history at the Mesquite deposit." Journal of Geochemical Exploration 41: 137-149.

Quinn, G.P. and M.J. Keough (2003). Experimental design and data analysis for biologists. University of Cambridge.

Ramette, A (2009). Quantitave community fingerprinting methods for estimating abundance of operational taxonomic units in microbial communities. Appl Environ Microboil. 75 (8): 2495-2505.

Reith, F., M. Durr, et al. (2008). Geomicrobiology of the regolith. Regolith Science. CSIRO.

Reith, F. and S. L. Rogers (2007). Exploration Geomicrobiology - The New Frontier.

Reith, F. and S. L. Rogers (2008). Assessment of Bacterial Communities in Auriferous and Non-Auriferous Soils Using Genetic and Functional Fingerprinting. Geomicrobiology Journal 25: 203-215.

REX MINERALS 2009. Hillside Copper Project, South Australia. ASX and Media Release: 24 August 2009.

REX MINERALS 2008. Rex confirms new copper-gold (IOCG) discovery with 100m intersection at Hillside, South Australia. ASX and Media Release: 30 June, 2008.

Scott, K.M., and C.F. Pain 2008. Regolith Science. CSIRO Publishing.

Singh, B. K. Dawson, L. A, Macdonald, C. A. and S. M. Buckland (2008). Impact of biotic and abiotic interaction on soil microbial communities and functions: A field study.

Taylor, J.P., Wilson, B., Mills, M.S., and R.G. Burns (2002). Comparison of microbial numbers and enzymatic activities in surface soils and subsoils using various techniques. Soil Biology and Biochemistry 34: 387-401.

Wakelin, S. A., G. Chu, et al. (2009). Structural and functional response of soil microbiota to addition of plant substrate are moderated by soil Cu levels. Biology and fertility of soils.

Wakelin, S. A., Anand, R. R, et al (2010). Bacterial communities associated with a mineral weathering profile at an arid, sulphidic mine tailing dump, Australia. [In-preparation].

Wakelin, S. A., Macfarlane, C, et al (2010). Biogeochemical surface expression over a deep, overburdened-covered VMS mineralisation. [In-preparation].

Webb, A.W., Thomson, B.P., Blissett, A.H., Daly, S.J., Flint, R.B and A.J. Parker (1986). Geochronology of Gawler Craton, South Australia. Australian Journal of Earth Science. 33: 119-143.

Wilford, J., Worral, L., and B, Minty (2009). Radiometric map of Australia provides new insights into uranium prospectivity. AusGeo 95.

Van-Hees, P.A.W., Rosling, A., Lundstrom, U.S., and R.D. Finlay (2006). The biogeochemical impact of ectomycorrhizal connifers on major soil elements (A, Fe, K and Si). Geoderma vol: 136, issue: 1-2: 364-377

Tables

Table 1: Geochemistry - Permanova results for the samples using Type I SS.

Source	*F*	*P*	*Unique perms*
Sample depth (m) [1]	2.1766	0.0005	9872

Table 2: Geochemistry - Permanova results for selected samples using Type III SS.

Source	*F*	*P*	*Unique perms*
Sample depth (m)	1.3281	0.1902	9917
Regolith[2]	2.2749	0.0082	9920

Table 3: Geochemistry - Permanova results for selected samples using Type I SS.

Source	*F*	*P*	*Unique perms*
Geophysics[3]	4.6545	0.0001	9924
Regional geology[4]	2.2108	0.0227	9903
Mineralisation_5x_rule[5]	1.2163	0.2672	9930

[1]Soil samples taken at different depths through the soil (0.1 – 1.0 (m))

[2] Soil samples taken from different regolith landform units

[3] Samples based on magnetic geophysical data

[4] Samples based on geological map of the Yorke Peninsula area

[5] Samples based on mineralisation

Table 4: Bacteria - Permanova results for all samples using Type III SS.

Source	*F*	*P*	*Unique perms*
Depth Compressed[6]	8.8167	0.0001	9937
Land-use[7]	2.0785	0.0563	9935

[6]Soil samples taken from shallow or deep measurements

[7]Soil samples taken from different land-use areas

Table 5: Bacteria – Permanova results for all samples using Type III SS

Source	*F*	*P*	*Unique perms*
Land-use	2.1378	0.0505	9934
Sample depth (m)	3.761	0.0001	9903

Table 6: Bacteria – Permanova results for surface samples using Type III SS

Source	*F*	*P*	*Unique perms*
Regional geology	3.7729	0.0107	9946
Mineralisation_5x_rule	1.362	0.1921	9922

Table 7: Bacteria – Permanova results for surface samples using Type III SS

Source	*F*	*P*	*Unique perms*
Regional geology	3.6099	0.0381	9927
Regolith	2.323	0.0411	9933

Table 8: Bacteria – Permanova results for all samples using Type III SS

Source	*F*	*P*	*Unique perms*
Sample depth (m)	4.1623	0.0001	9895
Mineralisation_5x_rule	1.4208	0.1042	9913

Table 9: Bacteria – Permanova results for selected samples using Type I SS

Source	*F*	*P*	*Unique perms*
Geophysics	3.9407	0.001	9939
Regional geology	6.4611	0.0002	9953
Mineralisation_5x_	2.7034	0.0086	9928

Table 10: Bacteria - Permanova results for surface samples using Type I SS.

Source	*F*	*P*	*Unique perms*
Regolith	2.8909	0.023	9945
Regional geology	4.2544	0.0383	9942
Geophysics	3.1738	0.0019	9944

Table 11: Bacteria – Permanova results for surface samples using Type I SS

Source	*F*	*P*	*Unique perms*
Regolith	2.8343	0.1019	9950
Geophysics[6]	2.6397	0.0775	9944
Mineralisation_5x_rule	1.6982	0.2444	9956

Table 12: Bacteria – Permanova results for all depth samples excluding Transect 1 samples using Type I SS

Source	*F*	*P*	*Unique perms*
Regional geology	3.8801	0.0079	9956
Mineralisation_5x_rule	1.4299	0.1481	9914

Table 13: Fungi – Permanova results for all samples using Type III SS

Source	*F*	*P*	*Unique perms*
Land-use	3.5961	0.0019	9956
Depth Compressed	14.622	0.0001	9952

Table 14: Fungi – Permanova results for all samples using Type III SS

Source	*F*	*P*	*Unique perms*
Regional Geology	2.0004	0.033	9928

Table 15: Fungi – Permanova results for all samples using Type III SS

Source	*F*	*P*	*Unique perms*
Land-use	3.2314	0.0044	9940
Sample depth (m)	4.3622	0.0001	9897

Table 16: Fungi – Permanova results for surface samples using Type III SS

Source	*F*	*P*	*Unique perms*
Regional geology	3.806	0.0382	9939
Regolith	3.6399	0.0004	9921

Table 17: Fungi – Permanova results for surface samples using Type III SS

Source	*F*	*P*	*Unique perms*
Regional geology	3.3139	0.0027	9959
Mineralisation_5x_rule	1.1614	0.3241	9910

Table 18: Fungi – Permanova results for all samples using Type III SS

Source	*F*	*P*	*Unique perms*
Sample depth (m)	4.1974	0.0001	9913
Mineralisation_5x_rule	1.8665	0.011	9902

Table 19: Fungi - Permanova results for all depth samples excluding Transect 1 using Type I SS.

Source	*F*	*P*	*Unique perms*
Regolith	1.9478	0.0349	9927
Geophysics	2.269	0.0091	9931
Mineralisation_5x_rule	2.0892	0.0057	9918

Table 20: Fungi – Permanova results for surface samples using Type I SS

Source	*F*	*P*	*Unique perms*
Regional geology	3.4455	0.0247	9955
Regolith	3.3544	0.001	9939
Mineralisation_5x_rule	0.84322	0.6577	9926

Table 21: Fungi – Permanova results for selected samples using Type I SS

Source	*F*	*P*	*Unique perms*
Geophysics	2.6043	0.0017	9914
Regional geology	0.89881	0.543	9912
Mineralisation_5x_	2.0171	0.0078	9915

Table 22: Archaea – Permanova results for all samples using Type III SS

Source	*F*	*P*	*Unique perms*
Depth compressed	3.1274	0.0136	9962
Land-use	2.8105	0.0206	9945

Table 23: Archaea – Permanova results for all samples using Type III SS

Source	*F*	*P*	*Unique perms*
Land-use	2.6242	0.0374	9953
Sample depth (m)	1.6528	0.0801	9914

Table 24: Archaea – Permanova results for surface samples using Type III SS

Source	*F*	*P*	*Unique perms*
Regional geology	4.0678	0.0177	9947
Regolith	2.3935	0.0483	9951

Table 25: Archaea – Permanova results for surface samples using Type I SS

Source	*F*	*P*	*Unique perms*
Regolith	3.3449	0.0363	9908
Geophysics	2.4922	0.072	9950
Mineralisation_5x_rule	1.8473	0.1572	9961

Table 26: Archaea – Permanova results for all depth samples excluding Transect 1 using Type I SS

Source	*F*	*P*	*Unique perms*
Regional geology	4.0892	0.0123	9953
Mineralisation_5x_rule	1.4671	0.1808	9941

Table 27: Archaea – Permanova results for surface samples using Type I SS

Source	*F*	*P*	*Unique perms*
Sample depth (m)		No test	
Mineralisation_5x_rule	1.8169	0.0757	9910

Table 28: Archaea – Permanova results for surface using III SS

Source	*F*	*P*	*Unique perms*
Sample depth (m)	2.6417	0.0036	9921
Mineralisation_5x_rule	1.5845	0.0702	9917

Table 29: Archaea – Permanova results for surface using Type I SS

Source	*F*	*P*	*Unique perms*
Regional geology	3.9418	0.0211	9945
Regolith	2.4177	0.0627	9957
Mineralisation_5x_rule	1.0787	0.4089	9949

Table 30: Archaea – Permanova results for selected samples using type I SS

Source	*F*	*P*	*Unique perms*
Geophysics	2.1515	0.1175	9970
Regional geology	4.5344	0.031	9974
Mineralisation_5x_	1.5405	0.2244	9942

Table 31: pH and EC measurements for the 93 samples laboratory analysed.

