

# Variation in chain-length of leaf wax *n*-alkanes in plants and soils across Australia

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## VARIATION IN CHAIN-LENGTH OF LEAF WAX N-ALKANES IN PLANTS AND SOILS ACROSS AUSTRALIA

### RUNNING TITLE

ACL of *n*-alkanes in plants and soils

### ABSTRACT

Long chain *n*-alkanes are produced as part of leaf epicuticular wax and are ideal biomarkers for palaeoclimatology and palaeoecology due to their persistence in soils and sediments. Sedimentary records often show shifts in average chain-lengths (ACL) of *n*-alkanes, both across geologic time and modern-day climate gradients and this shift may be climate driven.

Australia spans a broad range of different climate conditions providing an ideal study area for investigating the relationship of ACL to climate. The Terrestrial Ecosystem Research Network (TERN) has developed a network of biodiversity monitoring plots (AusPlots and TREND) at which plant and soil samples are collected and made available to the research community. By analysing *n*-alkane ACL present in plants and soils collected from these sites and comparing with each site's respective climatic conditions, this study examines whether ACL of leaf wax *n*-alkanes varies systematically in modern plants and soils in relation to climate over a N-S transect of Australia.

Specifically, this study examines whether:

- (1) ACL in plants correlates with different climate variables.
- (2) ACL measured in soil represents a weighted average of the ACL of the dominant plant species at each site.
- (3) ACL signature in the soils correlates to different climate variables.

This study finds no relationship between the different climate variables to ACL of modern. Further, the weighted average of the dominant plant species ACL from each site

analysed is a poor predictor of the actual ACL present in the soils. In contrast to ACL from plants, the ACL from the soils shows a strong relationship with temperature and aridity measures. Soils may correlate better with climate because they integrate a long-term average of highly variable ACL values from all contributing organisms. This study supports climate as a driver of ACL in sediments across space and time.

## **KEYWORDS**

VARIATION, N-ALKANE, SOILS, PLANTS, CLIMATE, PALAEOCLIMATE, AUSTRALIA, ACL, BIOMARKERS

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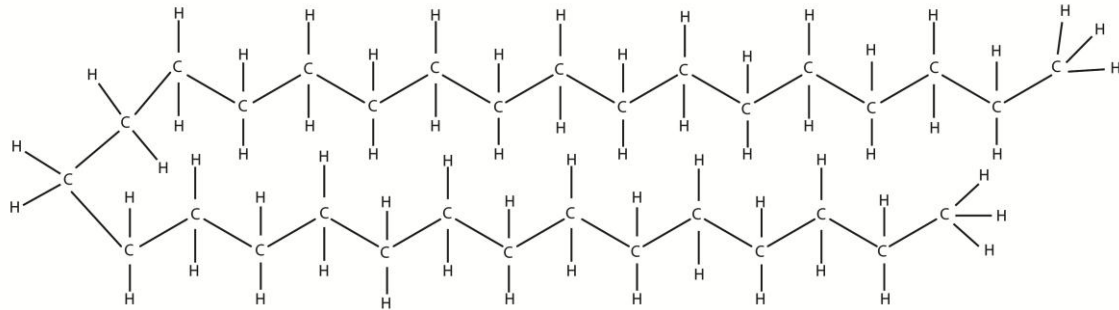
## INTRODUCTION

Human induced climate change due to increased CO<sub>2</sub> emissions from burning of fossil fuels and land use change is of great societal concern, with present day concentrations nearly 100ppm higher than they have been for the last 800,000 years (IPCC 2013, Masson-Delmotte et al. 2013). However, similar CO<sub>2</sub> induced greenhouse warming has occurred previously in the Earth's geologic history. For example, the Paleocene-Eocene Thermal Maximum (PETM) was a period of extreme and rapid warming driven by an increase in atmospheric CO<sub>2</sub> (Smith et al. 2007, McInerney and Wing 2011).

Reconstructing these analogous past climates is important for understanding how climate functions and what sort of environmental and socio-economic impacts we can expect as a result of climate change in to the future (Berger et al. 2012). There is therefore a need to develop new tools that can be used for reconstructing past terrestrial climates.

A number of proxies are available for reconstructing past climates, including chemical analyses of continuous lake and marine sedimentary records, ice cores and speleothems. Recent workers have proposed that certain plant biomarkers such as long chain *n*-alkanes may provide an effective proxy for climatic variability as they are sensitive to ambient climate conditions (Eglinton and Eglinton 2008, Bai et al. 2009, Castañeda and Schouten 2011) and are persistent in the sedimentary record on geologic timescales (Gagosian and Peltzer 1986). Long chain *n*-alkanes are non-polar, unbranched, straight chained hydrocarbon molecules that form a component of plant leaf waxes found on the leaf cuticle (Diefendorf et al. 2011). Each carbon atom contained within an *n*-alkane

forms four single bonds (Olah et al. 2011) resulting in the general saturated formula of  $C_nH_{2n+2}$  (Jones 2000) and take on a form as given in **Figure 1**.



**Figure 1: Structural diagram of an  $nC_{31}$  straight chain *n*-alkane, *n*-Hentriacontane ( $C_{31}H_{64}$ ), a common *n*-alkane found in the cuticular waxes of most higher plant species.**

The different *n*-alkane chain lengths demonstrate different physical properties, with longer chain lengths having greater hydrophobicity and higher melting point (1-3°C for each carbon unit) than shorter chain lengths (Gibbs 2002, Rommerskirchen et al. 2003). Plants use these compounds to regulate their water balance by preventing water loss through the surface of their leaves (Eglinton and Hamilton 1967, Dodd and Poveda 2003). They also form a photoprotective layer, limiting leaf tissue damage from UV radiation (Shepherd and Griffiths 2006, Koch et al. 2009), as well as helping to resist fungal infection and herbivory (Banthorpe 2006).

*n*-Alkanes are ideal for palaeoclimate reconstruction due to their continuous accumulation and relative persistence in soil and sediment records (Smith et al. 2007, Diefendorf et al. 2011), where they accumulate as a result of wind ablation and leaf fall (Rommerskirchen et al. 2006, Shepherd and Griffiths 2006, Zech et al. 2013). The decomposition of these molecules requires the presence of specific co-metabolising compounds and decomposer enzymes along with optimal soil properties, such as pH,



which may explain their persistence in sedimentary environments (Schmidt et al. 2011). While high quantities of *n*-alkanes are present in modern day soils, they have also been extracted from Cretaceous-Paleogene boundary sediments (Yamamoto et al. 2010) as well as Eocene (Smith et al. 2007), Miocene (Huang et al. 2001) and Holocene sediments (Schwark et al. 2002). *n*-Alkanes present in the sedimentary record are useful for reconstructing past climates because they are representative of the effects of climate on the organisms that contribute them.

The chain length of *n*-alkanes differs between different groups of organisms. Generally, short chained, even-numbered *n*-alkanes ( $nC_{12} - nC_{22}$ ) found in sediments are associated with bacteria, whereas odd-numbered, short-chained *n*-alkanes, particularly  $nC_{17}$ , are produced by algae or photosynthetic bacteria (Sachse et al. 2004). Medium chained, odd-numbered *n*-alkanes ( $nC_{21} - nC_{25}$ ) are associated with aquatic plants, and longer chained, odd-numbered *n*-alkanes ( $nC_{25} - nC_{31}$ ) are representative of leaf waxes from terrestrial plants (Sachse et al. 2004). Plants produce greater quantities of odd than even chain lengths due to synthesis by sequential elongation or condensation of a  $C_2$  primer, where even-numbered fatty acid chains become decarboxylated to produce odd chain length alkanes (Khan and Kolattukudy 1974, Shepherd and Griffiths 2006). Higher plants produce different chain lengths of *n*-alkanes, ranging from  $nC_{21}$  to  $nC_{35}$  (Sachse et al. 2004, Pu et al. 2011) and their distribution is best represented by the average chain length (ACL) parameter (Rommerskirchen et al. 2003). It is calculated using the below equation:

$$ACL = \frac{(25nC_{25} + 27nC_{27} + 29nC_{29} + 31nC_{31} + 33nC_{33} + 35nC_{35})}{(nC_{25} + nC_{27} + nC_{29} + nC_{31} + nC_{33} + nC_{35})} \quad (\text{Diefendorf et al. 2011}), \quad (1)$$

Where  $nC_x$  is the total chromatographic peak area of each *n*-alkane with x carbon atoms.

ACL was initially considered to provide information on plant type, such as woody species versus graminoids and this was the main way in which variation in ACL in the sedimentary record was interpreted (Brincat et al. 2000, Smith et al. 2007). Recent workers have investigated whether the ACL of plant *n*-alkanes is determined by plant functional type and have demonstrated no differentiation between woody species and graminoids, although *Sphagnum* mosses are distinct (Schefuß et al. 2003, Bush and McInerney 2013). A proposed alternative explanation for variation in ACL is that climate is an influencing factor (Bush and McInerney 2013, Tipple and Pagani 2013).

A number of different observations have been made in regards to the relationships between modern day climate and ACL. Light intensity and temperature affect leaf wax composition (Shepherd and Griffiths 2006), including ACL, as does aridity and humidity (Tipple and Pagani 2013). Studies have shown that ACL demonstrates a spatial variance with climate, with longer chain lengths ( $nC_{34} - nC_{37}$ ) being found in sediments from warmer and more arid regions than in those from cooler and more humid climate conditions (Dodd and Poveda 2003, Leider et al. 2013). Plants may increase *n*-alkane production in dry conditions to reduce their water loss (Hoffmann et al. 2013). The sensitivity of *n*-alkane ACL to changes in these parameters may thus provide a robust record of climate variability through time, in particular changes in temperature and aridity.

Similar systematic shifts in ACL distribution of *n*-alkanes have also been recorded in the past where they couple with other proxies supporting climatic perturbations. For

example, the PETM was a period of extreme warming that demonstrated an increase in ACL from 28.6 to 30.1 in the Bighorn Basin, Wyoming (Smith et al. 2007). Similarly, Lake Baikal sediments indicate a shift from longer chain lengths ( $nC_{31}$ ) in the last glacial maximum, to shorter chain lengths ( $nC_{27}$ ) in Holocene aged sediments (Brincat et al. 2000). Further developing our understanding of how ACL is influenced by climate variations in modern systems allows us to better characterise extreme climate perturbations in the geologic record.

Australia supports a broad range of climate conditions and thus provides an ideal study area in which to examine the relationship of ACL with climate. The Terrestrial Ecosystem Research Network (TERN) has developed a network of biodiversity monitoring plots (AusPlots) at which plant and soil samples are collected and made available to the research community (White et al 2012). By analysing the ACL of *n*-alkanes present in both the dominant plants and the soils collected from these sites and comparing with each site's respective climatic conditions, this study tests whether ACL of leaf wax *n*-alkanes varies systematically in modern plants and soils under a range of climate conditions over a N-S transect of Australia. The climate variables examined are mean annual precipitation (MAP), mean annual temperature (MAT), annual moisture index (MI), lowest quarter mean MI, radiation, driest month precipitation and maximum month vapour pressure deficit, in order to test the response of *n*-alkane ACL response. A relationship between ACL and latitude is also considered.

Specifically, this study examines:

- (1) Whether *n*-alkane ACL in plants correlates with each climate variable.

- (2) Whether the *n*-alkane ACL measured in soil represents a weighted average of the ACL of the dominant plant species at each site.
- (3) Whether the *n*-alkane ACL signature in the soils shows a relationship with each climate variable.

We show that although *n*-alkane ACL is highly variable in plants, *n*-alkane ACL in soils covaries with temperature and aridity and is suitable as a proxy for recording climate change in the sedimentary record.

### **Climate and ecological setting**

Australia's climate varies widely and encompasses tropical monsoonal in the north, to dry arid in the centre, and wet temperate conditions in the south. The Interim Biogeographic Regionalisation for Australia (IBRA), who work in conjunction with the Department of Sustainability, Environment, Water, Population and Communities, identifies 89 distinct bioregions across Australia, based on their climate, geology, landform, native vegetation and species information (Department of Sustainability Environment Water Population and Communities 2012). This study examines plants and soils from the Gulf Fall and Uplands, Darwin Coastal, Burt Plain and Finke bioregions in the Northern Territory and the Flinders Lofty Block, Kanmantoo and Stony Plains bioregions in South Australia.

## METHODS

### Selection of samples

Plant and soil samples from 20 AusPlots and TREND sites were all obtained from the Terrestrial Ecosystem Research Network (TERN), a national organisation that are involved in the collection, storage and use of ecosystem data for sharing with universities and government agencies for research purposes (White et al. 2012). Detailed descriptions of TERN's sampling procedures are provided in **Appendix A** to this study and in their survey protocols manual (White et al. 2012). Selection of AusPlots sites and TREND plots for subsampling was determined by plotting the MAT, MAP and MI data provided by TERN for each site, against one another to determine the broadest spread of this data, as per **Figure 2**. Subsequent subsampling of each plot was based on selection of the top three dominant plant species from each plot, where available. The information regarding percentage cover of each plant species was obtained from the Soils to Satellites website produced by TERN. Sample number five of the available nine soil samples was taken from each plot, for a total of 59 plant samples and 20 soil samples.

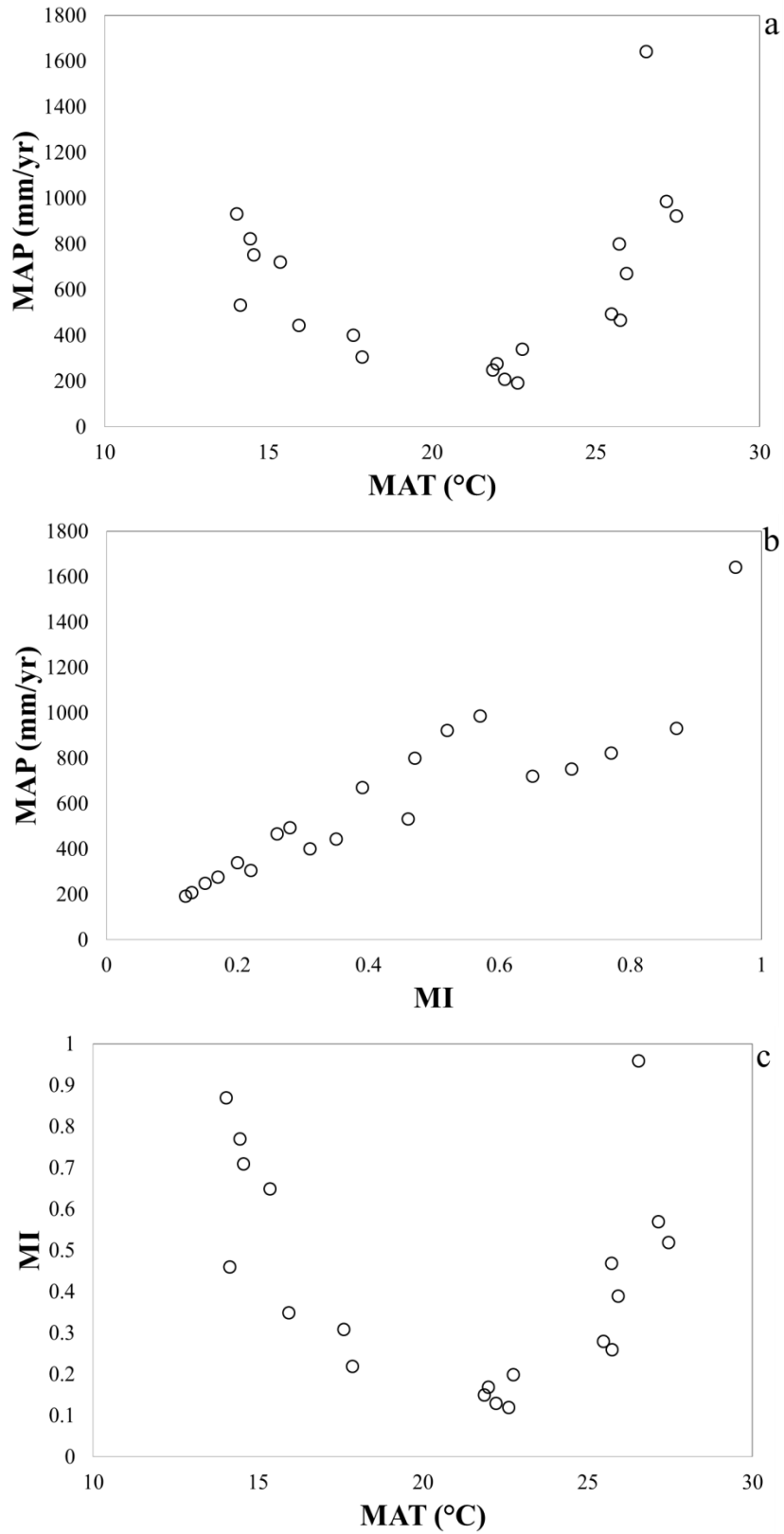


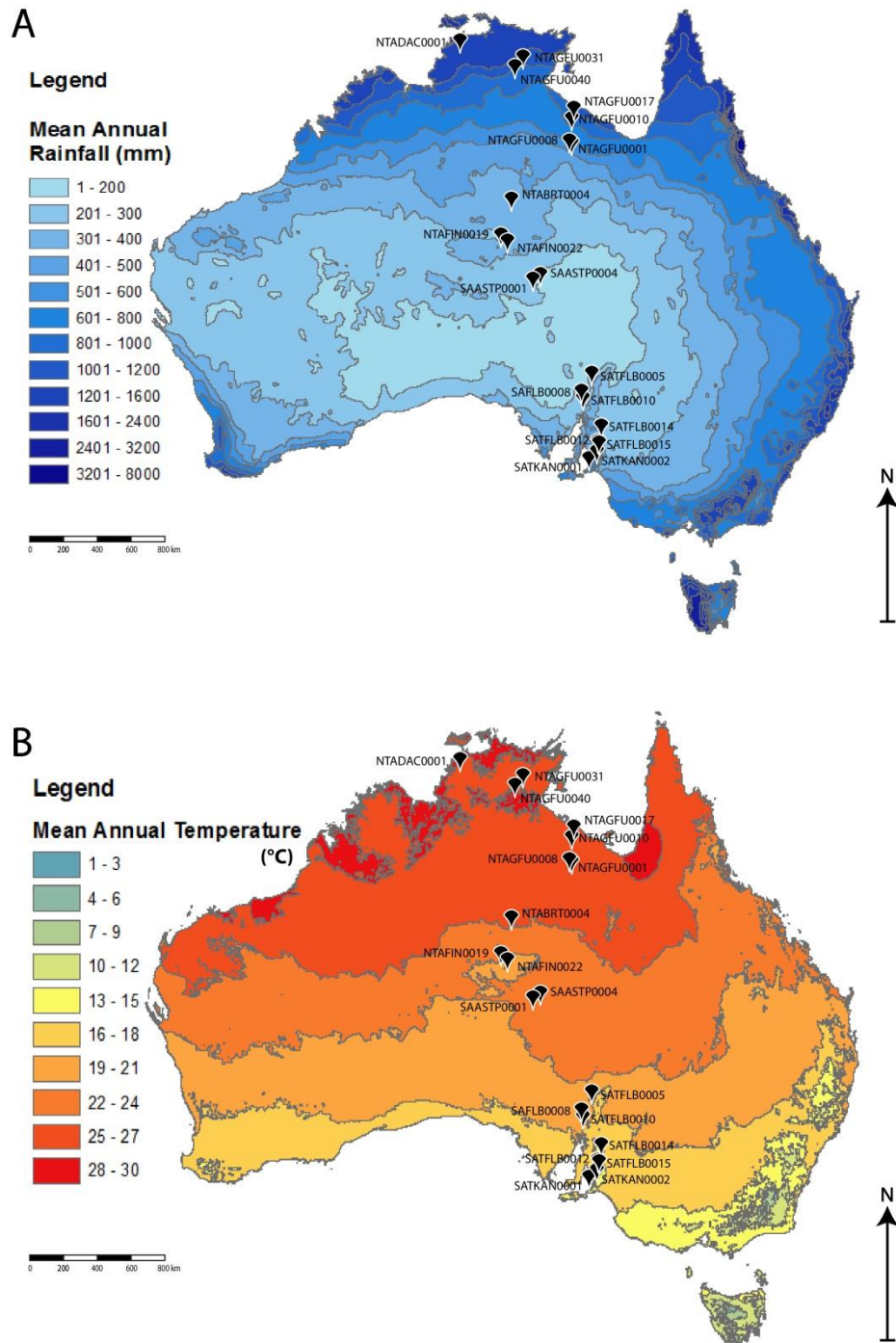
Figure 2: (a) Mean annual precipitation (MAP) versus mean annual temperature (MAT), (b) MAP versus moisture index (MI), and (c) MI versus MAT of selected sites.

## Climate data

TERN provided climate data including mean annual temperature (MAT), mean annual precipitation (MAP) and annual moisture index (MI) data as per **Table 1. Figure 3** shows the relationship between the selected sites and their position with respect to MAP and MAT data, obtained with permission from the Bureau of Meteorology. Further ANUCLIM climate data, including lowest quarter mean MI, highest period radiation and month maximum vapour pressure deficit (VPD) was obtained from the Atlas of Living Australia website, with kind permission from CSIRO (Williams et al. 2012) and the Fenner School of Environment and Society who worked together to produce the ANUCLIM data. The driest month precipitation data was obtained from the Atlas of Living Australia website and was produced and made freely available for academic use by WorldClim. **Table 1** describes each of the climate variables and **Table 2** provides all data for each climate variable.

**Table 9: Description of climate variables (Williams et al. 2012, Prentice et al. 2014).**

Climate Variable	Description
MAP	Mean annual precipitation (mm/yr)
MAT	Mean annual temperature (°C)
Annual MI	An annual average moisture index. Moisture index is a measure of relative soil moisture available to plants, calculated from precipitation and evaporation and in conjunction with soil type. Dimensionless values from 0.0-1.0.
Lowest Quarter Mean MI	The lowest yearly quarter MI. Dimensionless values from 0.0-1.0.
Radiation – highest period	Solar radiation is a function of longitude, latitude and rainfall. Rainfall is associated with cloud cover which reduces radiation (MJ/m <sup>2</sup> /day)
Precipitation – driest month	Amount of lowest month of rainfall (mm)
VPD – month maximum	Month of maximum vapour pressure deficit. Vapour pressure deficit is the difference between the amount of moisture in the air and how much moisture the air can hold when it is saturated (dew point). The dew point increases with temperature. This variable affects the ability of plants to transpire and with increased VPD, transpiration also increases (KPa).



**Figure 3: Location maps of selected Ausplots sites (black pins) provided by TERN, across Australia including the TREND sites located in the southern half of South Australia. (A) Shows where the selected sites sit with respect to mean annual rainfall and (B) shows where the selected sites sit with respect to the mean annual temperature. Climate data based on a standard 30-year climatology (1961-1990) and reproduced with permission from Bureau of Meteorology (© Commonwealth of Australia).**



**Table 10: Table showing the different sites with respect to their bioregion, along with the mean annual precipitation (MAP), mean annual temperature (MAT), annual moisture index (MI), lowest quarter MI, aridity index, radiation, highest month precipitation and vapour pressure deficit for each site.**

<b>SITE</b>	<b>Bioregion</b>	<b>MAP (mm/yr)</b>	<b>MAT (°C)</b>	<b>MI (annual) (dimensionless)</b>	<b>MI - lowest quarter mean (dimensionless)</b>	<b>Aridity index - month max (dimensionless)</b>	<b>Radiation - highest period (MJ/m<sup>2</sup>/day)</b>	<b>Precipitation driest month (mm)</b>	<b>Vapour Pressure Deficit - month max (KPa)</b>	<b>Presence of Cryptogams</b>
NTAGFU0001	Gulf fall and uplands	468.81	25.73	0.26	0.01	0.45	27.9	1	2.27	Y
NTAGFU0008	Gulf fall and uplands	494.62	25.47	0.28	0.02	0.48	27.8	1	2.24	Y
NTAGFU0010	Gulf fall and uplands	673.05	25.92	0.39	0.01	0.76	27.5	1	2.02	Y
NTAGFU0017	Gulf fall and uplands	800.91	25.71	0.47	0.02	0.98	27.3	1	1.84	Y
NTAGFU0031	Gulf fall and uplands	988.65	27.14	0.57	0.01	1.35	26.2	1	1.85	Y
NTAGFU0040	Gulf fall and uplands	923.53	27.44	0.52	0.01	1.23	26.1	0	2.11	Y
NTABRT0004	Burt plain	341.05	22.74	0.20	0.04	0.20	29	7	2.30	Y
NTAFIN0019	Finke	278.92	21.97	0.17	0.04	0.13	29.4	10	2.39	Y
NTAFIN0022	Finke	251.51	21.85	0.15	0.04	0.13	29.5	9	2.34	Y
SATFLB0005	Flinders lofty block	306.95	17.85	0.22	0.07	0.54	28.9	18	1.50	Y
SATFLB0008	Flinders lofty block	446.71	15.92	0.35	0.07	0.82	28.9	22	1.33	Y
SATFLB0010	Flinders lofty block	402.76	17.59	0.31	0.06	0.68	28.6	19	1.29	Y
SATFLB0012	Flinders lofty block	722.62	15.35	0.65	0.11	3.05	27.4	21	0.97	N
SATFLB0014	Flinders lofty block	533.39	14.14	0.46	0.10	1.68	27.7	22	1.02	Y
SATFLB0015	Flinders lofty block	933.83	14.03	0.87	0.16	3.85	27	26	0.76	Y
SATKAN0001	Kanmantoo	753.76	14.55	0.71	0.13	2.87	27.1	23	0.45	Y
SATKAN0002	Kanmantoo	823.48	14.44	0.77	0.14	2.97	27.2	27	0.65	N
SAASTP0001	Stony plains	209.25	22.21	0.13	0.05	0.11	29.7	5	2.42	Y
SAASTP0004	Stony plains	194.65	22.60	0.12	0.04	0.10	29.7	3	2.48	N
NTADAC0001	Darwin Coastal	1642.88	26.53	0.96	0.02	2.41	24.2	2	1.21	N/A

### **Preparation of plant samples**

Plant samples were ground with a mortar and pestle in liquid nitrogen and stored in ashed scintillation vials ready for lipid extraction. The lipids were extracted from the plant samples using a 9:1 optima grade DCM:MeOH eluent. Ground sample was used for extraction with weights ranging from 5.8 – 52.3mg; with 51 of the 59 plant samples  $\geq 50$ mg. Approximately 5mL of eluent was added to the ground samples and was then sonicated in a Soniclean 250TD for 15 minutes. The resulting total lipid extract (TLE) was then pipetted off and filtered through ashed glass fibre filter paper. This process was repeated two times, for a total of three extractions. For the final extraction, the ground plant sample was also tipped in to the filter paper and rinsed with 9:1 DCM:MeOH. The TLE solvent was evaporated in a stream of 5.0 N<sub>2</sub> using a FlexiVap and transferred to 4ml vials with optima grade DCM and refrigerated in readiness for short column chromatography.

### **Preparation of soil samples**

Soil samples were sieved with 1000 and 250  $\mu\text{m}$  sieves to remove any obvious plant matter, such as leaves, bark and roots, and to remove any pebbles or other lithified material. Samples were then stored in labelled falcon tubes. The lipid extraction of the  $<250$   $\mu\text{m}$  soil fraction was conducted using a Thermo Scientific Dionex ASE 350 using a 9:1 optima grade DCM:MeOH solvent solution. TLE solvent was evaporated in a stream of 5.0 N<sub>2</sub> using a FlexiVap and transferred to 4ml vials with optima grade DCM and refrigerated in readiness for short column chromatography.

### **Short column chromatography and GCMS analysis**

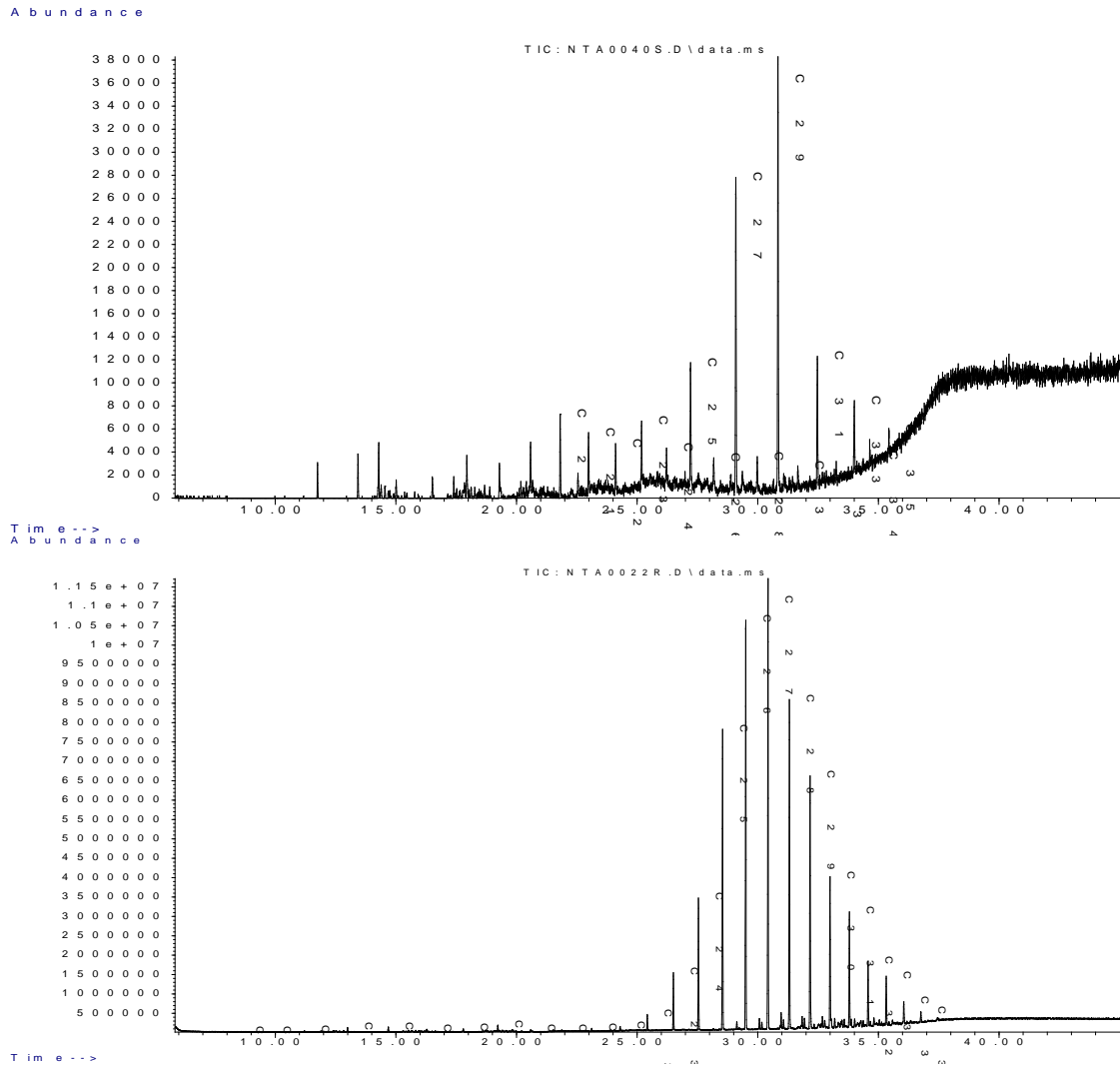
The polar and non-polar fractions of both the plant and the soil TLEs were separated by eluting them with, firstly, 4ml optima grade hexane to collect the non-polar, aliphatic hydrocarbon fraction, followed by 4ml 1:1 DCM:MeOH eluent to collect the polar fraction, through a silica gel glass short column. A Pasteur pipette was plugged with a small amount of glass wool was ashed and then filled with a slurry of activated silica gel and optima grade hexane (Bastow et al. 2007). The non-polar eluate was then quantitatively transferred to 2ml vials and dried on the FlexiVap and resuspended in 100 $\mu$ L of optima grade hexane Gas chromatograph mass spectrometry (GCMS) analysis was conducted using either a HP5973 MS coupled to a HP6890 GC (MS operated in scanning mode from 45 to 500Da), or by a Perkin Elmer Clarus 500 GCMS. Both machines had the following specifications: The capillary was an SGE CPSil-5MS, 60m (length) x 0.25mm (internal diameter) x 0.25 $\mu$ m (phase thickness). The carrier gas was helium with a 1ml/min constant flow. The injection temperature was 300°C, with a temperature program set to 50°C and held for 1 minute, then ramped at 8°C/min to 340°C and held for 7.75mins. Injection was set to 1 $\mu$ l in either split mode, with a 50:1 split for higher concentration samples, or pulsed splitless for low sample concentrations. The majority of samples were run on the HP5973 MS coupled to a HP6890 GC, and four samples that had previously been run on the Perkin Elmer Clarus 500 GCMS were re-run on the HP5973 MS coupled to a HP6890 GC to ensure there was no difference in the results between the two machines. Chromatograms and peak areas were integrated using Chemstation for the HP5973 MS coupled to a HP6890 GC, and Turbomass for the Perkin Elmer Clarus 500 GCMS.

## Calculations

From the GCMS data, relative abundances of *n*-alkane chain lengths were characterised by calculating average chain length (ACL). See equation (1). Soil sample data used for regression analysis was selected based on the carbon preference index (CPI) for each sample, calculated using the below equation:

$$CPI = \frac{[\Sigma_{odd}(C_{21-33}) + \Sigma_{odd}(C_{23-35})]}{(2 \Sigma_{even} C_{22-34})} \quad (\text{Bush and McInerney 2013}) \quad (2)$$

Where  $\Sigma_{odd}C_{x-y}$  is the sum of the peak area for *n*-alkanes with an odd carbon chain length inclusive of that range and  $\Sigma_{even}C_{x-y}$  is the sum of the peak area for *n*-alkanes with an even number of carbon chain lengths inclusive of that range. Values where  $CPI > 1.5$  were considered to represent an *n*-alkane source of primarily plant origin (Bush and McInerney 2013). Soils that had a  $CPI < 1.5$  were analysed separately and in comparison to soils that had a  $CPI < 1.5$  because the source of the low CPI is unknown. **Figure 4** shows examples of GC results for soils with a  $CPI < 1.5$  and  $> 1.5$ . ACL for both the plants and soils and CPI of the soils were plotted against the different climate variables and least squares regression analysis was conducted using Excel.



**Figure 4: Two chromatograms of the GC results for two soils. NTAGFU0040, at the top, shows a high CPI=6.07 and NTAFIN0022 at the bottom has a CPI=1.1. NTAFIN0022 has a normal distribution of chain lengths and does not show a clear odd-over-even predominance of chain lengths as would be expected for a higher plant *n*-alkane source.**

Predicted soil ACL was calculated from an average of the ACL of the plant samples for each site, weighted by their percentage cover (% cover).

$$\text{Predicted Soil ACL} = \frac{[(ACL_{Dom1} \times \%_{Dom1}) + (ACL_{Dom2} \times \%_{Dom2}) + (ACL_{Dom3} \times \%_{Dom3})]}{(\%_{Dom1} + \%_{Dom2} + \%_{Dom3})} \quad (3)$$

Where  $ACL_{Domx}$  is the ACL for the dominant plants species and  $\%_{Domx}$  is the percentage cover of that dominant species. The calculated results were used to compare ACL with

the different climate variables and latitude. More detailed methods can be found in

**Appendix B.**

**RESULTS**

Plant samples show a clear odd-over-even carbon number preference, ranging from 1.5 – 238.3, and tend to have highest concentrations of chain lengths ranging C<sub>27</sub>-C<sub>33</sub>, with the most dominant chain length being C<sub>31</sub>. These results are consistent with those chain lengths of a terrestrial higher plant origin for *n*-alkanes (Zhang et al. 2006). The average chain lengths for all plants ranges from 26.6 to 33.3, whereas the predicted soil ACL values range from 26.8 to 31.9 and the actual soil ACL values range from 27.7 to 31.1 (CPI of >1.5). (**Table 3**).

**Table 11: ACL for plant and soil samples. The total plant cover (%) is the sum of the top 3 dominant plants % cover. Soil samples used for further analysis were determined based on their carbon preference index (CPI>1.5). The predicted soil ACL is a weighted average of the ACL of the top three dominant plants for each site, based on percentage cover. See Equation (3). There is no predicted soil ACL for NTADAC0001 because percentage cover data was not available for this site.**

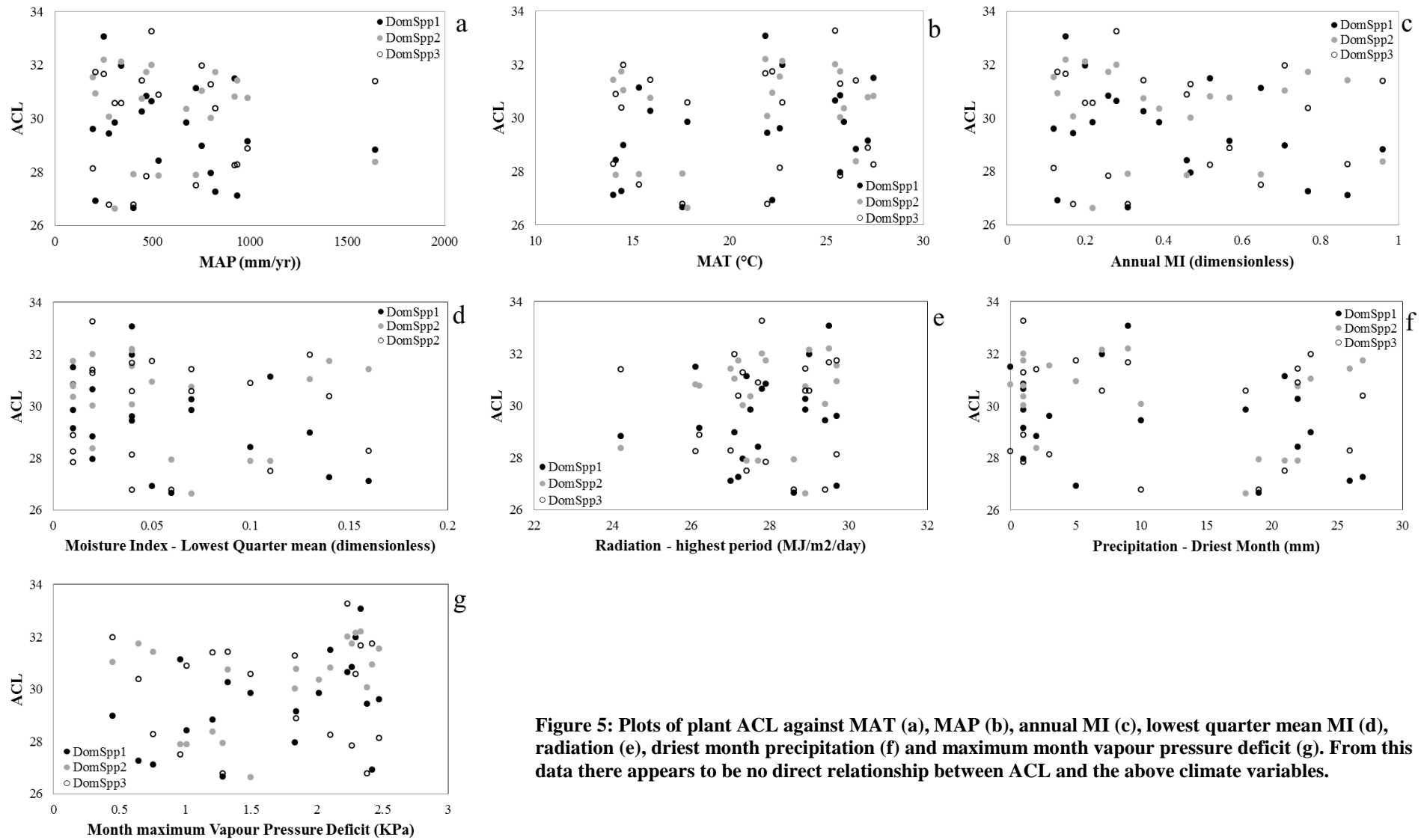
SITE	Dominant Plant Species 1	% Cover	ACL	Dominant Plant Species 2	% Cover	ACL	Dominant Plant Species 3	% Cover	ACL	Total Plant Cover (%)	Soil CPI	Predicted Soil ACL	Actual Soil ACL
NTAGFU0001	<i>Aristida pruinosa</i>	17.4	30.9	<i>Enneapogon polyphyllus</i>	13.3	31.7	<i>Eucalyptus pruinosa</i>	13.2	27.9	43.9	2.60	30.2	30.3
NTAGFU0008	<i>Triodia pungens</i>	45.4	30.7	<i>Aristida contorta</i>	19.5	32.0	<i>Fimbristylis doctotoma</i>	14.4	33.3	79.3	3.33	31.5	29.2
NTAGFU0010	<i>Triodia pungens</i>	62.7	29.9	<i>Eucalyptus leucophloia</i>	36.4	30.4	N/A	N/A	N/A	99.1	7.87	30.0	29.8
NTAGFU0017	<i>Melaleuca viridiflora</i>	34.5	28.0	<i>Chrysopogon fallax</i>	10.4	30.0	<i>Schizachyrium fragile</i>	7.7	31.2	52.6	5.19	28.9	30.3
NTAGFU0031	<i>Melaleuca viridiflora</i>	30.5	29.1	<i>Schizachyrium pachyarthron</i>	28.3	30.8	<i>Petalostigma banksii</i>	9.2	28.9	68	0.86	29.8	28.2
NTAGFU0040	<i>Acacia dimidiata</i>	26.8	31.5	<i>Heteropogon contorus</i>	15.9	30.8	<i>Eucalyptus tectifera</i>	9.7	28.3	52.4	6.07	30.7	28.8
NTABRT0004	<i>Acacia aptaneura</i>	56.8	32.0	<i>Aristida holathera</i>	24.4	32.1	<i>Triodia schinzii</i>	7.4	30.6	88.6	5.08	31.9	31.1
NTAFIN0019	<i>Cenchrus ciliaris</i>	68.6	29.4	<i>Acacia estrophiolata</i>	19.2	30.1	<i>Enchylaena tomentosa</i>	2.4	26.8	90.2	2.67	29.5	29.8
NTAFIN0022	<i>Eremophila freelingii</i>	50.5	33.1	<i>Enneapogon polyphyllus</i>	15	32.2	<i>Aristida contorta</i>	7.7	31.7	73.2	1.11	32.7	27.9
SATFLB0005	<i>Dodonaea viscosa subsp. angustissima</i>	21.9	29.8	<i>Eucalyptus flindersii</i>	18.8	26.6	<i>Chrysocephalum semipapposum</i>	13.2	30.6	53.9	2.32	28.9	28.5
SATFLB0008	<i>Triodia scariosa</i>	47.6	30.3	<i>Cassinia laevis</i>	23.7	30.8	<i>Casuarina pauper</i>	12.6	31.4	83.9	2.00	30.6	28.4
SATFLB0010	<i>Eucalyptus odorata</i>	67	26.7	<i>Rhagodia paradoxa</i>	10.1	27.9	<i>Enchylaena tomentosa var. tomentosa</i>	6.1	26.8	83.2	2.00	26.8	28.4
SATFLB0012	<i>Allocasuarina muelleriana subsp. Muellieriana</i>	42.1	31.1	<i>Hibbertia crinita</i>	15.5	27.9	<i>Eucalyptus fasciculosa</i>	12.6	27.5	70.2	1.44	29.8	28.1
SATFLB0014	<i>Eucalyptus odorata</i>	33	28.4	<i>Xanthorrhoea quadrangulata</i>	18.5	27.9	<i>Allocasuarina verticillata</i>	14	30.9	65.5	1.58	28.8	28.3
SATFLB0015	<i>Eucalyptus obliqua</i>	61.2	27.1	<i>Lepidosperma semiteres</i>	8.5	31.4	<i>Hibbertia crinita</i>	6.6	28.3	76.3	2.69	27.7	27.7
SATKAN0001	<i>Eucalyptus baxteri</i>	42.9	29.0	<i>Lepidosperma semiteres</i>	11.3	31.0	<i>Pultenaea involucrata</i>	10.3	32.0	64.5	6.21	29.8	28.7
SATKAN0002	<i>Eucalyptus obliqua</i>	55.2	27.3	<i>Lepidosperma semiteres</i>	9.2	31.7	<i>Hakea rostrata</i>	8.2	30.4	72.6	3.07	28.2	28.0
SAASTP0001	<i>Maireana aphylla</i>	34.6	26.9	<i>Eragrostis setifolia</i>	12.8	30.9	<i>Acacia aneura var. tenuis</i>	8.5	31.7	55.9	1.28	28.6	27.7
SAASTP0004	<i>Malvastrum americanum var. americanum</i>	25.6	29.6	<i>Rutidosia helichrysoides subsp. Helichrysoides</i>	18.5	31.6	<i>Sida fulbilifera</i>	11.7	28.1	55.8	1.26	29.9	28.0
NTADAC0001	<i>Eucalyptus tetradonta</i>	N/A	28.8	<i>Eucalyptus miniata</i>	N/A	28.4	<i>Sorghum plumosum</i>	N/A	31.4	N/A	1.31	N/A	28.0

**Figure 5** shows all plant ACL data plotted against each of the climate variables. Plant ACL does not show a significant relationship to MAP, MAT, annual MI, Radiation, Driest Month Precipitation or Vapour Pressure Deficit ( $p < 0.05$ ). This is the case regardless of whether the plant is the top 1, top 2 or top 3 dominant species present at that site. **Table 4** shows the p-values and  $r^2$  for each climate variable versus ACL and shows that all of the relationships with the climate variables are not significant ( $p > 0.05$ ). To further explore any relationships between chain length and climate, ratios between  $C_{27}/C_{31}$  and  $C_{29}/C_{31}$  for each plant species were both plotted against the different climate variables yet still no clear relationship was apparent. Eucalyptus genus ACL values were analysed separately, however there appeared to be no relationship between ACL and the different climate variables for this genus. Data for the  $C_{27}/C_{31}$  and  $C_{29}/C_{31}$  ratio results and the Eucalyptus genus results can be found in **Appendix B** to this document.