Sample number	pH	EC us	Sample number	pH	EC us	Sample number	pH	EC us	Sample number	pH	EC us
100 D	8.65	146.4	110 S	8.4	159.6	114 D	8.64	157.1	201 S	8.8	121.1
102 D	9.14	169.3	105 D	8.7	330	116 D	8.94	119.2	204 S	8.51	196.4
103 D	9.01	192.5	106 D	8.82	134	118 D	8.71	206.9	205 S	8.64	126.4
214 D	9.4	282	110 D	8.8	105.4	119 D	9.71	1754	206 S	8.8	132.9
215 D	9.16	280	107 D	8.32	140.4	121 D	8.9	121.3	209 S	8.6	162
216 D	9.02	112.8	109 D	8.6	101.8	126 D	8.5	499	203 D	9.23	166.7
218 D	9.61	7.59	151 D	8.81	133.7	217 D	8.92	115.5	204 D	9.26	139.9
100 S	8.58	635	235 D	8.76	307	227 D	9.94	1297	205 D	9.11	129
101 S	8.52	283	152 D	9.02	161	122 D	8.43	125.6	206 D	9.25	187.2
102 S	8.21	197.4	236 D	9.1	248	123 D	9.09	124.9	207 D	8.7	231
103 S	8.5	153.1	153 D	9.18	155.9	124 D	9.25	166.2	208 D	9.01	139.4
215 S	7.64	169	155 D	8.96	126.1	125 D	8.4	146	209 D	9.07	145.1
213 S	8.99	57.2	156 D	9.08	141.6	1 D	9.4	207.2	1 S	8.41	226
214 S	8.13	87.2	157 D	9.34	160.5	2 D	9.03	149.6	5 S	8.5	97.3
216 S	6.75	118.7	158 D	8.84	170.1	9 D	8.65	1568	9 S	8.5	194.8
218 S	7.21	180.2	159 D	8.94	142.1	10 D	9.25	189.6	10 S	8.63	258
219 S	7.65	180.7	147 D	9.05	147.4	11 D	9.17	149.3	17 S	9	362
104 S	7.5	103.5	148 D	9.41	147	12 D	8.9	151	20 S	8.5	418
105 S	7.22	450	149 D	8.89	126	16 D	8.9	3090	107 D T1	8.76	111
106 S	7.79	107.2	233 D	8.51	186.2	19 D	8.9	540	109 D T1	8.46	178.1
107 S	6.4	187	150 D	9.05	116.3	20 D	8.9	172.1	215 D T1	9.44	205.3
108 S	7.3	43.6	234 D	8.97	110.1	197 D	8.68	115.9	213 D T1	8.99	57.2
109 S	7.72	75.4	113 D	9.67	268	245 D	9.01	141.4	102 D T1	8.65	85
									219 D T1	8.05	98.9

Table 32: primer and labelling of PCR primers

Primer	Fluorescent label	Sequence (5' to 3')	Concentrations added to PCR	Target and size
63F	None	AGGCCTAACACATGCAAGTC	0.2uM	Bacteria, 1024bp
1087R	VIC (HEX)	CTCGTTGCGGGACTTACCCC	0.2uM	
Ar3F	None	TTCCGGTTGATCCTGCCGGA	0.4uM	Archaea, 924bp
Ar927R	NED (TET)	CCCGCCAATTCCTTTAAGTTTC	0.4uM	
ITS 1F	FAM	CTTGGTCATTTTAGAGGAAGTAA	0.4uM	Fungi, varies, approx 600bp
ITS 4R	None	TCCTCCGCTTATTGATATGC	0.4uM	

Figure captions

Figure 1: Map of the Hillside area located near Ardrossan on the Yorke Peninsula with modification

(http://www.ppbh-marionbay.com/PPBH/images/MapYorkePeninsulaLarge.jpg)

Figure 2: Geological map of the Hillside area

Figure 3: Map of Hillside Mine area with sample numbers.

Figure 4: Geochemistry - MDS plot of sample depth (m)

Figure 5: Geochemistry - CAP analysis of regolith on all samples

Figure 6: Geochemistry - CAP analysis of geophysics on selected samples

Figure 7: Geochemistry - CLUSTER analysis of elements on selected samples

Figure 8: RELATE analysis of the geochemistry and microbiology data sets

Figure 9: Bacteria - CAP analysis on depth compressed for all samples

Figure 10: Bacteria - CAP analysis on regional geology of surface samples

Figure 11: Bacteria - CAP analysis on regolith of surface samples

Figure 12: Bacteria - CAP analysis on Geophysics surface samples

Figure 13: Bacteria - CAP analysis mineralisation_5x surface samples (yes meaning there is mineralisation of Cu, Au and U. No meaning there are no elements present, no mineralisation)

Figure 14: Fungi - CAP analysis on regolith surface samples

Figure 15: Fungi - CAP analysis on sample depth (m) all samples

Figure 16: Archaea - CAP analysis on sample depth (m) all samples

Figure 17: Archaea - CAP analysis on regional geology surface samples

Figures

Figure 1.

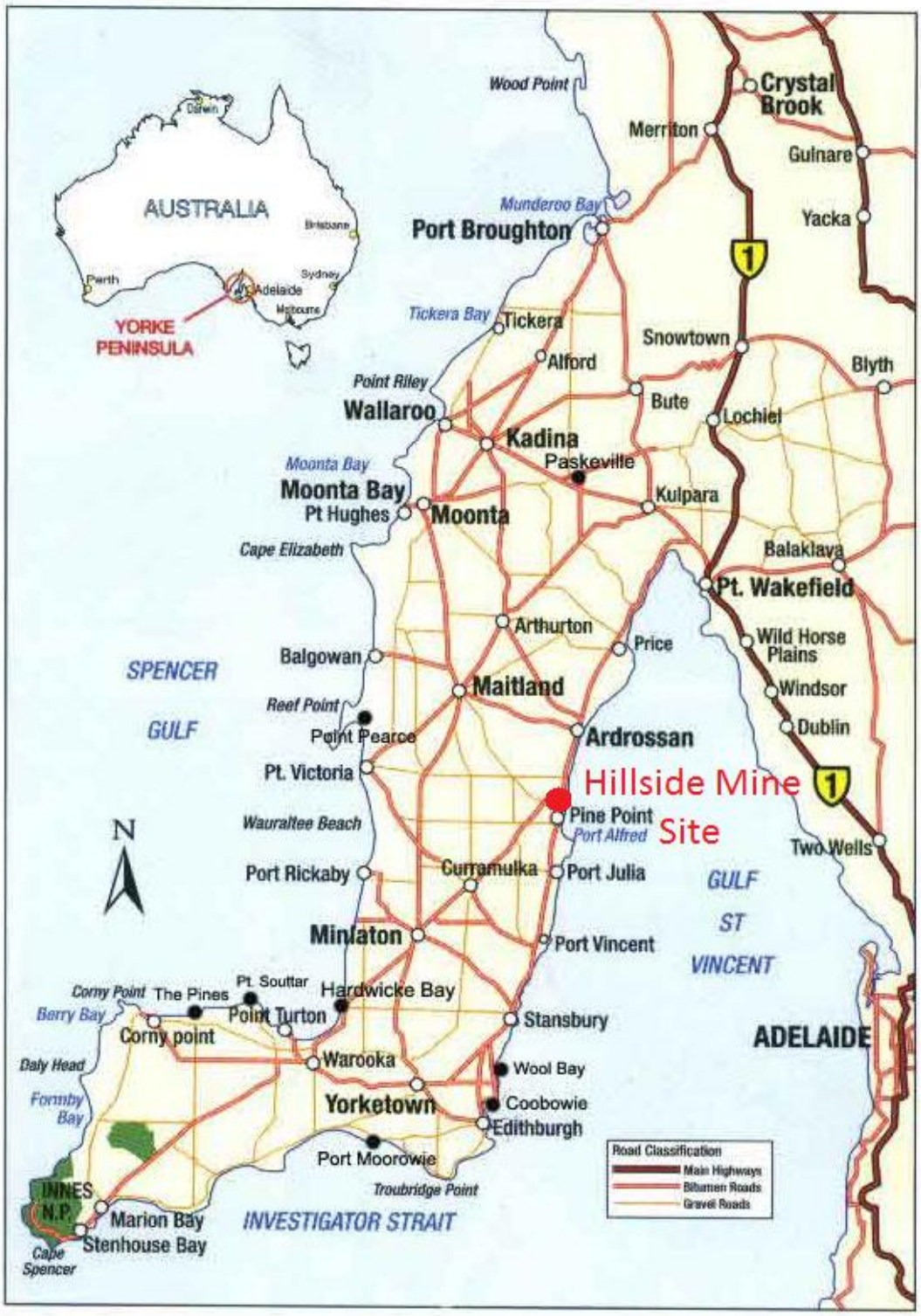

AUSTRALIA
Darwin
Brisbane
Perth
Adelaide
Sydney
Melbourne
YORKE PENINSULA
Wood Point
Crystal Brook
Merriton
Guinare
Yacka
Munderoo Bay
Port Broughton
1
Tickera Bay
Tickera
Snowtown
Alford
Blyth
Point Riley
Wallaroo
Bute
Lochiel
Kadina
Paskeville
Moonta Bay
Moonta Bay
Pt Hughes
Moonta
Kulpara
Cape Elizabeth
Balaklava
Pt. Wakefield
Arthurton
Price
Wild Horse Plains
Balgowan
SPENCER
GULF
Maitland
Windsor
Reef Point
Point Pearce
Ardrossan
Dublin
Pt. Victoria
Hillside Mine Site
Wauraltee Beach
Pine Point
Port Alfred
Two Wells
N
Port Rickaby
Curramulka
Port Julia
GULF ST VINCENT
Minlaton
Port Vincent
Corny Point
The Pines
Pt. Souttar
Hardwicke Bay
Berry Bay
Point Turton
Stansbury
Corny point
ADELAIDE
Daly Head
Warooka
Wool Bay
Formby Bay
Yorketown
Coobowie
Edithburgh
Port Moorowie
Troubridge Point
Road Classification
Main Highways
Bitumen Roads
Gravel Roads
INNES N.P.
Marion Bay
INVESTIGATOR STRAIT
Stenhouse Bay
Cape Spencer

Figure 2.

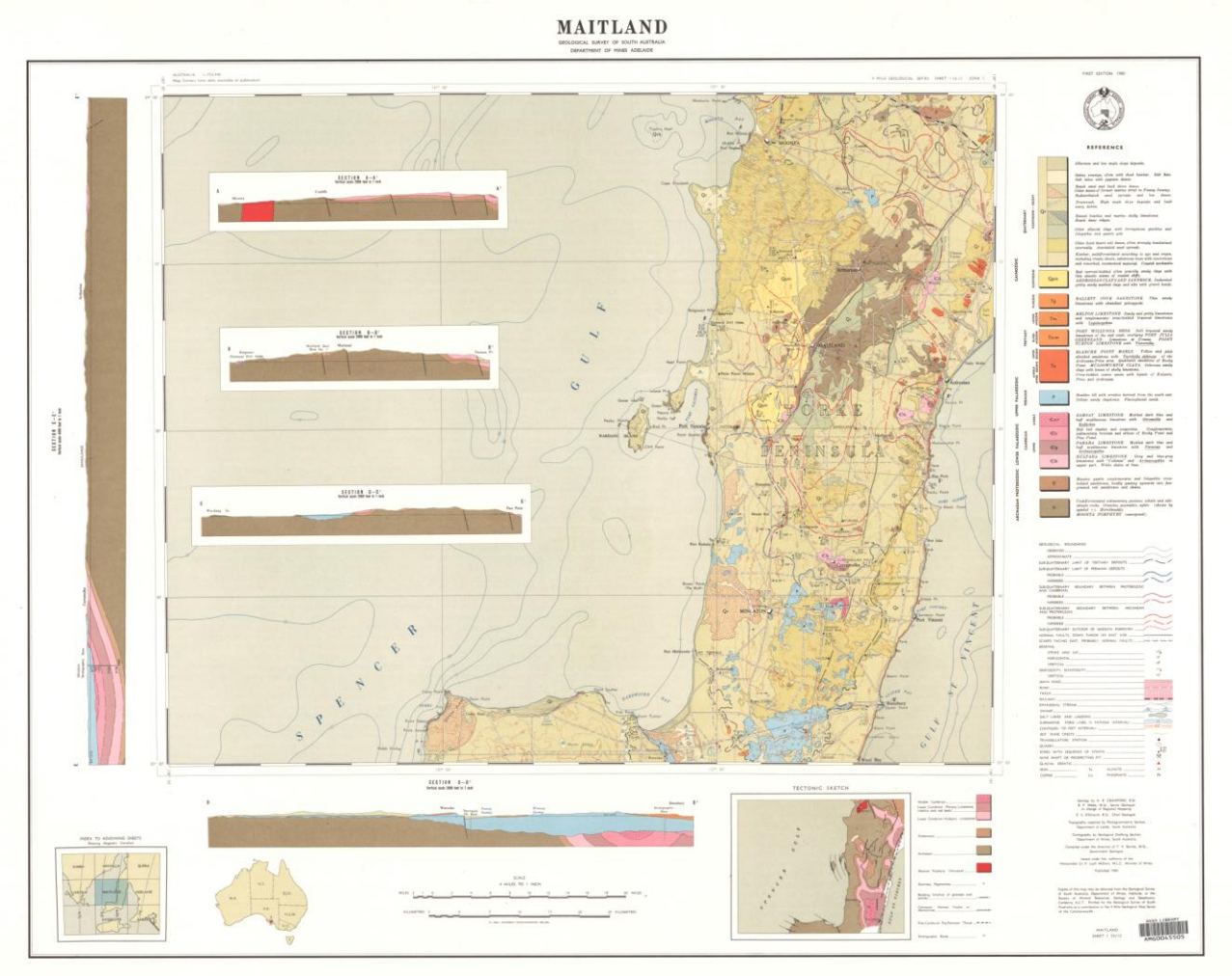

MAITLAND
GEOLOGICAL SURVEY OF SOUTH AUSTRALIA
DEPARTMENT OF MINES ADELAIDE
GULF
SPENCER
YORKE
PENINSULA
GULF ST VINCENT
REFERENCE
SECTION A–A'
SECTION B–B'
SECTION C–C'
SECTION D–D'
TECTONIC SKETCH
INDEX TO ADJOINING SHEETS

Figure 3.


763000
763250
763500
763750
764000
764250
764500
764750
765000
6175500
6175250
6175000
6174750
6174500
6174250
6174000
6173750
6173500
6173250
6173000
6172750
0 50100 200 300 400
N

Figure 4.

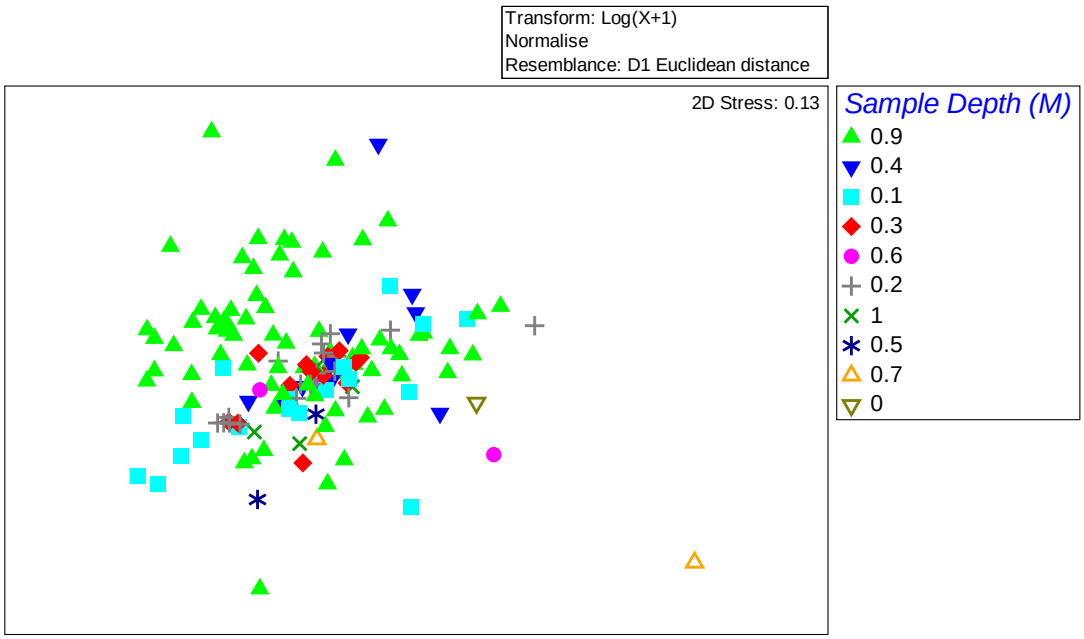

Transform: Log(X+1)
Normalise
Resemblance: D1 Euclidean distance
2D Stress: 0.13
Sample Depth (M)
0.9
0.4
0.1
0.3
0.6
0.2
1
0.5
0.7
0

Figure 5.