**Table 12: Results of least squares regression analysis for the plant ACL**

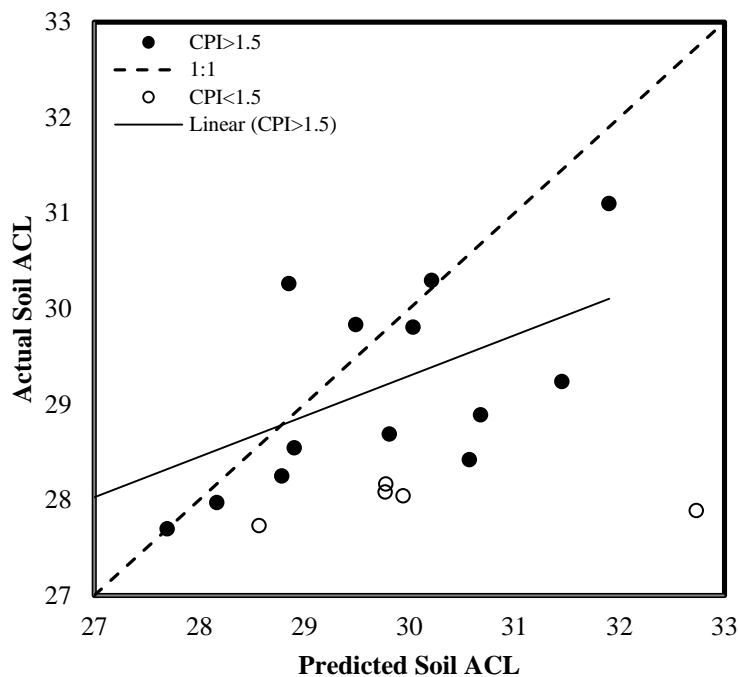
Climate Variable	$r^2$	P-value
MAP	0.01	0.48
MAT	0.04	0.13
Annual MI	0.02	0.25
Lowest quarter mean MI	0.03	0.20
Radiation – highest period	0.01	0.54
Precipitation – driest month	0.05	0.10
VPD – month max	0.06	0.07





**Figure 5: Plots of plant ACL against MAT (a), MAP (b), annual MI (c), lowest quarter mean MI (d), radiation (e), driest month precipitation (f) and maximum month vapour pressure deficit (g). From this data there appears to be no direct relationship between ACL and the above climate variables.**

The total cover % of the top three dominant species at each site range from 43.9% to 99.1%, with 18 out of the 19 sites with a total % cover being represented by >50% of cover from these three top dominant plants. Predicted soil ACL values calculated from the top three dominant plants ranges from 26.8 to 31.9. The difference between predicted soil ACL and actual soil ACL range from 0.0006 – 2.22. Least squares analysis for the actual soil ACL versus the predicted soil ACL produced a P-value that is not significant ( $p>0.05$ ). **Figure 6** shows the relationship between the predicted soil ACL and the actual soil ACL, with most predicted soil ACL results lower than the actual soil ACL results. All available soil results are included, including those samples with a  $CPI<1.5$ , in order to capture whether or not the dominant *n*-alkane contributors are the plants.



**Figure 6:** Predicted Soil ACL calculated from the weighted average of the top three dominant plant species at each site versus the actual ACL of the soils. The dashed line represents the 1:1 line. Most data points fall below this 1:1 line, showing that actual ACL is lower than predicted ACL. The slope of the trendline is much lower than 1.

Least squares regression analysis on the soil ACL data is presented in **Tables 5** and **6**. These show that where all soils are analysed (**Table 5**), the p-value is not significant ( $p>0.05$ ) for all climate variables, except for the lowest quarter mean MI. However, for the soils with a  $CPI>1.5$  (**Table 6**) all climate variables except for MAP and radiation – highest period, have significant p-values ( $p<0.05$ ).

**Table 13: Results of least squares regression analysis for the actual soil ACL for all soils. Rows in bold indicate variables with statistical significance ( $p<0.05$ ).**

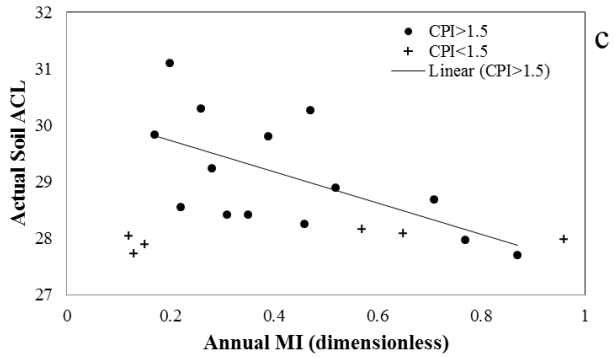
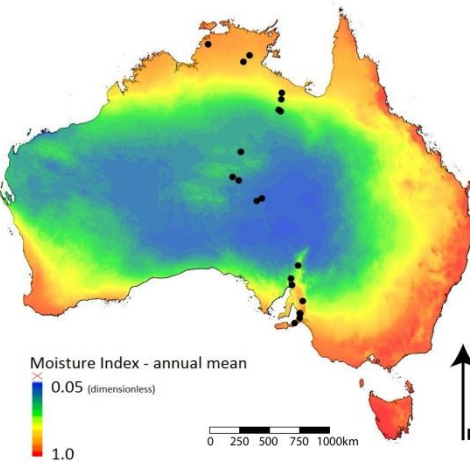
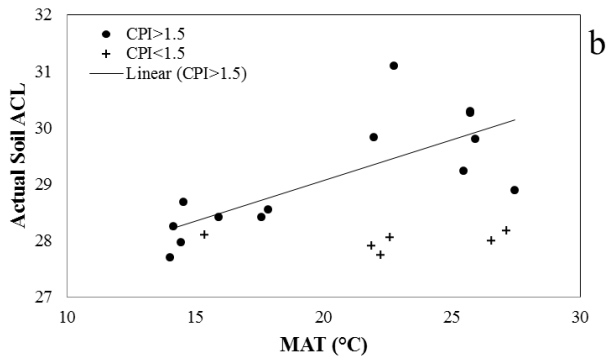
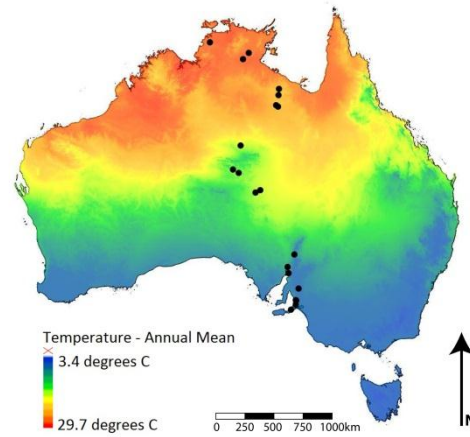
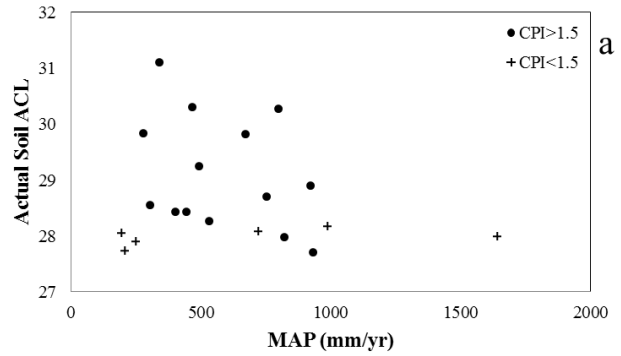
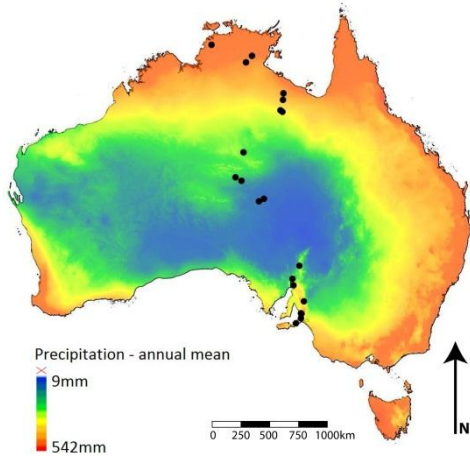
Climate Variable	$r^2$	P-value	Equation
MAP	0.03	0.43	
MAT	0.19	0.06	
Annual MI	0.12	0.14	
<b>Lowest quarter mean MI</b>	<b>0.22</b>	<b>0.04</b>	<b><math>y=-9.87x + 29.33</math></b>
Radiation – highest period	0.01	0.62	
Precipitation – driest month	0.18	0.06	
VPD – month max	0.18	0.06	

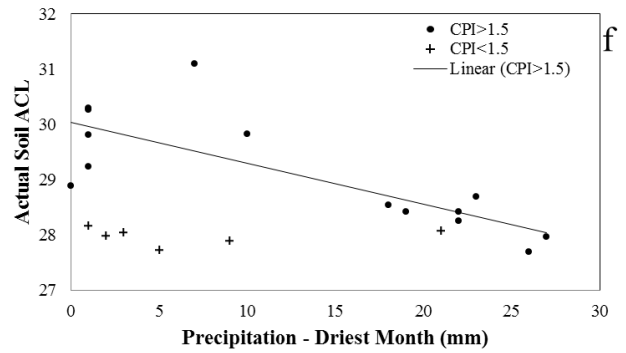
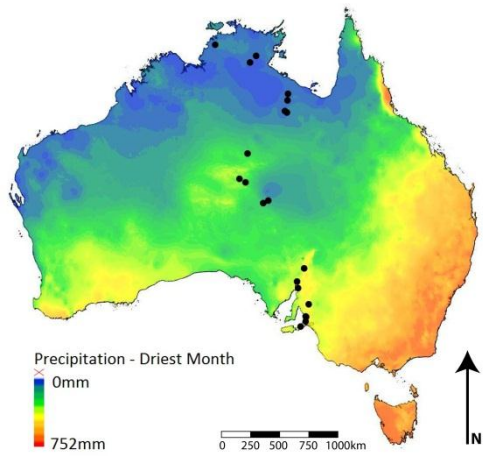
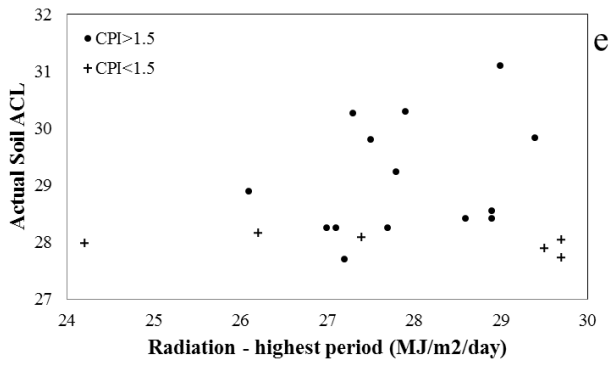
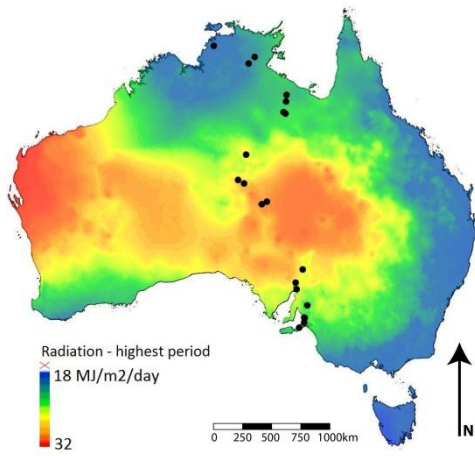
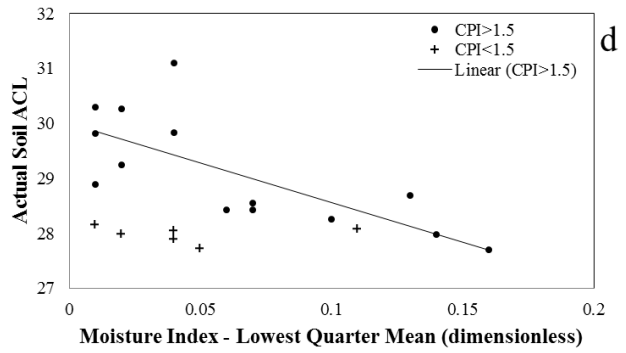
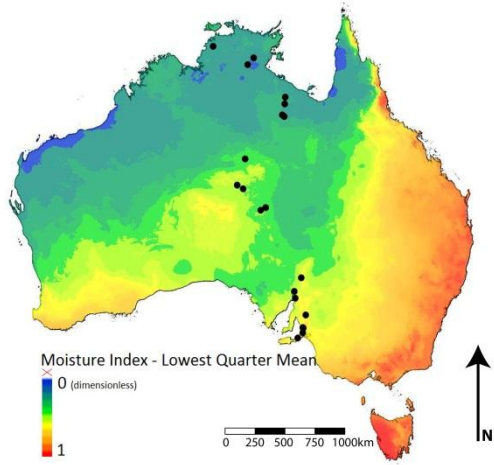
**Table 14: Results of least squares regression analysis for the actual soil ACL for soils with a  $CPI>1.5$ . Rows in bold indicate variables with statistical significance ( $p<0.05$ ).**

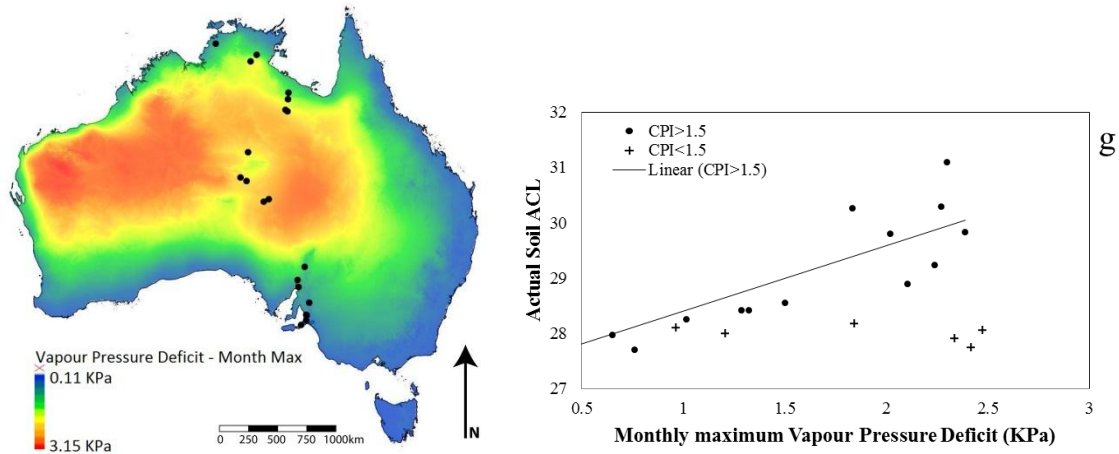
Climate Variable	$r^2$	P-value	Equation
MAP	0.12	0.23	
<b>MAT</b>	<b>0.56</b>	<b>0.002</b>	<b><math>y=0.14x + 26.17</math></b>
<b>Annual MI</b>	<b>0.37</b>	<b>0.021</b>	<b><math>y=-2.77 + 30.28</math></b>
<b>Lowest quarter mean MI</b>	<b>0.54</b>	<b>0.003</b>	<b><math>y=-14.42x + 30.01</math></b>
Radiation - highest period	0.08	0.33	
<b>Precipitation - driest month</b>	<b>0.60</b>	<b>0.001</b>	<b><math>y=-0.07x + 30.04</math></b>
<b>VPD - month max</b>	<b>0.63</b>	<b>0.001</b>	<b><math>y=1.19x + 27.22</math></b>

**Figure 7** shows both the soils with a  $CPI>1.5$  and the soils with a  $CPI<1.5$ . Maps obtained from the Atlas of Living Australia website show the locations of the sites with respect to the different climate variables. When looking at the samples with a  $CPI>1.5$ , the samples that have a significant p-value ( $p<0.05$ ) have been plotted with their regression line. As MAT and monthly maximum VPD increase, so does ACL. In

contrast, as annual mean MI, lowest quarter mean MI and driest month precipitation increase, ACL decreases.

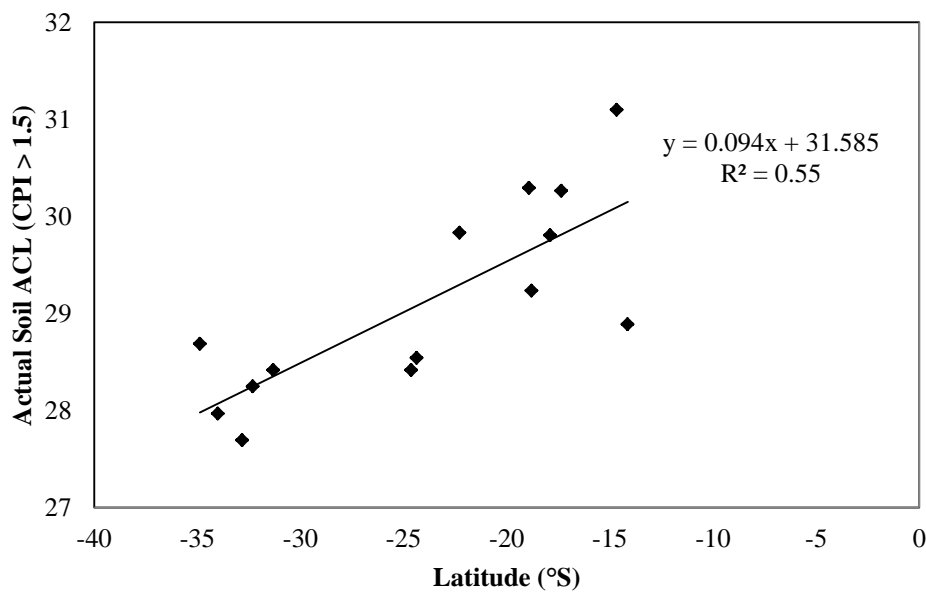




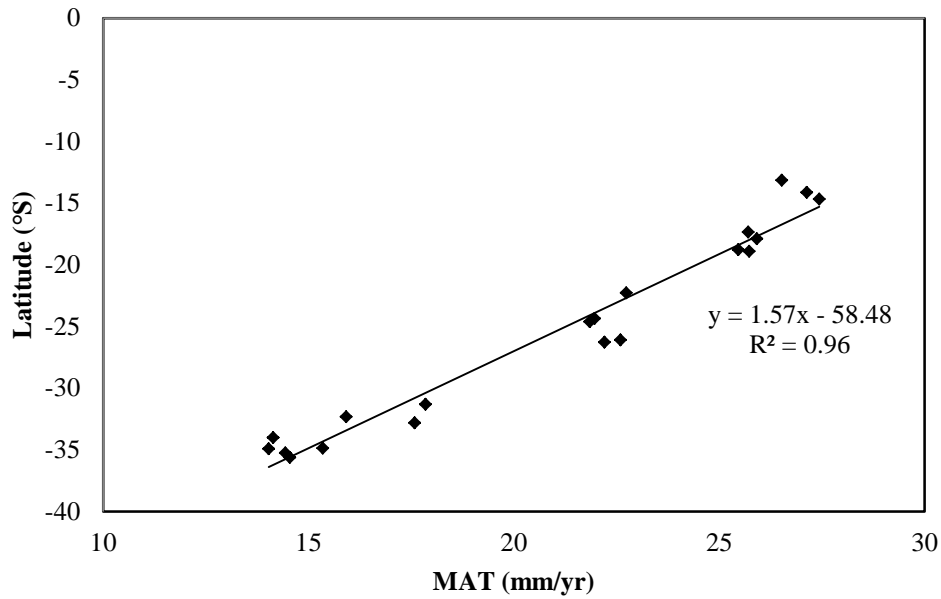


**Figure 7:** Plots demonstrating the relationship between actual soil ACL and MAT (a), MAP (b), annual MI (c), lowest quarter mean MI (d), highest period radiation (e), driest month precipitation (f) and vapour pressure deficit (g). Maps of the location of sites (black dots) with respect to the various climate variables reproduced with permission from CSIRO (Williams et al. 2012) and the Fenner School of Environment and Society at ANU. Regression lines are displayed for significant ( $p < 0.05$ ) relationships.

A plot of actual soil ACL and latitude (**Figure 8**) shows that ACL increases towards the equator. Least squares regression analysis shows that the  $r^2 = 0.55$  and the  $p$ -value = 0.003 for this relationship. A comparison of latitude with MAT has an  $r^2 = 0.959$  and a  $p$ -value =  $6.23 \times 10^{-14}$  as shown in **Figure 9**.

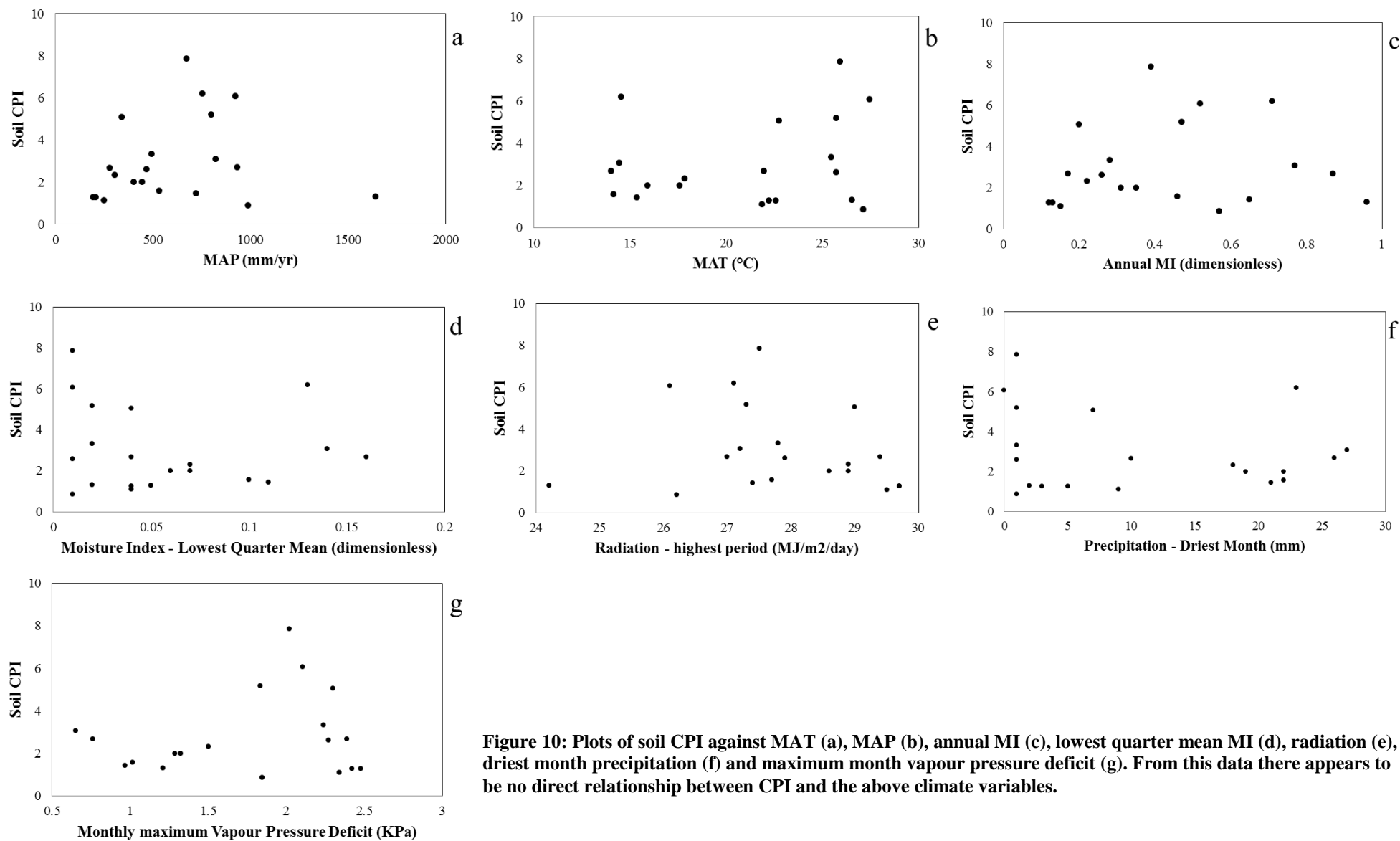


**Figure 8:** Plot of actual soil ACL (CPI > 1.5) with respect to latitude.



**Figure 9:** Plot showing the relationship between latitude and MAT.

**Figure 10** shows all soil CPI data plotted against each of the climate variables. Soil CPI does not show a significant relationship to MAP, MAT, annual MI, Radiation, Driest Month Precipitation or Vapour Pressure Deficit ( $p < 0.05$ ). **Table 7** shows the  $p$ -values and  $r^2$  for each climate variable versus CPI and shows that all of the relationships with the climate variables are not significant ( $p > 0.05$ ).



**Figure 10:** Plots of soil CPI against MAT (a), MAP (b), annual MI (c), lowest quarter mean MI (d), radiation (e), driest month precipitation (f) and maximum month vapour pressure deficit (g). From this data there appears to be no direct relationship between CPI and the above climate variables.



**Table 15: Results of least squares regression analysis for the soil CPI for all soils**

Climate Variable	$r^2$	P-value
MAP	0.02	0.60
MAT	0.04	0.39
Annual MI	0.01	0.69
Lowest quarter mean MI	0.01	0.64
Radiation - highest period	0.04	0.43
Precipitation - driest month	0.03	0.46
VPD - month max	0.00	0.99

## DISCUSSION

### Plant ACL response to climate

This study examines whether variation in *n*-alkane ACL distributions in different plants is dependent on different climate variables. It tests whether annual averages, as well as periods of extreme conditions drive the *n*-alkane distribution in plants. It is expected that plants are more likely to need to protect themselves from climatic extremes than moderate climate conditions. These relationships are expected because of the role that leaf epicuticular waxes play in protecting the plant against water loss and limiting damage against UV radiation. In particular, work by Shepherd and Griffiths (2006) shows that light intensity and temperature affect leaf epicuticular wax composition. Other work has also found evidence that ACL in plants is affected by temperature, humidity and VPD (Tipple and Pagani 2013). Results from this study show that plant ACL has no relationship with any of the climate variables tested.

There may be a number of reasons why the ACL of plants shows no relationship to the climate variables tested, for example, the timing of initial production of *n*-alkanes in plants. Recent work has identified that there is limited variation in *n*-alkane chain length distribution across a growing season in trees sampled near Chicago, US (Bush and McInerney 2013). Similarly, Gülz and Müller (1992) also showed that *n*-alkane concentrations remain fairly constant over a two year period for *Quercus robur* leaves growing at the University of

Cologne in Germany. Tipple et al. (2013) found that *n*-alkane ACL increased during the leaf-flush interval in *Populus angustifolia*, but once the leaf was fully expanded *n*-alkane distributions did not vary for the remainder of the growing season. This indicates that any climatic parameters that affect ACL in terrestrial plants must mainly do so during the leaf-flush interval. Timing of this event in plants may vary from species to species. Production of *n*-alkanes at different times of the year may result in variation in ACL between plants, because of the different timings of the leaf flush interval, as a response to the climate conditions at that moment in time. This may help to explain why the ACL of the plants does not covary with any of the climate variables tested. Plants represent a snapshot in time, which show both seasonal and year-to-year variation in growth. The different sites were each sampled on different days across 2011 and 2012, which means that any seasonal influences on the *n*-alkane production of the plants have not been controlled for.

Leaf life-span may also affect the *n*-alkane production in plants. Sachse et al. (2006) have suggested that deciduous trees that have a long vegetation period that are subject to high incoming radiation protect their leaves by producing longer chained *n*-alkanes. Diefendorf et al. (2011) further identify that evergreen angiosperm and gymnosperm species have a higher abundance of *n*-alkanes than their deciduous counterparts, indicating that a longer leaf life span is potentially exposed to greater extremes and needs to protect against that. As well as this, Sachse et al. (2009) observed variation in *n*-alkane concentrations in *Acer pseudoplatanus* as a result of wind and water ablation, resulting in the constant production of *n*-alkanes over the life of the leaf in this particular species in response to damaging conditions. Different types of plants have different leaf life times. This study examines many different species, with few species replicated and these results indicate that between species

variation is high. This may explain why there is no relationship between the ACL of the different species and the climate variables.

In addition, different plant species or genera may respond differently to one another in response to different climate variables. Hoffmann et al. (2013) found that measuring *Acacia* and *Eucalyptus* genera along a hydrological gradient across the Northern Territory exhibited an opposite trend in ACL to one another. While they were not able to identify specifically why this occurred, they suggested that perhaps different plant species or genera exhibit different responses in ACL because of variation in leaf functional traits or because of evolutionary differences. However, results from this study do not show a relationship within *Eucalyptus* genus between ACL and the climate variables, indicating that within genera trends are not always consistent.

Recent work regarding a study of South African flora, however, found that there was no statistically significant relationship between *n*-alkane distribution as it related to mean or extreme climate conditions, specifically MAT and maximum temperature of the warmest month (Carr et al. 2014). Similarly, results from this study show that neither extreme nor average conditions have a greater influence on the ACL of the plants. It is possible that the relationships between plant ACL and climate in Australia are very similar to that observed in South Africa, due to the comparable arid and hot climate conditions experienced in both.

### **Predicted soil ACL versus actual soil ACL**

This study also sets out to examine whether the ACL measured in soil represents a weighted average of the ACL of the dominant plants species. Results from this study show that the predicted soil ACL is not a reliable indicator for actual soil ACL. The calculation method

used to predict the ACL for each site was based on the percentage of cover of the top three dominant plant species. The range of total percentage cover that the top three dominant plant species represented, however, was variable, from 43.9 to 99.1%. Furthermore, percentage cover does not necessarily equal biomass. In many ecosystems, percentage cover may not be representative of percentage biomass as a tree contains more biomass than a grass covering the same area. Moreover, it is possible that this selection method may not have captured the dominant *n*-alkane producers at each site. Different plant functional types, such as trees and graminoids, as well as different plant species each produce different concentrations of *n*-alkanes per kg of biomass. Research has identified that deciduous angiosperms produce 200 times more *n*-alkanes than deciduous gymnosperms (Diefendorf et al. 2011, Bush and McInerney 2013). Sachse et al. (2006) also identified that deciduous angiosperm trees are major contributors compared with conifers and mosses. Plant cover may be a poor predictor of the source of *n*-alkanes found in soils. Different species and different plant functional types are all represented in this study and results indicate that relying on the top plant cover alone is insufficient information for predicting the actual ACL of the soil.

The soils represent a temporal average of all of the different contributing organisms and so it is necessary to consider other contributors as well as plants. Different organisms all produce different concentrations of *n*-alkanes, as well as different chain lengths, which in turn affects the ACL of the soil. Generally, short-chained *n*-alkanes with even numbers are associated with bacteria and odd numbers are associated with algae or photosynthetic bacteria. *n*-Alkanes with medium, odd numbered chain lengths are associated with aquatic plants, whereas longer odd numbered chain lengths are representative of leaf waxes from land plants (Sachse et al. 2004). A particular group of organisms that has not been accounted for in this analysis are the cryptogams. Cryptogams form soil crusts, are common in arid regions, and

consist of a number of different species including lichens, bryophytes, algae, cyanobacteria, fungi and bacteria. These organisms have been observed and recorded by TERN for each of the sites and recorded on the Soils to Satellites website. Most of the selected sites have observed cryptogam substrate cover which is expected in Australia where an arid climate predominates. It is possible that the presence of the cryptogams has an effect on the ACL of the soils. Little data exists for ACL of lichens, however Sachse et al. (2006) found that analysis of a small number of samples of the genus of moss-like lichens, *Cladonia spp*, in northern Finland and southern Italy yielded varying CPI between 0.9 – 5.0 and average chain lengths between 22.6 – 26.4. Huang et al. (2012) found that lichen species analysed in the Hubei province in China showed a CPI ranging between 3.5 – 8.2 and slightly longer average chain lengths ranging from 27.2 – 28.8. Results from this study show that it is important to consider all contributing species and not just those species which are dominant in terms of cover. High values of ACL in sediments may indicate a higher percentage of vascular plants contributing *n*-alkanes, as compared to non-vascular contributors such as lichens and, likewise, a low ACL may indicate an *n*-alkane source other than higher plants. The weighted average of the top three dominant plant species alone is not reliable for predicting ACL in soil.

### **Soil ACL response to climate**

Although ACL in plants does not show a relationship with climate, the ACL signature in the soils does show a relationship with a number of the different climate variables. Soils with a  $CPI < 1.5$  were excluded from this analysis because a low CPI indicates a low odd-over-even carbon number and the source of the *n*-alkanes cannot be clearly identified. It is possible that this low CPI is due to petroleum contamination (Hughen et al. 2004, Douglas et al. 2012), which can conflate results. However, the soils with a  $CPI > 1.5$  are likely to indicate an *n*-

alkane source of lichens and higher plants that are locally derived and subject to the local climate conditions. There has been some research investigating the CPI of *n*-alkanes and its relationship to humidity, precipitation and temperature in sediments in south-eastern China to the northern margin of the Loess Plateau (Luo et al. 2012). Luo et al. (2012) found that high CPI values were associated with aridity and that a decrease in CPI was potentially caused by enhanced biodegradation in more humid climates. In this study, however, there was no statistically significant relationship between soil CPI and climate. This study has utilised CPI primarily as an indicator for determining the potential source of the contributing *n*-alkanes.

Soils with a  $CPI > 1.5$  show a statistically significant relationship exists between ACL and MAT, annual MI, lowest quarter mean MI, driest month precipitation and maximum month VPD, but do not show a strong relationship with radiation or MAP. Both maximum month VPD and driest month of precipitation show a strong relationship with ACL, with ACL increasing with greater aridity. Similarly a decrease in MI, both annually and the lowest quarter mean, correlate with an increase in ACL in soils. Andersson et al. (2011) also demonstrated that the *n*-alkane ACL of a peat bog in the north-east European Russian Arctic also demonstrated a positive correlation with drier conditions. Our results suggest that aridity is a significant driver of ACL in soils.

In addition, ACL in soils increases as VPD increases. Warmer air results in a higher VPD, which in turn results in increased transpiration in the leaf. This indicates that VPD is an indicator of temperature also and it may be that temperature is the main driver of increased ACL found in the soils with increasing VPD. Similarly, MAT shows a strong relationship to the ACL of soils with a  $CPI > 1.5$ , with ACL increasing as MAT increases. A strong relationship between ACL in soils and temperature was also found by Bush et al. (In Review)

from their measurements from soils across the mid-continental US which also showed an increase in ACL with MAT. Our results show that temperature is also a significant driver of ACL in soils.

The strong relationship between latitude and ACL appears to be strongly related to MAT. Similar to the findings here, Tipple and Pagani (2013) also found that ACL is inversely related to latitude, also with strong correlations between ACL and MAT. While it is also expected that radiation also varies along a latitudinal gradient, this study shows that radiation appears to show no relationship with latitude. However, this may be because the radiation measured in this instance accounts for cloud cover, as well as longitude and latitude.

The findings from this study are similar to the findings from other work (**Table 8**), with comparable  $r^2$  values for latitude, temperature and VPD as they relate to ACL in soils and sediments. Although this study used different metrics for aridity than other studies, the climate variables annual MI, lowest quarter mean MI and driest month of precipitation each reflect available water, and each show an increase in ACL with drier conditions as Carr (2014) also showed.

**Table 16:  $r^2$  values for different climate variables and the ACL found in soils and sediments from other work compared with the findings of this study.**

Climate variable	Other workers	This study
	$r^2=0.69$	
Latitude	Terrestrial and marine sediments from Italy (Leider et al. 2013)	$r^2=0.55$
	$r^2=0.65$	
MAT	Soils from the east coast of the US (Tipple and Pagani 2013)	$r^2=0.56$
Annual MI		$r^2=0.37$
Lowest quarter mean MI		$r^2=0.54$
Precipitation – driest month		$r^2=0.54$
	$r^2=0.45$	
VPD	Soils from the east coast of the US (Tipple and Pagani 2013)	$r^2=0.63$
	$r^2=0.35$	
Aridity	Soils from South Africa (Carr et al. 2014)	

Significant relationships exist between climate and ACL in the soils but not in the plants because the soil integrates the highly variable ACL of all contributing organisms over time. As well as accounting for different organism inputs, plant waxes can also be transported long distances by air or water so the ACL found in sediments integrates not only the local sources, but also regional inputs (Leider et al. 2013). Similar to our results, Sachse et al. (2006) found that *n*-alkane ACL distribution was less variable in sediments than in plant biomass, with their research investigating *n*-alkanes in lake sediments in Finland and Italy. Carr et al. (2014) also found that the soil represented an average of all of the plant variation in their study of leaf wax *n*-alkane distributions in sediments from South Africa. Bush and McInerney (In Review) also showed that the soils represent a pooled and averaged chain length distribution. This study demonstrates that *n*-alkane ACL in soils covaries with temperature and aridity and is thus suitable as a proxy for recording climate change in the sedimentary record.

## **CONCLUSIONS**

This study demonstrates the strong correlation between both mean and extreme climate conditions relating to temperature and aridity and the ACL of soils across Australia. In particular, the mean conditions of interest are MAT and annual MI and the extreme conditions include lowest quarter mean MI, driest month of precipitation and the maximum month VPD. Interestingly, there is also a strong relationship between the ACL in the soils and latitude, and further investigation reveals that this relationship is driven by temperature rather than radiation. The soils show a much stronger relationship with the climate variables than the plants do and this is likely to be because the soils represent a temporal integration of all *n*-alkane contributing organisms. The plants, on the other hand, are subject to different rates and timing of growth and are more susceptible to climate variations on a much smaller



timescale. This timescale does not necessarily represent the overall climate conditions, and the production of *n*-alkanes in the plants may instead be more closely related to seasonal variation. Overall, these results show that aridity and temperature are significant drivers of ACL found in soils. Coupled with their persistence in the sedimentary record, these results confirm that *n*-alkane ACL in soils is suitable as a proxy for recording climate variation in the sedimentary record.

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## APPENDIX A: EXTENDED METHODOLOGY

### AusPlots and TREND

Samples were taken from AusPlots and TREND plots provided by the services of the Terrestrial Ecosystem Research Network (TERN). The TERN plot selection process can be found in their AusPlots Rangelands Survey Protocols Manual (White et al. 2012). The process consists of four stages, with the first three stages being desktop exercises:

1. Bioregional stratification

Hierarchical cluster analysis of Australia's different bioregions, to create groups of similar bioregions.

2. Selecting representative bioregions to sample

The main goal is to sample at least one bioregion in each group.

3. Stratifying areas of sampling interest within bioregions

Hierarchical analysis to a greater resolution to that of Stage 1, based on scientific and environmental information, historic information, logistic considerations and political considerations.

4. Choosing plot locations in the field based on areas of interest

Precise sites are chosen based on a consistent and constant mix of vegetation, slope, relief and soil, with plots being 1 hectare in size and having a N/S, E/W orientation.

Once plots had been selected, field work planned and the plot layout positioned, field workers then conducted a number of different methods at each plot. These include a plot description, photo panoramas, collection of vascular plant samples, collection of point intercept data, determination of basal area of trees and shrubs, determination of plant structural summary, leaf area index and soil descriptions and soil metagenomic sampling.

For the purposes of this project, the plant samples and soil metagenomics samples were those required for subsampling. Plant samples were collected by trimming off plant material with secateurs and placing in a labelled paper bag and then barcoded. At the end of each day, plant samples were then placed in a plant press to assist with preservation and identification.

Once brought back from the trip all plant samples were sent to a local herbarium for identification. Once identification was complete, plant samples were then transferred to synthetic tea bags and stored with silica granules in an airtight plastic lunch box.

At each plot, 9 soil sampling locations were identified, with cores to 30cm deep being taken. As well as this, surface soil is also sampled for soil metagenomics. This involved scraping aside any loose plant material and animal waste and taking a soil sample with a small clean trowel to 3cm depth. This soil was then placed in a calico bag and barcoded. Each calico bag was then placed in a larger snaplock bag with silica granules for storage.

### Site and Sample Selection

Sites for subsampling were initially selected based on the immediate availability of plant and soil samples. To further narrow down which sites were to be selected, Mean Annual Temperature (MAT) and Mean Annual Precipitation (MAP) for each site were plotted against one another in Excel to help select sites that provide a broad spread of these two variables. Information, including MAT and MAP, for each site was provided in spreadsheet format directly from TERN.

Once the sites had been narrowed down to 19 through the above process, the top three dominant plant species was selected from each site. This process was made simple by the Soils to Satellite website, found at <http://soils2sat.ala.org.au:8080/ala-soils2sat/>, provided by TERN. By selecting the Study Location>Point Intercept>Herbarium Determination, amongst

other things, a simple pie chart is presented that provides the percentage cover of all plant species present at that site, allowing selection of the top three dominant species. Soil sample selection was a little more arbitrary than the plant sampling, with “Sample 5” being selected for each site. Initially it was assumed that Sample 5 represented the central sample of a total of 9 having been taken at each site; however this may or may not be the case for each site.