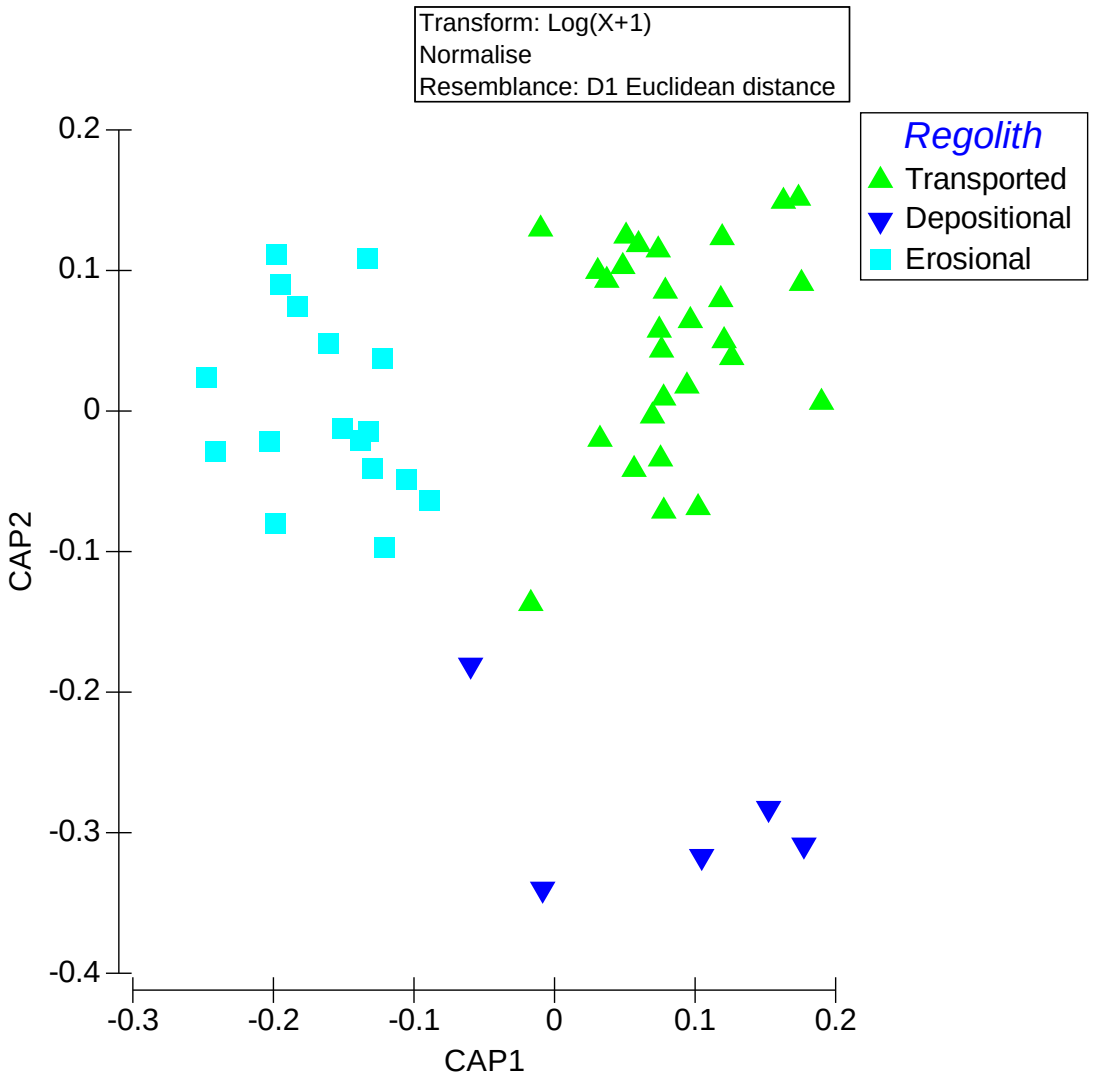

Transform: Log(X+1)
Normalise
Resemblance: D1 Euclidean distance
Regolith
Transported
Depositional
Erosional
CAP2
CAP1
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.3
-0.2
-0.1
0
0.1
0.2

Figure 6.

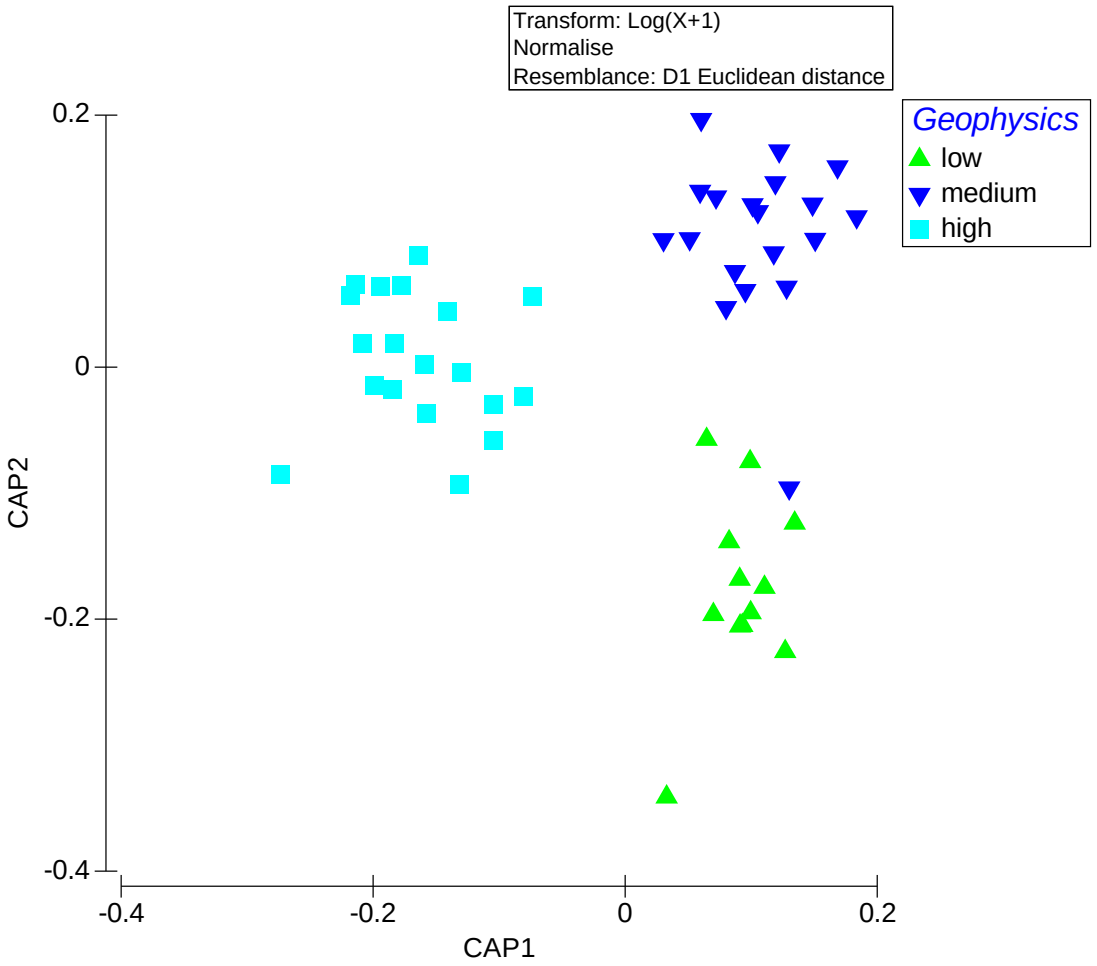

Transform: Log(X+1)
Normalise
Resemblance: D1 Euclidean distance
Geophysics
low
medium
high
0.2
0
-0.2
-0.4
CAP2
-0.4
-0.2
0
0.2
CAP1

Figure 7.

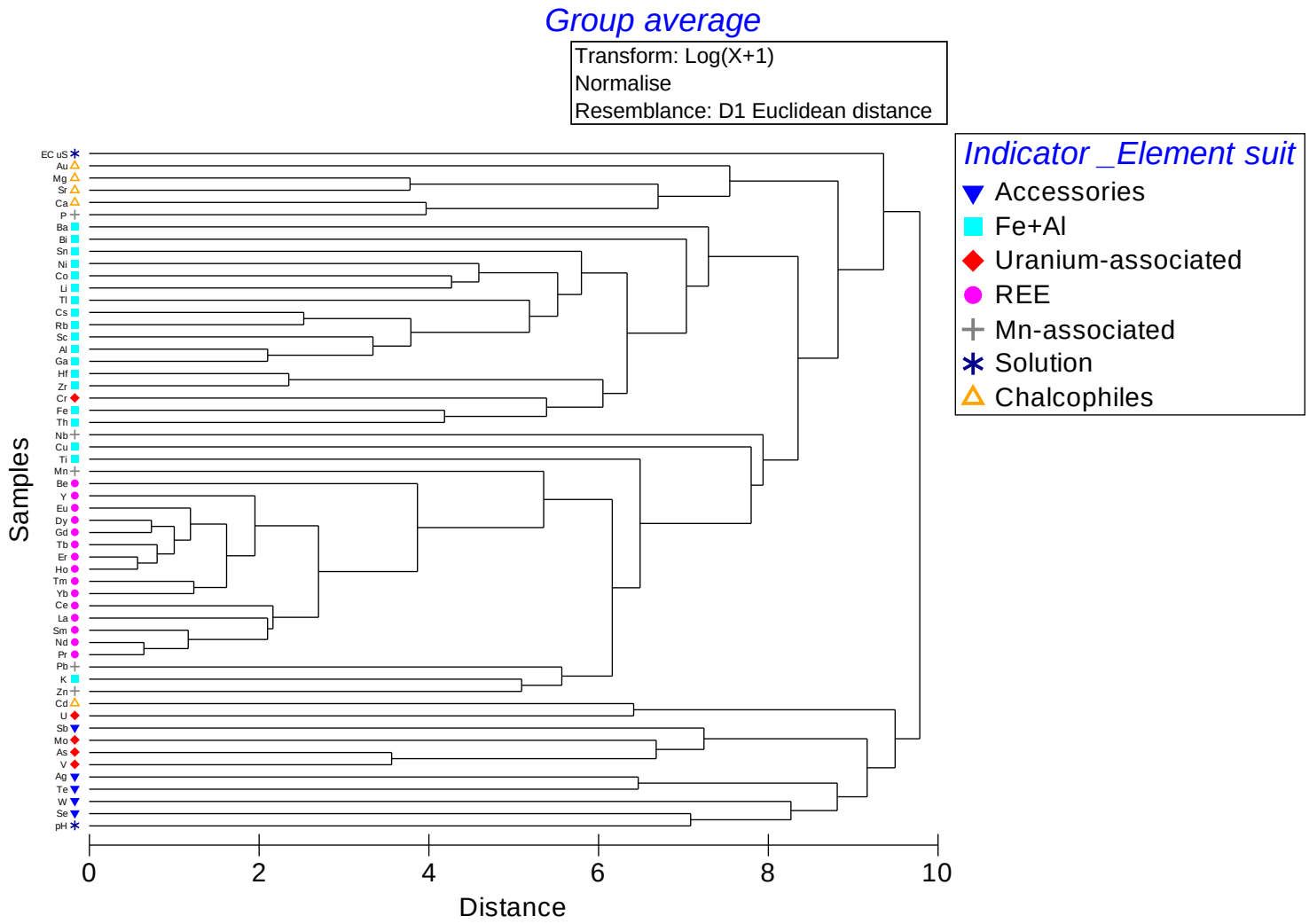

Group average
Transform: Log(X+1)
Normalise
Resemblance: D1 Euclidean distance
Indicator _Element suit
Accessories
Fe+Al
Uranium-associated
REE
Mn-associated
Solution
Chalcophiles
Samples
EC uS
Au
Mg
Sr
Ca
P
Ba
Bi
Sn
Ni
Co
Li
Tl
Cs
Rb
Sc
Al
Ga
Hf
Zr
Cr
Fe
Th
Nb
Cu
Ti
Mn
Be
Y
Eu
Dy
Gd
Tb
Er
Ho
Tm
Yb
Ce
La
Sm
Nd
Pr
Pb
K
Zn
Cd
U
Sb
Mo
As
V
Ag
Te
W
Se
pH
0
2
4
6
8
10
Distance

Figure 8.

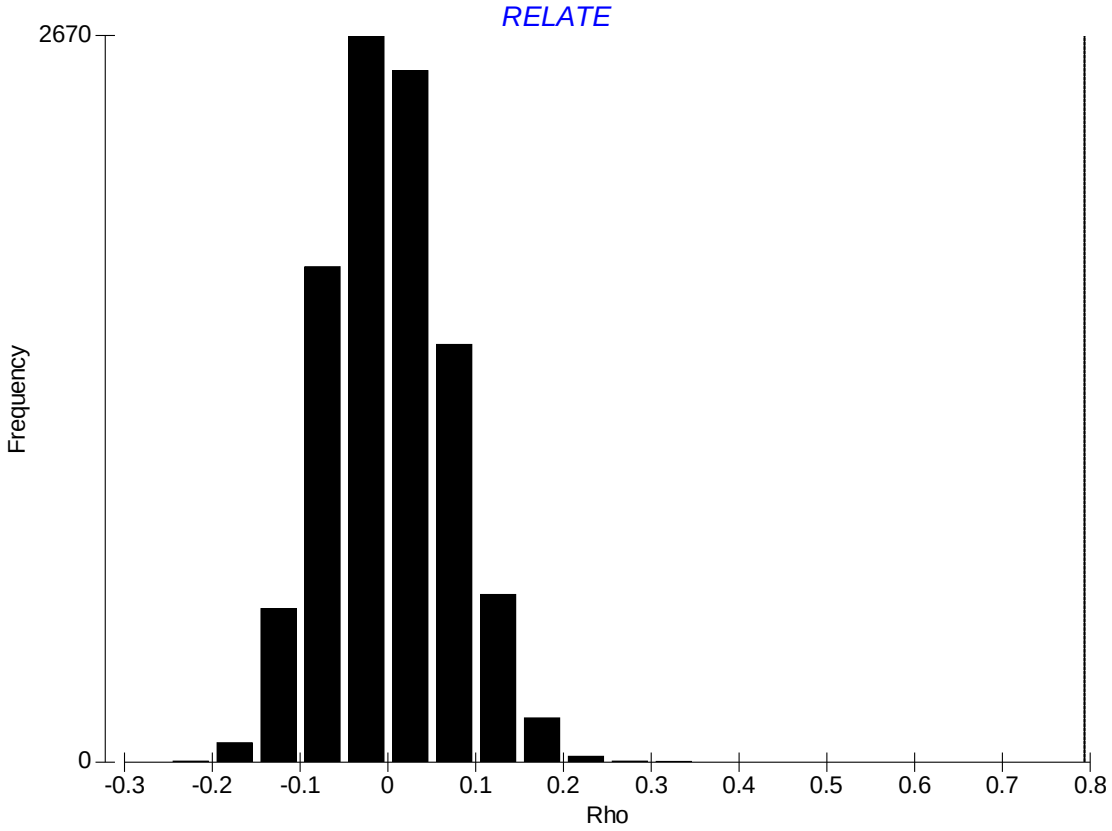


Figure 9.

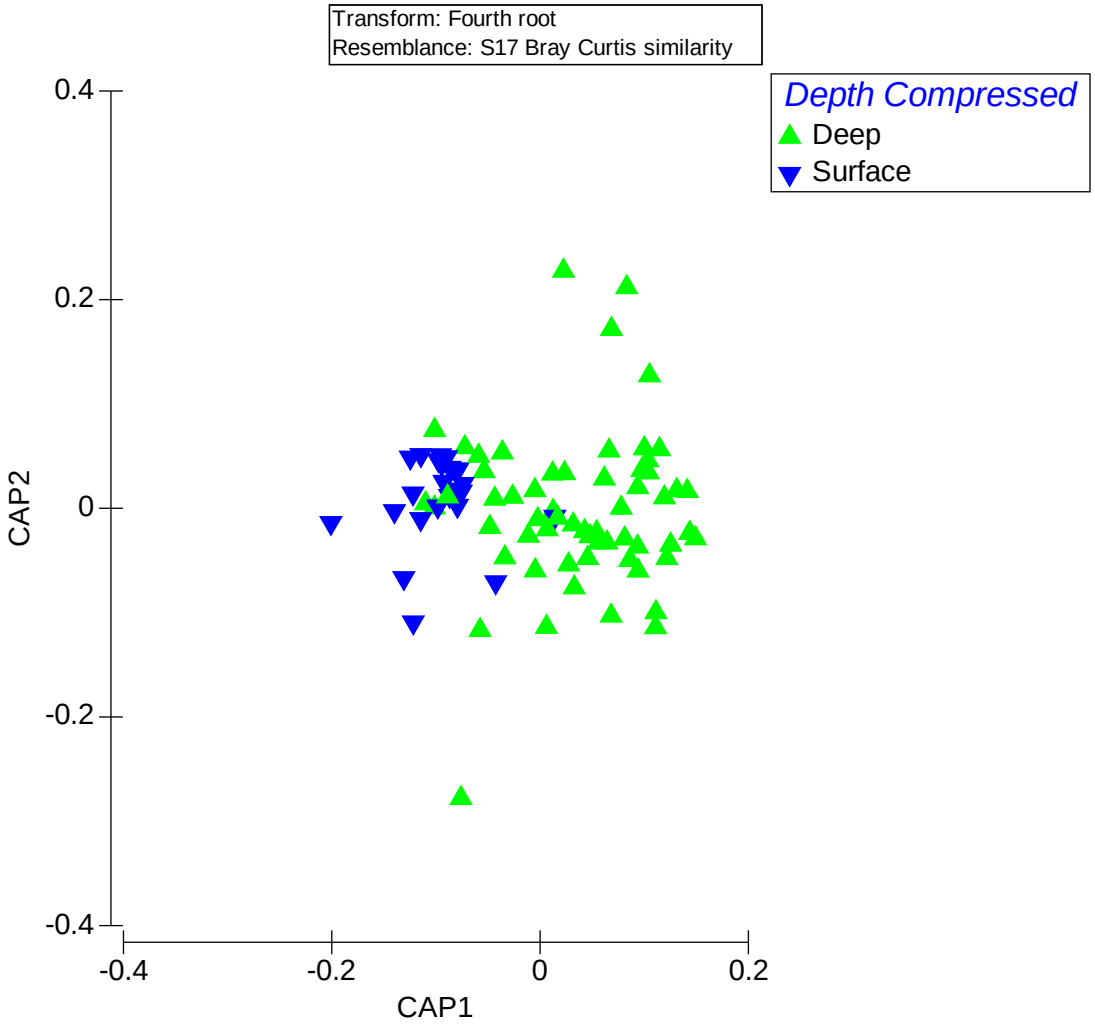

Transform: Fourth root
Resemblance: S17 Bray Curtis similarity
Depth Compressed
Deep
Surface
0.4
0.2
0
-0.2
-0.4
CAP2
-0.4
-0.2
0
0.2
CAP1

Figure 10.