Metadata for Atlas of Living Australia Website (for both maps and data)

**Precipitation - annual mean**

Description: Mean annual rainfall (mm)

Short Name: rainm

Metadata

contact [CSIRO Ecosystem Sciences](#)

organization:

Organisation

role:

Metadata date: 2010-07

Reference

date:

Resource constraints:

- Licence level: 1
- Licence info:

Licence notes: Permission required to re-distribute derivative works. Please contact Dr. Kristen Williams - [kristen.williams@csiro.au](mailto:kristen.williams@csiro.au)

Type: Environmental (gridded) 0.01 degree (~1km)

Classification: Climate ⇒ Precipitation

Units: mm

Data language: eng

Scope:

Notes:

Keywords: rain

More information: <http://spatial.ala.org.au/geonetwork/srv/en/metadata.show?uuid=64c0fb3f-b9c9-4ff1-bbaa-df7cba45e1b7>

View in spatial portal : [Click to view this layer](#)

**Temperature - annual mean (Bio01)**

Description: Temperature - annual mean (Bio01)

Short Name: bioclim\_bio1

Metadata contact organization: [CSIRO Ecosystem Sciences](#)

Organisation

role:

Metadata date: 2010-08

Reference date: 2008-02

Resource constraints:

- Licence level: 1
- Licence info:

Licence notes: Permission to re-distribute ANUCLIM outputs should be obtained from Prof. Michael Hutchinson - <http://fennerschool.anu.edu.au/publications/software/>

Type: Environmental (gridded) 0.01 degree (~1km)

Classification: Climate ⇒ Temperature

Units: degrees C

Data language: eng

Scope:

Notes: Data derived using ANUCLIM v6 (beta) with the new set of climate surfaces (centred on 1990), by Dr. Kristen Williams.

Keywords:

More information: <http://fennerschool.anu.edu.au/publications/software/>

View in spatial portal : [Click to view this layer](#)

**Moisture Index - annual mean (Bio28)**

Description: Moisture Index - annual mean (Bio28)

Short Name: bioclim\_bio28

Metadata contact organization: [CSIRO Ecosystem Sciences](#)

Organisation role:

Metadata date: 2010-08

Reference date: 2008-02

Resource constraints:

- Licence level: 1
- Licence info:

Licence notes: Permission to re-distribute ANUCLIM outputs should be obtained from Prof. Michael Hutchinson - <http://fennerschool.anu.edu.au/publications/software/>

Type: Environmental (gridded) 0.01 degree (~1km)

Classification: Substrate ⇒ Moisture

Units: Dimensionless

Data language: eng

Scope:

Notes: Data derived using ANUCLIM v6 (beta) with the new set of climate surfaces (centred on 1990), by Dr. Kristen Williams.

Keywords: soil, water, saturation

More <http://fennerschool.anu.edu.au/publications/software/>

information:

View in spatial  
portal : [Click to view this layer](#)

### **Moisture Index - lowest quarter mean (Bio33)**

Description: Moisture Index - lowest quarter mean (Bio33)

Short Name: bioclim\_bio33

Metadata contact  
organization: [CSIRO Ecosystem Sciences](#)

Organisation  
role:

Metadata date: 2010-08

Reference date: 2008-02

Resource constraints: 

- Licence level: 1
- Licence info:

Licence notes: Permission to re-distribute ANUCLIM outputs should be obtained from  
Prof. Michael Hutchinson -  
<http://fennerschool.anu.edu.au/publications/software/>

Type: Environmental (gridded) 0.01 degree (~1km)

Classification: Substrate ⇒ Moisture

Units: Dimensionless

Data language: eng

Scope:

Notes: Data derived using ANUCLIM v6 (beta) with the new set of climate  
surfaces (centred on 1990), by Dr. Kristen Williams.

Keywords: soil, water, saturation

More  
information: <http://fennerschool.anu.edu.au/publications/software/>

View in spatial  
portal : [Click to view this layer](#)

### **Aridity index - month max**

Description: Maximum month aridity index

Short Name: arid\_max

Metadata  
contact organization: [CSIRO Ecosystem Sciences](#)

Organisation  
role:

Metadata  
date: 2010-07

Reference  
date:



Resource constraints: 

- Licence level: 1
- Licence info:

Licence notes: Permission required to re-distribute derivative works. Please contact Dr. Kristen Williams - [kristen.williams@csiro.au](mailto:kristen.williams@csiro.au)

Type: Environmental (gridded) 0.01 degree (~1km)

Classification: Climate ⇒ Precipitation

Units: dimensionless

Data language: eng

Scope:

Notes: The monthly ratio of precipitation to potential evaporation (pan, free-water surface). A numerical indicator of the degree of dryness of the climate at a given location. Adapted from the index proposed by UNEP (1992; cited in Middleton and Thomas (1997)).

Keywords: evaporation, rain, precipitation, temperature

More information: <http://spatial.ala.org.au/geonetwork/srv/en/metadata.show?uuid=057e11df-fc1c-4d20-ad54-19dc0345e969>

View in spatial portal : [Click to view this layer](#)

### **Radiation - highest period (Bio21)**

Description: Radiation - highest period (Bio21)

Short Name: bioclim\_bio21

Metadata contact organization: [CSIRO Ecosystem Sciences](#)

Organisation role:

Metadata date: 2010-08

Reference date: 2008-02

Resource constraints: 

- Licence level: 1
- Licence info:

Licence notes: Permission to re-distribute ANUCLIM outputs should be obtained from Prof. Michael Hutchinson - <http://fennerschool.anu.edu.au/publications/software/>

Type: Environmental (gridded) 0.01 degree (~1km)

Classification: Climate ⇒ Solar radiation

Units: MJ/m<sup>2</sup>/day

Data language: eng

Scope:

Notes: Data derived using ANUCLIM v6 (beta) with the new set of climate surfaces (centred on 1990), by Dr. Kristen Williams.

Keywords: solar, sun

More information: <http://fennergchool.anu.edu.au/publications/software/>  
View in spatial portal : [Click to view this layer](#)

### **WorldClim: Precipitation - driest month**

Description: Precipitation of Driest Month

Short Name: worldclim\_bio\_14

Metadata contact organization: [WorldClim](#)

Organisation role: custodian

Metadata date: 2010-07

Reference date:

Resource constraints:

- Licence level: 2
- Licence info: <http://www.worldclim.org/current>

Licence notes: This dataset is freely available for academic and other non-commercial use. Redistribution, or commercial use, is not allowed without prior permission.

Type: Environmental (gridded) 0.01 degree (~1km)

Classification: Climate ⇒ Precipitation

Units: mm

Data language: eng

Scope:

Notes: (From <http://www.worldclim.org/methods>) - For a complete description, see: Hijmans, R.J., S.E. Cameron, J.L. Parra, P.G. Jones and A. Jarvis, 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25: 1965-1978. The data layers were generated through interpolation of average monthly climate data from weather stations on a 30 arc-second resolution grid (often referred to as 1 km<sup>2</sup> resolution). Variables included are monthly total precipitation, and monthly mean, minimum and maximum temperature, and 19 derived bioclimatic variables. The WorldClim interpolated climate layers were made using: \* Major climate databases compiled by the Global Historical Climatology Network (GHCN), the FAO, the WMO, the International Center for Tropical Agriculture (CIAT), R-HYdronet, and a number of additional minor databases for Australia, New Zealand, the Nordic European Countries, Ecuador, Peru, Bolivia, among others. \* The SRTM elevation database (aggregated to 30 arc-seconds, 1 km) \* The ANUSPLIN software. ANUSPLIN is a program for interpolating noisy multi-variate data using thin plate smoothing splines. We used latitude, longitude, and elevation as independent variables.

Keywords: rain, bio14

More information: <https://gist.github.com/tucotuco/1152668>

View in spatial  
portal : [Click to view this layer](#)

### Vapour pressure deficit - month max

Description: Maximum month vapour pressure deficit (KPa)

Short Name: vpd2max

Metadata

contact [CSIRO Ecosystem Sciences](#)

organization:

Organisation

role:

Metadata date: 2010-07

Reference

date:

Resource constraints:

- Licence level: 1
- Licence info:

Licence notes: Permission required to re-distribute derivative works. Please contact Dr. Kristen Williams - kristen.williams@csiro.au

Type: Environmental (gridded) 0.01 degree (~1km)

Classification: Climate ⇒ Humidity

Units: KPa

Data language: eng

Scope:

Notes:

Keywords: temperature, moisture

More information: <http://spatial.ala.org.au/geonetwork/srv/en/metadata.show?uuid=b0da1579-7cc6-4fff-8d56-d2bf1fae3d74>

View in spatial  
portal : [Click to view this layer](#)

### Email from Dr Kristen William granting permission for use of climate data

**From:** Kristen.Williams@csiro.au [mailto:Kristen.Williams@csiro.au]

**Sent:** Saturday, 11 October 2014 8:06 PM

**To:** Siân Howard

**Subject:** RE: Use of maps made available on Atlas of Living Australia  
Hi Siân,

Thank you for your enquiry.

I can help you with:

- Temperature: MINT and MAXT
- Precipitation: RAIN
- Radiation: RADN
- Aridity Index: ARID

- Vapour pressure deficit: VPD

For the moisture index, I can provide water deficit (P-E): ADEF.

postfix on naming: I = min, X – max; M = mean annual; A = annual total

1960 series includes VPD

1990 series includes RH (relative humidity)

All of above are custom derivatives of monthly variables generated using ANUCLIM software.

See XML metadata for details.

Will send data via cloudstor with license and acknowledgement/attribution requirements.

Use of this data in reports and publications requires citation of my paper describing the data collection: Williams et al. 2012 in the International Journal of GIS (attached).

This data is provided for your personal research use only.

You'll need help from someone with GIS skills to assist with mapping.

regards,  
Kristen

**Kristen J Williams, PhD, GISP-AP**

Senior Research Scientist - Ecological Geographer  
Group Leader Biodiversity Assessment and Conservation  
Biodiversity, Ecosystem Knowledge and Services Research Program

CSIRO Land & Water National Research Flagship  
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[kristen.williams@csiro.au](mailto:kristen.williams@csiro.au) | [www.csiro.au](http://www.csiro.au) | <http://www.csiro.au/people/Kristen.Williams.html>  
<http://www.researcherid.com/rid/B-9941-2008> | <http://orcid.org/0000-0002-7324-5880>

Address: GPO Box 1700, Canberra, ACT 2601  
Location: Black Mountain Laboratories, Clunies Ross Road, Acton

Email from BOM granting permission for use of climate data

**From:** climatedata@bom.gov.au [mailto:climatedata@bom.gov.au]  
**Sent:** Friday, 1 August 2014 11:47 AM  
**To:** Sian Howard  
**Subject:** Bureau of Meteorology Climate Data: Ticket# E7WG664726 - Use of maps for Honours thesis [SEC=UNCLASSIFIED]



**In reply please quote: E7WG664726**

Dear Sian,

Thank you for your enquiry. You can use the maps and data on our website as you wish - you just need to acknowledge the Bureau of Meteorology as the source.

### Feedback

We are constantly working to improve our service and appreciate your feedback. If you would like to contribute, please complete our 2 minute survey at

[http://www.bom.gov.au/climate/surveys/customer\\_feedback.shtml](http://www.bom.gov.au/climate/surveys/customer_feedback.shtml) .

Regards,

Melanie Harris

Climate Data Services  
Bureau of Meteorology

Contact details:

Monday to Friday: 10am – 12noon & 2pm – 4pm

Head office: 03 9669 4082

To avoid interstate call charges please use the appropriate number below:

NSW: 02 9296 1627

NT: 08 8920 3921

QLD: 07 3239 8727

SA: 08 8366 2746

TAS: 03 6221 2027

VIC: 03 9669 4082

WA: 08 9263 2228

<http://www.bom.gov.au/climate/data-services/>

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### Sample Collection and Weighing

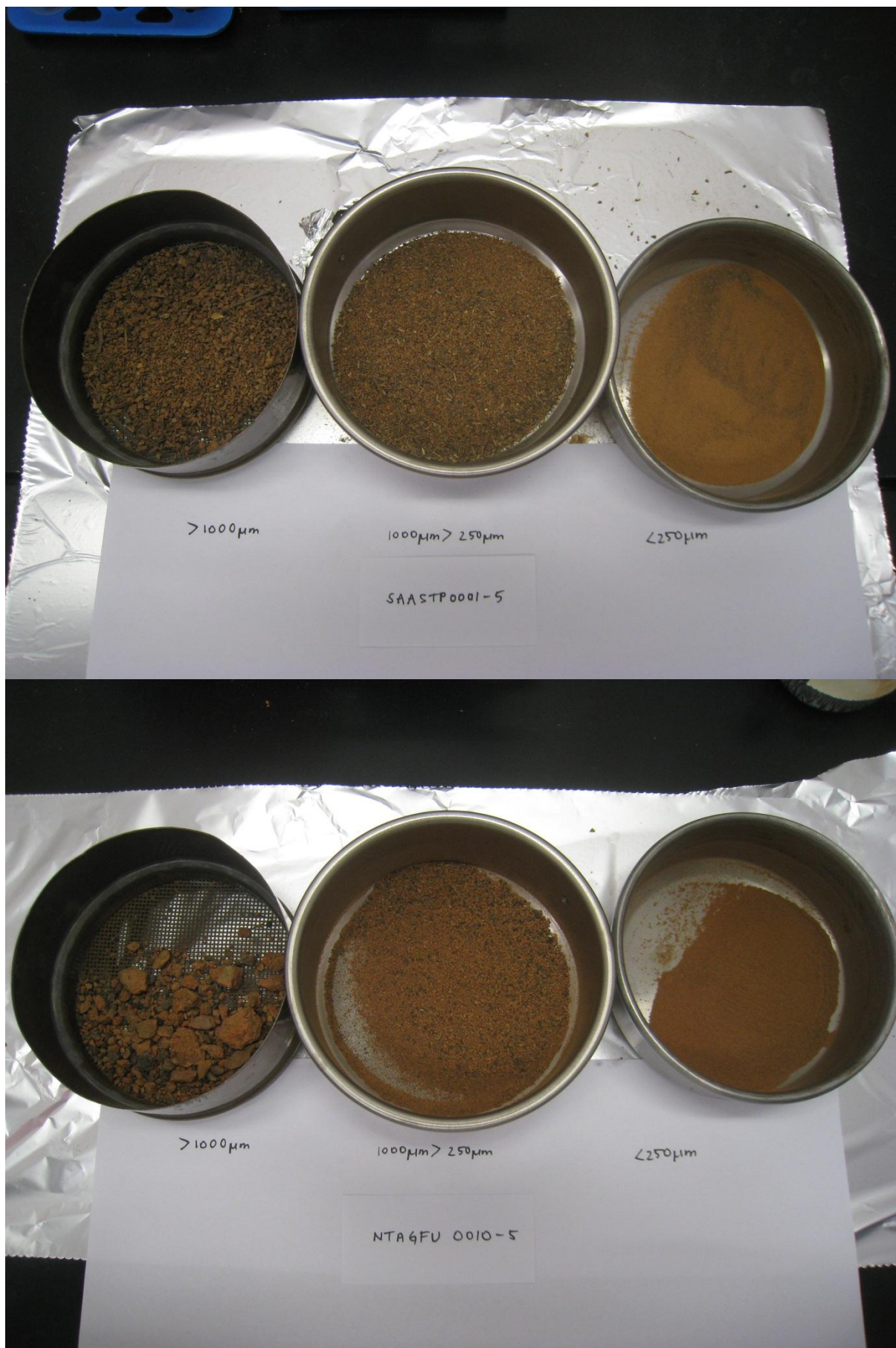
Plant samples were weighed using a Sartorius Analytical Microbalance. To clean tweezers, used to handle the plant samples, they are rinsed with solvents from Teflon squeeze bottles in the following order: three rinses with methanol, three rinses with dichloromethane, and three rinses of hexane, in order to remove any hydrocarbons present. All solvents are of Optima grade. Tweezers are cleaned before handling each sample. Nitrile gloves are also worn. A clean sheet of aluminium foil is placed on the bench, shiny surface facing down, and used as the surface for working from. This sheet of foil was replaced between handling of each sample, in the event that it came in to contact with any plant sample, to avoid cross contamination. A small clean beaker was placed on to the scales and a new, labelled and open plastic falcon tube rested inside it. These were tared on the scales. Using the tweezers, each “tea bag” containing the plant samples was opened and between 0.1-0.2g of plant sample grasped with the tweezers and weighed on the Sartorius Analytical Microbalance, making sure to avoid the sample came in to contact with anything except for the inside of the uncontaminated falcon tube. For larger samples, solvent rinsed scissors (see above process for solvent rinsing tweezers) were used to cut the plant sample into smaller pieces before being weighed. Once each sample had been weighed, the falcon tube was removed from the scales, capped, and the caps then labelled. The capped falcon tubes were then stored in a test tube rack until grinding occurred.

Soil samples were collected from storage at the TERN warehouse. The sample bag labelled “5” was subsampled from each site. Wearing nitrile gloves, a new clean and opened falcon tube was used to scoop out one tubeful of soil. A fresh pair of nitrile gloves was used for each soil sample taken. Once soil had been scooped out of the sample bag, the falcon tube was immediately capped, the outside wiped with Kimwipes to remove any residual material, labelled and then stored in a test tube rack until total lipid extraction occurred.

#### Sample Grinding and Sieving

Plant samples were ground into finer material in order to maximise the amount of lipids that could be extracted from them. These were ground using a ceramic mortar and pestle. Each sample was ground in a clean mortar and pestle, that had been washed with a 1:50 solution of decon90:water, followed by rinsing with tap water three times, and then rinsed with RO water three times, dried and then thoroughly solvent rinsed with Optima grade solvents from Teflon squeeze bottles in the following order: three rinses with methanol, three rinses with dichloromethane and then three rinses with hexane. Liquid Nitrogen was used to help grind the samples, and was collected in a thermal flask, following the regulation Safe Operating Procedures of wearing protective eyewear, labcoat and insulated gloves. Each plant sample was removed from its falcon tube, either by pouring directly in to the mortar, or by using clean, solvent rinsed tweezers, and then placed into the mortar. The mortar was then approximately 1/3 filled with liquid nitrogen, to speed up the crushing and grinding process by freezing the sample and making it more brittle. Using the pestle and attempting to avoid spillage, the plant was pulverised and ground until fine. Once all the liquid nitrogen had evaporated, the ground plant sample was then carefully scraped in to an ashed scintillation vial with a clean and solvent rinsed steel scoopula. The scintillation vials were then capped and labelled. In the event that the plant material was not entirely dry at this point, the scintillation vial was loosely covered with alfoil instead of being capped, and left in the fume cupboard so that the sample could dry out, in order to avoid and mould or fungal growth from occurring. Each sample was ground with a clean and solvent rinsed mortar and pestle and transferred with clean and solvent rinsed tweezers and scoopulas. Labelled scintillation vials were stored until sample was to undergo total lipid extraction.

Soil samples need to be sieved prior to total lipid extraction to remove any visible plant detritus including leaves, bark and root material, and to also remove any small pebbles. The soil sample was placed in an ashed aluminium sample boat and gently pressed with a solvent rinsed scoopula or tweezers to break up any clods. Two sieves, 530 micron and 1000 micron, were scrubbed with a 1:50 decon90:water mixture, rinsed three times with tap water, rinsed three times with RO water, sonicated in acetone for 15 minutes, followed by triple rinsing with Optima grade solvents from Teflon squeeze bottles in the following order: three times with Methanol, three times with dichloromethane and then three times with hexane. The sieves were stacked on top of a solvent rinsed catcher bowl, with the 1000 micron sieve on the top, and the soil sample poured onto the top sieve and gently shaken through. The sieved material collected in the catcher bowl was poured into a new, labelled falcon tube in readiness for total lipid extraction in an ASE, and the residual material placed into the original falcon tube and labelled with the site location and lab user initials.









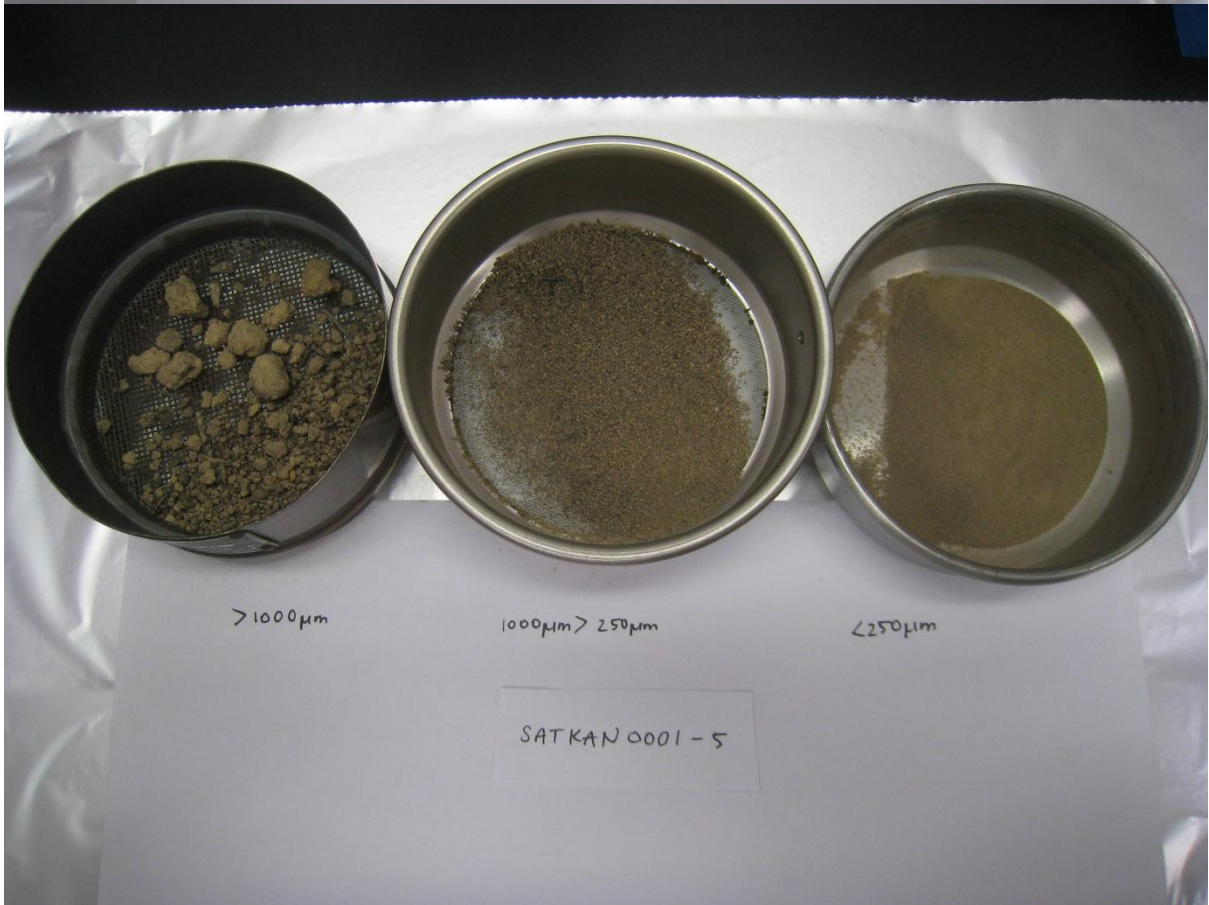
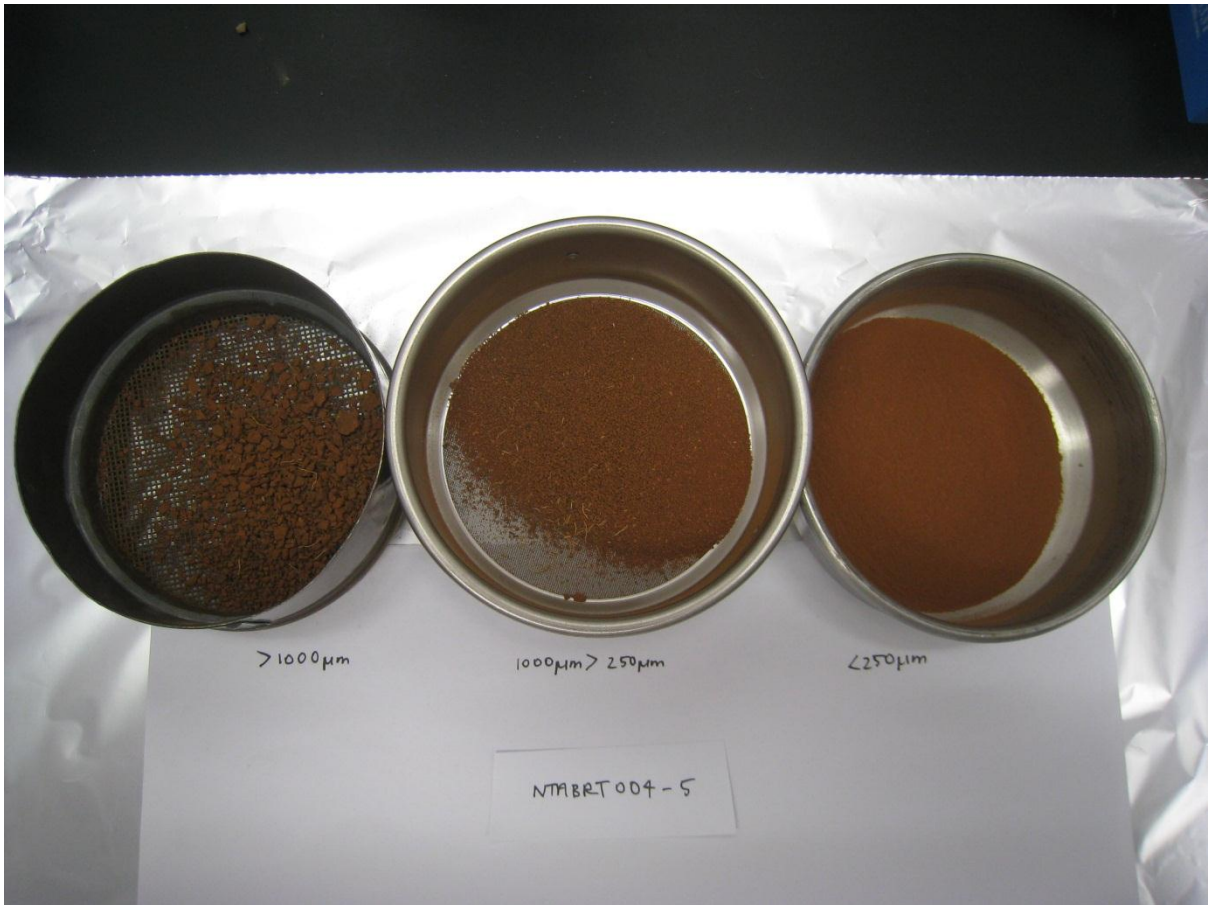














**Figure 1. Photos of sieved soil samples.**  
Total Lipid Extraction (TLE)



## 1. Sonication

Because it is relatively easy to extract lipids from plant samples, sonication in a Soniclean 250TD using solvents is sufficient for conducting a total lipid extraction. Using a sonication bath filled with RO water, dried and ground plant samples are added to an ashed test tube and covered with a 9:1 DCM:MeOH solution (approximately 5ml). Each test tube is covered with ashed alfoil and sonicated in the sonication bath for 15 minutes. During the sonication process, a clean set of ashed test tubes is arranged in a test tube rack, one per sample. An ashed glass funnel is placed in each one and using solvent rinsed tweezers, an ashed glass fibre filter is folded in half then half again, and opened up into a cone and placed in the funnel. Each funnel is covered with ashed alfoil until ready to use. Once sonication is complete, samples were left to stand to allow most sediment to settle. The sonicated sample is then decanted through the filter in the funnel. An ashed pipette can be used to assist with this. After transfer is complete, add a further amount of 9:1 solvent solution to cover the sample (approximately 5ml) and sonicate for 15 minutes. Decant this extract into the funnel. Repeat this process for a total of 3 extractions. The filtered extract is then dried down under N<sub>2</sub> in the FlexiVap until almost dry. The TLE is then quantitatively transferred using an ashed pipette and rinsing and transferring three times with DCM to ashed 4ml vials for refrigerated storage until ready for polar and non-polar fraction separation.

## 2. ASE

A Thermo Scientific Dionex Acceleration Solvent Extraction (ASE) 350 is used for total lipid extracts from soils. This process is suitable for soils because it uses heat and pressure in the extraction, and is therefore a quicker and more thorough means of extracting these compounds from soils than sonication.

The 22ml cell components, including PEEK seals and frits are cleaned with 1:50 decon90:water solution and then rinsed three times with tap water, followed by three rinses with RO water. Components are then placed in a 2L ashed beaker and covered with Histologic grade acetone. The beaker is then placed in to a sonicating bath and the components are sonicated for 15 minutes. This acetone is then replaced with Methanol, and the cells are again sonicated for 15 minutes. After the second sonication, the cell components are then left to soak in the methanol for a further 15 minutes. Each solvent can be reused a maximum of 6 times. Using clean, solvent rinsed tweezers, the components are removed from the beaker and placed on to ashed alfoil to dry. Using only solvent rinsed tweezers to handle them, two 27mm ashed glass fibre filters are inserted in the bottom end of the cell and the cell body was then screwed on to this.

Using the correct sized solvent rinsed funnel for the cells, between 4.5-26g of the <250µm soil sample was added to each 22ml cell and topped up to fill line with diatomaceous earth. Another 27mm ashed glass fibre filter paper was placed on top of the cell body, and the top cell end was screwed on. The cells were then labelled and placed in their respective slots on the ASE. Collection vials (60ml) that had been topped with alfoil and then ashed are capped with solvent rinsed caps and septa were labelled and placed in their respective slots on the ASE.

One of the ASE reservoirs contains Optima Grade DCM, and a second reservoir contains Optima Grade MeOH. A ratio of 9:1 DCM:MeOH is to be used for the extraction. The ASE sequence is set to preheat for 12 minutes up to 100°C and held at that temperature for 5 minutes, with this heating process repeated three times. The cell is then

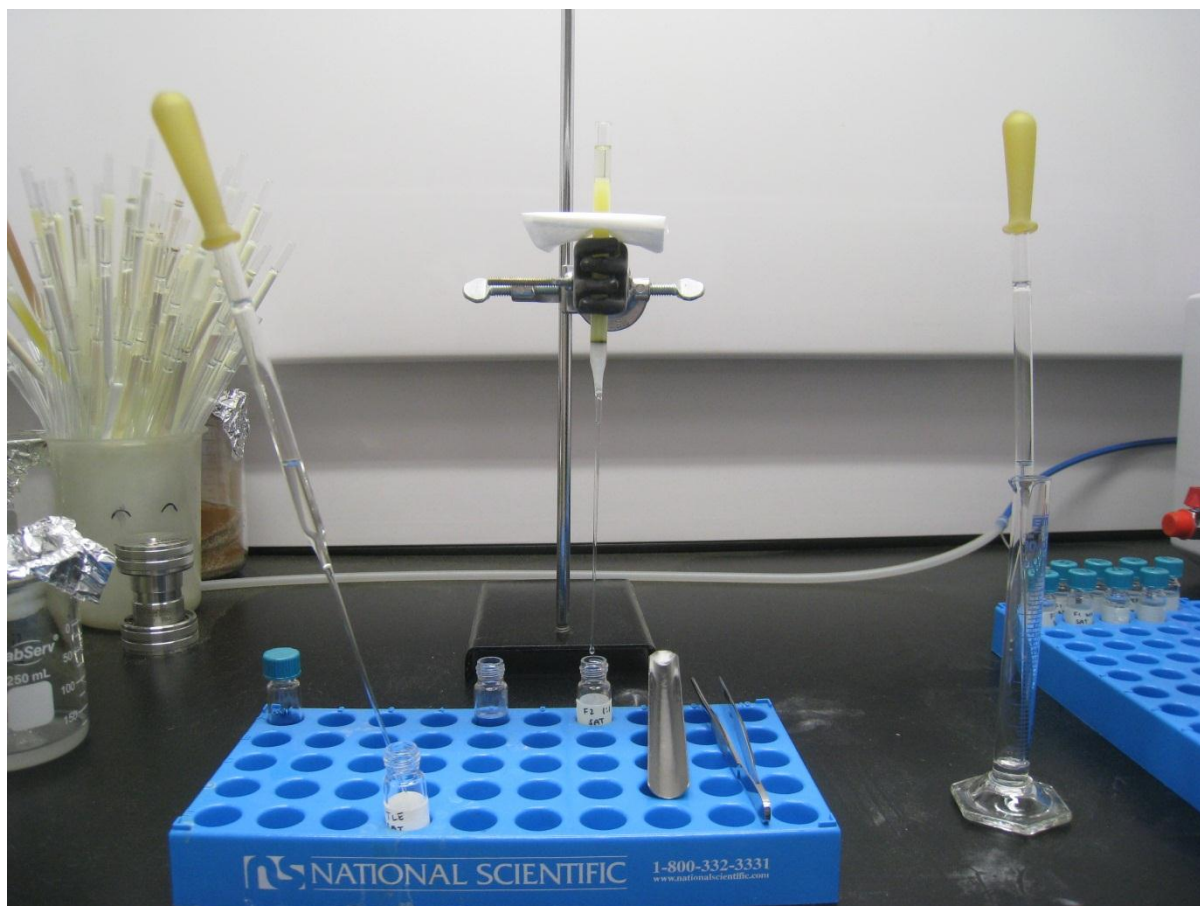
rinsed with 5ml of solvent solution a total of three times. The rinse volume was set to 60%, with a purge time of 120 seconds.

The total lipid extract is then dried down under N<sub>2</sub> in the FlexiVap until almost dry. The TLE is then quantitatively transferred using an ashed pipette and rinsing and transferring three times with DCM to ashed 4ml vials for refrigerated storage until ready for polar and non-polar fraction separation.

#### Polar and non-Polar Lipid Fraction Separation using short column chromatography

Separating the non-polar and polar fractions of the total lipid extract (TLE) is necessary for subsequent GC-MS analysis. The silica gel used in the chromatography columns is slightly polar, and the initial pass of a non-polar solvent allows the non-polar fraction to be removed and collected, while the polar fraction remains bonded to the silica gel. Following this with the addition of a solvent with greater polarity than that of the silica gel allows the polar fraction to then be removed and collected. Long-tipped pipettes were stuffed with a small amount of glass wool at their base, before their narrow tip, and then ashed. One of these glass wool pipettes was set up on a retort stand, and 4ml vial set up underneath it. A slurry of oven dried silica gel and hexane was combined in a small beaker and using a short-tipped, ashed pipette, the slurry was transferred to the glass wool pipette to produce a chromatography column. The silica gel was allowed to settle in the glass wool pipette until it reached the level of the indent near the top. Hexane was continually added to ensure that the top level of the silica gel was not exposed to air. Underneath the chromatography column, a new ashed 4ml vial labelled as Fraction 1 (F1) was set up underneath. The total lipid extract (TLE), which had been completely dried down, was diluted with a couple of drops of hexane, and transferred to the top of the chromatography column using a new ashed pipette. 4ml of hexane was used to continue rinsing the vial that originally held the TLE, and this 4ml was continually added to the top of the chromatography column and captured in the 4ml vial beneath. After the last of the 4ml of hexane was used, a new 4ml collection vial, labelled Fraction 2 (F2) was set up underneath and 4ml of 1:1 DCM:MeOH solution was then used to rinse the original TLE vial and was then transferred to the top of the chromatography column. Once the chromatography column ceased dripping the polar fraction into the 4ml collection vial, the two collection vials (F1 and F2) were then capped and stored in the fridge.

Prior to GC-MS being conducted, the F1 samples were dried down under nitrogen using a Flexivap. These samples then had a small amount of Optima grade hexane (7-8 drops), the hexane was rinsed down the sides of the vial using an ashed pipette and was transferred to a bottom spring insert in a 2ml vial. This quantitative transfer was repeated another two times, for a total of three rinses and transfers. Once the samples were transferred to the insert in the 2ml vial, they were dried down under nitrogen using a Flexivap. Once the samples were dried down fully, 50µl of Optima grade hexane was added using a 50µl syringe that had been fully cleaned and rinsed with hexane prior to use. Samples were then labelled with their sample number and F1, and stored in the fridge in preparation for GC-MS analysis.



**Figure 2: Silica gel chromatography column.**

GC-MS

Instrument: HP5973 MS coupled to a HP6890 GC (MS operated in scanning mode from 45 to 500Da)

Capillary: SGE CPSil-5MS, 60m (length) x 0.25mm (internal diameter) x 0.25 $\mu$ m (phase thickness)

Carrier Gas: Helium at 1ml/min constant flow

Temperature program: 50°C held for 1 min ramped at 8°C/min to 340°C held for 7.75mins

Injection: 1 $\mu$ l in either split mode with a 50:1 split or pulsed splitless depending on sample concentration.

Injection temperature: 300°C

Software: Chemstation

Using Chemstation software:

A quant package was set up that enabled automatic quantitation of peak areas in each samples' chromatogram. For each run of samples, they were opened and the quant package set to run by hitting Method>Load Method>[name of quant package method]. Then select Quantitate>Calculate. Mass 57 was selected. QUANT files were saved for each sample and opened up in Excel in order to copy the "NAME", "TIME" and "PEAK AREA" columns into a new spreadsheet, in order to calculate ACL for each soil sample.

Instrument: Perkin Elmer Clarus 500 GCMS

Capillary: SGE CPSil-5MS, 60m (length) x 0.25mm (internal diameter) x 0.25 $\mu$ m (phase thickness)

Carrier Gas: Helium at 1ml/min constant flow

Temperature program: 50°C held for 1 min ramped at 8°C/min to 340°C held for 7.75mins  
Injection: 1µl in either split mode with a 50:1 split or splitless depending on sample concentration.

Injection temperature: 300°C

Software: Turbomass

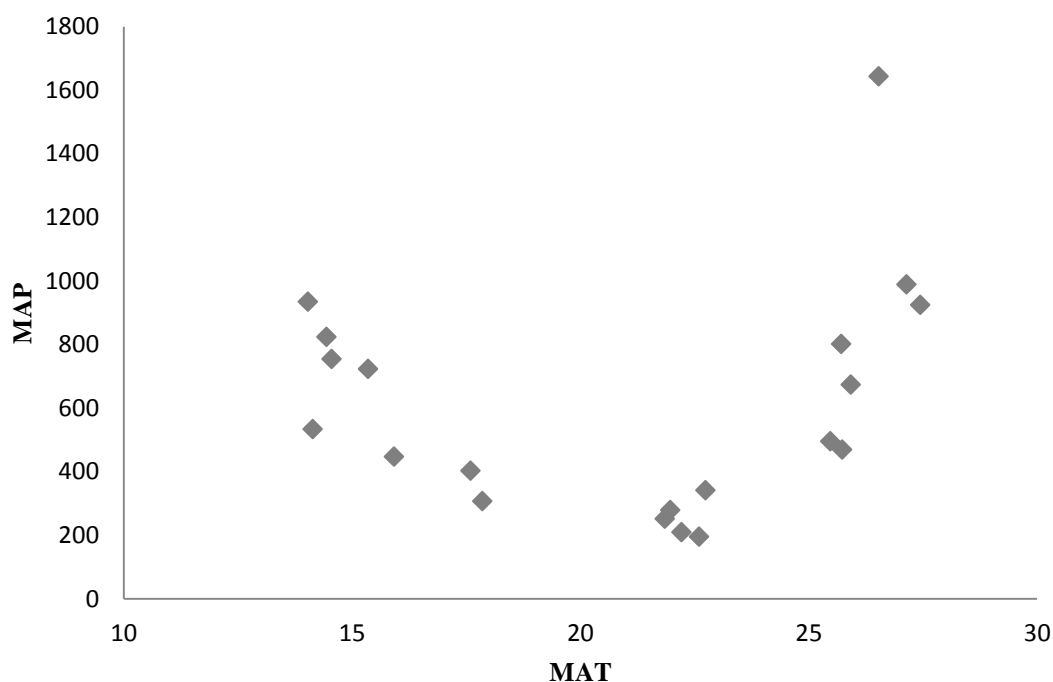
Using Turbomass software:

Open up chromatogram for the standard in order to determine which peak is associated with which *n*-alkane chain length for the sample, and then pick out Mass 57. Add chromatogram for sample and pick out Mass 57. Hit Edit>Integrated Peaks. Then Edit>Peak List Write. Create a file, name as the sample name>Open>Append All>Exit. The .pdb files created were opened in Excel and the “NAME”, “FOUND RT” and “AREA” columns were copied and pasted into a new Excel spreadsheet, in order to calculate ACL for each soil sample.

Standard: an in-house hydrocarbon standard with even *n*-alkanes from C<sub>14</sub> to C<sub>32</sub> without C<sub>28</sub>.

### Statistical Analysis

Regression analysis of the soil samples GC data was conducted using the Data Analysis Add-on in Excel. The ACL of the soil samples was given as the Input Y Range, with MAP, MAT and MI separately given as the Input X Range.



**Figure 3.** Mean annual precipitation (MAP) versus mean annual temperature (MAT) of selected sites. This allows for comparison of similar MAP with differing MAT as well as comparison of differing MAP with similar MAT.