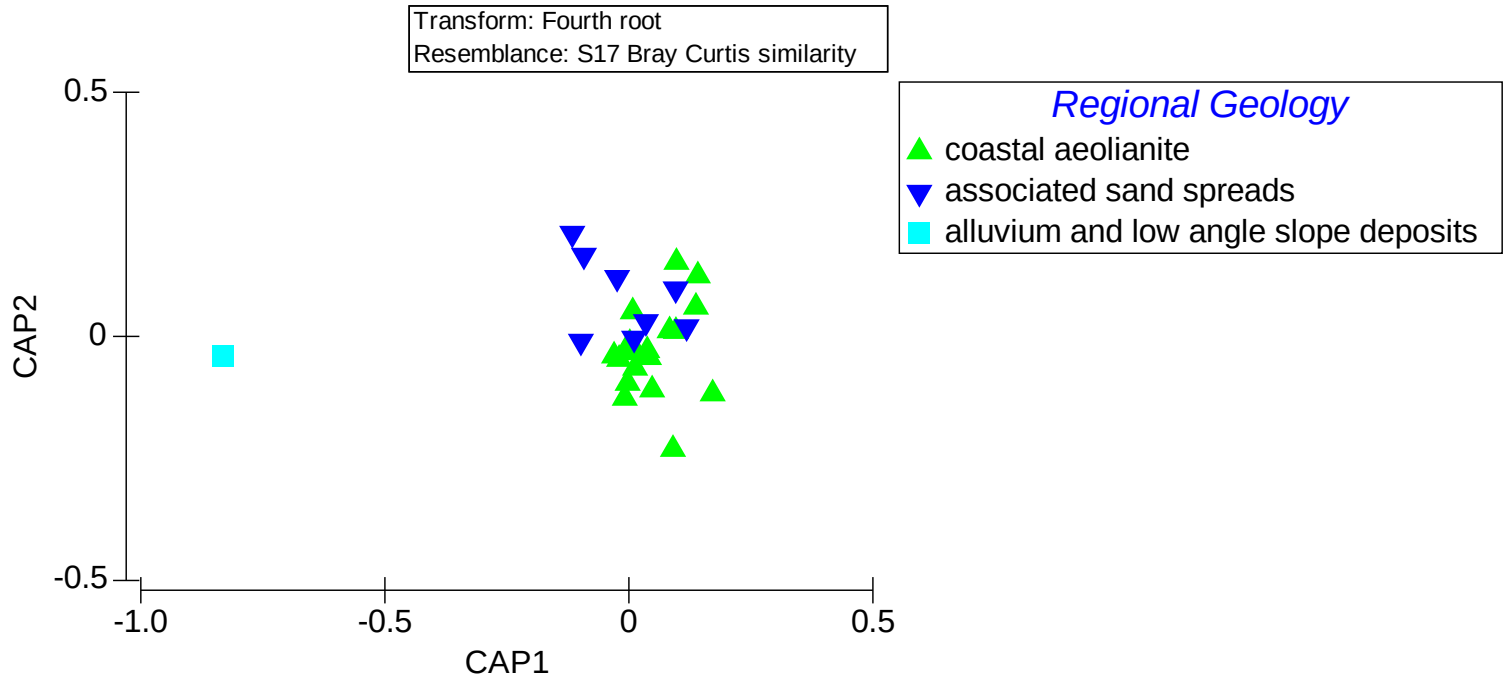

Transform: Fourth root
Resemblance: S17 Bray Curtis similarity
Regional Geology
coastal aeolianite
associated sand spreads
alluvium and low angle slope deposits
0.5
0
-0.5
CAP2
-1.0
-0.5
0
0.5
CAP1

Figure 11.

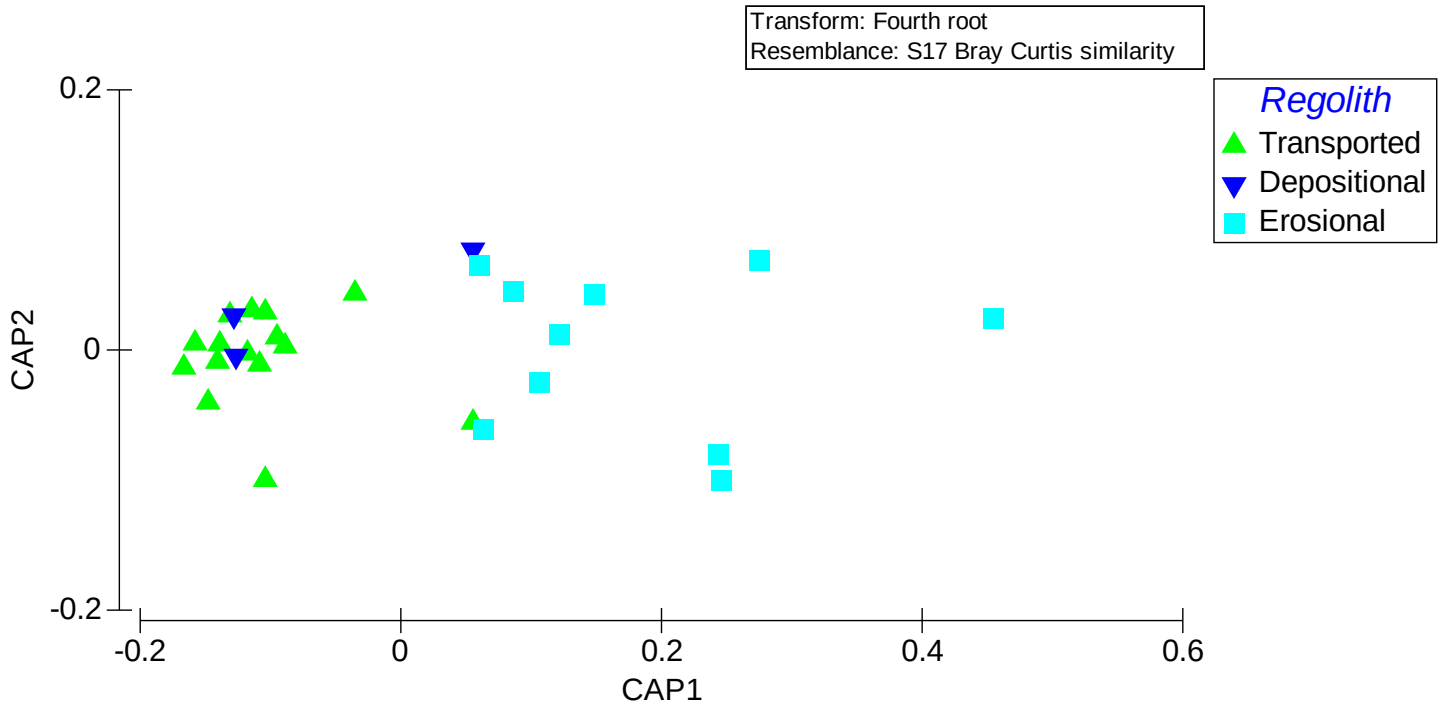

Transform: Fourth root
Resemblance: S17 Bray Curtis similarity
Regolith
Transported
Depositional
Erosional
0.2
0
-0.2
CAP2
-0.2
0
0.2
0.4
0.6
CAP1

Figure 12.