**Table 1: Data regarding the growth form, genetic voucher, percentage cover and amounts weighed out for analysis for each plant sample (cont'd on next two pages).**

Site	Bioregion	Dominant Spp 1	Growth form	Genetic Voucher No	Amount Subsampled from teabag (g)	Amount subsampled for sonication (mg)	% cover
NTAGFU0001	Gulf fall and uplands	<i>Aristida pruinosa</i>	Tree Mallee	NTA 001524	0.101	46.8	17.4
NTAGFU0008	Gulf fall and uplands	<i>Triodia pungens</i>	Hummock grass	NTA 002012	0.187	50.8	45.4
NTAGFU0010	Gulf fall and uplands	<i>Triodia pungens</i>	Hummock grass	NTA 002136	0.147	52	62.7
NTAGFU0017	Gulf fall and uplands	<i>Melaleuca viridiflora</i>	Shrub	NTA 002634	0.172	50.4	34.5
NTAGFU0031	Gulf fall and uplands	<i>Melaleuca viridiflora</i>	Shrub	NTA 003622	0.182	50.5	30.5
NTAGFU0040	Gulf fall and uplands	<i>Acacia dimidiata</i>	Shrub	NTA 004200	0.117	50.4	26.8
NTABRT0004	Burt plain	<i>Acacia aptaneura</i>	Shrub	NTA 001301	0.19	50.5	56.8
NTAFIN0019	Finke	<i>Cenchrus ciliaris</i>	Tussock Grass	NTA 000754	0.066	34.8	68.6
NTAFIN0022	Finke	<i>Eremophila freelingii</i>	Shrub	NTA 000964	0.125	51.3	50.5
SATFLB0005	Flinders lofty block	<i>Dodonaea viscosa</i> subsp. <i>angustissima</i>	Shrub	SAT 000316	0.113	50.5	21.9
SATFLB0008	Flinders lofty block	<i>Triodia scariosa</i>	Hummock grass	SAT 000424	0.149	50.4	47.6
SATFLB0010	Flinders lofty block	<i>Eucalyptus odorata</i>	Tree/Palm	SAT 000535	0.152	51.1	67
SATFLB0012	Flinders lofty block	<i>Allocasuarina muelleriana</i> subsp. <i>Muelleriana</i>	Shrub	SAT 000649	0.178	52	42.1
SATFLB0014	Flinders lofty block	<i>Eucalyptus odorata</i>	Tree Mallee	SAT 000746	0.172	51	33
SATFLB0015	Flinders lofty block	<i>Eucalyptus obliqua</i>	Tree/Palm	SAT 000816	0.12	51.4	61.2
SATKAN0001	Kanmantoo	<i>Eucalyptus baxteri</i>	Tree/Palm	SAT 000122	0.136	50.4	42.9
SATKAN0002	Kanmantoo	<i>Eucalyptus obliqua</i>	Tree/Palm	SAT 000191	0.139	50.3	55.2
SAASTP0001	Stony plains	<i>Maireana aphylla</i>	Chenopod	SAA 000250	0.189	50.4	34.6
SAASTP0004	Stony plains	<i>Malvastrum americanum</i> var. <i>americanum</i>	Forb	SAA 000019	0.062	36.2	25.6
NTADAC0001	Darwin Coastal	<i>Eucalyptus tetrodonta</i>		NTA 006020	0.169	52.3	

Site	Bioregion	Dominant Spp 2	Growth form	Genetic Voucher No	Amount Subsampled from teabag (g)	Amount subsampled for sonication (mg)	% Cover
NTAGFU0001	Gulf fall and uplands	Enneapogon polyphyllus	Tussock Grass	NTA 001525	0.124	50	13.3
NTAGFU0008	Gulf fall and uplands	Aristida contorta	Tussock Grass	NTA 002011	0.135	50	19.5
NTAGFU0010	Gulf fall and uplands	Eucalyptus leucophloia	Tree Mallee	NTA 002140	0.188	50.6	36.4
NTAGFU0017	Gulf fall and uplands	Chrysopogon fallax	Tussock Grass	NTA 002610	0.166	50.6	10.4
NTAGFU0031	Gulf fall and uplands	Schizachyrium pachyarthron	Tussock Grass	NTA 003588	0.063	23.5	28.3
NTAGFU0040	Gulf fall and uplands	Heteropogon contorus	Tussock Grass	NTA 003995	0.091	22.8	15.9
NTABRT0004	Burt plain	Aristida holathera	Tussock Grass	NTA 001318	0.17	51	24.4
NTAFIN0019	Finke	Acacia estrophiolata	Tree/Palm	NTA 000784	0.123	50	19.2
NTAFIN0022	Finke	Enneapogon polyphyllus	Tussock Grass	NTA 000962	0.118	51.6	15
SATFLB0005	Flinders lofty block	Eucalyptus flindersii	Tree Mallee	SAT 000286	0.196	52	18.8
SATFLB0008	Flinders lofty block	Cassinia laevis	Shrub	SAT 000419	0.105	50.2	23.7
SATFLB0010	Flinders lofty block	Rhagodia paradoxa	Chenopod	SAT 000552	0.13	51.3	10.1
SATFLB0012	Flinders lofty block	Hibbertia crinita	Shrub	SAT 000657	0.112	51.2	15.5
SATFLB0014	Flinders lofty block	Xanthorrhoea quadrangulata	Shrub	SAT 000791	0.208	51.6	18.5
SATFLB0015	Flinders lofty block	Lepidosperma semiteres	Sedge	SAT 000860	0.123	51.4	8.5
SATKAN0001	Kanmantoo	Lepidosperma semiteres	Sedge	SAT 000167	0.218	50.5	11.3
SATKAN0002	Kanmantoo	Lepidosperma semiteres	Sedge	SAT 000218	0.16	50.1	9.2
SAASTP0001	Stony plains	Eragrostis setifolia	Tussock Grass	SAA 000294	0.136	50.6	12.8
SAASTP0004	Stony plains	Rutidosis helichrysoides subsp. Helichrysoides	Forb	SAA 000016	0.017	5.8	18.5
NTADAC0001	Darwin Coastal	Eucalyptus miniata		NTA 006042	0.144	51.1	

Site	Bioregion	Dominant Spp 3	Growth form	Genetic Voucher No	Amount subsampled from teabag (g)	Amount subsampled for sonication (mg)	% Cover
NTAGFU0001	Gulf fall and uplands	<i>Eucalyptus pruinosa</i>	Tree Mallee	NTA 001531	0.139	50.2	13.2
NTAGFU0008	Gulf fall and uplands	<i>Fimbristylis dochotoma</i>	Sedge	NTA 002018	0.118	51	14.4
NTAGFU0010	Gulf fall and uplands	N/A	N/A	N/A	N/A	N/A	N/A
NTAGFU0017	Gulf fall and uplands	<i>Schizachyrium fragile</i>	Tussock Grass	NTA 002681	0.124	50.3	7.7
NTAGFU0031	Gulf fall and uplands	<i>Petalostigma banksii</i>	Shrub	NTA 003613	0.147	50.2	9.2
NTAGFU0040	Gulf fall and uplands	<i>Eucalyptus tectifica</i>	Tree/Palm	NTA 003965	0.137	49.9	9.7
NTABRT0004	Burt plain	<i>Triodia schinzii</i>	Hummock Grass	NTA 001317	0.17	52.6	7.4
NTAFIN0019	Finke	<i>Enchylaena tomentosa</i>	Tussock Grass	NTA 000761	0.014	8	2.4
NTAFIN0022	Finke	<i>Aristida contorta</i>	Tussock Grass	NTA 000960	0.106	50.6	7.7
SATFLB0005	Flinders lofty block	<i>Chrysocephalum semipapposum</i>	Forb	SAT 000287	0.09	50.1	13.2
SATFLB0008	Flinders lofty block	<i>Casuarina pauper</i>	Shrub	SAT 000401	0.165	50.4	12.6
SATFLB0010	Flinders lofty block	<i>Enchylaena tomentosa</i> var. <i>tomentosa</i>	Chenopod	SAT 000550	0.11	50.8	6.1
SATFLB0012	Flinders lofty block	<i>Eucalyptus fasciculosa</i>	Tree Mallee	SAT 000630	0.15	50.6	12.6
SATFLB0014	Flinders lofty block	<i>Allocasuarina verticillata</i>	Shrub	SAT 000775	0.123	50.5	14
SATFLB0015	Flinders lofty block	<i>Hibbertia crinita</i>	Shrub	SAT 000866	0.112	51.5	6.6
SATKAN0001	Kanmantoo	<i>Pultenaea involucreta</i>	Shrub	SAT 000124	0.181	50.9	10.3
SATKAN0002	Kanmantoo	<i>Hakea rostrata</i>	Shrub	SAT 000207	0.187	51	8.2
SAASTP0001	Stony plains	<i>Acacia aneura</i> var. <i>tenuis</i>	Shrub	SAA 000338	0.186	51.4	8.5
SAASTP0004	Stony plains	<i>Sida fubulifera</i>	Forb	SAA 000022	0.049	29.8	11.7
NTADAC0001	Darwin Coastal	<i>Sorghum plumosum</i>		NTA 005954	0.118	49.9	

Sample displays some fungal growth in scintillation vial after grinding

Data not on S2S - no information available about % cover or growth form available

**Table 2: Amount of soil weighed out for extraction of lipids in the ASE 350**

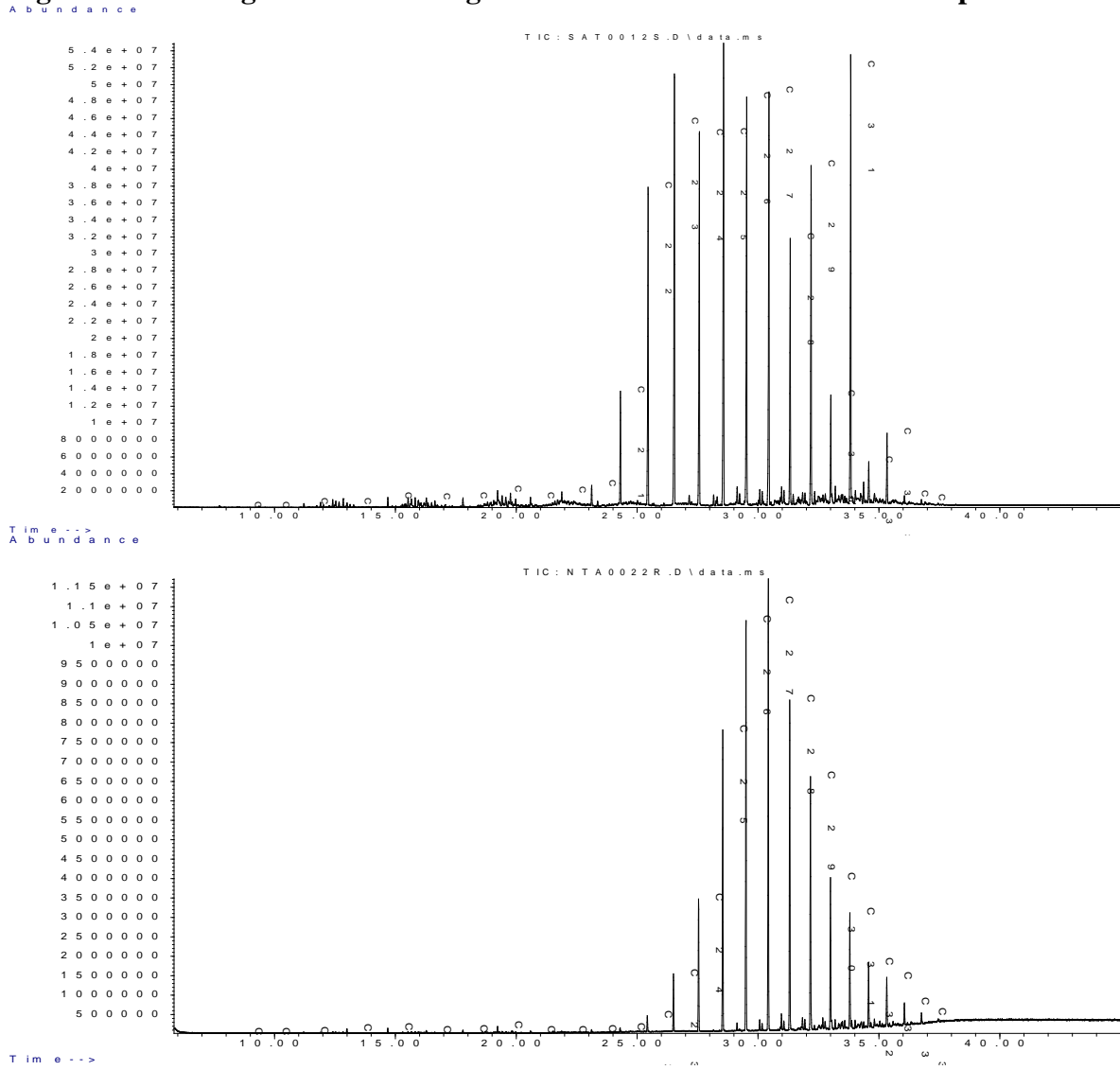
Site	Bioregion	Amount subsampled for ASE (g)
NTAGFU0001	Gulf fall and uplands	13.222
NTAGFU0008	Gulf fall and uplands	18.709
NTAGFU0010	Gulf fall and uplands	8.257
NTAGFU0017	Gulf fall and uplands	16.513
NTAGFU0031	Gulf fall and uplands	14.521
NTAGFU0040	Gulf fall and uplands	8.625
NTABRT0004	Burt plain	19.371
NTAFIN0019	Finke	16.781
NTAFIN0022	Finke	26.635
SATFLB0005	Flinders lofty block	15.934
SATFLB0008	Flinders lofty block	18.487
SATFLB0010	Flinders lofty block	12.185
SATFLB0012	Flinders lofty block	15.854
SATFLB0014	Flinders lofty block	12.559
SATFLB0015	Flinders lofty block	4.475
SATKAN0001	Kanmantoo	5.891
SATKAN0002	Kanmantoo	6.818
SAASTP0001	Stony plains	11.365
SAASTP0004	Stony plains	21.287
NTADAC0001	Darwin Coastal	9.976



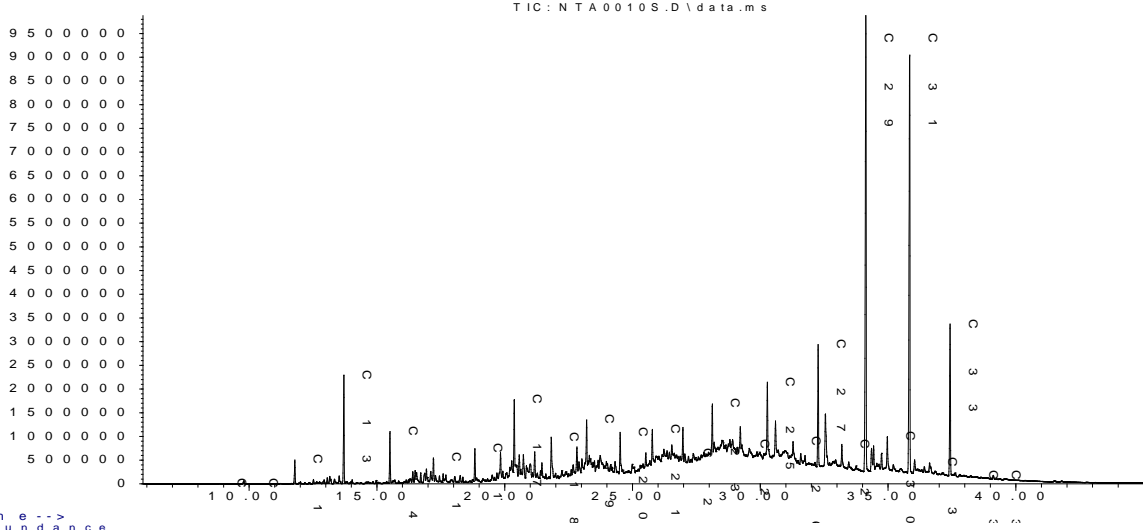
## APPENDIX B: ADDITIONAL DATA

### Appendix B – Additional Data

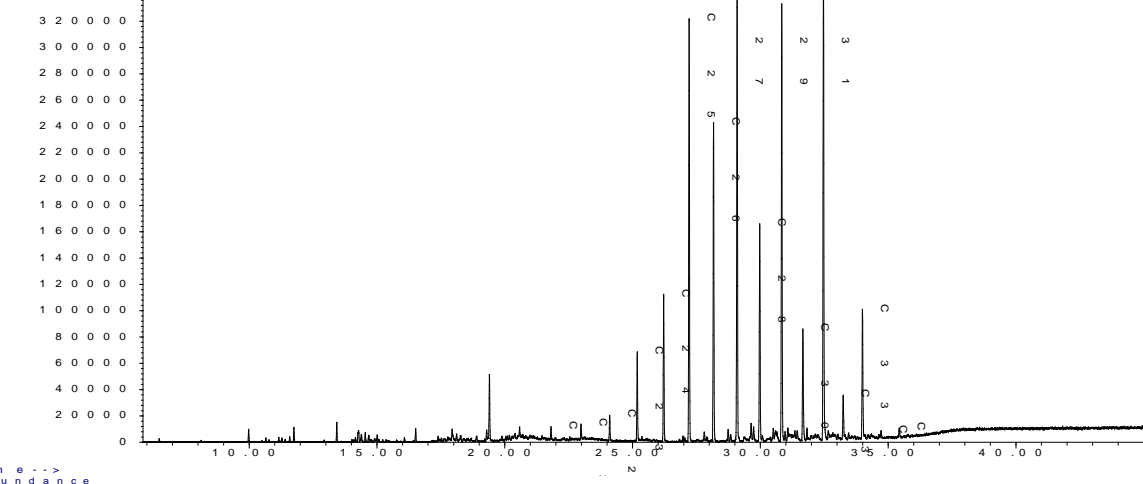
**Figure 1: Below figures – chromatograms for GCMS results for soils and plants**



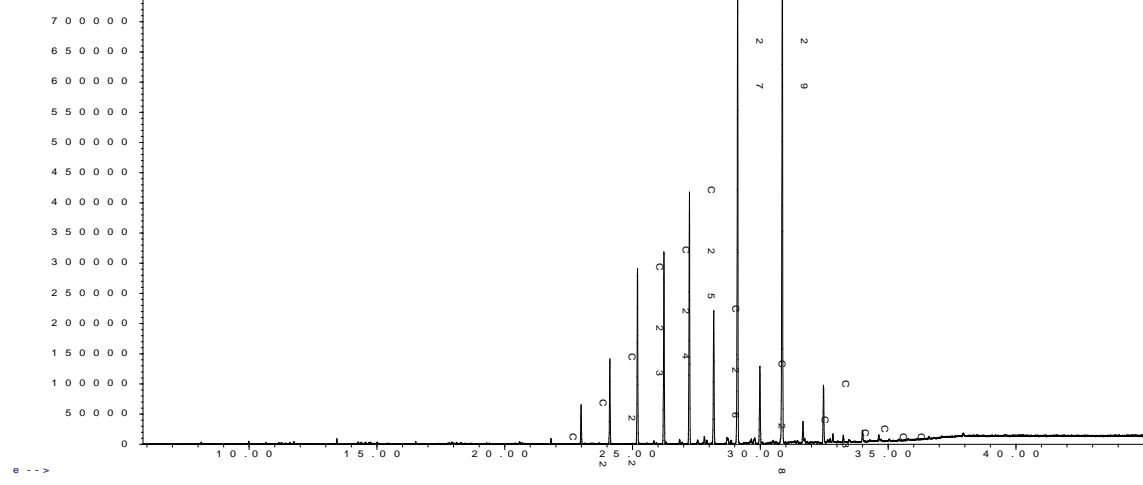
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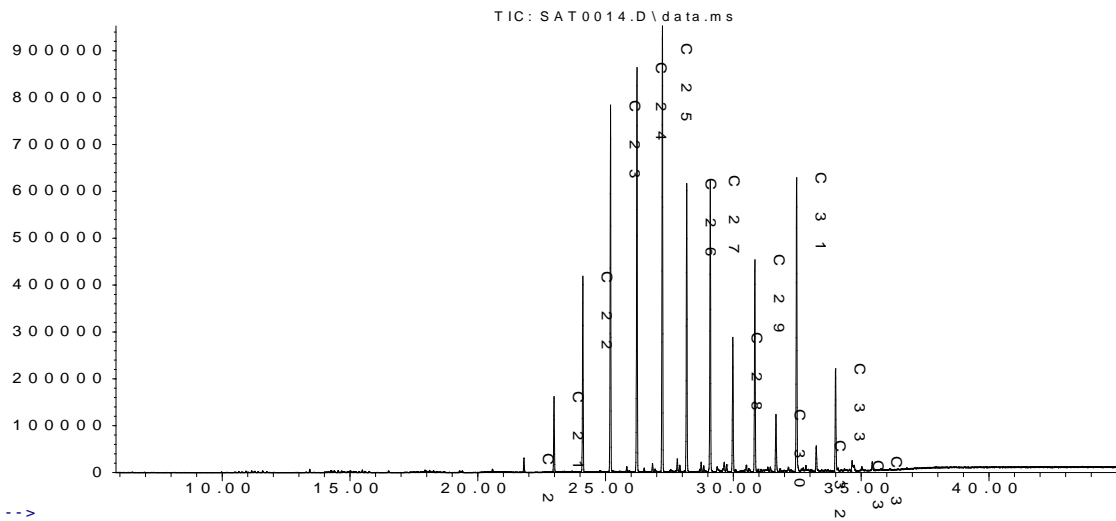
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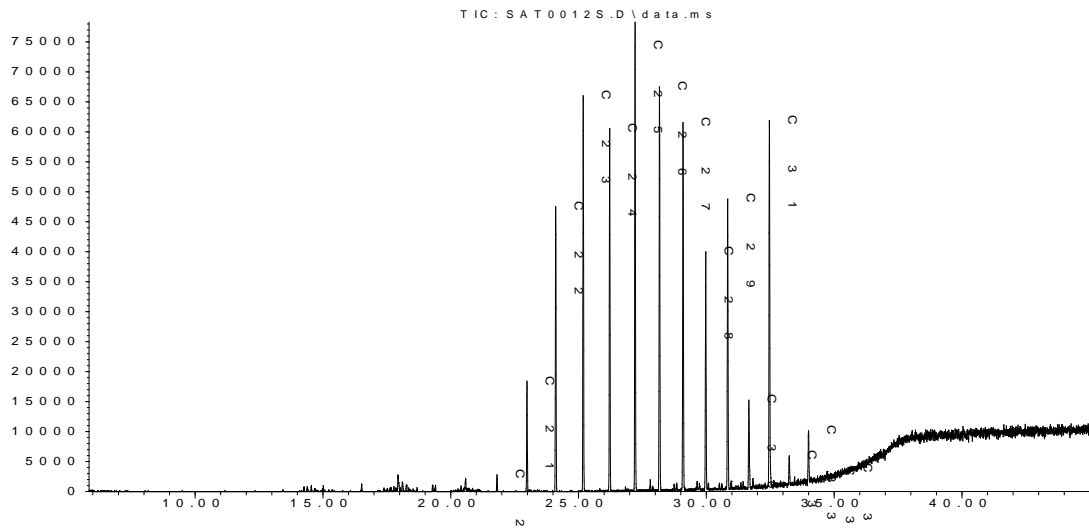
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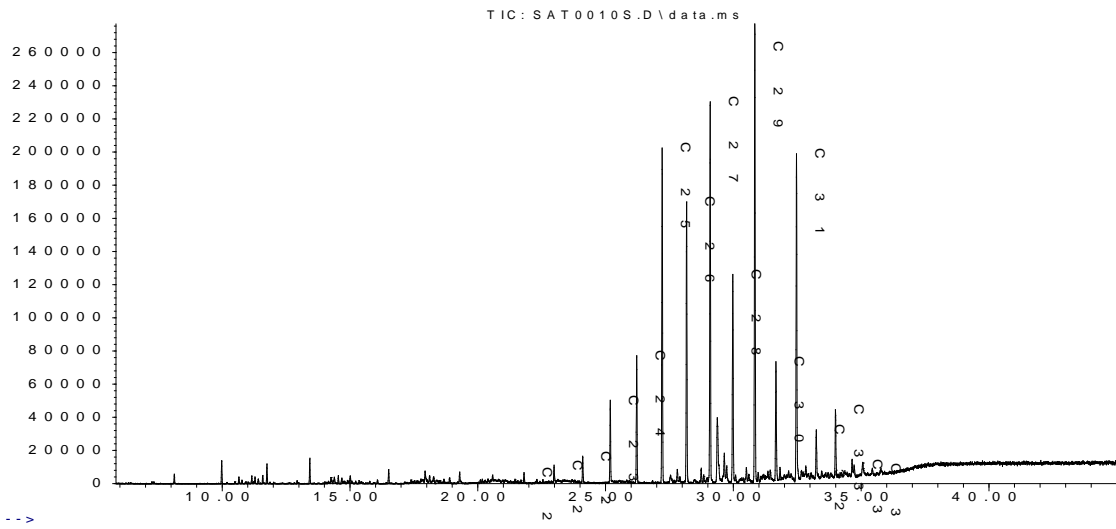
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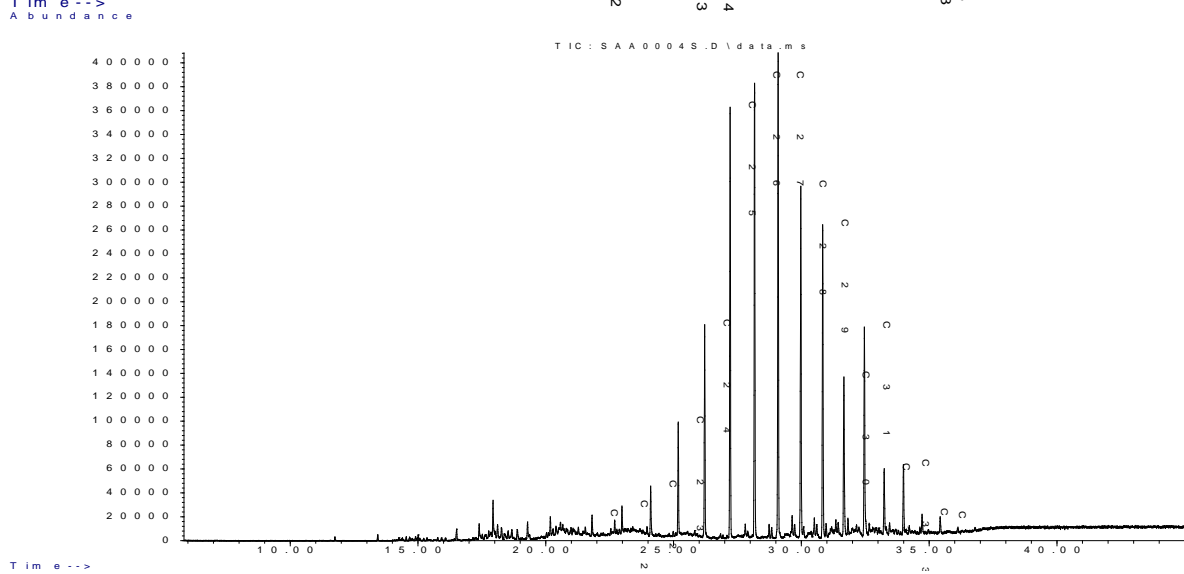
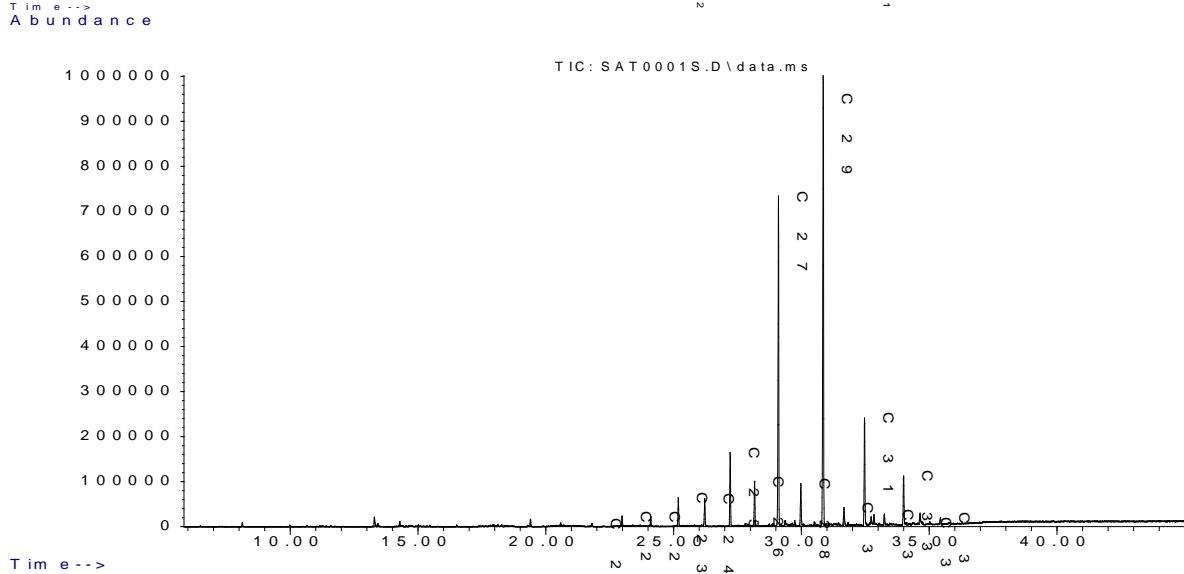
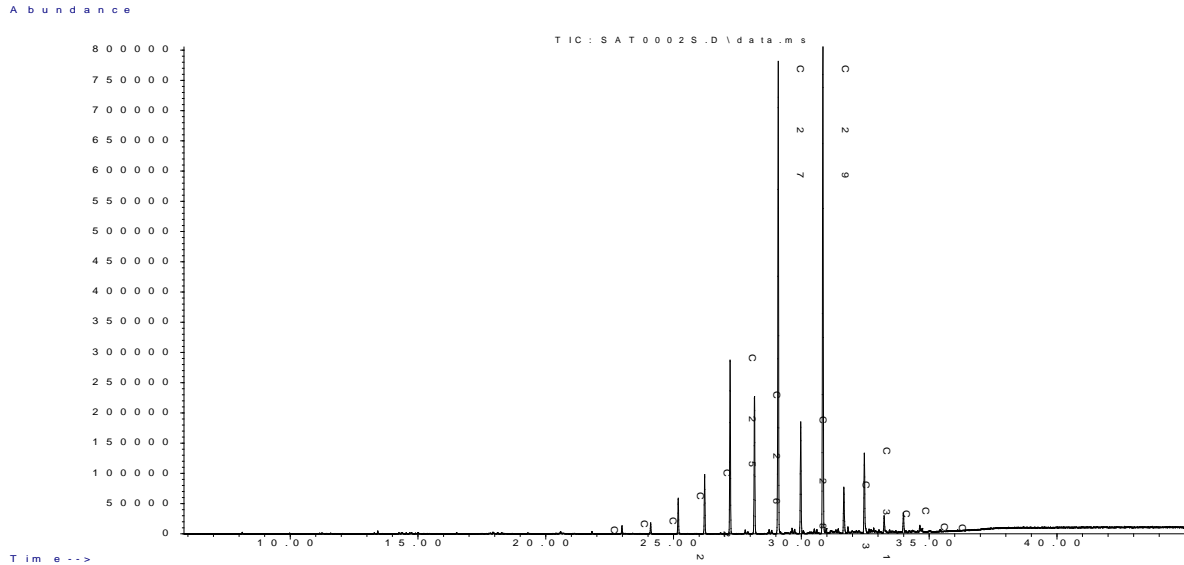
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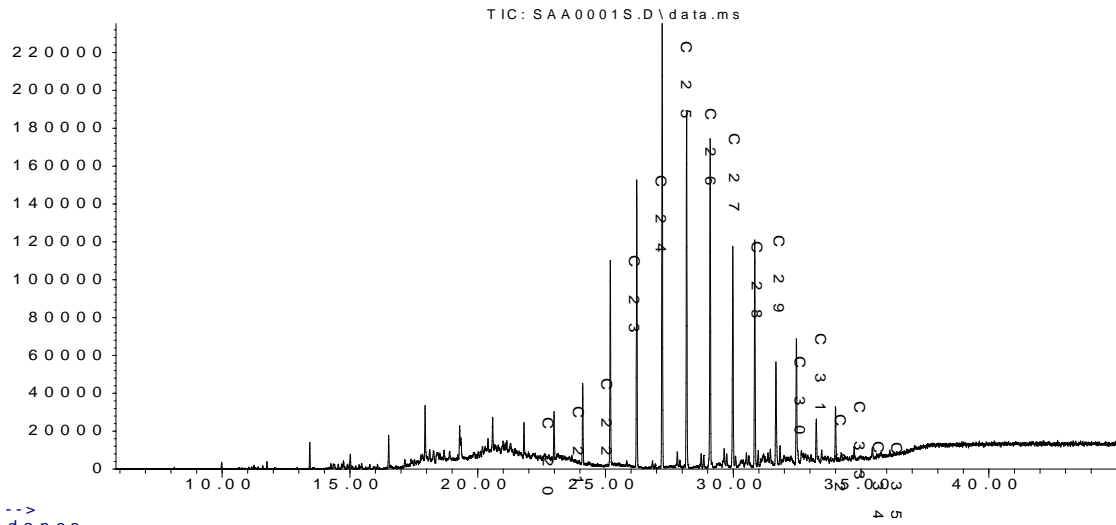
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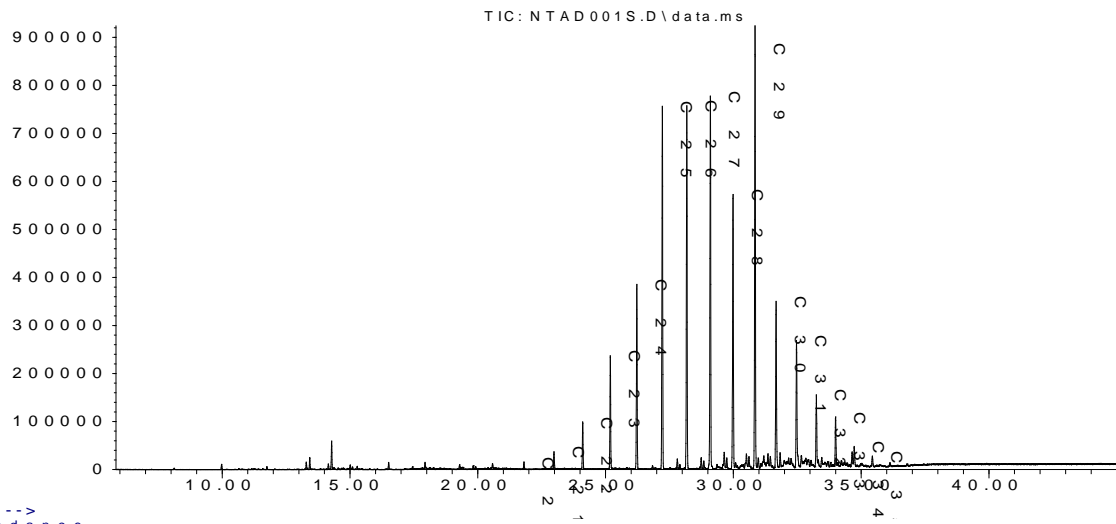
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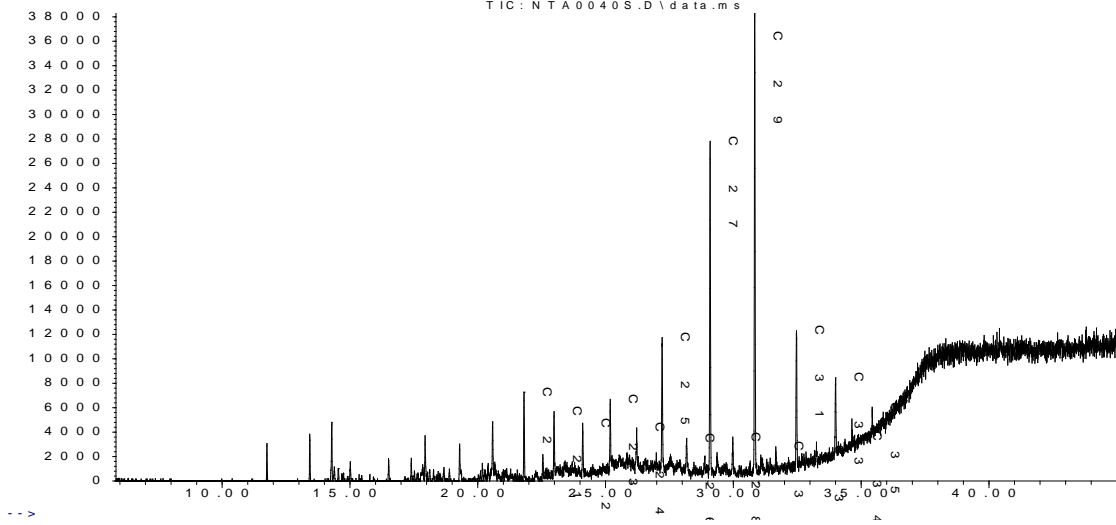
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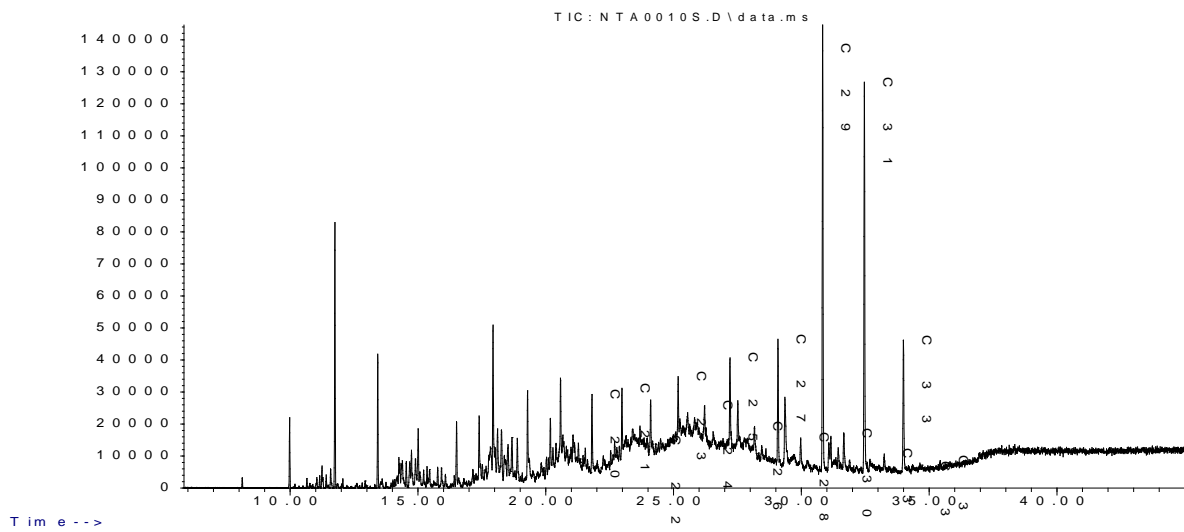
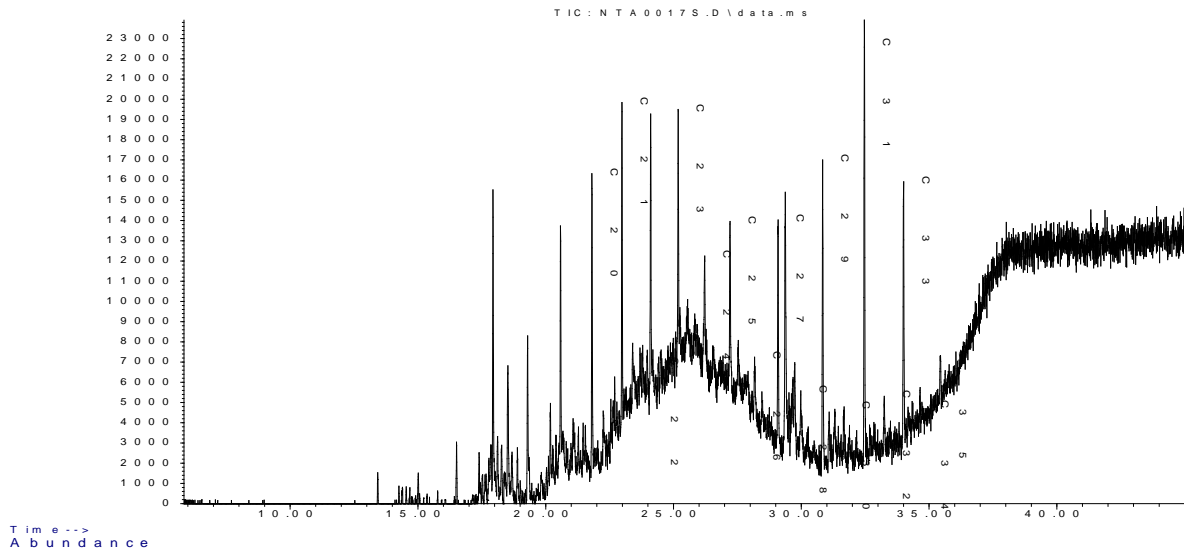
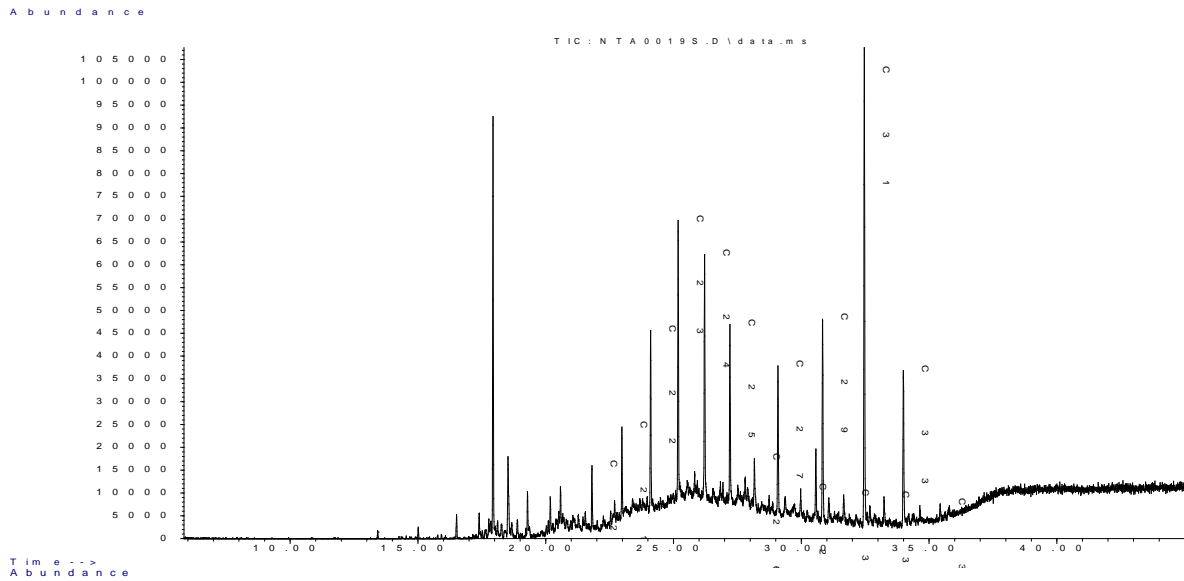
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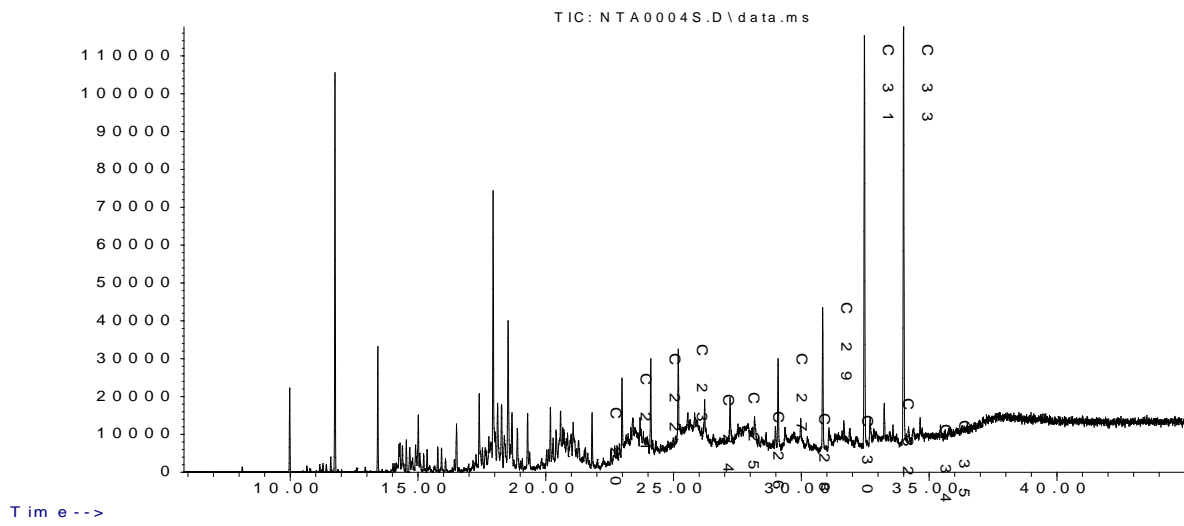
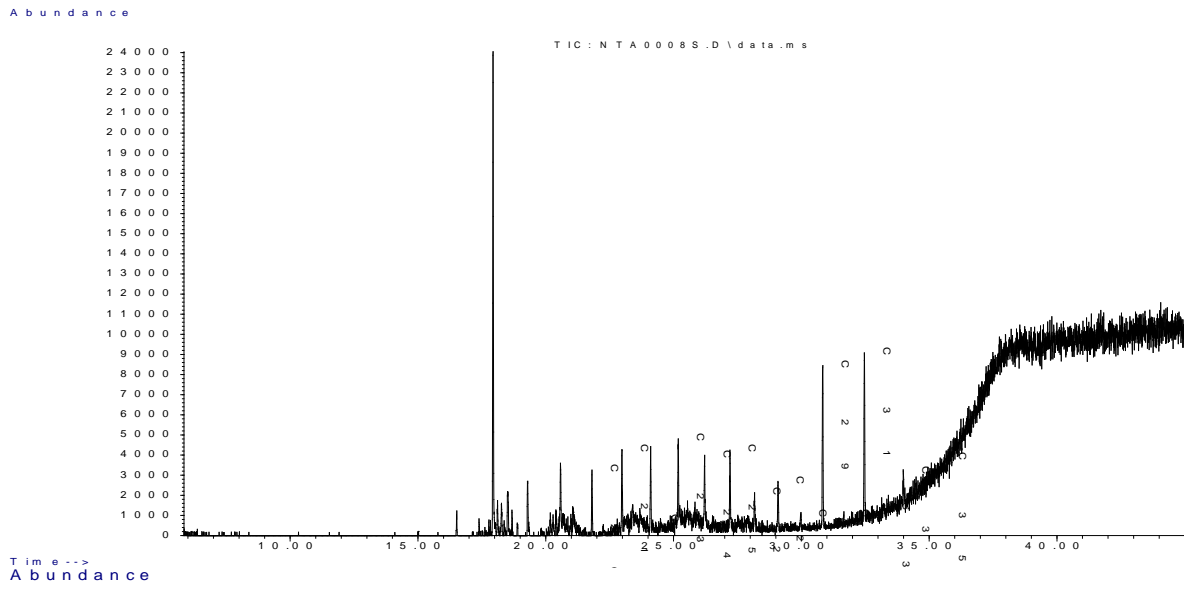