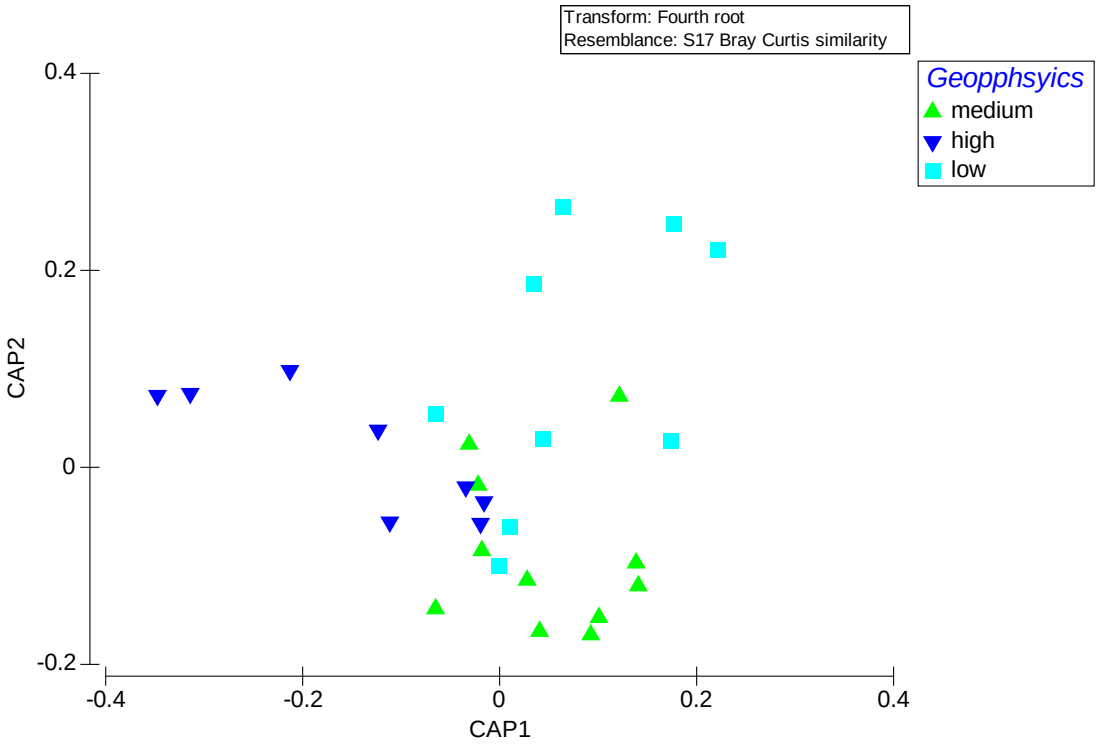

Transform: Fourth root
Resemblance: S17 Bray Curtis similarity
Geopphsyics
medium
high
low
0.4
0.2
0
-0.2
CAP2
-0.4
-0.2
0
0.2
0.4
CAP1

Figure 13.

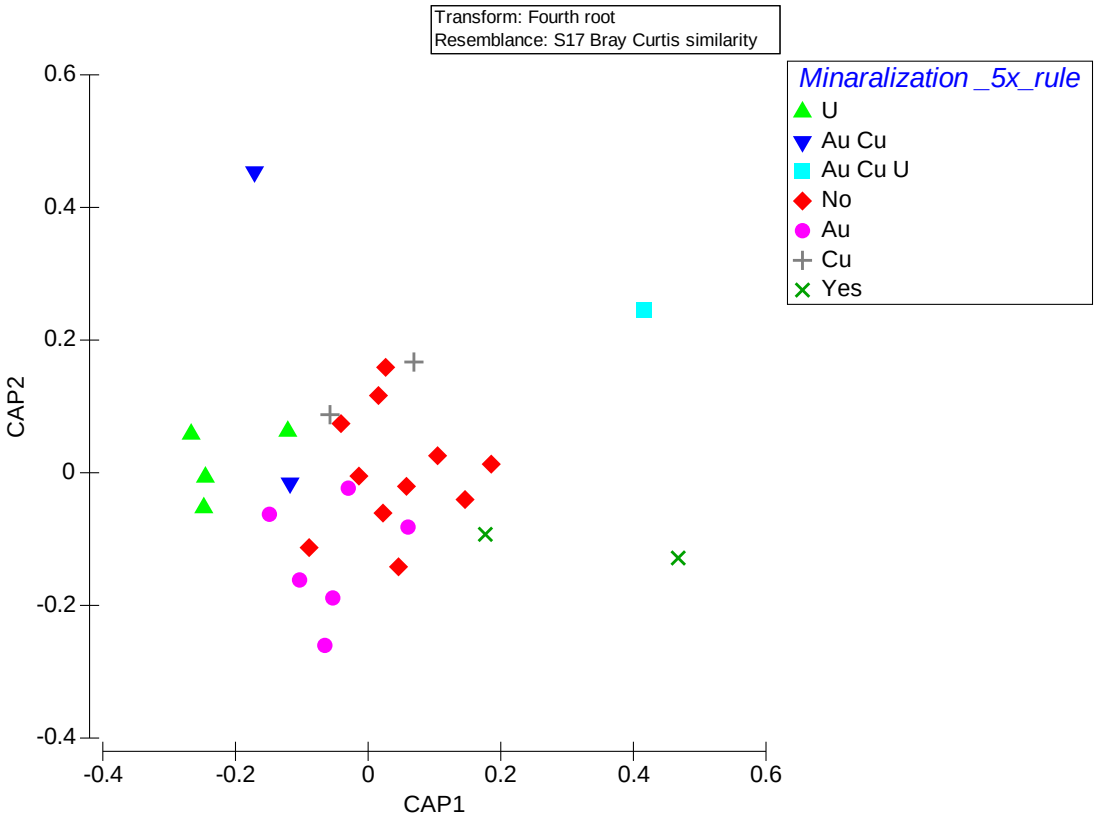

Transform: Fourth root
Resemblance: S17 Bray Curtis similarity
Minaralization _5x_rule
U
Au Cu
Au Cu U
No
Au
Cu
Yes
CAP2
CAP1
0.6
0.4
0.2
0
-0.2
-0.4
-0.4
-0.2
0
0.2
0.4
0.6

Figure 14.

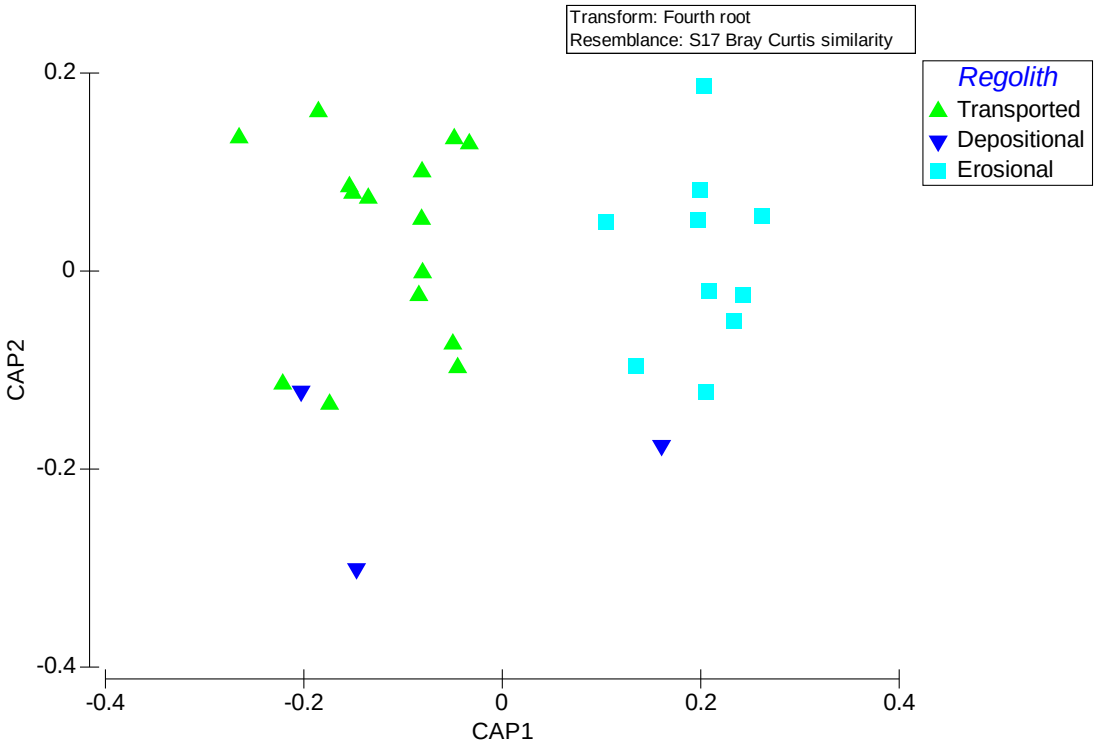

Transform: Fourth root
Resemblance: S17 Bray Curtis similarity
Regolith
Transported
Depositional
Erosional
0.2
0
-0.2
-0.4
CAP2
-0.4
-0.2
0
0.2
0.4
CAP1

Figure 15.

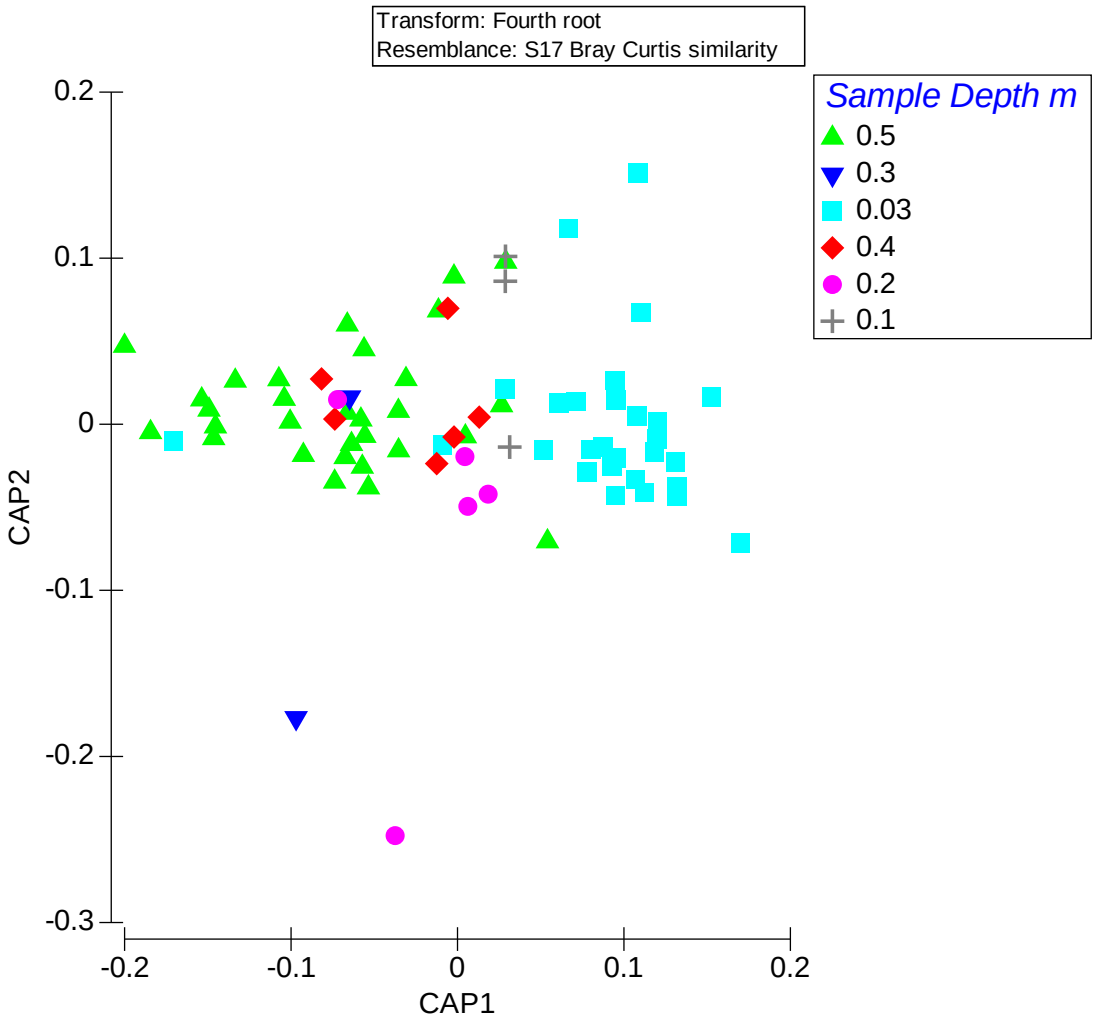

Transform: Fourth root
Resemblance: S17 Bray Curtis similarity
Sample Depth m
0.5
0.3
0.03
0.4
0.2
0.1
CAP2
CAP1
0.2
0.1
0
-0.1
-0.2
-0.3
-0.2
-0.1
0
0.1
0.2

Figure 16.

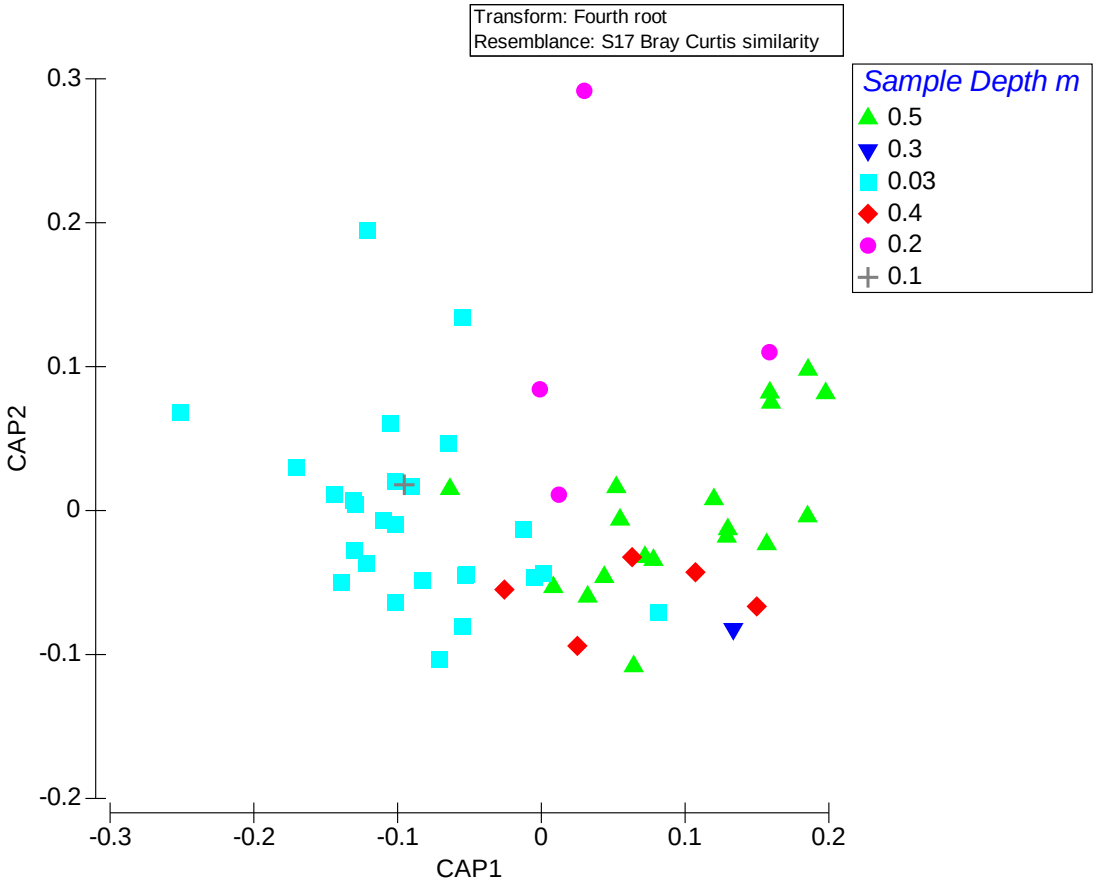
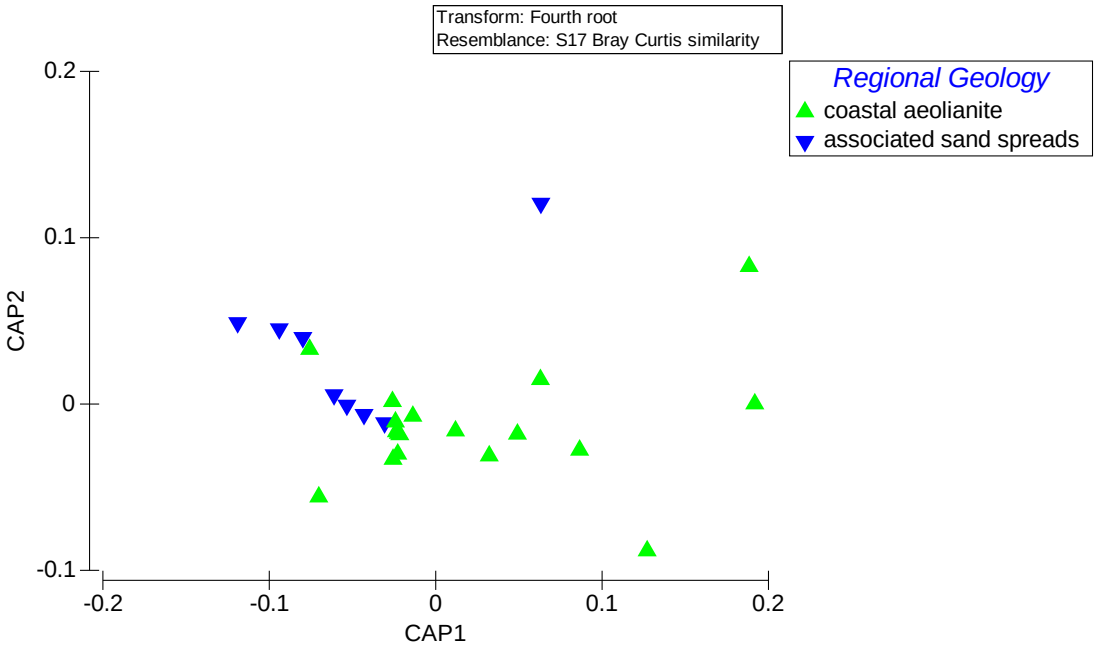

Transform: Fourth root
Resemblance: S17 Bray Curtis similarity
Sample Depth m
0.5
0.3
0.03
0.4
0.2
0.1
CAP2
CAP1
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.2
-0.1
0
0.1
0.2

Figure 17.


Transform: Fourth root
Resemblance: S17 Bray Curtis similarity
Regional Geology
coastal aeolianite
associated sand spreads
0.2
0.1
0
-0.1
CAP2
-0.2
-0.1
0
0.1
0.2
CAP1