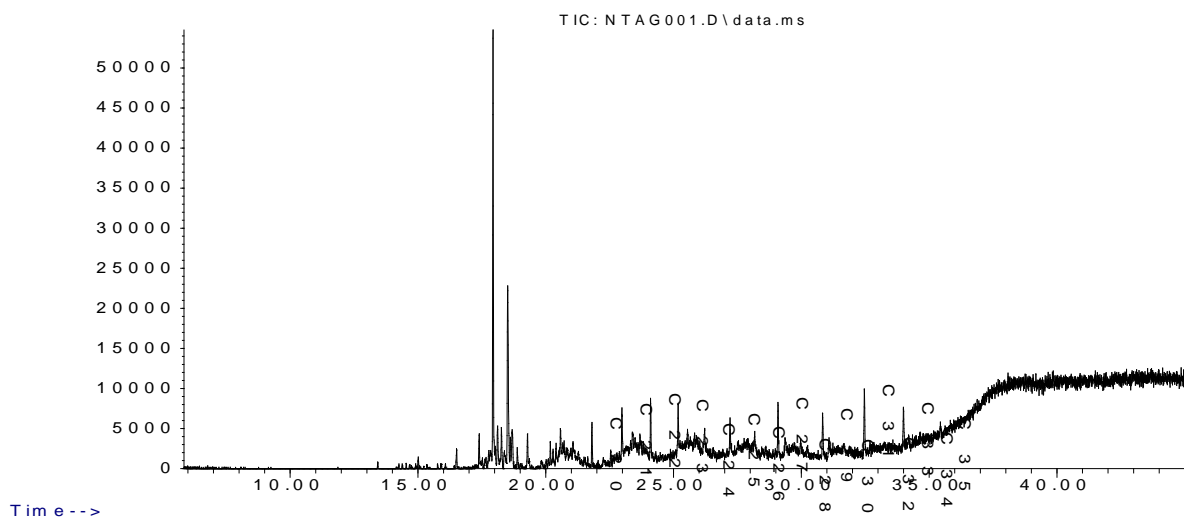
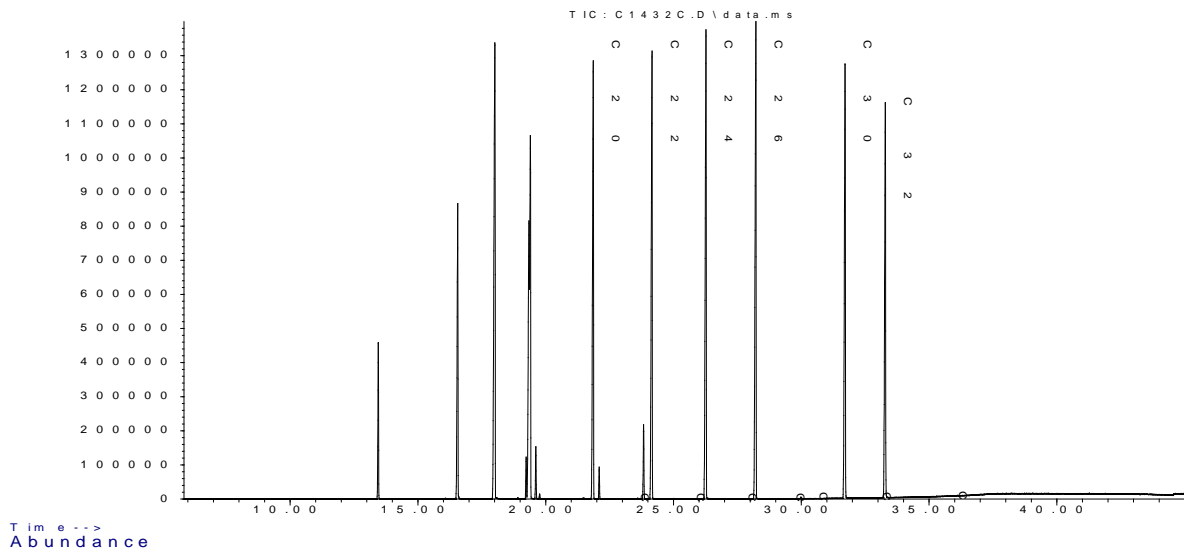
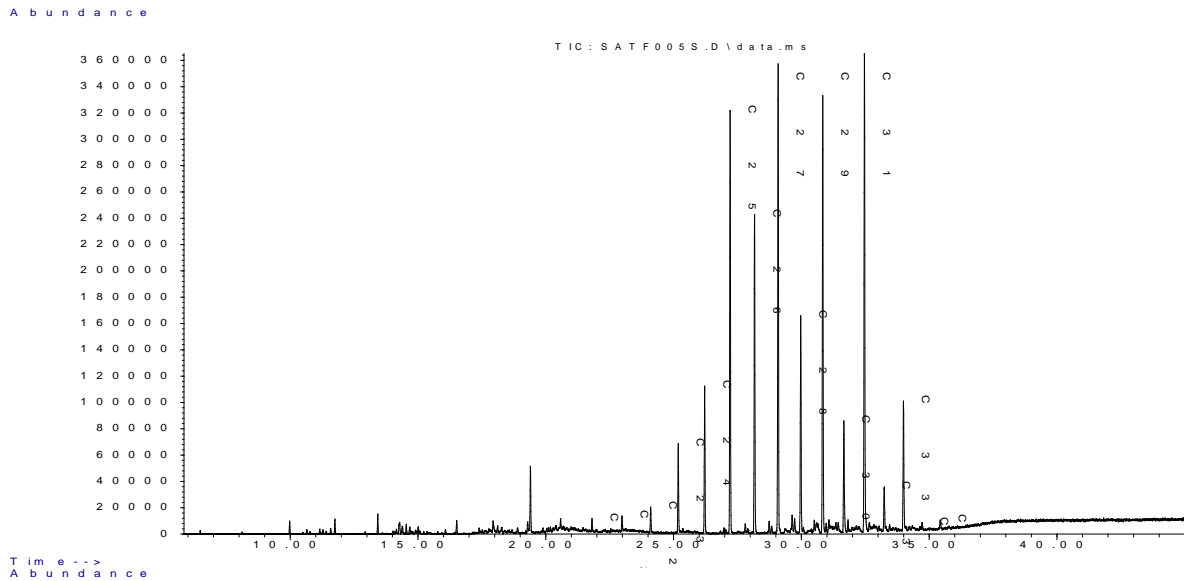
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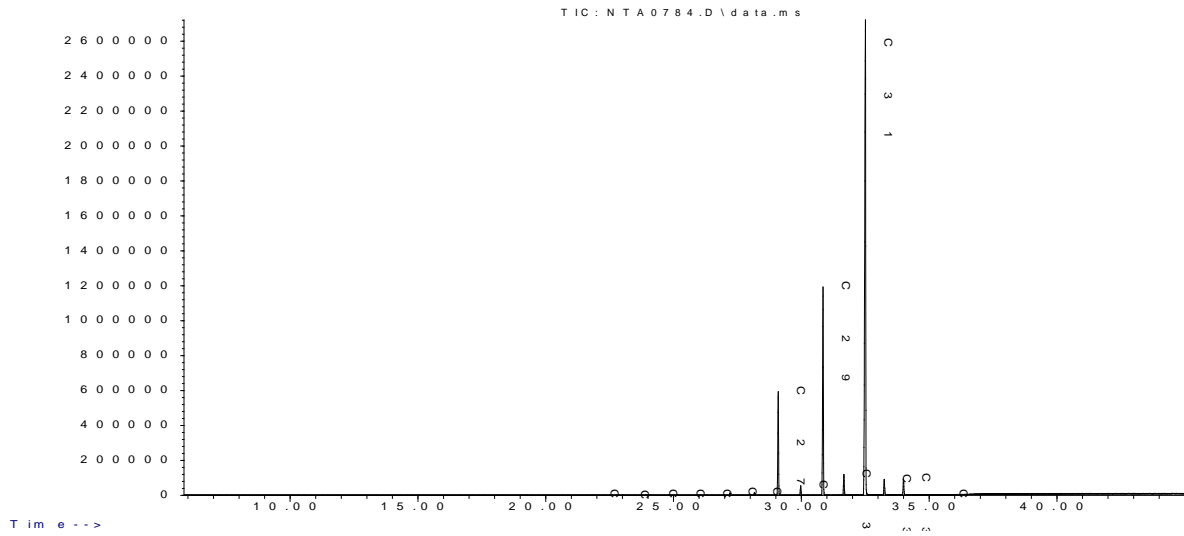
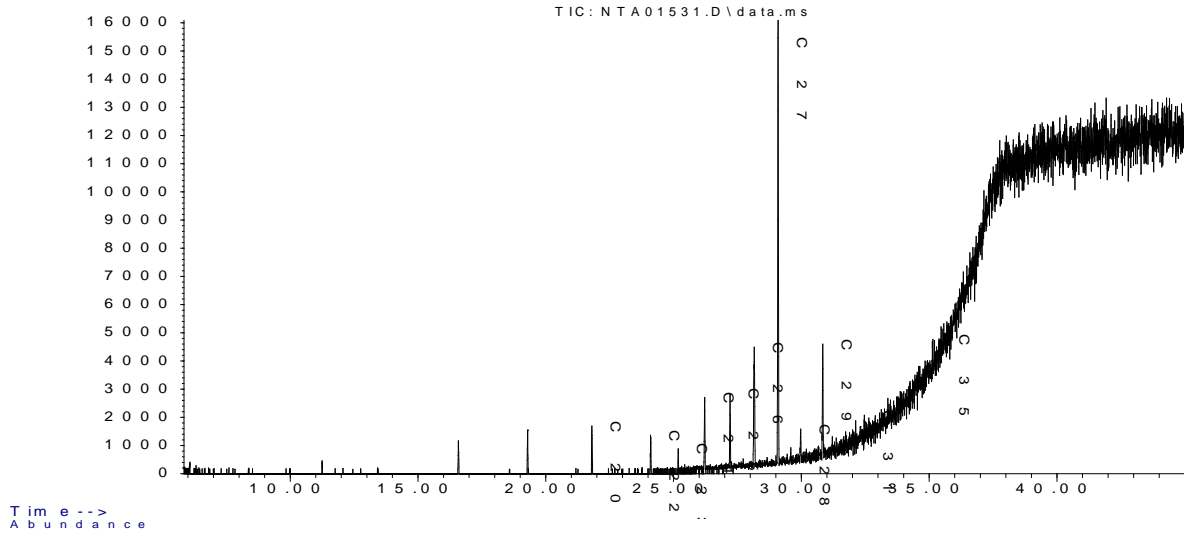
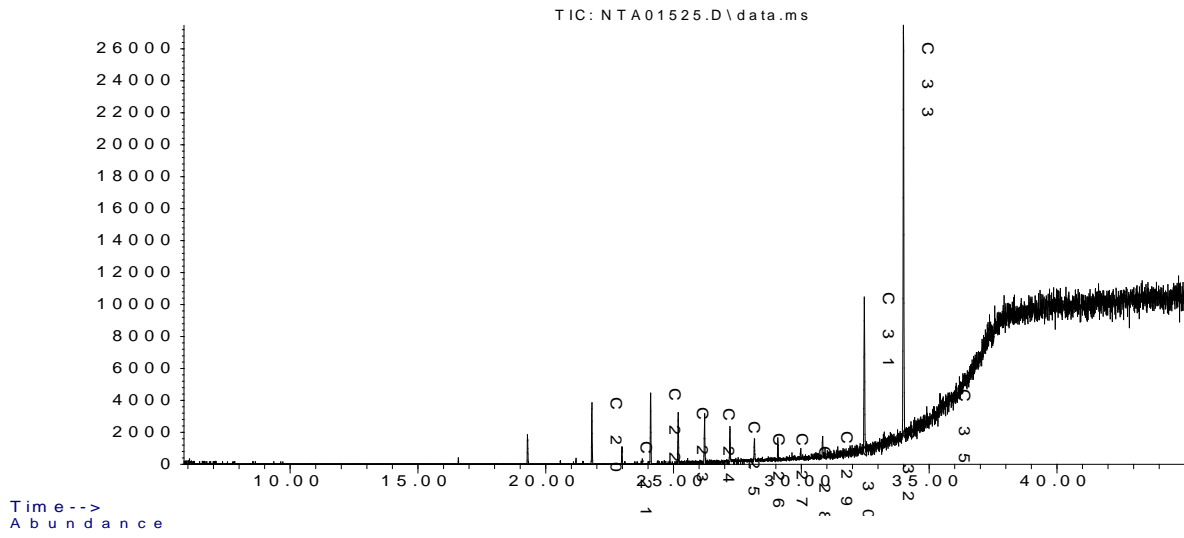


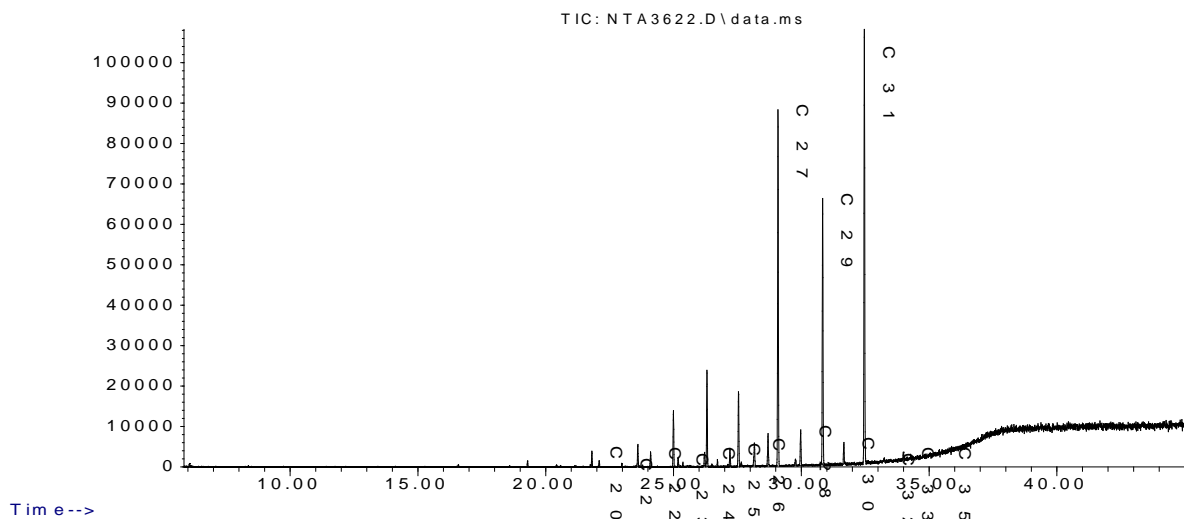
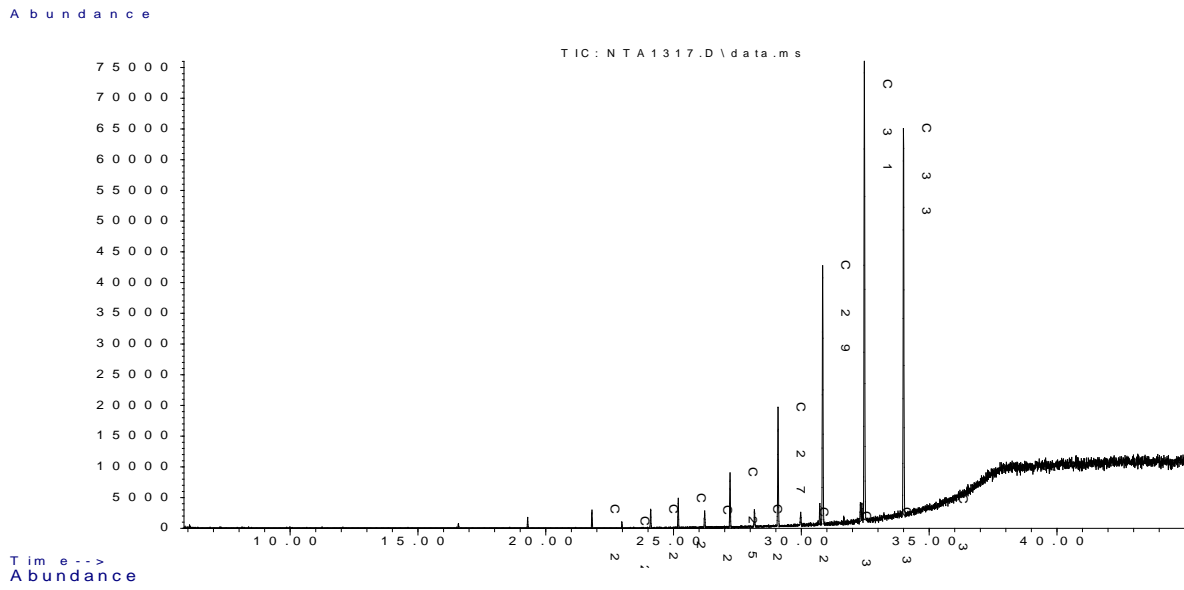


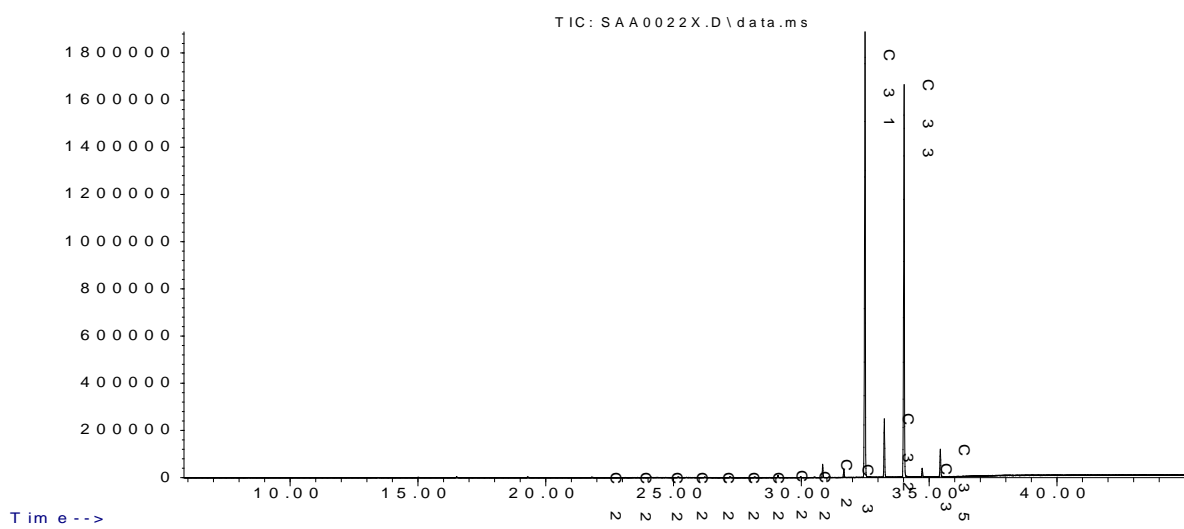
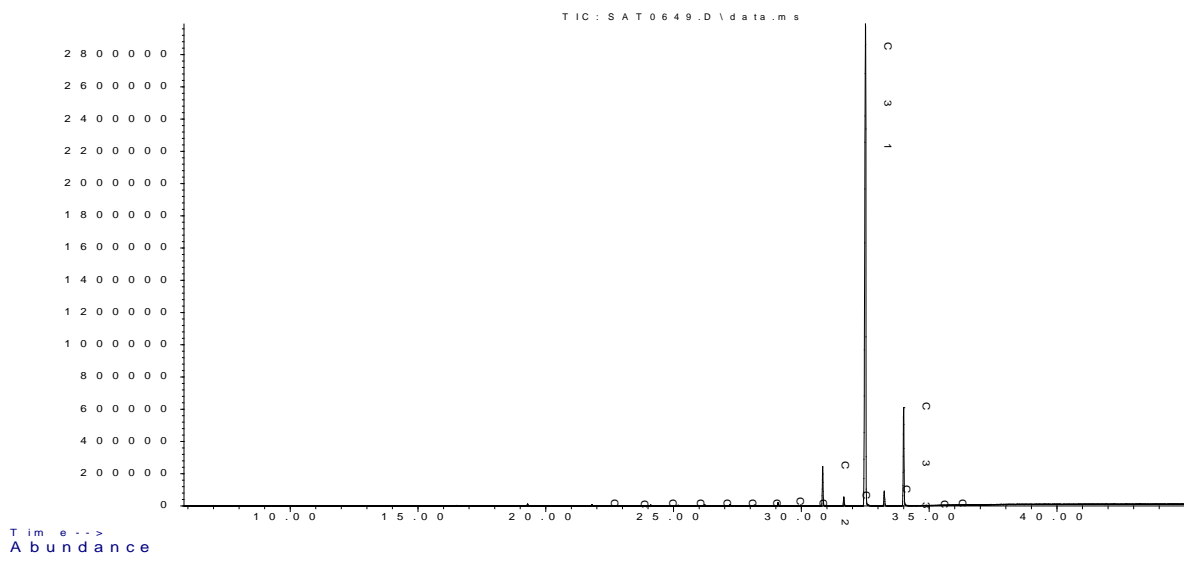
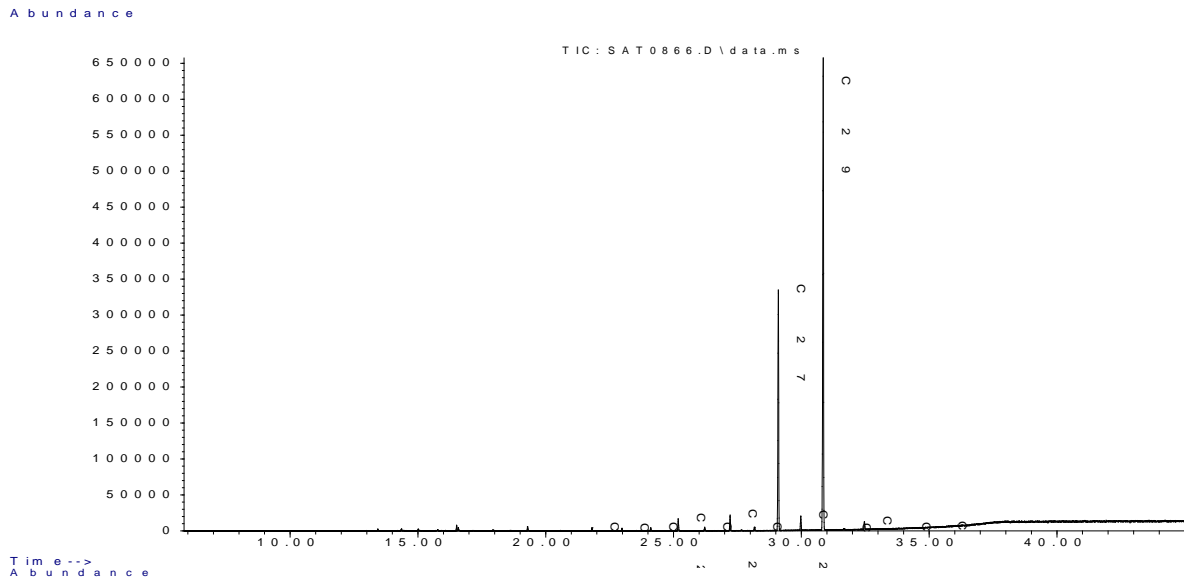


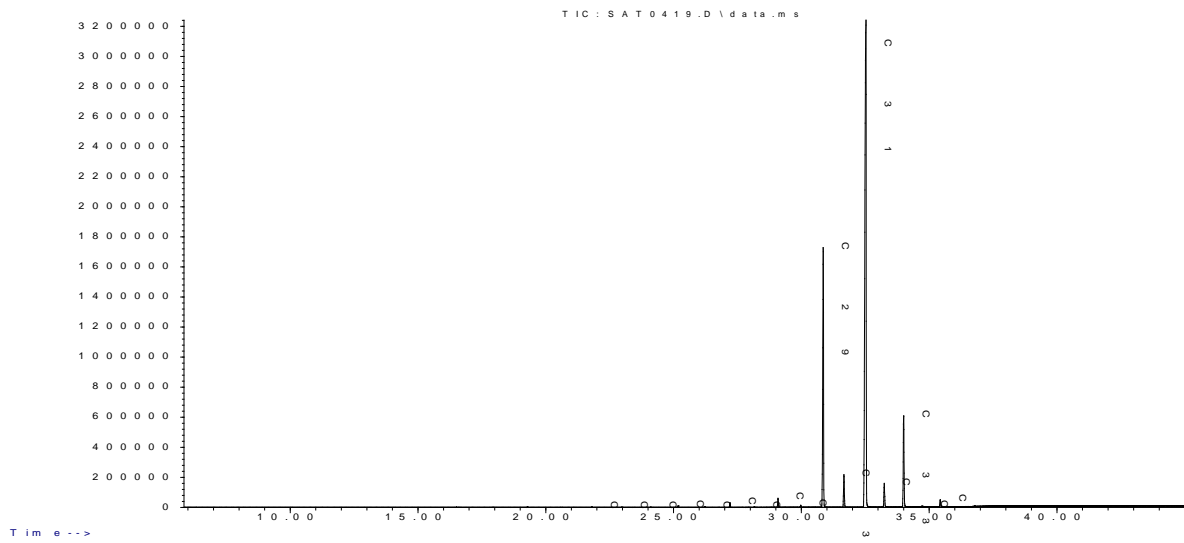
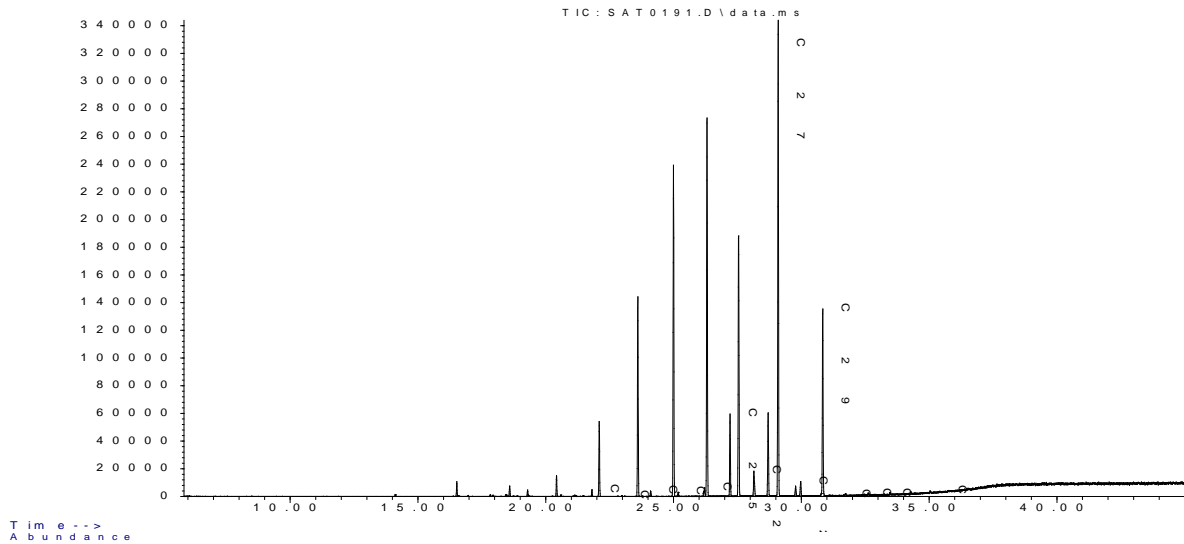
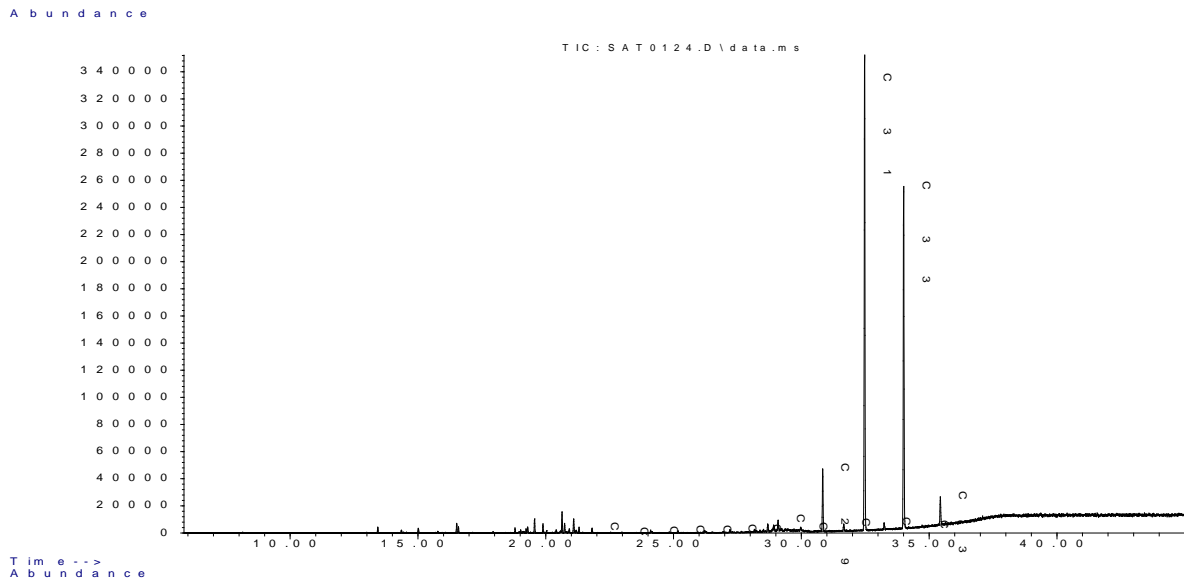


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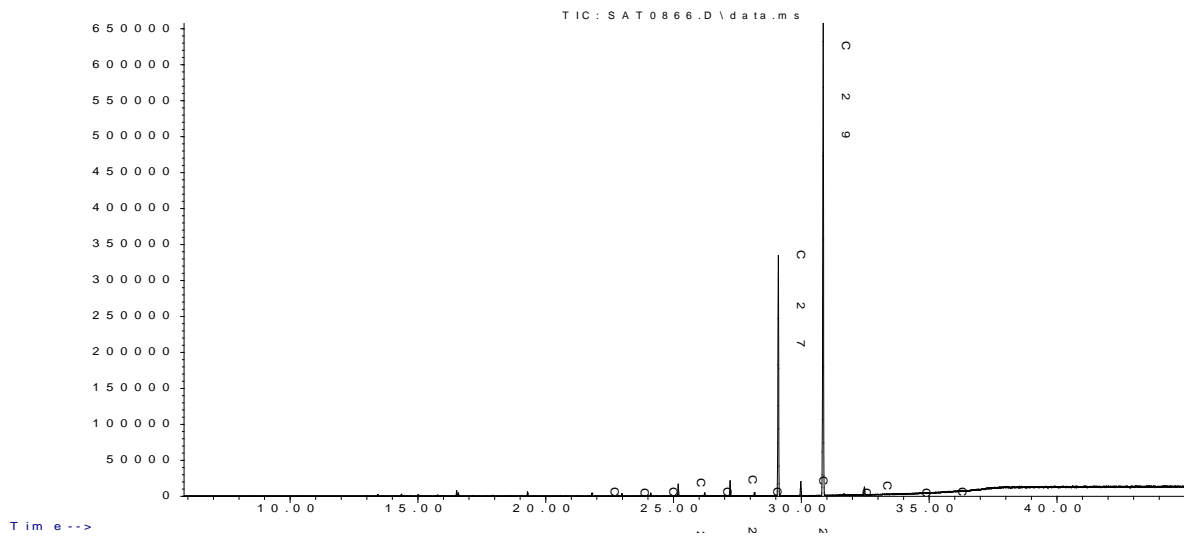
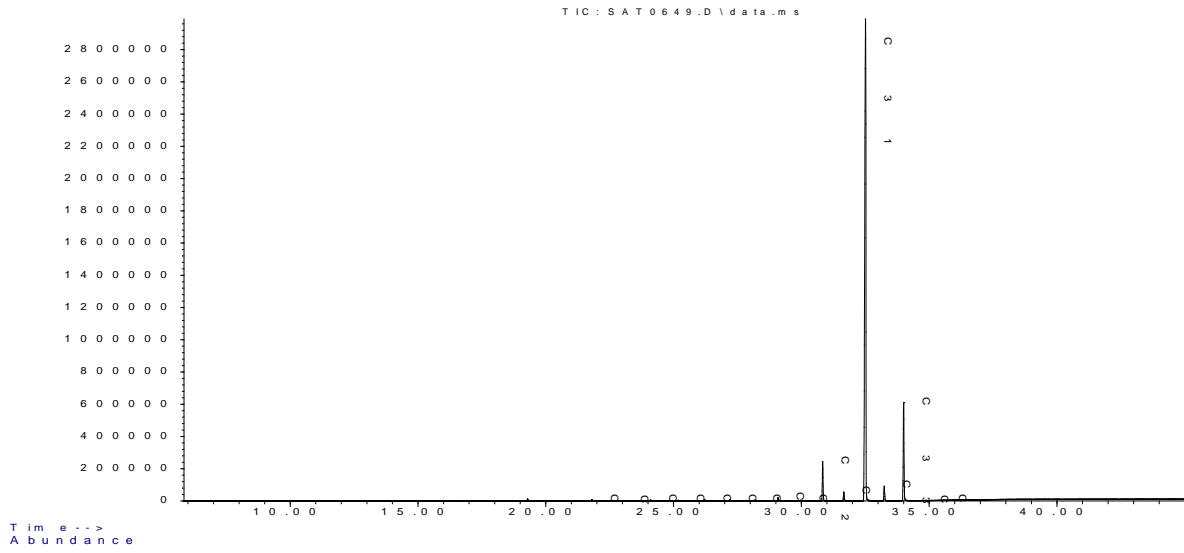
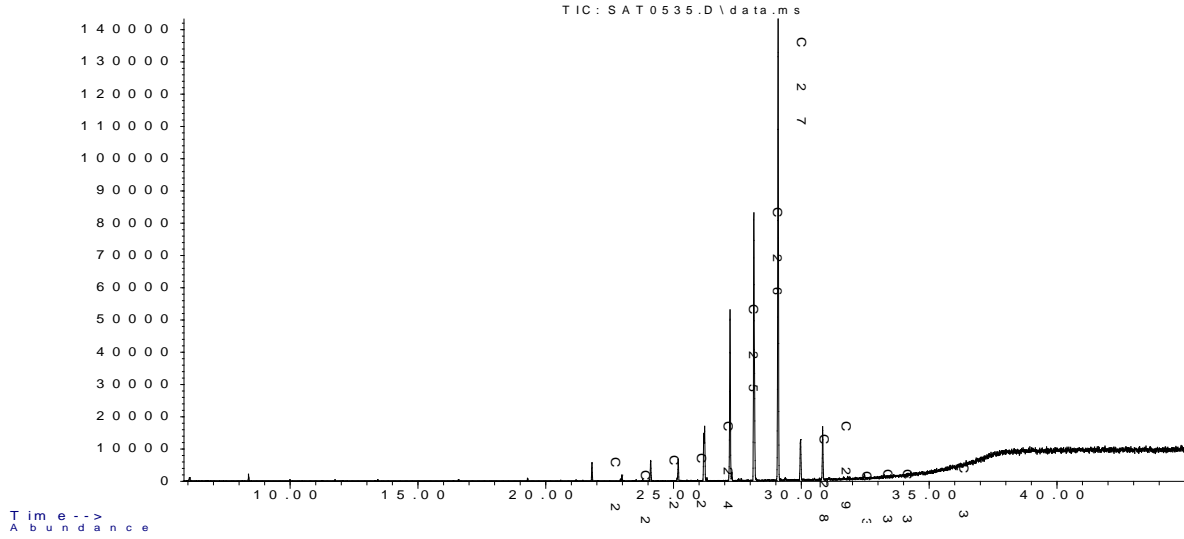






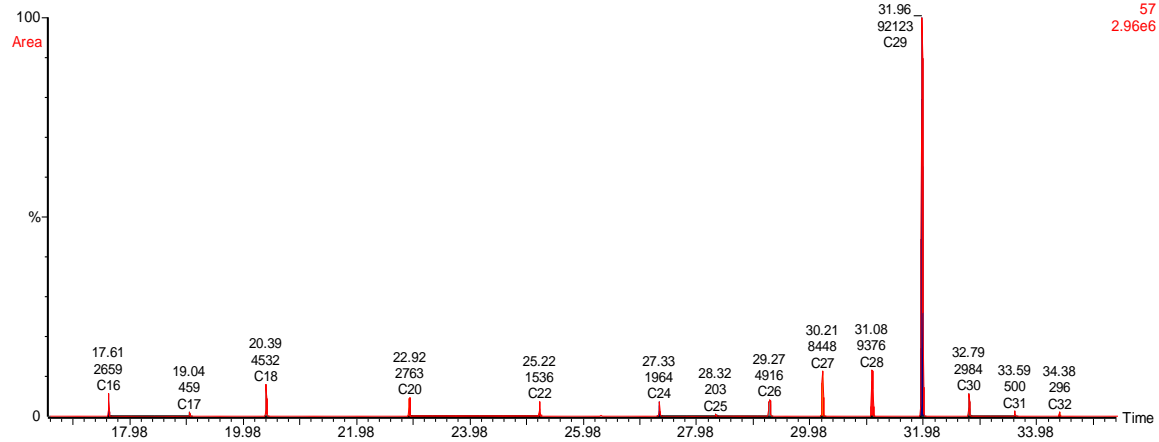


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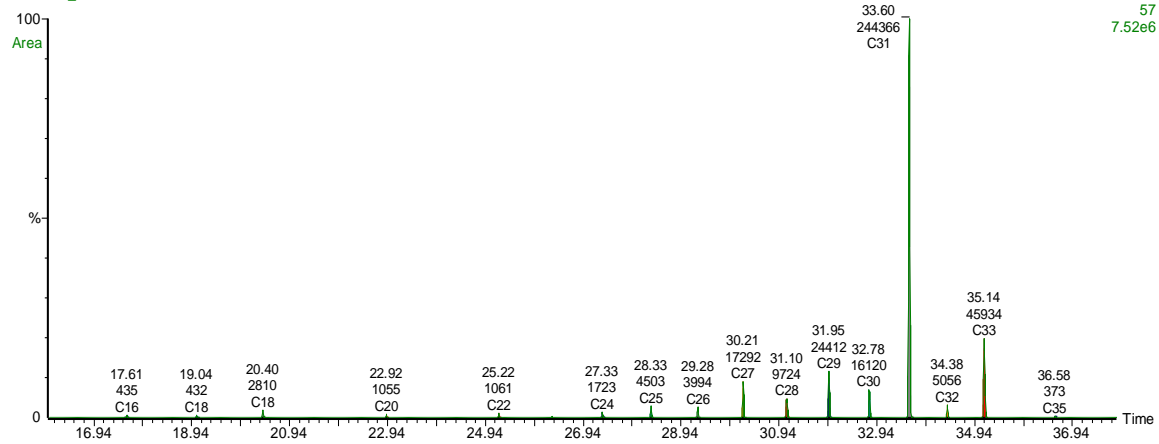
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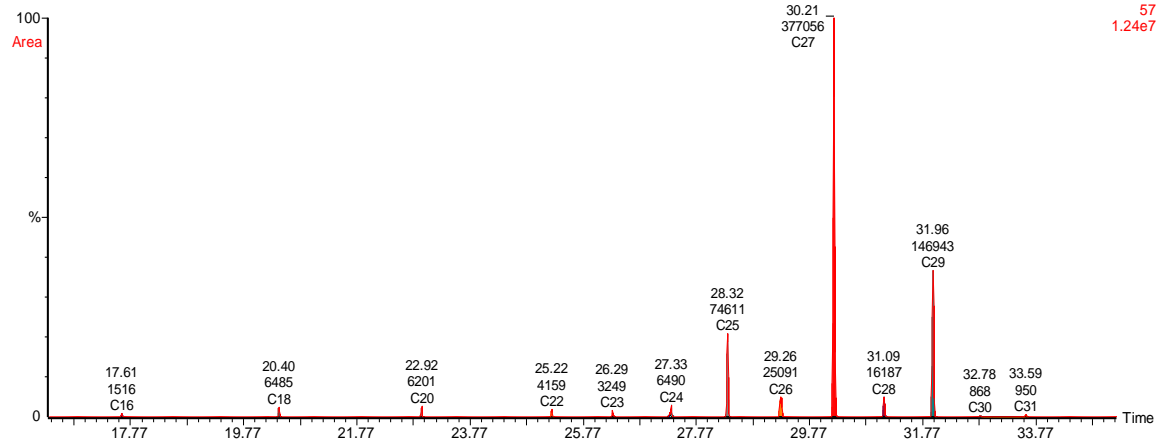
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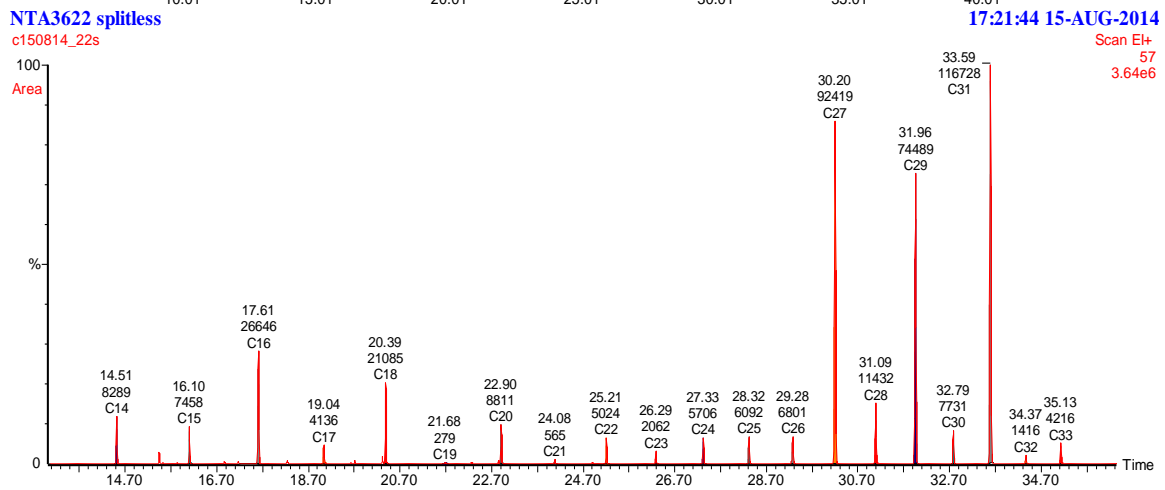
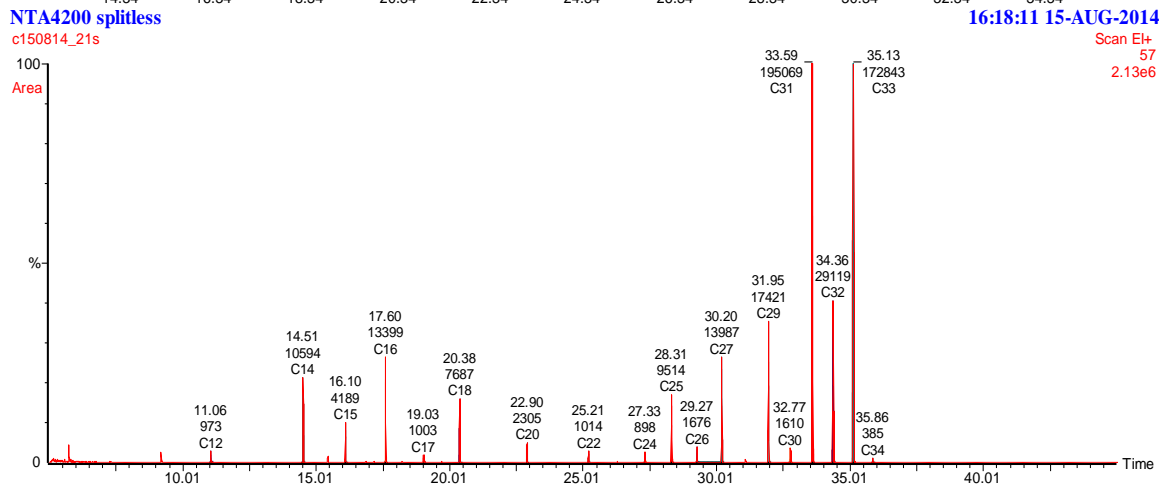
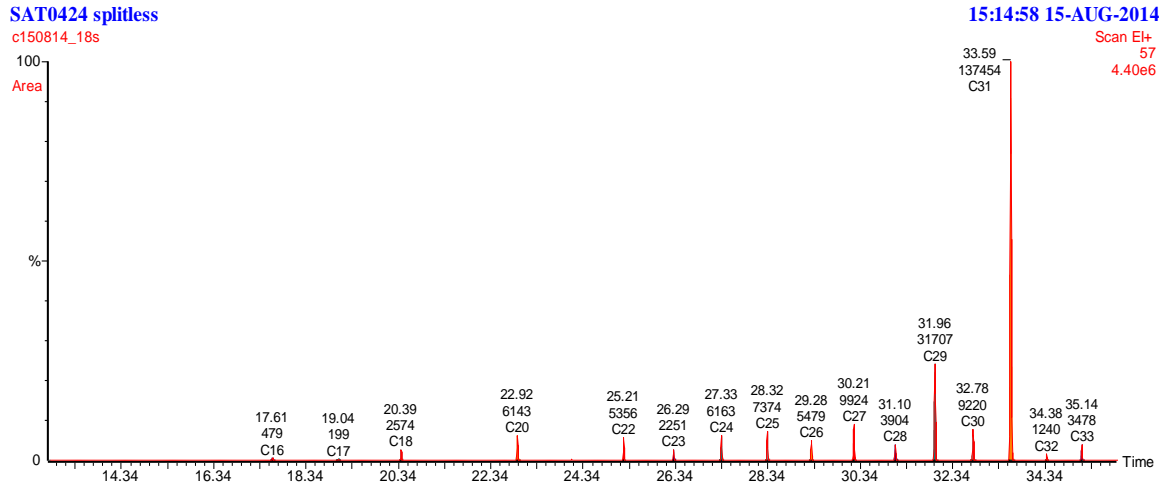
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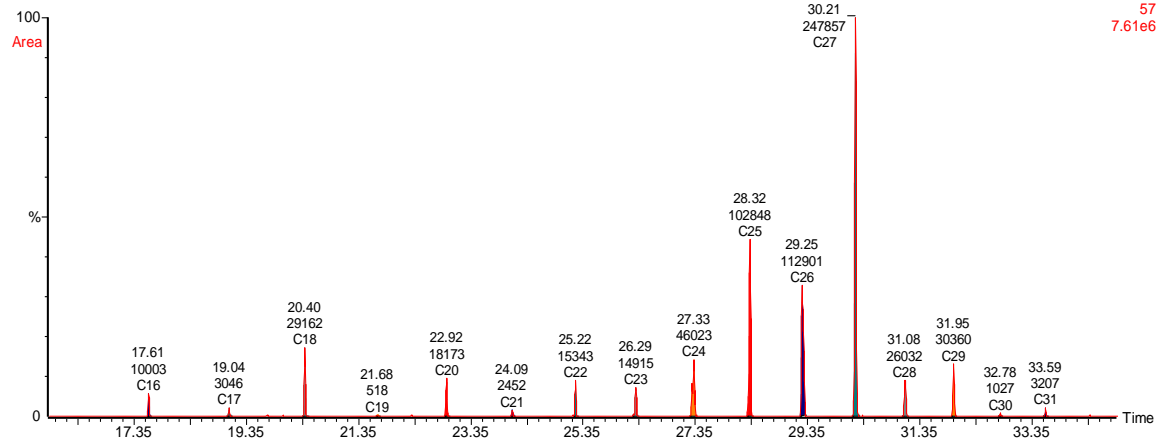
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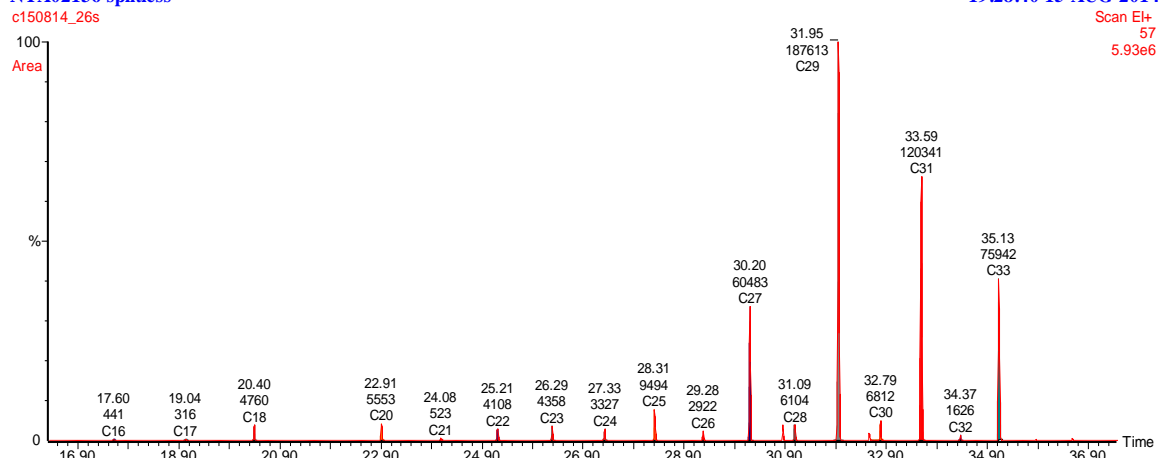
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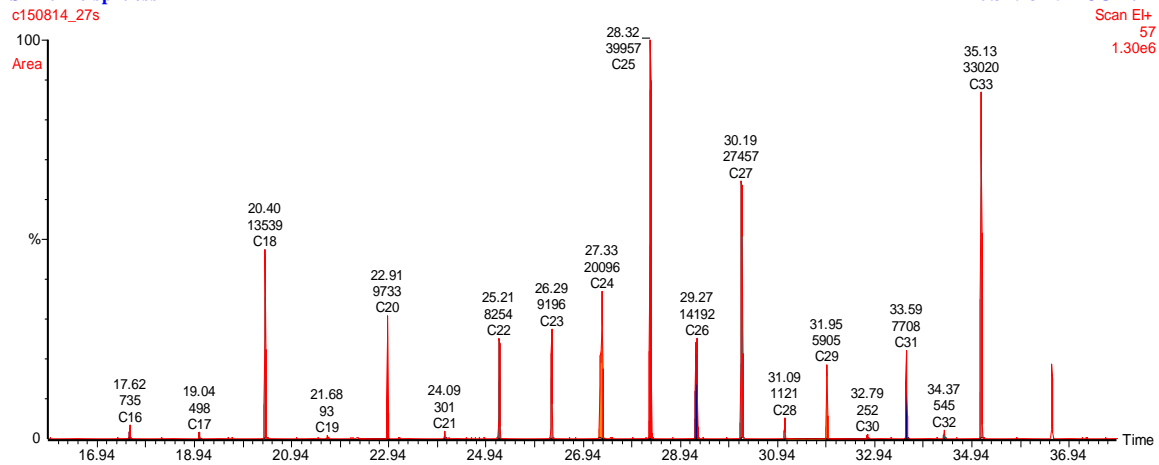
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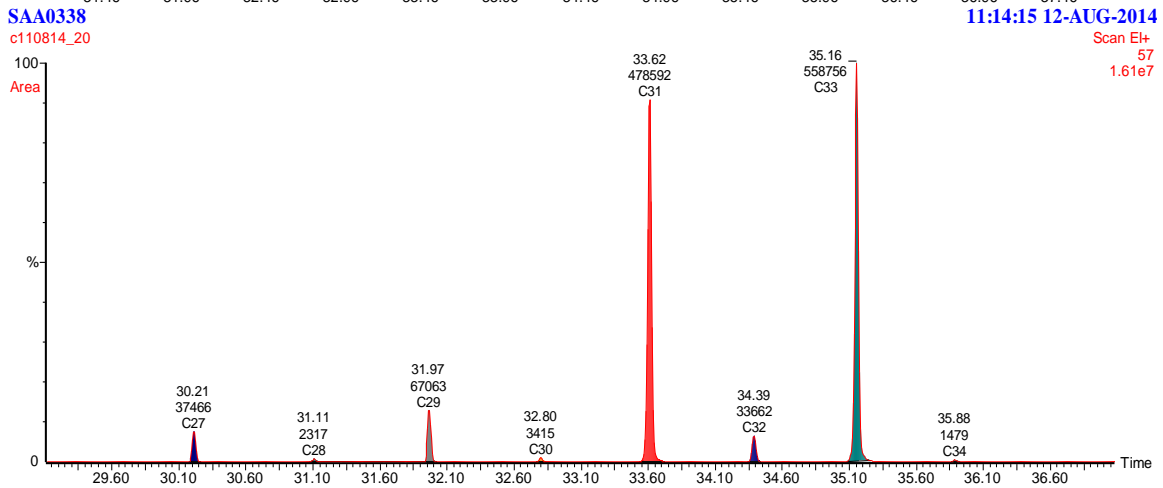
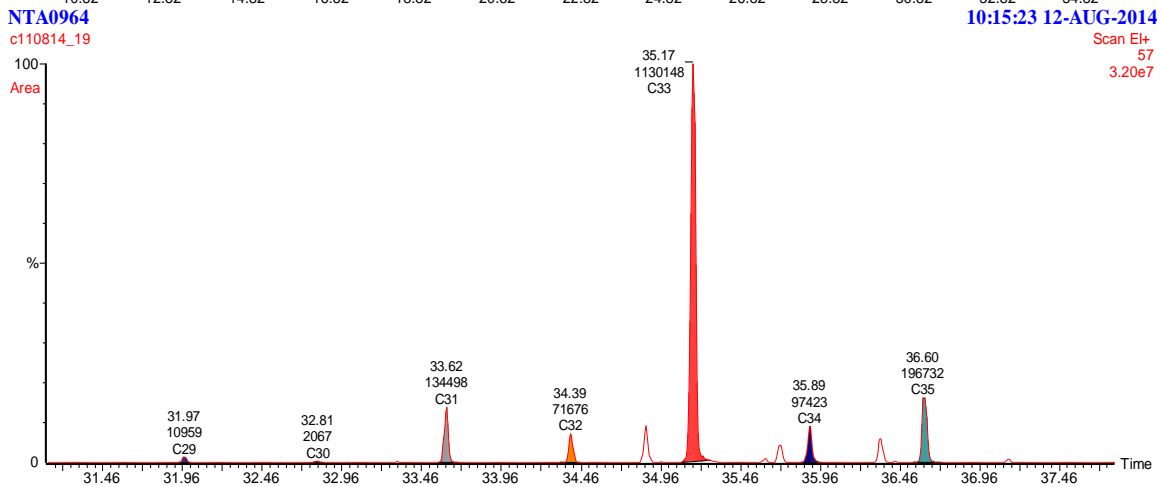
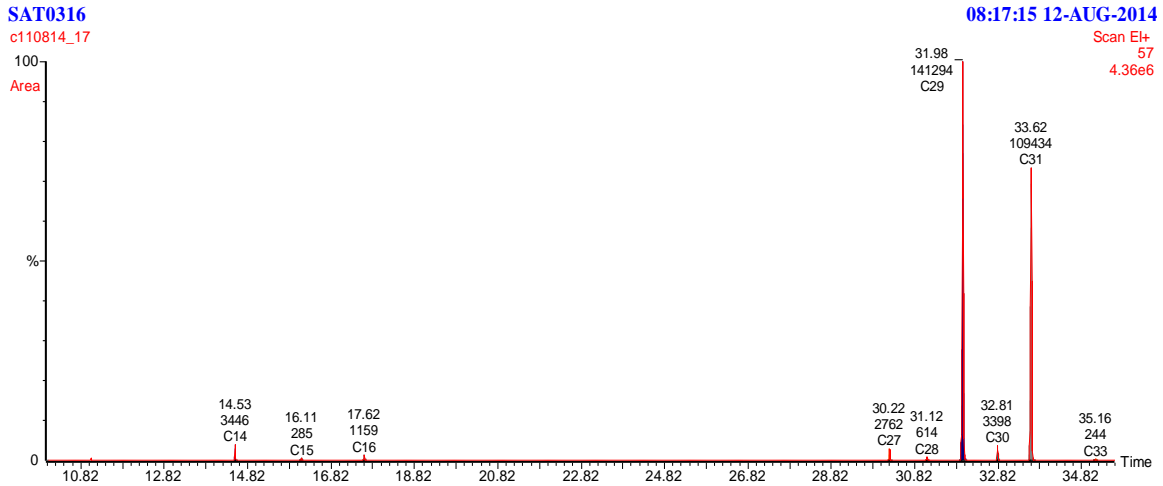


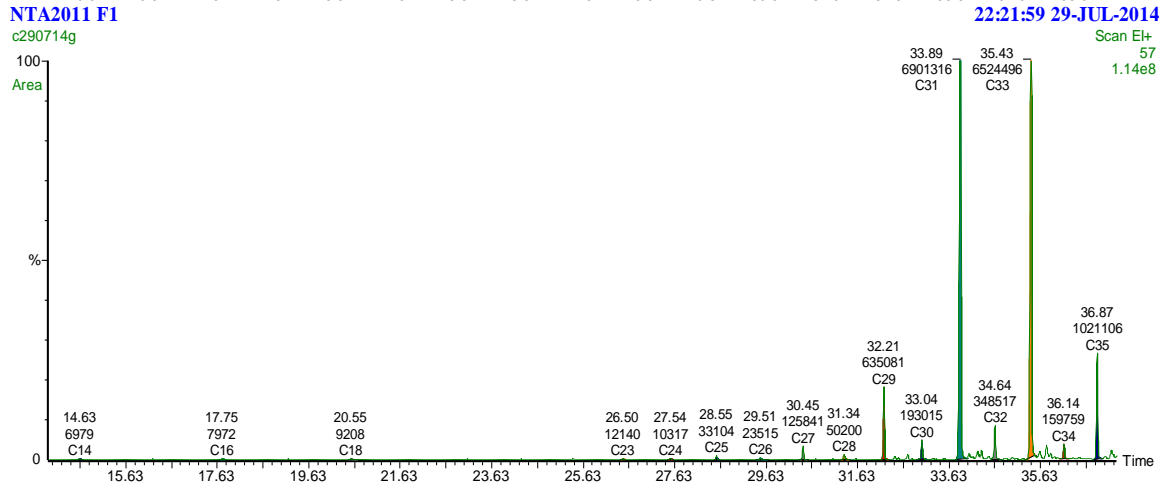
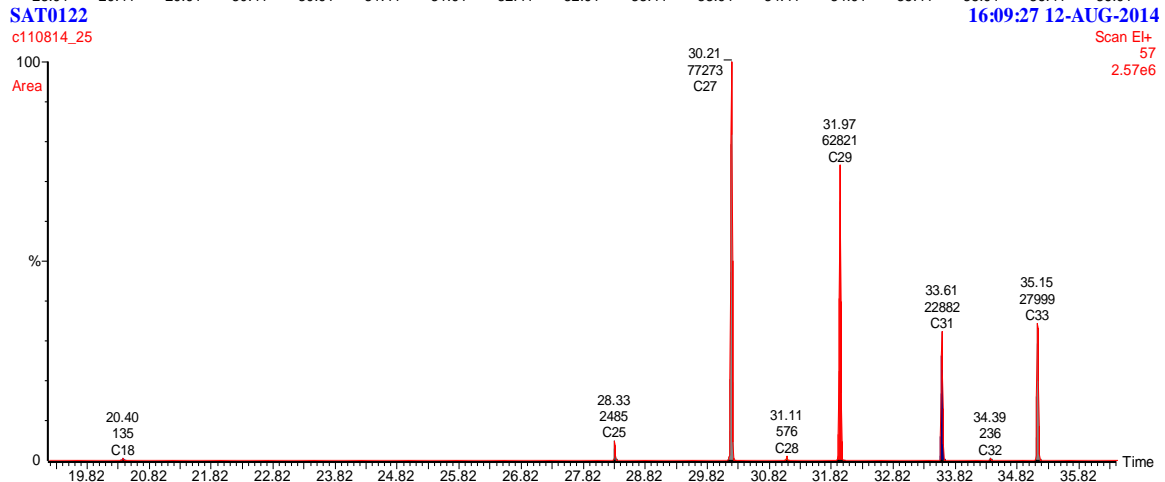
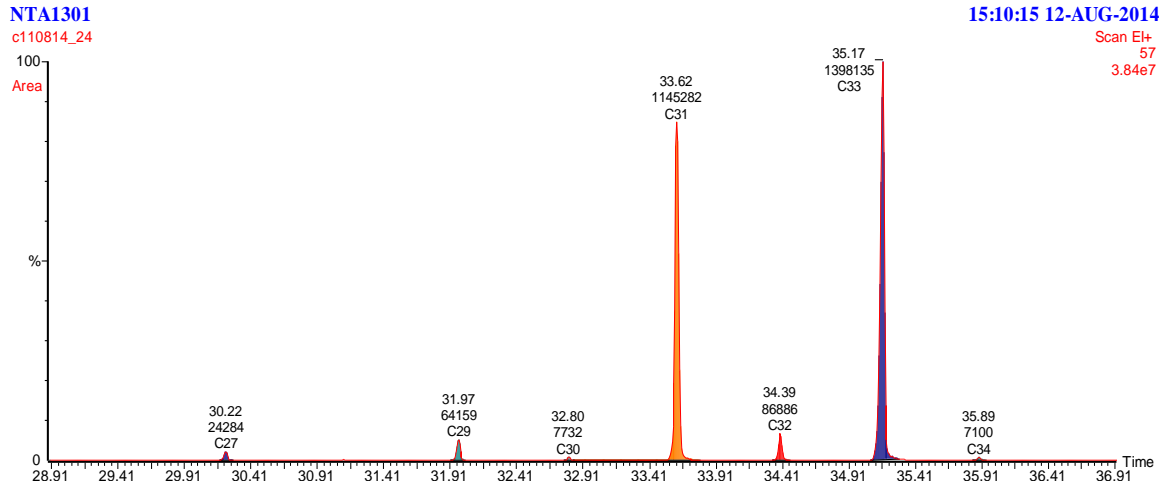
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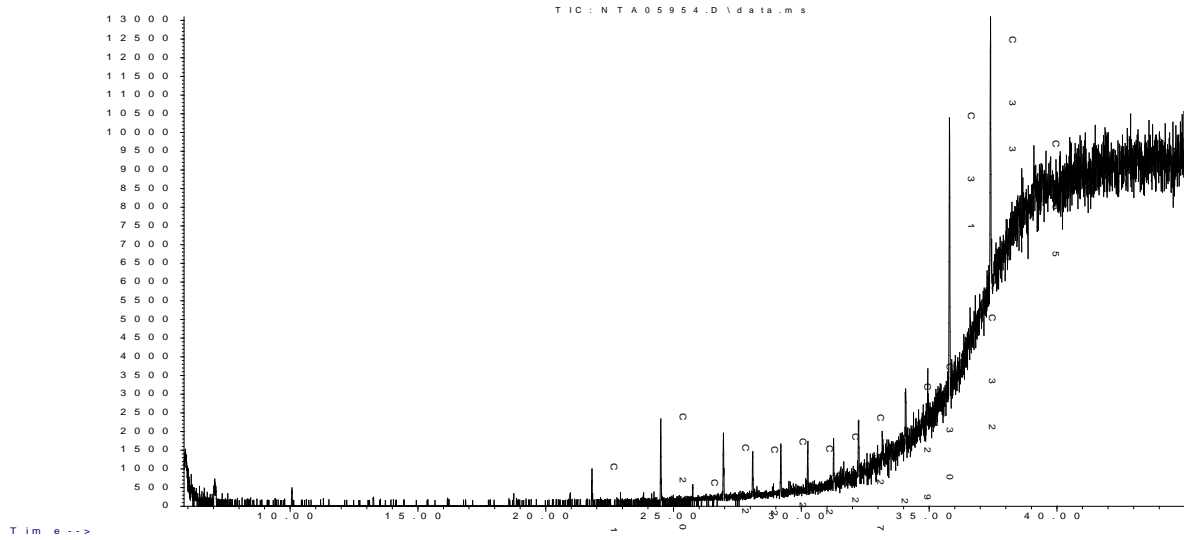
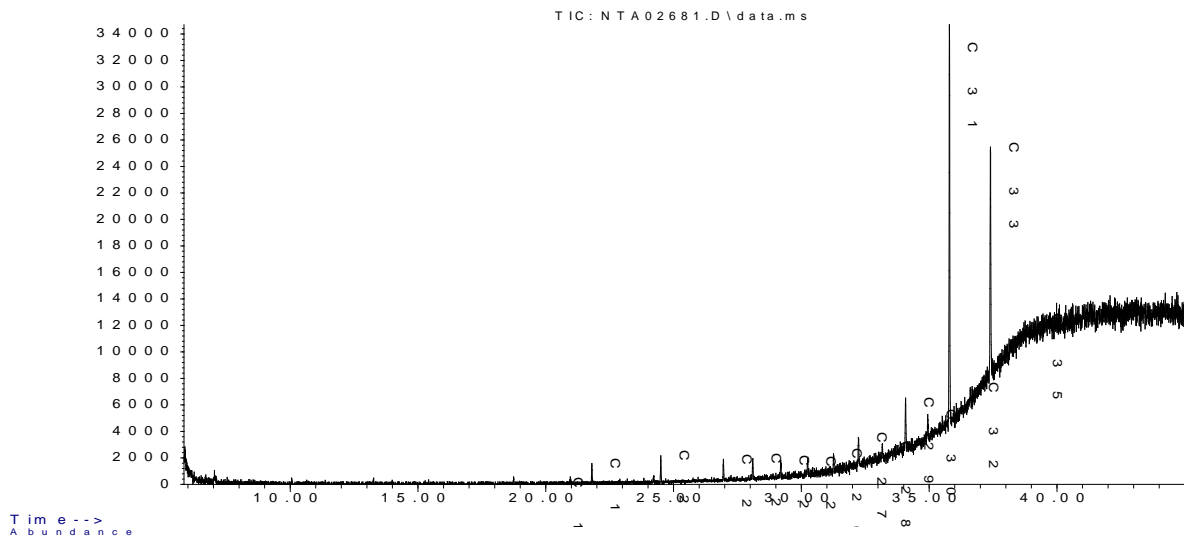
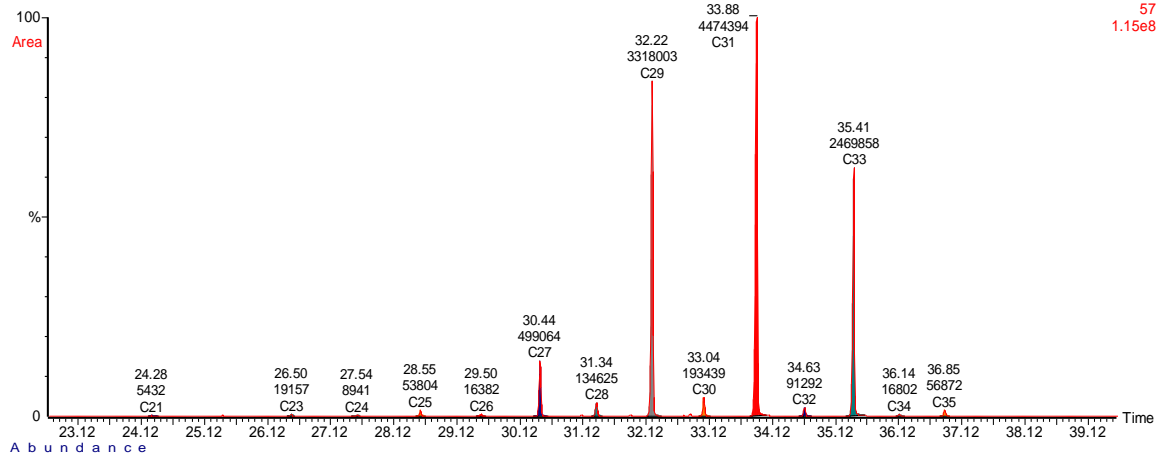




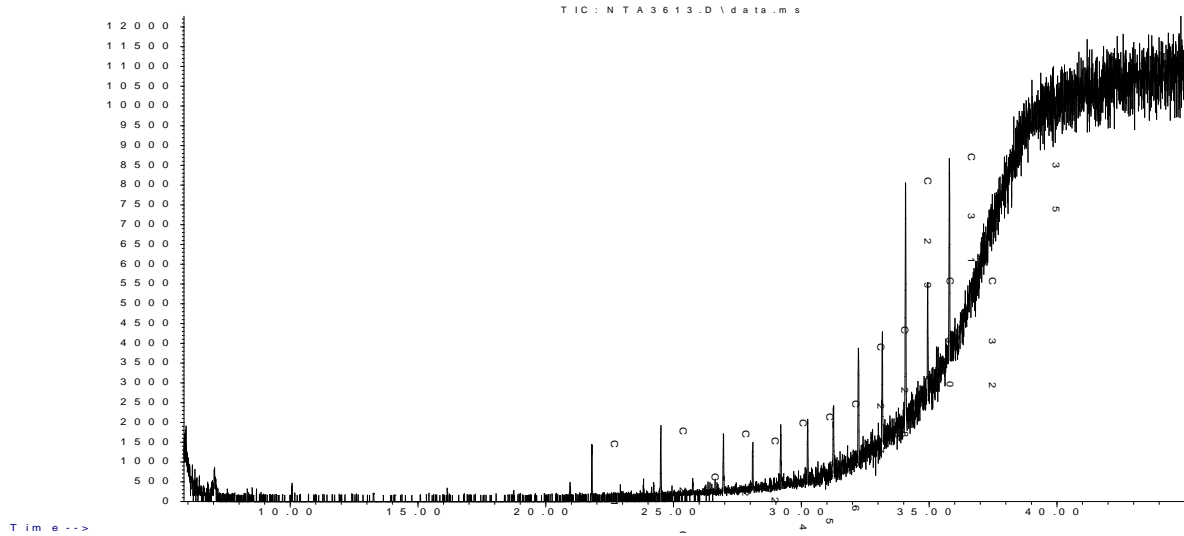
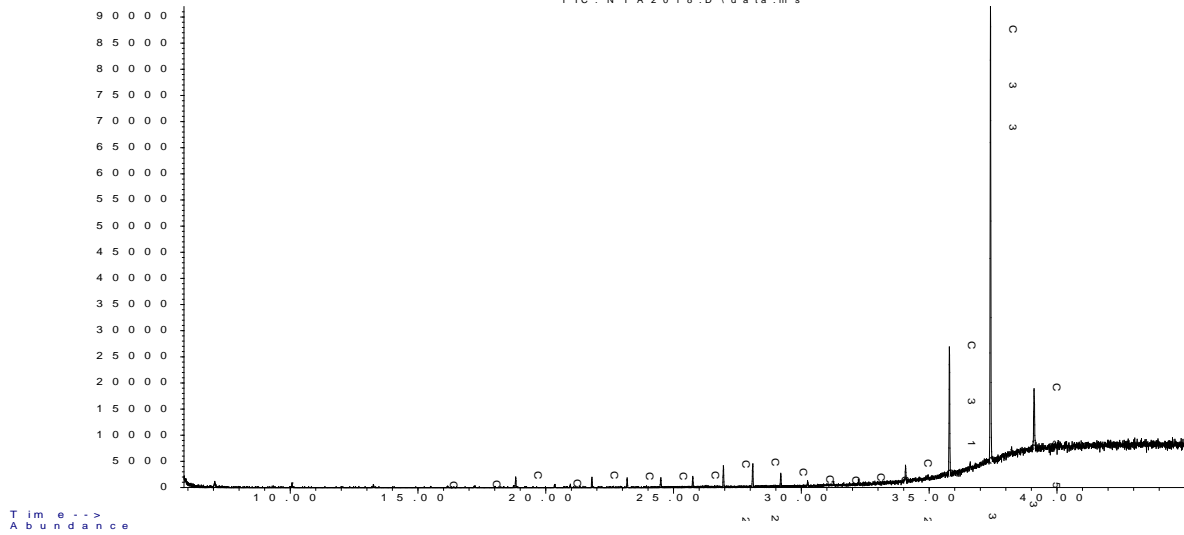
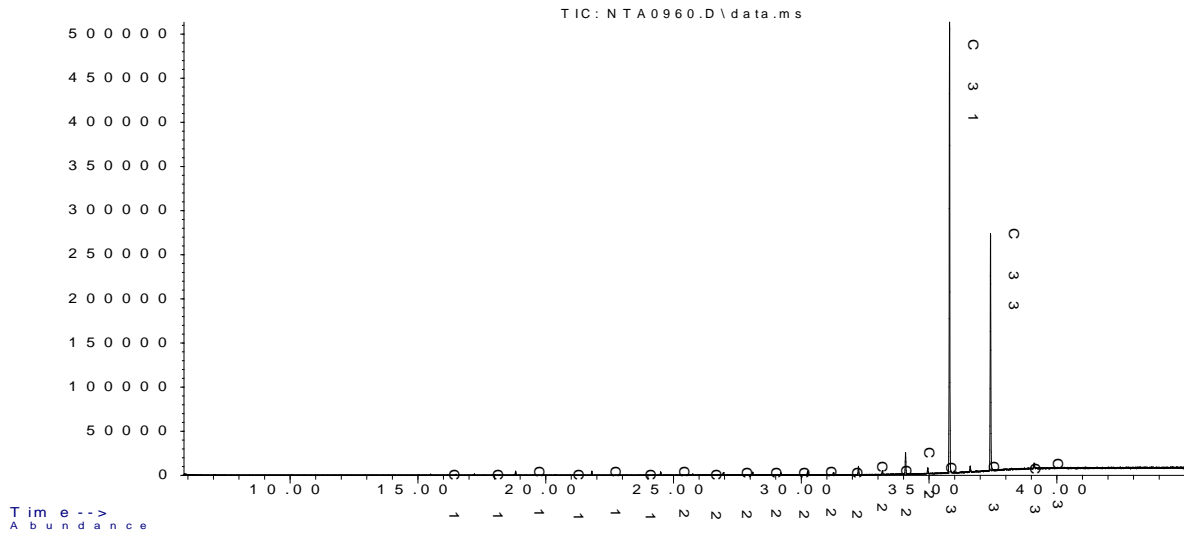


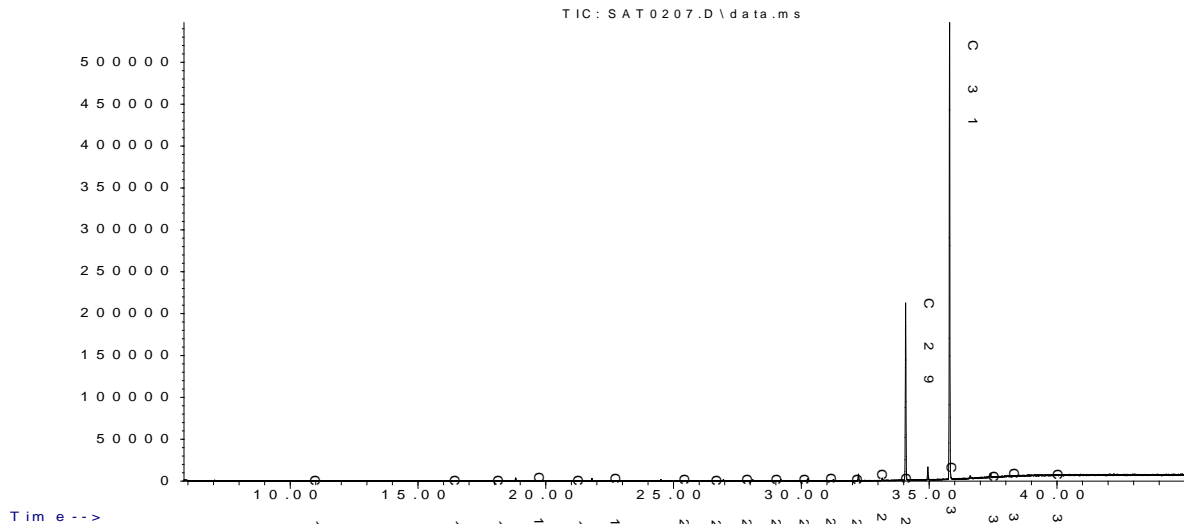
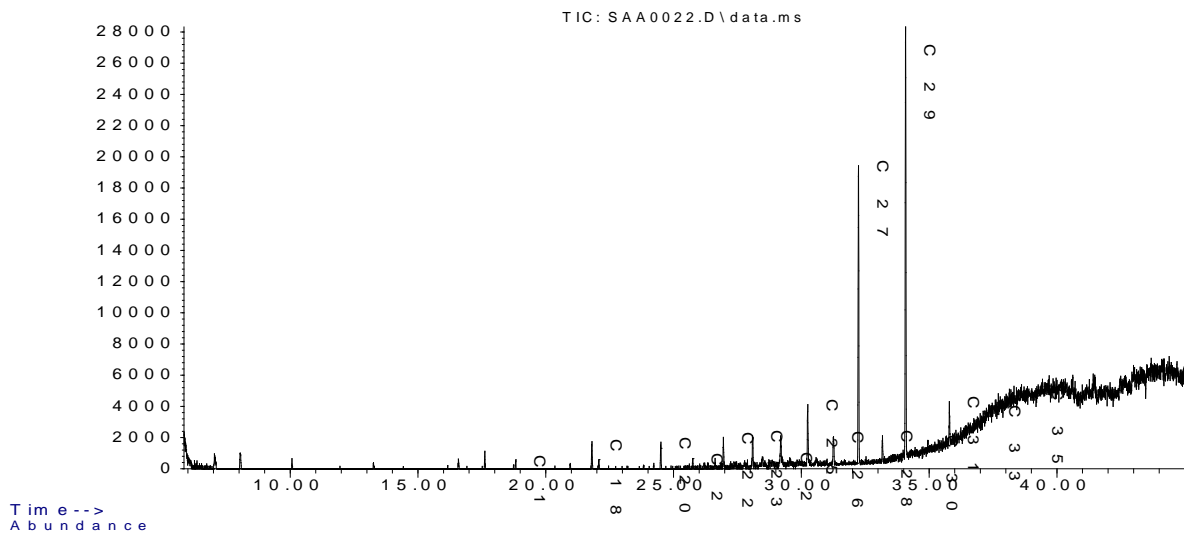
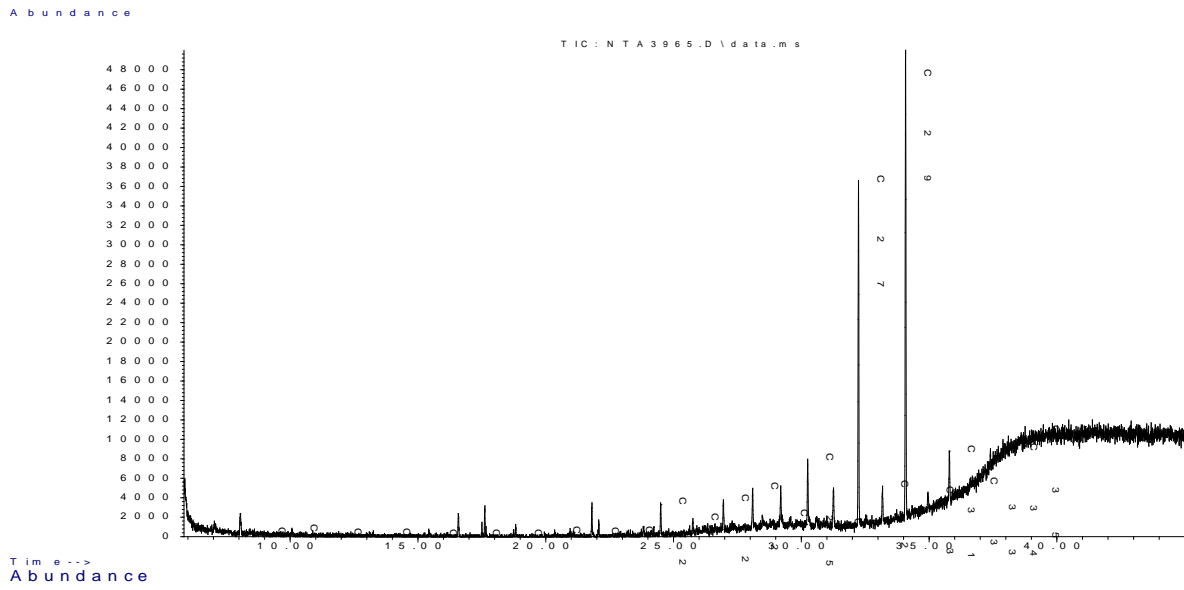
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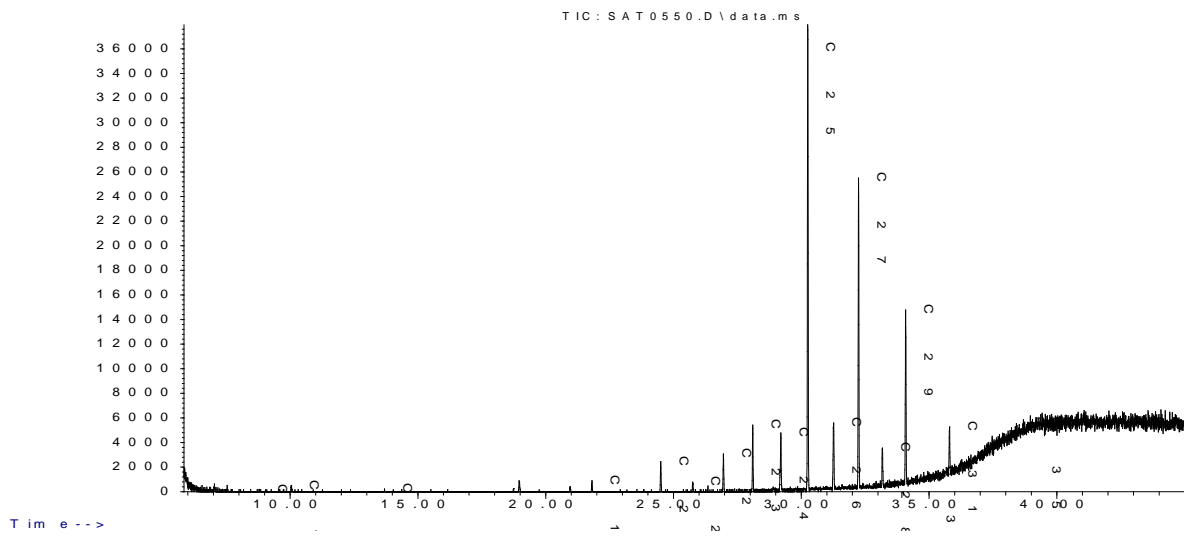
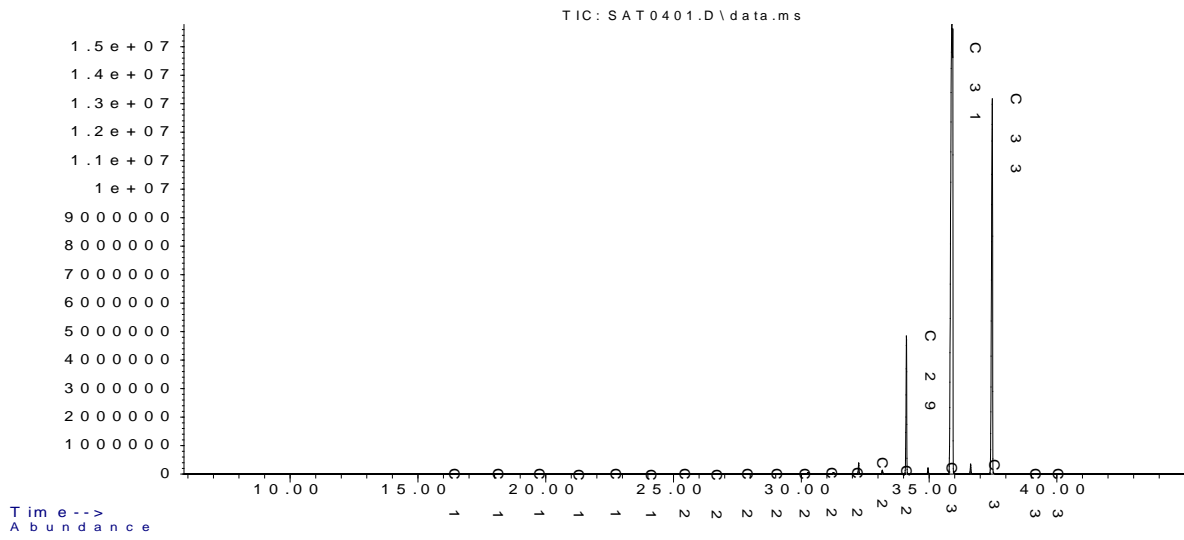
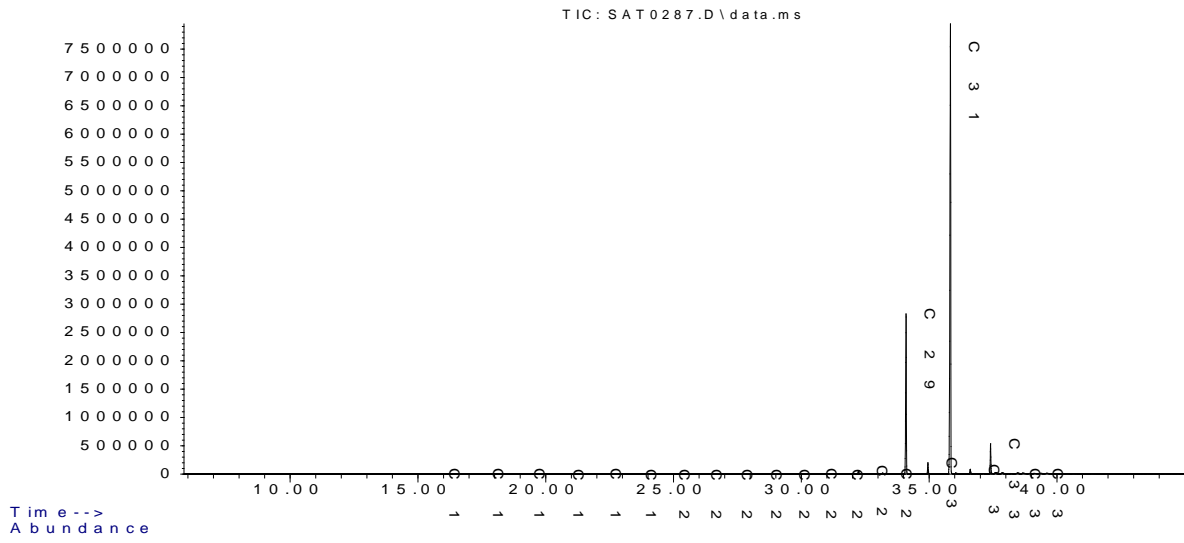


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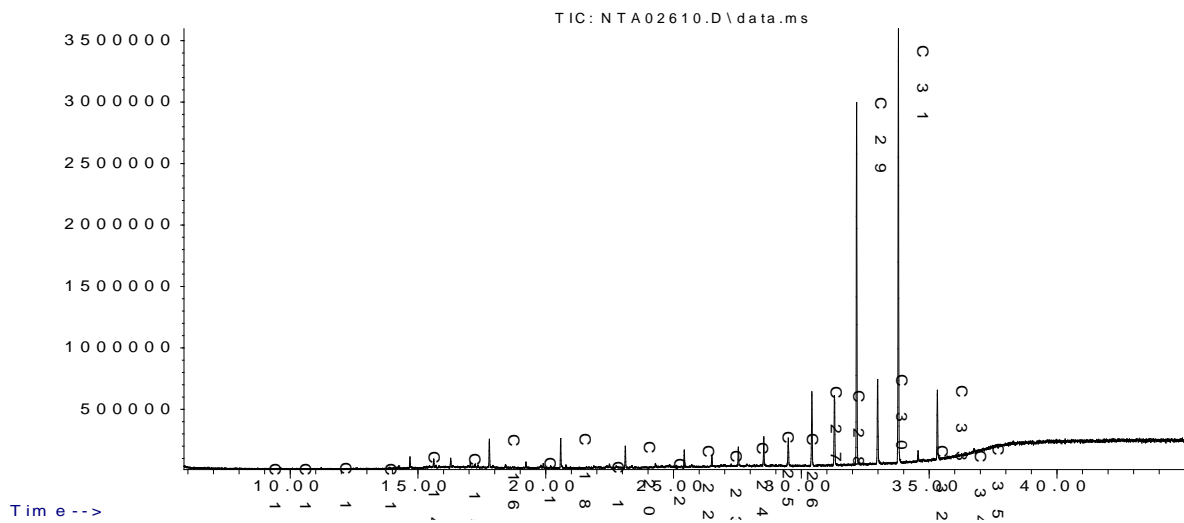
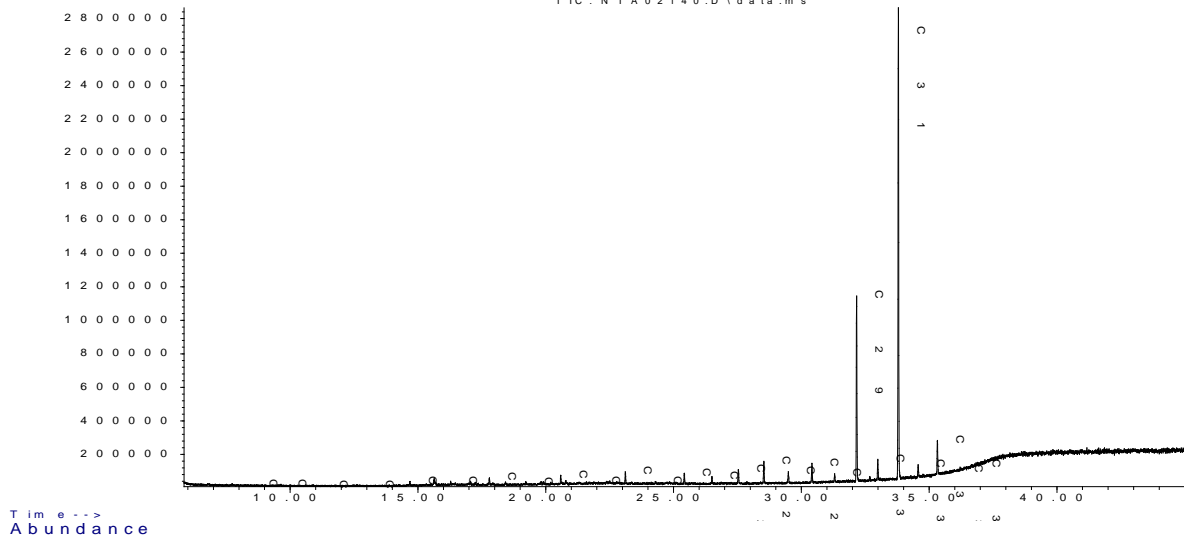
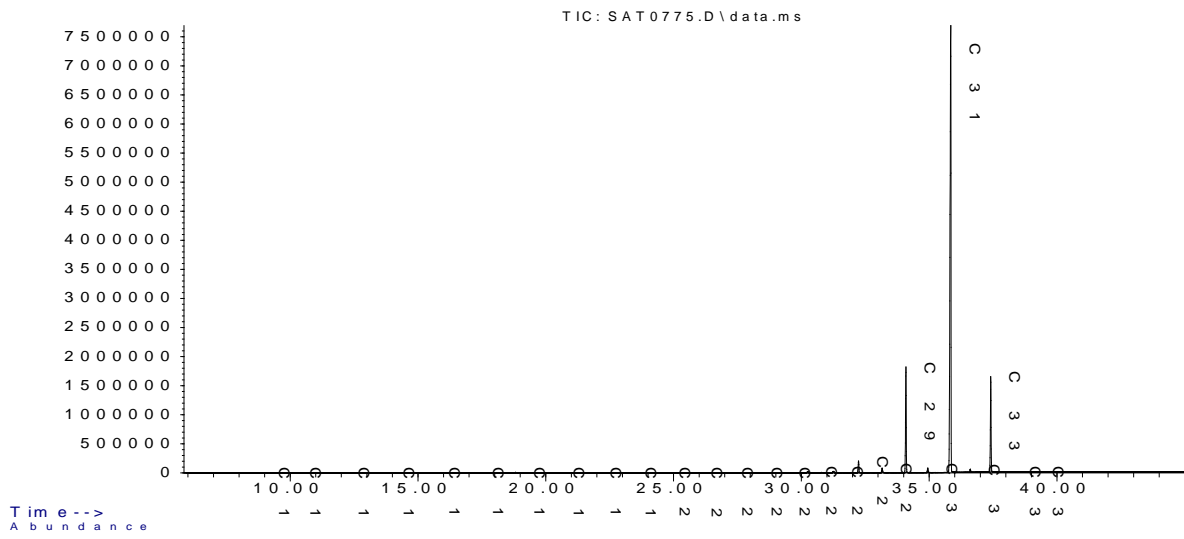


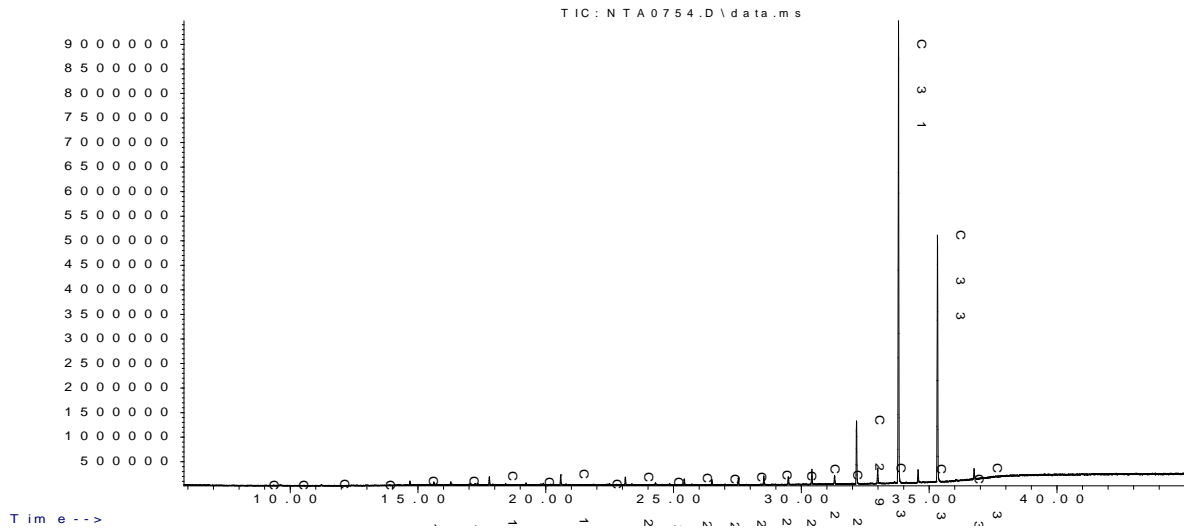
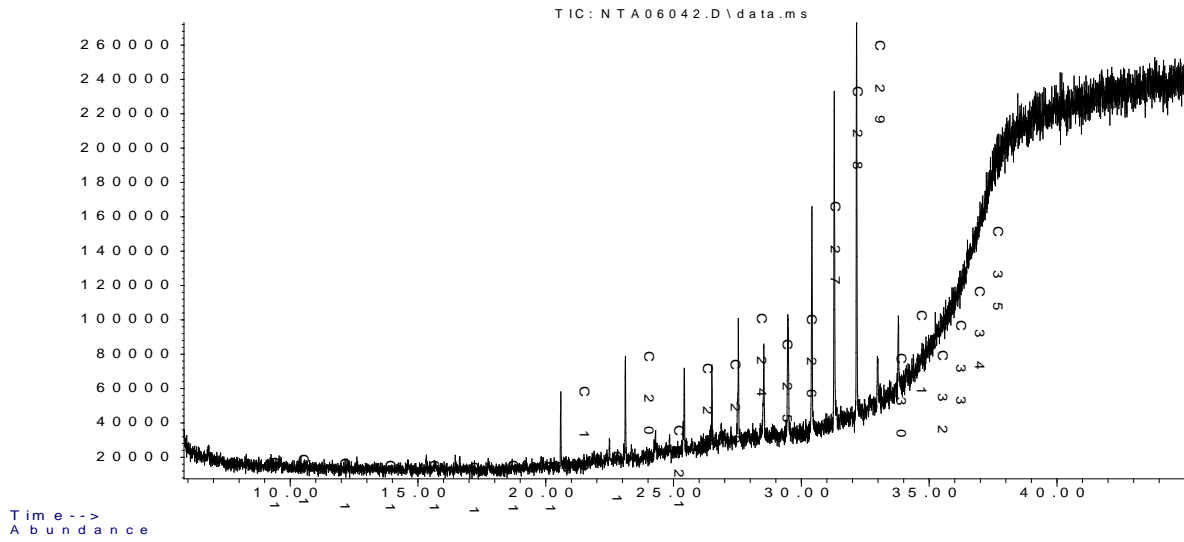
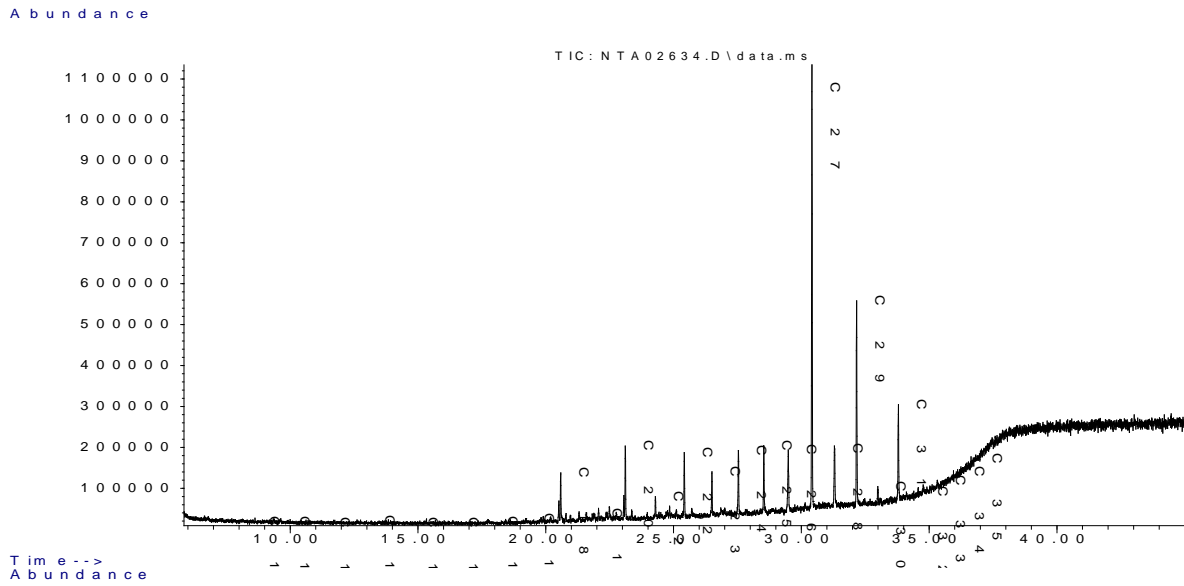


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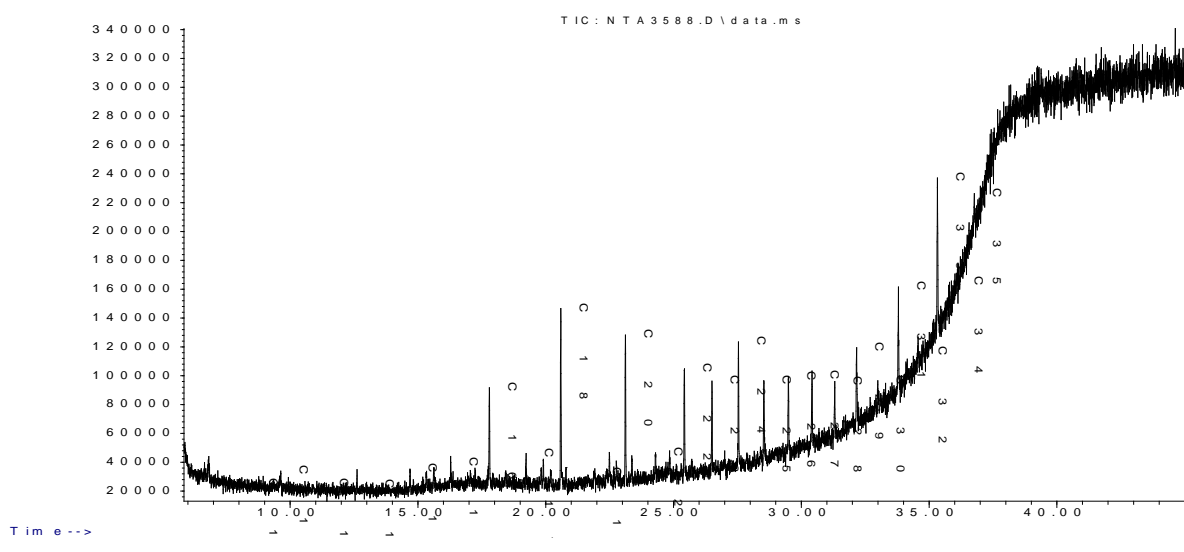
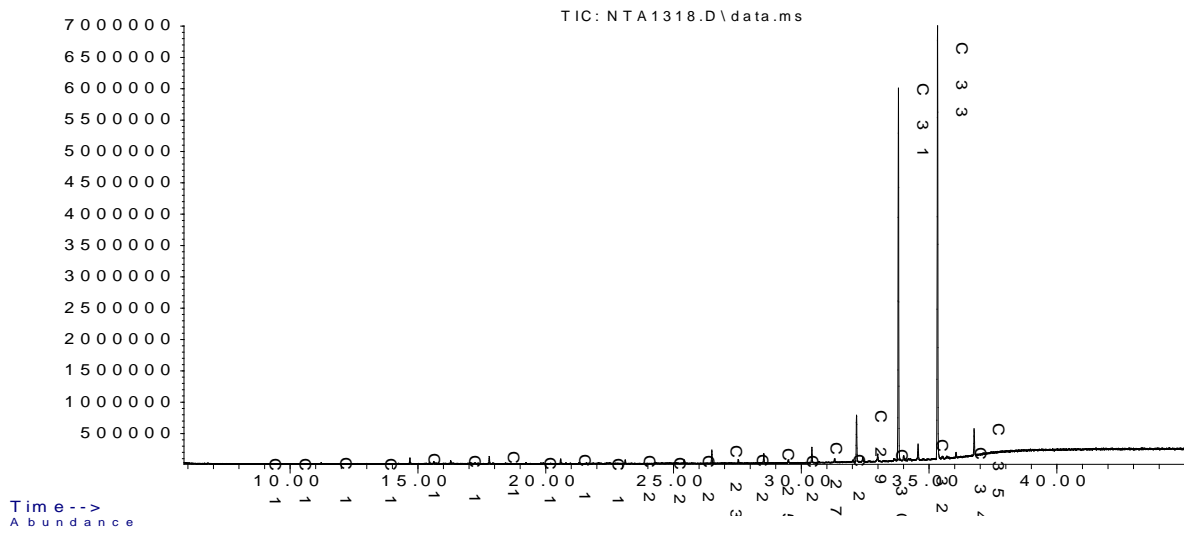
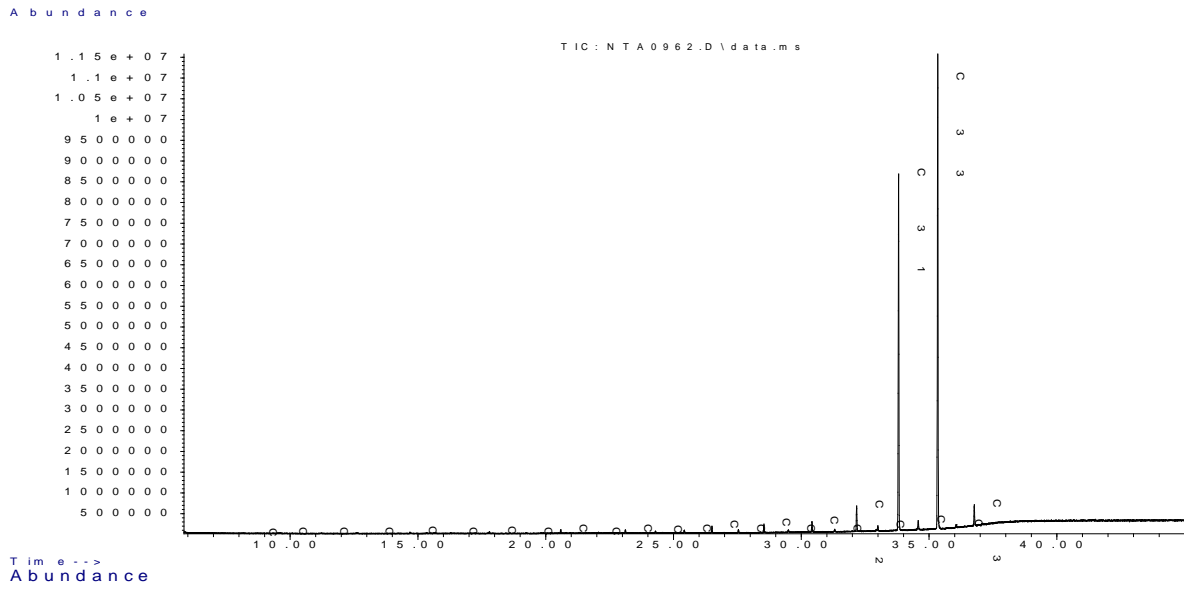


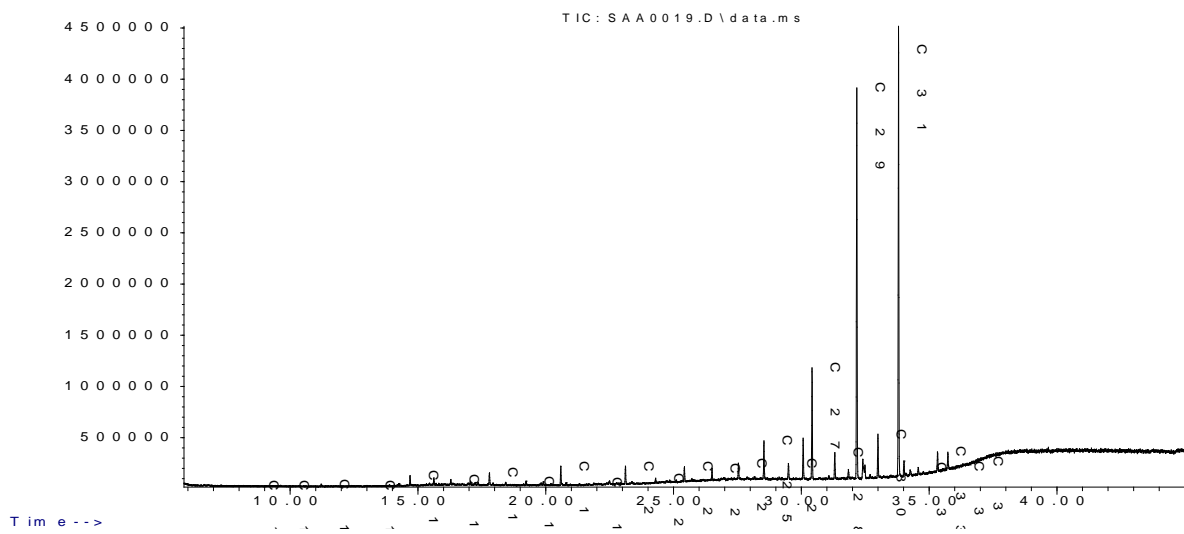
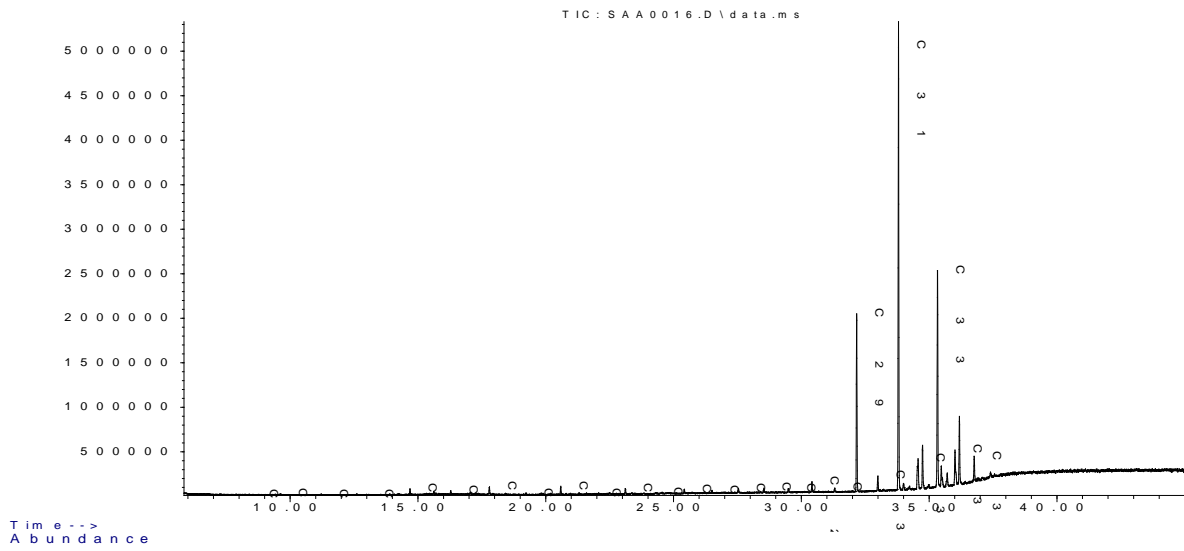
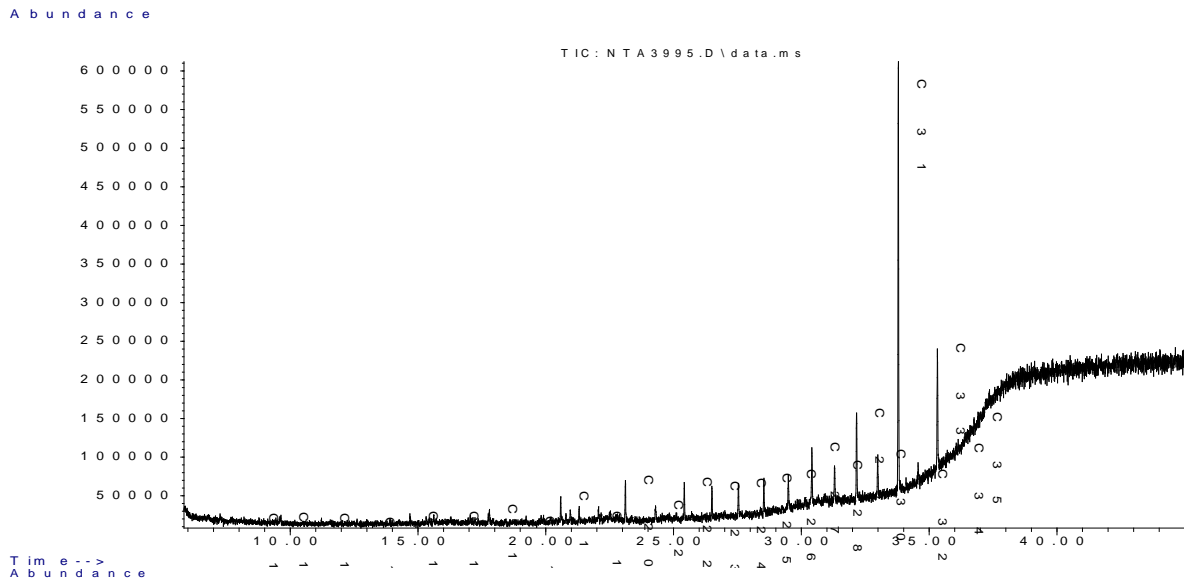
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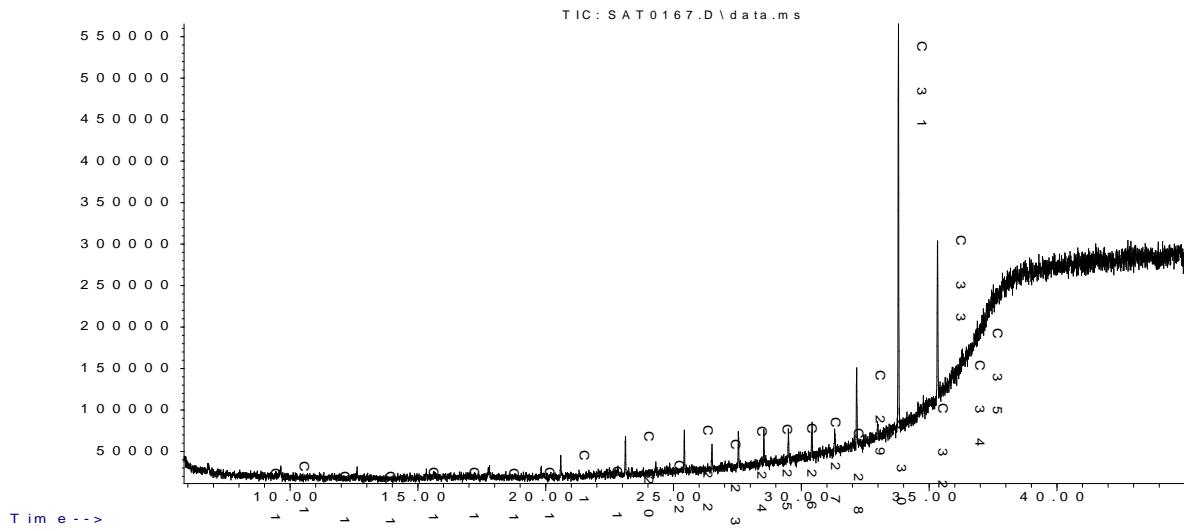
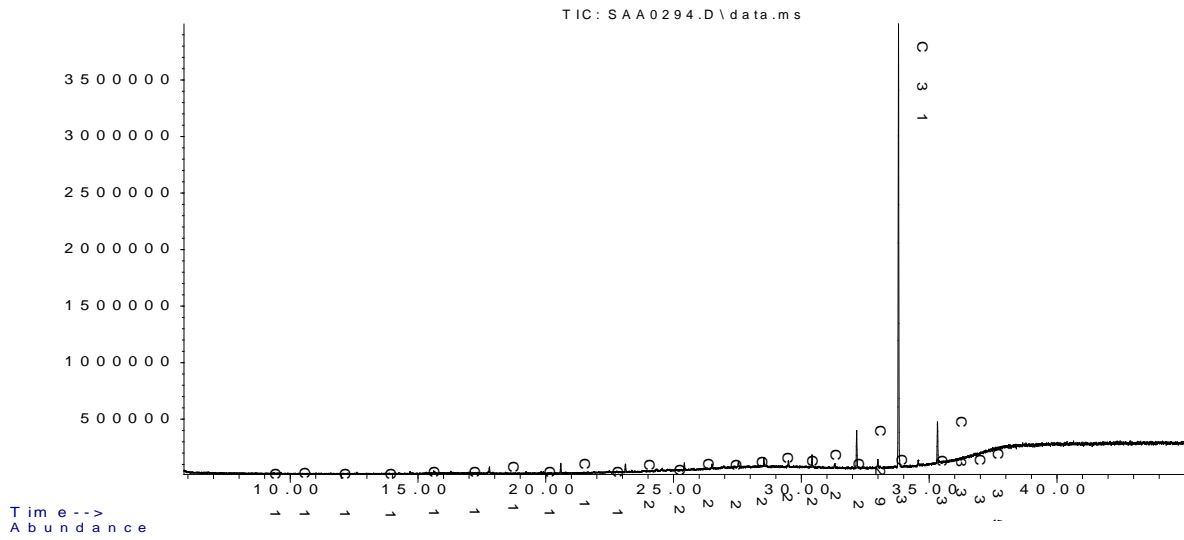
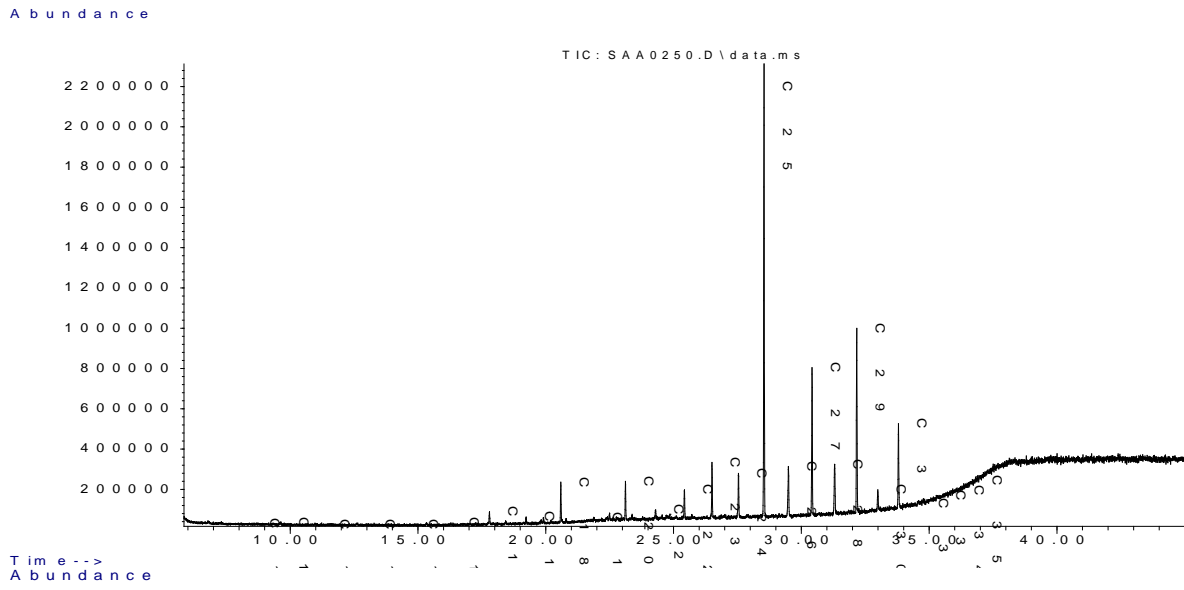


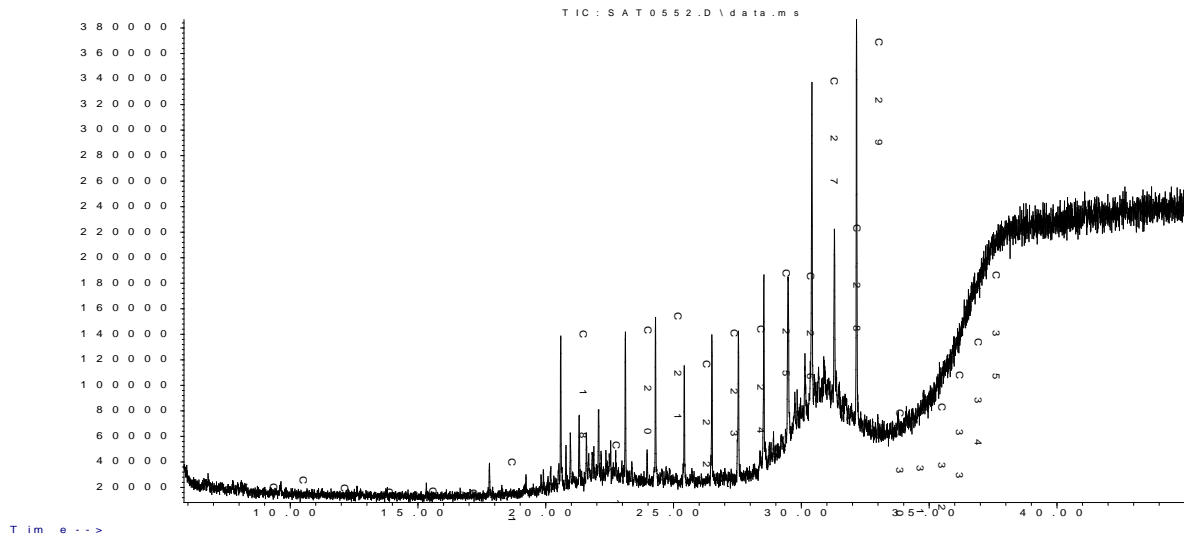
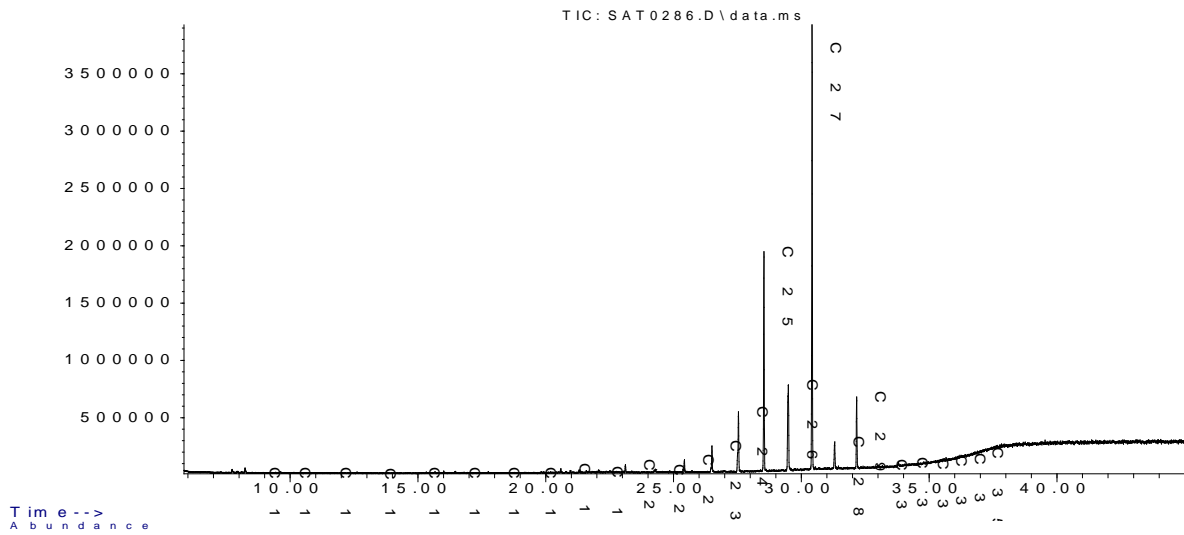
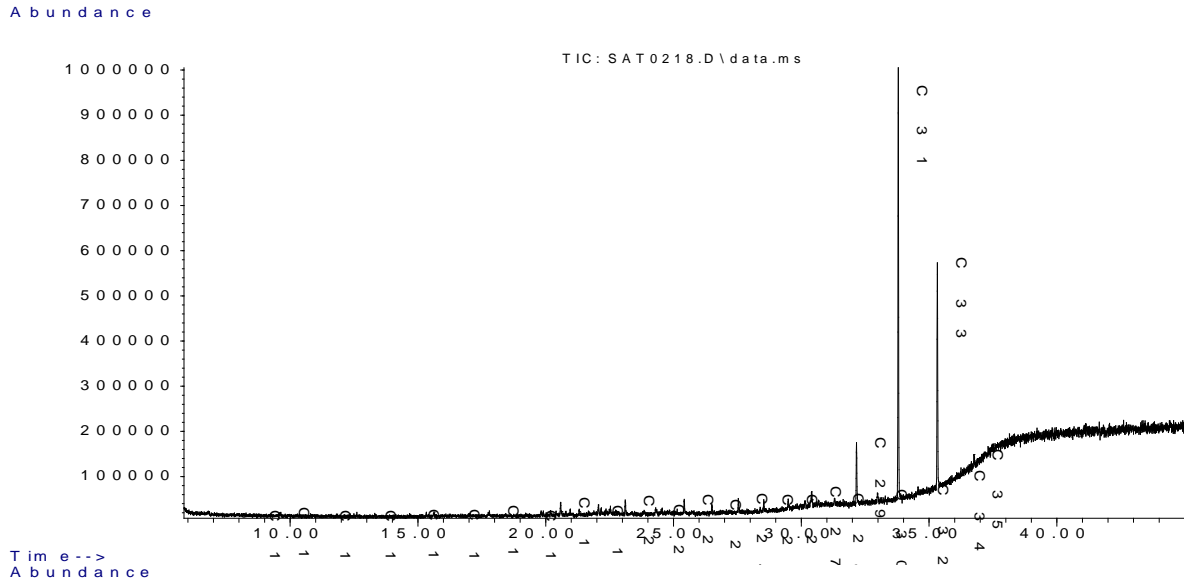




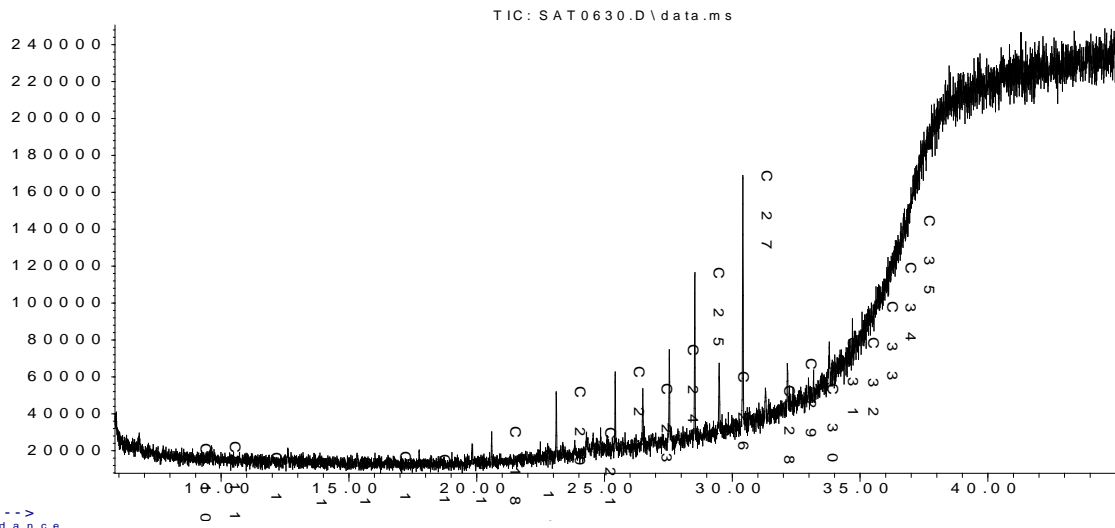




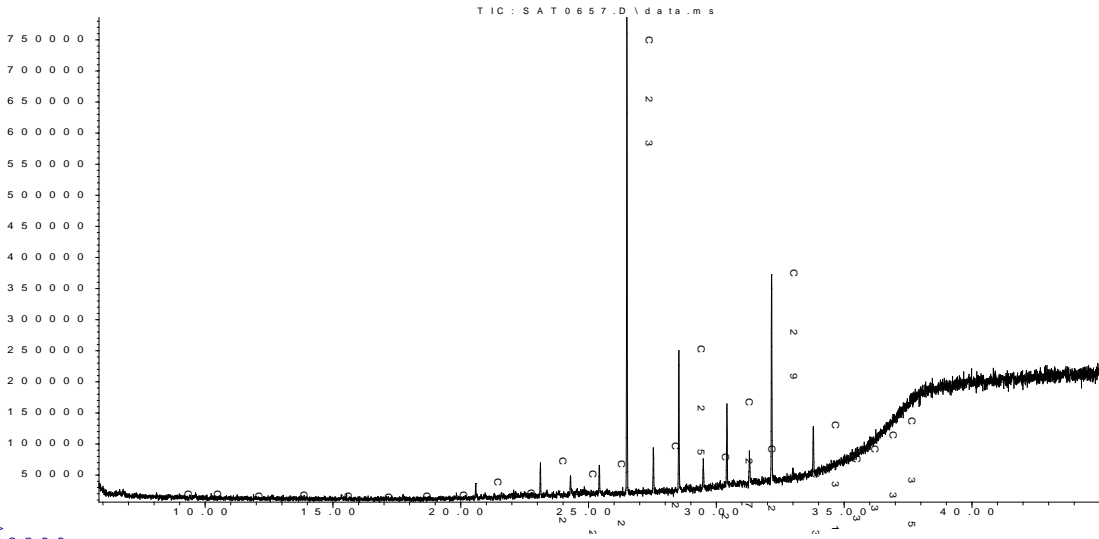




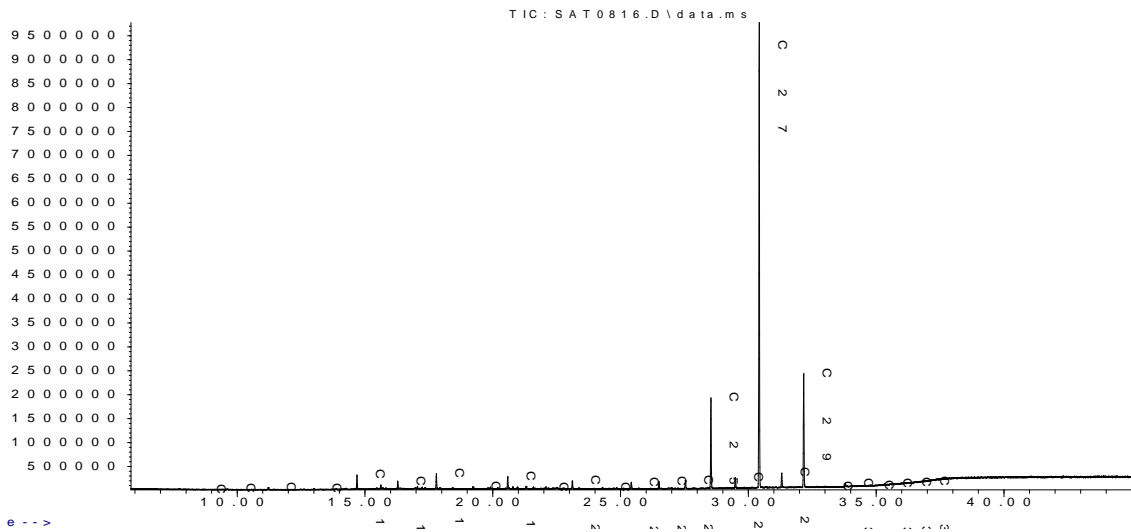
Abundance



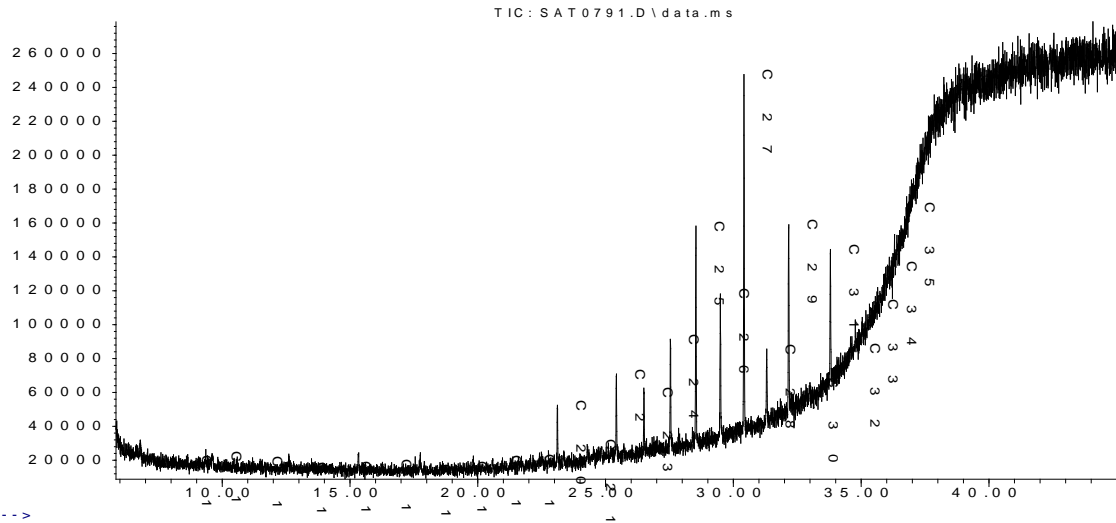
Time-->  
Abundance



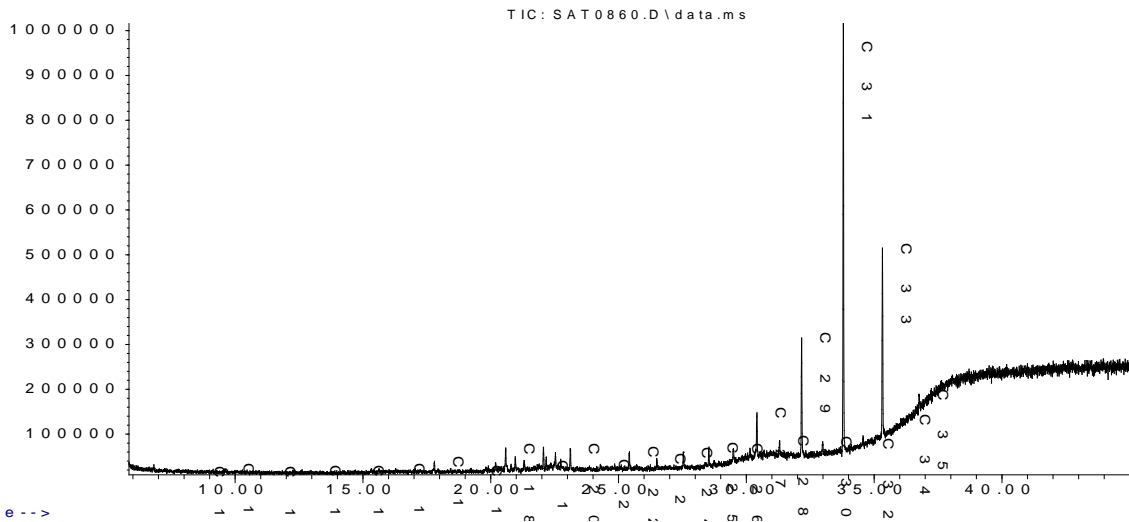
Time-->  
Abundance



Abundance



Time-->  
Abundance



Time-->

Following Tables: Chain length peak areas for each sample (both plants and soils) obtained from GC results with calculations for CPI, ACL, C27/C31 and C29/33

Site	Bioregion	Dominant Spp 1	Growth form	Genetic Voucher No	Dominant Spp 2	Growth form	Genetic Voucher No	Dominant Spp 3	Growth form	Genetic Voucher No	Soil		
NTAGFU0001	Gulf fall and uplands	Aristida pruinosa	Tree Mallee	NTA.001524	Eneapogon polyphyllus	Tussock Grass	NTA.001525	Eucalyptus pruinosa	Tree Mallee	NTA.001531	NTAGFU0001	Gulf fall and uplands	
		NAME	FOUND_RT	AREA	NAME	FOUND_RT	AREA	NAME	FOUND_RT	AREA	NAME	FOUND_RT	AREA
		C16	17.610	434.566	C20	21.802	2726	C20	21.806	1299	C20	21.808	3845
		C18	19.043	432.281	C21	22.98	982	C21	0	0	C21	22.975	4825
		C18	20.395	2810.149	C22	24.101	3108	C22	24.105	1084	C22	24.101	5036
		C20	22.919	1054.922	C23	25.179	2341	C23	25.178	557	C23	25.179	4623
		C22	25.217	1060.543	C24	26.216	2273	C24	26.215	2077	C24	26.216	2622
		C24	27.326	1723.095	C25	27.205	1752	C25	27.209	1742	C25	27.211	2780
		C25	28.329	4502.880	C26	28.168	1208	C26	28.162	3010	C26	28.174	1473
		C26	29.282	3993.667	C27	29.079	1053	C27	29.089	9496	C27	29.09	4184
		C27	30.213	17291.709	C28	29.959	329	C28	29.974	874	C28	29.985	906
		C28	31.100	9724.136	C29	30.828	1107	C29	30.838	2567	C29	30.833	3796
		C29	31.951	24412.240	C30	31.671	60	C30	0	0	C30	31.655	775
		C30	32.780	16119.520	C31	32.462	6210	C31	32.461	292	C31	32.462	5231
		C31	33.602	244365.578	C32	33.236	282	C32	0	0	C32	33.237	422
		C32	34.380	5055.872	C33	33.996	15930	C33	0	0	C33	34.001	4506
		C33	35.136	45933.559	C34	0	0	C34	0	0	C34	34.739	54
		C35	36.583	372.683	C35	35.435	1301	C35	35.439	1271	C35	35.441	3720
		ACL	30.847	Average	ACL	31.736	Average	ACL	27.845	Average	ACL	30.293	Average
		C27/C31	0.73566	30.73566	C27/C31	0.170	30.42007	C27/C31	32.521	27.11933	C27/C31	0.800	29.22241
		C29/C33	0.531	31.61187	C29/C33	0.069	32.7401	C29/C33	INDIV/OI	29	C29/C33	0.842	31.17104
		CPI	8.326748907		CPI	4.068112948		CPI	2.170262598		CPI	2.603871368	
NTAGFU0008	Gulf fall and uplands	Triodia pungens	Hummock grass	NTA.002012	Aristida contorta	Tussock Grass	NTA.002011	Fimbristylis dochotoma	Sedge	NTA.002018	NTAGFU0008	Gulf fall and uplands	
		NAME	FOUND_RT	AREA	NAME	FOUND_RT	AREA	NAME	TIME	PEAK AREA	NAME	FOUND_RT	AREA
		C21	24.279	5432.154	C14	14.629	6979.258	C10	0	0	C20	21.808	2690
		C25	26.5	19156.979	C16	17.748	7971.676	C11	0	0	C21	22.98	3716
		C24	27.538	8940.808	C18	20.553	9207.618	C12	0	0	C22	24.185	635
		C25	28.546	53803.813	C23	26.5	12139.624	C13	0	0	C23	25.185	3278
		C26	29.503	16381.983	C24	27.545	10316.734	C14	15.509	48	C24	26.211	2400
		C27	30.438	499063.969	C25	28.546	33103.785	C15	17.205	382	C25	27.206	2907
		C28	31.336	134624.813	C26	29.51	23514.857	C16	18.828	1415	C26	28.169	1890
		C29	32.22	3318002.75	C27	30.445	125840.734	C17	20.352	775	C27	29.09	2032
		C30	33.039	193438.5	C28	31.336	50200.406	C18	21.802	1616	C28	29.965	1244
		C31	33.879	4474394	C29	32.206	635080.75	C19	23.179	1464	C29	30.839	5979
		C32	34.631	91291.695	C30	33.039	193015.188	C20	24.503	1494	C30	31.656	611
		C33	35.413	2469857.5	C31	33.886	6901315.5	C21	25.749	1499	C31	32.467	5504
		C34	36.136	16801.893	C32	34.639	348517.281	C22	26.953	2763	C32	33.237	162
		C35	36.852	56871.539	C33	35.435	6524495.5	C23	28.1	3175	C33	33.996	1199
		ACL	30.65159259	Average	ACL	31.99477227	Average	C24	29.194	1963	C34	0	0
		C27/C31	0.111537779	30.59862	C27/C31	0.018234311	30.92837	C25	30.241	546	C35	35.43	699
		C29/C33	1.343398455	30.70692	C29/C33	0.097337909	32.64519	C26	31.257	667	ACL	29.235	Average
		CPI	23.54476491		CPI	18.77255932		C27	32.231	628	C27/C31	0.369	29.92144
								C28	33.178	180	C29/C33	4.987	29.66815
								C29	34.074	2013	CPI	3.328507635	
								C30	34.958	420			
								C31	35.796	13042			
								C32	36.608	760			
								C33	37.403	42991			
								C34	38.21	922			
								C35	39.105	32702			
								ACL	33.25164814	Average			
								C27/C31	0.048152124	30.81624			
								C29/C33	0.046823754	32.82108			
								CPI	10.35771987				





Site	Bioregion	Dominant Spp 1	Growth form	Genetic Voucher No	Dominant Spp 2	Growth form	Genetic Voucher No	Dominant Spp 3	Growth form	Genetic Voucher No	Soil			
NTAGFU0017	Gulf fall and uplands	Melaleuca viridiflora	Shrub	NTA 002634	Chrysopogon fallax	Tussock Grass	NTA 002610	Schizachyrium fragile	Tussock Grass	NTA 002681	NTAGFU0017			
		<b>NAME</b>	<b>TIME</b>	<b>PEAK AREA</b>	<b>NAME</b>	<b>TIME</b>	<b>PEAK AREA</b>	<b>NAME</b>	<b>TIME</b>	<b>PEAK AREA</b>	<b>NAME</b>	<b>TIME</b>	<b>PEAK AREA</b>	
		C10	8.483	1199	C10	8.462	831	C10	0	0	C20	21.804	8792	
		C11	9.661	294	C11	9.645	262	C11	0	0	C21	22.982	9554	
		C12	11.247	474	C12	11.226	6823	C12	0	0	C22	24.171	1248	
		C13	12.99	1239	C13	12.985	2627	C13	0	0	C23	25.186	6915	
		C14	14.687	1212	C14	14.687	50926	C14	0	0	C24	26.218	4435	
		C15	16.268	333	C15	16.278	42275	C15	0	0	C25	27.212	5883	
		C16	17.796	2680	C16	17.791	118359	C16	0	0	C26	28.171	1179	
		C17	19.226	6632	C17	19.221	23127	C17	20.357	106	C27	29.087	7171	
		C18	20.582	57080	C18	20.582	114216	C18	21.802	1191	C28	29.977	1720	
		C19	21.88	6588	C19	21.886	7981	C19	0	0	C29	30.835	8824	
		C20	23.11	84286	C20	23.111	81614	C20	24.503	1517	C30	31.673	1295	
		C21	24.288	23283	C21	24.294	15426	C21	0	0	C31	32.469	13101	
		C22	25.419	71383	C22	25.414	66308	C22	26.954	1184	C32	33.244	1084	
		C23	26.503	52515	C23	26.498	37141	C23	28.095	1232	C33	34.003	7437	
		C24	27.54	73056	C24	27.529	67254	C24	29.194	1088	C34	34.746	184	
		C25	28.529	79616	C25	28.529	111757	C25	30.241	1073	C35	35.448	7488	
		C26	29.487	74378	C26	29.488	107288	C26	31.252	439		<b>ACL</b>	<b>30.263</b>	Average
		C27	30.414	526718	C27	30.409	288556	C27	32.236	1438		<b>C27/C31</b>	<b>0.547</b>	29.58504
		C28	31.304	80270	C28	31.299	290281	C28	33.179	916		<b>C29/C33</b>	<b>1.186</b>	30.82941
		C29	32.163	223566	C29	32.163	1366131	C29	34.069	2593		<b>CPI</b>	<b>5.190847914</b>	
		C30	32.985	18339	C30	32.985	293784	C30	34.948	1166				
		C31	33.791	107959	C31	33.796	1668989	C31	35.791	15380				
		C32	34.618	810	C32	34.571	33311	C32	36.613	529				
		C33	35.32	3487	C33	35.32	282131	C33	37.393	9232				
		C34	36.058	748	C34	36.048	3002	C34	0	0				
		C35	36.754	23461	C35	36.754	69869	C35	39.11	1924				
			<b>ACL</b>	<b>27.96220902</b>	Average		<b>ACL</b>	<b>30.01957606</b>	Average		<b>ACL</b>	<b>31.27762326</b>	Average	
			<b>C27/C31</b>	<b>4.878870682</b>	27.6804		<b>C27/C31</b>	<b>0.172892691</b>	30.41037		<b>C27/C31</b>	<b>0.093498049</b>	30.65799	
			<b>C29/C33</b>	<b>64.11413823</b>	29.06143		<b>C29/C33</b>	<b>4.842186786</b>	29.68468		<b>C29/C33</b>	<b>0.280870884</b>	32.12288	
			<b>CPI</b>	<b>3.188978131</b>			<b>CPI</b>	<b>4.409230192</b>			<b>CPI</b>	<b>5.995866216</b>		









Site	Bioregion	Dominant Spp 1	Growth form	Genetic Voucher No	Dominant Spp 2	Growth form	Genetic Voucher No	Dominant Spp 3	Growth form	Genetic Voucher No	Soil		
SATFLB0005	Flinders lofty block	<i>Dodonaea viscosa</i> subsp. ar Shrub		SAT 000316	<i>Eucalyptus flindersii</i>	Tree Mallee	SAT 000286	<i>Chrysocephalum semipapp</i> Forb		SAT 000287	SATFLB0005	Flinders lofty block	
		NAME	FOUND_RT	AREA	NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA
		C14	14.526	3445.533	C10	8.467	420	C10	0	0	C20	21.807	5309
		C15	16.112	285.478	C11	9.651	315	C11	0	0	C21	22.985	7949
		C16	17.624	1158.885	C12	11.232	567	C12	0	0	C22	24.106	12086
		C27	30.22	2762.435	C13	12.986	424	C13	0	0	C23	25.184	38788
		C28	31.122	614.363	C14	14.698	311	C14	15.499	2615	C24	26.215	66156
		C29	31.98	141293.922	C15	16.289	283	C15	17.205	2382	C25	27.215	185953
		C30	32.809	3397.923	C16	17.823	369	C16	18.818	4309	C26	28.168	139367
		C31	33.616	109433.781	C17	19.258	629	C17	20.357	603	C27	29.09	211334
		C33	35.158	244.056	C18	20.582	16343	C18	21.813	2161	C28	29.974	97570
		ACL	29.84465918	Average	C19	21.875	3435	C19	23.184	203	C29	30.838	184920
		C27/C31	0.025242982	30.90151	C20	23.116	32413	C20	24.498	1590	C30	31.66	51355
		C29/C33	578.9405792	29.0069	C21	24.294	12486	C21	25.75	634	C31	32.467	216314
		CPI	63.23930896		C22	25.425	49453	C22	26.949	1145	C32	33.241	21328
					C23	26.504	105446	C23	28.1	1427	C33	33.995	59389
					C24	27.54	268200	C24	29.195	1189	C34	34.718	3725
					C25	28.535	909930	C25	30.247	3672	C35	35.43	16181
					C26	29.493	449406	C26	31.257	1590	ACL	28.54328554	Average
					C27	30.42	1799995	C27	32.236	29766	C27/C31	0.976977912	29.02329
					C28	31.305	114210	C28	33.174	9778	C29/C33	3.113707926	29.97236
					C29	32.163	285938	C29	34.095	1249753	CPI	2.320718001	
					C30	33.001	7861	C30	34.948	93881			
					C31	33.802	13975	C31	35.833	3636459			
					C32	34.608	582	C32	36.608	42122			
					C33	35.325	2190	C33	37.404	214231			
					C34	36.048	673	C34	38.221	3305			
					C35	36.755	9518	C35	39.105	15636			
					ACL	26.63502128	Average	ACL	30.58256357	Average			
					C27/C31	128.8010733	27.03082	C27/C31	0.008185435	30.96752			
					C29/C33	130.5652968	29.0304	C29/C33	5.833670197	29.58534			
					CPI	3.513621636		CPI	33.61507745				

Site	Bioregion	Dominant Spp 1	Growth form	Genetic Voucher No		Dominant Spp 2	Growth form	Genetic Voucher No		Dominant Spp 3	Growth form	Genetic Voucher No		Soil		
SATFLB0008	Flinders lofty block	Triodia scariosa	Hummock grass	SAT 000424		Cassinia laevis	Shrub	SAT 000419		Casuarina pauper	Shrub	SAT 000401		SATFLB0008		
		NAME	FOUND_RT	AREA		NAME	FOUND_RT	AREA		NAME	TIME	PEAK AREA		NAME	TIME	PEAK AREA
		C16	17.61	478.964		C20	21.806	2282		C10	0	0		C20	21.792	17758
		C17	19.035	199.162		C21	22.979	1450		C11	0	0		C21	22.876	8626
		C18	20.388	2573.548		C22	24.11	1696		C12	0	0		C22	24.09	74485
		C20	22.919	6143.172		C23	25.178	6669		C13	0	0		C23	25.043	2151
		C22	25.209	5356.313		C24	26.22	3155		C14	15.5	4650		C24	26.2	335580
		C23	26.293	2250.673		C25	27.209	18274		C15	17.207	4113		C25	27.2	731521
		C24	27.326	6162.694		C26	28.167	2326		C16	18.83	9045		C26	28.153	654838
		C25	28.322	7374.059		C27	29.083	34297		C17	20.353	1956		C27	29.074	661925
		C26	29.282	5478.503		C28	29.973	8873		C18	21.809	7210		C28	29.964	390513
		C27	30.205	9924.186		C29	30.853	938087		C19	23.191	598		C29	30.823	627299
		C28	31.1	3904.074		C30	31.664	130169		C20	24.505	4718		C30	31.655	182060
		C29	31.958	31706.822		C31	32.518	2377226		C21	25.756	1288		C31	32.456	1115695
		C30	32.78	9219.813		C32	33.24	93439		C22	26.95	3897		C32	33.231	49664
		C31	33.594	137454.375		C33	33.999	349549		C23	28.096	5161		C33	33.99	102767
		C32	34.38	1239.889		C34	34.727	4937		C24	29.201	8648		C34	35.802	1600
		C33	35.136	3478.314		C35	35.434	116321		C25	30.253	28572		C35	35.781	9560
		ACL	30.2608204	Average		ACL	30.74995161	Average		C26	31.264	28285		ACL	28.52286021	Average
		C27/C31	0.072199855	30.73065		C27/C31	0.01442732	30.94311		C27	32.243	188350		C27/C31	0.593284903	29.51054
		C29/C33	9.115572085	29.39543		C29/C33	2.683706719	30.08586		C28	33.17	54619		C29/C33	6.104089834	29.56306
		CPI	6.128206254			CPI	15.4663321			C29	34.112	2275896		CPI	1.924778829	
										C30	34.955	86633				
										C31	35.871	13251550				
										C32	36.625	159715				
										C33	37.473	7463781				
										C34	38.227	4417				
										C35	39.112	45033				
										ACL	31.41418288	Average				
										C27/C31	0.014213432	30.94394				
										C29/C33	0.304925345	32.06531				
										CPI	67.11591819					

Site	Bioregion	Dominant Spp 1	Growth form	Genetic Voucher No	Dominant Spp 2	Growth form	Genetic Voucher No	Dominant Spp 3	Growth form	Genetic Voucher No	Soil			
SATFLB0010	Flinders lofty block	Eucalyptus odorata	Tree/Palm	SAT 000535	Rhagodia paradoxa	Chenopod	SAT 000552	Enchylaena tomentosa var. Chenopod		SAT 000550	SATFLB0010	Flinders lofty block		
		NAME	FOUND_RT	AREA	NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA	
		C16	17.61	10003.089	C10	8.472	621	C10	8.805	107	C20	21.806	4581	
		C17	19.043	3046.466	C11	9.645	448	C11	10.045	199	C21	22.984	6327	
		C18	20.395	29162.174	C12	11.242	126	C12	0	0	C22	24.105	8890	
		C19	21.682	517.617	C13	13.006	630	C13	13.695	121	C23	25.183	27046	
		C20	22.919	18172.85	C14	14.707	196	C14	0	0	C24	26.215	42035	
		C21	24.089	2451.81	C15	16.288	884	C15	0	0	C25	27.215	109774	
		C22	25.217	15343.378	C16	17.802	13600	C16	0	0	C26	28.168	91206	
		C23	26.293	14915.36	C17	19.262	718	C17	0	0	C27	29.094	124117	
		C24	27.326	46022.891	C18	20.582	62718	C18	21.81	908	C28	29.979	71558	
		C25	28.322	102848.398	C19	21.88	9838	C19	0	0	C29	30.838	149004	
		C26	29.253	112901.305	C20	23.11	51829	C20	24.501	1814	C30	31.665	40599	
		C27	30.205	247856.609	C21	24.288	53706	C21	25.747	737	C31	32.471	107104	
		C28	31.078	26031.719	C22	25.419	36861	C22	26.956	2269	C32	33.246	16598	
		C29	31.951	30360.369	C23	26.498	55844	C23	28.097	3605	C33	33.995	23495	
		C30	32.78	1026.928	C24	27.54	60564	C24	29.192	3708	C34	34.722	4334	
		C31	33.587	3206.943	C25	28.529	72405	C25	30.254	20983	C35	35.44	12198	
			ACL	26.65610771	Average			C26	31.265	4135		ACL	28.41799761	Average
			C27/C31	77.28750059	27.05109			C27	32.244	14271		C27/C31	1.158845608	28.85284
			C29/C33		29			C28	33.176	2177		C29/C33	6.341945095	29.54481
			CPI	1.988879452				C29	34.081	8961		CPI	1.997683671	
								C30	34.951	401				
								C31	35.799	2692				
								C32	0	0				
								C33	0	0				
								C34	0	0				
								C35	39.092	303				
			ACL	27.9225188	Average				ACL	26.77013345	Average			
			C27/C31	18.10403361	27.20938				C27/C31	5.301263001	27.63479			
			C29/C33	89.32738095	29.04428				C29/C33	-	29			
			CPI	1.775156695					CPI	4.0214342				







Site	Bioregion	Dominant Spp 1	Growth form	Genetic Voucher No	Dominant Spp 2	Growth form	Genetic Voucher No	Dominant Spp 3	Growth form	Genetic Voucher No	Soil			
SATFLB0015	Flinders lofty block	Eucalyptus obliqua	Tree/Palm	SAT 000816	Lepidosperma semiteres	Sedge	SAT 000860	Hibbertia crinita	Shrub	SAT 000866	SATFLB0015	Flinders lofty block		
		<b>NAME</b>	<b>TIME</b>	<b>PEAK AREA</b>	<b>NAME</b>	<b>TIME</b>	<b>PEAK AREA</b>	<b>NAME</b>	<b>TIME</b>	<b>PEAK AREA</b>	<b>NAME</b>	<b>TIME</b>	<b>PEAK AREA</b>	
		C10	8.467	450	C10	8.463	460	C20	21.805	3003	C20	21.813	5940	
		C11	9.624	1027	C11	9.599	1187	C21	22.988	2570	C21	22.986	35028	
		C12	11.215	28188	C12	11.227	135	C22	24.108	2773	C22	24.111	73878	
		C13	12.995	11060	C13	12.986	624	C23	25.182	9295	C23	25.195	150538	
		C14	14.687	153012	C14	14.693	2946	C24	26.218	2934	C24	26.227	170542	
		C15	16.283	82567	C15	16.285	3415	C25	27.213	11958	C25	27.227	206709	
		C16	17.791	161749	C16	17.798	9999	C26	28.166	2964	C26	28.174	117779	
		C17	19.22	27370	C17	19.3	1282	C27	29.098	177661	C27	29.111	377398	
		C18	20.587	124591	C18	20.583	26425	C28	29.977	11479	C28	29.98	69997	
		C19	21.88	7696	C19	21.881	2983	C29	30.857	337029	C29	30.86	418056	
		C20	23.11	78591	C20	23.117	22323	C30	31.668	1279	C30	31.666	20149	
		C21	24.294	16111	C21	24.29	5874	C31	32.464	6396	C31	32.472	53210	
		C22	25.419	61948	C22	25.426	20783	C32	0	0	C32	33.247	6323	
		C23	26.503	67014	C23	26.494	13373	C33	33.998	101	C33	33.996	12290	
		C24	27.54	74205	C24	27.536	17981	C34	0	0	C34	34.729	1778	
		C25	28.534	883171	C25	28.536	19991	C35	35.412	353	C35	35.431	4520	
		C26	29.487	100433	C26	29.494	15914							
		C27	30.424	4430991	C27	30.41	42859							
		C28	31.304	137263	C28	31.3	16611							
		C29	32.168	1107100	C29	32.164	114433							
		C30	32.984	9531	C30	32.986	11791							
		C31	33.796	30970	C31	33.797	447281							
		C32	34.618	630	C32	34.624	460							
		C33	35.314	8878	C33	35.326	197379							
		C34	36.063	1287	C34	36.059	942							
		C35	36.764	16958	C35	36.761	129347							
			<b>ACL</b>	<b>27.11742235</b>	<b>Average</b>		<b>ACL</b>	<b>31.41196481</b>	<b>Average</b>			<b>ACL</b>	<b>27.69524885</b>	<b>Average</b>
			<b>C27/C31</b>	<b>143.0736519</b>	<b>27.02776</b>		<b>C27/C31</b>	<b>0.095821195</b>	<b>30.65023</b>			<b>C27/C31</b>	<b>7.09261417</b>	<b>27.49428</b>
			<b>C29/C33</b>	<b>124.7015093</b>	<b>29.03182</b>		<b>C29/C33</b>	<b>0.579762791</b>	<b>31.53203</b>			<b>C29/C33</b>	<b>34.01594793</b>	<b>29.11423</b>
			<b>CPI</b>	<b>16.98600949</b>			<b>CPI</b>	<b>25.38156237</b>				<b>CPI</b>	<b>2.688643185</b>	

Site	Bioregion	Dominant Spp 1	Growth form	Genetic Voucher No	Dominant Spp 2	Growth form	Genetic Voucher No	Dominant Spp 3	Growth form	Genetic Voucher No	Soil		
SATKAN001	Kanmantoo	Eucalyptus baxteri	Tree/Palm	SAT 000122	Lepidosperma semiteres	Sedge	SAT 000167	Pultenaea involucreta	Shrub	SAT 000124	SATKAN001	Kanmantoo	
		NAME	FOUND_RT	AREA	NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA
		C18	20.395	135.159	C10	8.517	186	C20	21.813	2558	C20	21.811	3775
		C25	28.329	2485.209	C11	9.627	234	C21	22.98	663	C21	22.983	12104
		C27	30.213	77272.953	C12	11.229	1586	C22	24.137	571	C22	24.109	13289
		C28	31.107	576.377	C13	13.009	1071	C23	25.174	888	C23	25.182	35128
		C29	31.965	62820.504	C14	14.69	3558	C24	26.216	1682	C24	26.219	35722
		C31	33.609	22881.779	C15	16.292	3069	C25	27.211	1596	C25	27.214	90750
		C32	34.394	235.546	C16	17.842	572	C26	28.163	1787	C26	28.167	57957
		C33	35.151	27998.75	C17	19.224	3413	C27	29.09	4690	C27	29.104	377555
		ACL	28.96522169	Average	C18	20.59	12309	C28	29.975	2001	C28	29.978	55893
		C27/C31	3.377051802	27.91386	C19	21.878	2121	C29	30.834	25476	C29	30.852	541364
		C29/C33	2.243689593	30.23316	C20	23.114	23752	C30	31.656	2996	C30	31.664	23944
		CPI	238.272835		C21	24.297	5532	C31	32.477	180401	C31	32.47	133756
					C22	25.423	20384	C32	33.237	2827	C32	33.245	14277
					C23	26.501	16103	C33	34.001	136195	C33	33.999	64296
					C24	27.538	20466	C34	34.713	185	C34	34.727	2756
					C25	28.527	18302	C35	35.436	49734	C35	35.439	34052
					C26	29.496	15358	ACL	31.98479246	Average	ACL	28.6866561	Average
					C27	30.423	17070	C27/C31	0.025997639	30.89864	C27/C31	2.822714495	28.04638
					C28	31.313	12197	C29/C33	0.187055325	32.36968	C29/C33	8.419870598	29.42463
					C29	32.155	44421	CPI	31.07681135		CPI	6.210456343	
					C30	32.993	7610						
					C31	33.799	222951						
					C32	34.621	549						
					C33	35.328	93157						
					C34	36.056	1254						
					C35	36.752	24634						
					ACL	31.04260287	Average						
					C27/C31	0.076563909	30.71552						
					C29/C33	0.476840173	31.70849						
					CPI	5.488280346							

Site	Bioregion	Dominant Spp 1	Growth form	Genetic Voucher No	Dominant Spp 2	Growth form	Genetic Voucher No	Dominant Spp 3	Growth form	Genetic Voucher No	Soil		
SATKAN002	Kanmantoo	Eucalyptus obliqua	Tree/Palm	SAT 000191	Lepidosperma semiteres	Sedge	SAT 000218	Hekea rostrata	Shrub	SAT 000207	SATKAN0002	Kanmantoo	
		NAME	FOUND_RT	AREA	NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA
		C16	17.61	1515.702	C10	8.475	594	C10	0	0	C20	21.807	2812
		C18	20.395	6484.721	C11	9.611	1427	C11	10.053	99	C21	22.985	7617
		C20	22.919	6201.095	C12	11.228	622	C12	0	0	C22	24.1	10797
		C22	25.217	4159.367	C13	12.993	697	C13	0	0	C23	25.179	35353
		C23	26.293	3248.991	C14	14.684	1509	C14	15.508	351	C24	26.215	57568
		C24	27.297	705.974	C15	16.281	1742	C15	17.215	423	C25	27.21	160753
		C24	27.326	6489.75	C16	17.794	5006	C16	18.822	2936	C26	28.168	135714
		C25	28.322	74610.914	C17	19.27	741	C17	20.34	233	C27	29.095	425304
		C26	29.26	25091.488	C18	20.589	16587	C18	21.806	2361	C28	29.974	108480
		C27	30.205	377055.563	C19	21.883	3377	C19	0	0	C29	30.844	448177
		C28	31.085	16187.328	C20	23.113	18572	C20	24.503	1489	C30	31.66	48957
		C29	31.958	146942.688	C21	24.291	6345	C21	25.749	184	C31	32.461	84612
		C30	32.78	868.014	C22	25.417	15990	C22	26.953	1232	C32	33.236	18449
		C31	33.594	950.179	C23	26.495	9406	C23	28.099	1015	C33	33.995	20089
		ACL	27.2476223	Average	C24	27.537	13593	C24	29.188	1288	C34	34.728	4936
		C27/C31	396.8258223	27.01005	C25	28.532	10913	C25	30.246	1814	C35	35.43	9941
		C29/C33	-	29	C26	29.49	11863	C26	31.256	1062	ACL	27.96908457	Average
		CPI	14.750266		C27	30.411	12805	C27	32.235	4890	C27/C31	5.026521061	27.66373
					C28	31.296	7664	C28	33.167	2043	C29/C33	22.3095724	29.1716
					C29	32.16	58660	C29	34.078	97442	CPI	3.073691677	
					C30	32.997	9416	C30	34.947	8232			
					C31	33.793	426614	C31	35.801	236536			
					C32	34.605	812	C32	36.607	1825			
					C33	35.322	237749	C33	37.397	2698			
					C34	36.05	1627	C34	0	0			
					C35	36.757	92392	C35	39.104	1412			
					ACL	31.72818969	Average	ACL	30.37851226	Average			
					C27/C31	0.030015424	30.88344	C27/C31	0.020673386	30.91898			
					C29/C33	0.246730796	32.20839	C29/C33	36.11638251	29.10777			
					CPI	13.21275322		CPI	22.01205203				

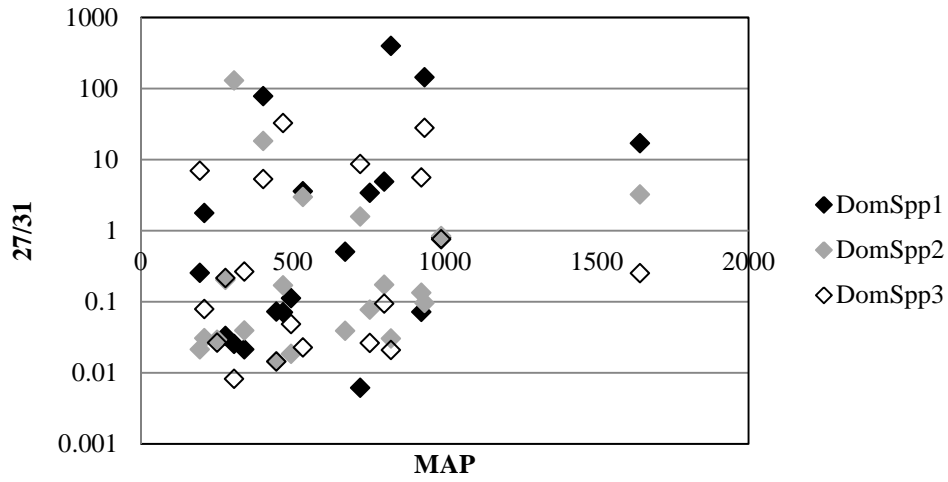
Site	Bioregion	Dominant Spp 1	Growth form	Genetic Voucher No	Dominant Spp 2	Growth form	Genetic Voucher No	Dominant Spp 3	Growth form	Genetic Voucher No	Soil				
SAASTP0001	Stony plains	Maireana aphylla	Chenopod	SAA 000250	Eragrostis setifolia	Tussock Grass	SAA 000294	Acacia aneura var. tenuis	Shrub	SAA 000338	SAASTP0001 Stony plains				
		<b>NAME</b>	<b>TIME</b>	<b>PEAK AREA</b>	<b>NAME</b>	<b>TIME</b>	<b>PEAK AREA</b>	<b>NAME</b>	<b>FOUND_RT</b>	<b>AREA</b>	<b>NAME</b>	<b>FOUND_RT</b>	<b>AREA</b>		
		C10	8.483	1450	C10	8.482	658	C27	30.213	37465.891	C20	21.809	10493		
		C11	9.619	1015	C11	9.628	391	C28	31.107	2316.905	C21	22.987	14012		
		C12	11.221	1054	C12	11.215	645	C29	31.965	67062.852	C22	24.107	23673		
		C13	13.001	1298	C13	12.99	590	C30	32.802	3415.121	C23	25.186	59744		
		C14	14.703	562	C14	14.691	10037	C31	33.616	478592.156	C24	26.222	79654		
		C15	16.289	4265	C15	16.283	7762	C32	34.394	33662.105	C25	27.217	121279		
		C16	17.797	30181	C16	17.791	27935	C33	35.158	558756.188	C26	28.175	99897		
		C17	19.226	20335	C17	19.22	8789	C34	35.885	1478.545	C27	29.092	93862		
		C18	20.587	95505	C18	20.592	44911		<b>ACL</b>	<b>31.72995869</b>	<b>Average</b>	C28	29.982	63607	
		C19	21.891	11707	C19	21.879	3458		<b>C27/C31</b>	<b>0.078283546</b>	<b>30.7096</b>	C29	30.84	61038	
		C20	23.116	84853	C20	23.115	37934		<b>C29/C33</b>	<b>0.120021672</b>	<b>32.57136</b>	C30	31.667	32517	
		C21	24.289	25598	C21	24.298	10965		<b>CPI</b>	<b>27.93741929</b>		C31	32.468	39882	
		C22	25.425	68877	C22	25.424	30367					C32	33.243	13651	
		C23	26.509	129020	C23	26.502	20356					C33	34.002	15686	
		C24	27.54	104415	C24	27.534	30810					C34	34.725	3822	
		C25	28.54	1071819	C25	28.534	32264					C35	35.442	14986	
		C26	29.493	139183	C26	29.487	25885						<b>ACL</b>	<b>27.72981228</b>	<b>Average</b>
		C27	30.419	357702	C27	30.413	55108						<b>C27/C31</b>	<b>2.353492804</b>	<b>28.19279</b>
		C28	31.304	126898	C28	31.303	17886						<b>C29/C33</b>	<b>3.891240597</b>	<b>29.81779</b>
		C29	32.168	425130	C29	32.162	147976						<b>CPI</b>	<b>1.281449146</b>	
		C30	32.99	45860	C30	32.989	34732								
		C31	33.796	203971	C31	33.801	1803911								
		C32	34.644	849	C32	34.565	21651								
		C33	35.32	6702	C33	35.324	177797								
		C34	36.037	1560	C34	36.052	2531								
		C35	36.744	33440	C35	36.759	55315								
			<b>ACL</b>	<b>26.91911334</b>	<b>Average</b>		<b>ACL</b>	<b>30.94142066</b>	<b>Average</b>						
			<b>C27/C31</b>	<b>1.753690476</b>	<b>28.4526</b>		<b>C27/C31</b>	<b>0.030549179</b>	<b>30.88143</b>						
			<b>C29/C33</b>	<b>63.43330349</b>	<b>29.06208</b>		<b>C29/C33</b>	<b>0.83227501</b>	<b>31.18308</b>						
			<b>CPI</b>	<b>4.560441882</b>			<b>CPI</b>	<b>13.856489</b>							

Site	Bioregion	Dominant Spp 1	Growth form	Genetic Voucher No	Dominant Spp 2	Growth form	Genetic Voucher No	Dominant Spp 3	Growth form	Genetic Voucher No	Soil			
SAASTP0004	Stony plains	<i>Malvastrum americanum</i> va Forb		SAA 000019	<i>Rutidosis helichrysoides</i> subsp Forb		SAA 000016	<i>Sida fubulifera</i>	Forb	SAA 000022	SAASTP0004 Stony plains			
		<b>NAME</b>	<b>TIME</b>	<b>PEAK AREA</b>	<b>NAME</b>	<b>TIME</b>	<b>PEAK AREA</b>	<b>NAME</b>	<b>TIME</b>	<b>PEAK AREA</b>	<b>NAME</b>	<b>TIME</b>	<b>PEAK AREA</b>	
		C10	8.446	384	C10	8.479	673	C10	0	0	C20	21.807	11124	
		C11	9.635	917	C11	9.609	1741	C11	0	0	C21	22.98	15045	
		C12	11.216	11775	C12	11.217	3017	C12	0	0	C22	24.1	26360	
		C13	12.996	2338	C13	12.997	3652	C13	0	0	C23	25.184	56157	
		C14	14.692	46846	C14	14.688	35594	C14	0	0	C24	26.215	103310	
		C15	16.284	27882	C15	16.279	20433	C15	0	0	C25	27.215	206761	
		C16	17.792	62889	C16	17.798	46197	C16	18.818	458	C26	28.168	221183	
		C17	19.226	23866	C17	19.227	8432	C17	0	0	C27	29.089	237569	
		C18	20.588	93883	C18	20.583	40806	C18	21.797	1498	C28	29.979	170842	
		C19	21.881	10092	C19	21.876	3891	C19	0	0	C29	30.833	148488	
		C20	23.121	84877	C20	23.112	30028	C20	24.498	1381	C30	31.665	80646	
		C21	24.299	20628	C21	24.3	6430	C21	25.755	524	C31	32.466	108762	
		C22	25.425	69453	C22	25.415	22929	C22	26.954	1430	C32	33.241	35558	
		C23	26.504	62511	C23	26.499	14050	C23	28.09	1763	C33	33.99	37119	
		C24	27.54	73483	C24	27.541	20405	C24	29.252	511	C34	34.718	10367	
		C25	28.535	178626	C25	28.53	22813	C25	30.252	3295	C35	35.43	32650	
		C26	29.498	77015	C26	29.494	19091	C26	31.252	1908				
		C27	30.42	512838	C27	30.41	51425	C27	32.236	10689		<b>ACL</b>	<b>28.04027619</b>	<b>Average</b>
		C28	31.31	117003	C28	31.3	19133	C28	33.173	1185		<b>C27/C31</b>	<b>2.184301502</b>	<b>28.25616</b>
		C29	32.168	1740982	C29	32.164	929720	C29	34.079	15287		<b>C29/C33</b>	<b>4.000323285</b>	<b>29.79995</b>
		C30	32.996	202182	C30	32.996	79294	C30	34.943	501		<b>CPI</b>	<b>1.262912909</b>	
		C31	33.802	2032081	C31	33.802	2419093	C31	35.791	1548				
		C32	34.577	36098	C32	34.567	146253	C32	0	0				
		C33	35.325	97370	C33	35.326	1239854	C33	37.404	255				
		C34	36.037	604	C34	36.017	252058	C34	0	0				
		C35	36.786	9884	C35	36.761	663899	C35	39.079	511				
			<b>ACL</b>	<b>29.60649581</b>	<b>Average</b>		<b>ACL</b>	<b>31.55066528</b>	<b>Average</b>		<b>ACL</b>	<b>28.13325946</b>	<b>Average</b>	
			<b>C27/C31</b>	<b>0.252370845</b>	<b>30.19394</b>		<b>C27/C31</b>	<b>0.021257967</b>	<b>30.91674</b>		<b>C27/C31</b>	<b>6.90503876</b>	<b>27.50601</b>	
			<b>C29/C33</b>	<b>17.88006573</b>	<b>29.21186</b>		<b>C29/C33</b>	<b>0.749862484</b>	<b>31.28589</b>		<b>C29/C33</b>	<b>59.94901961</b>	<b>29.06563</b>	
			<b>CPI</b>	<b>8.05723832</b>			<b>CPI</b>	<b>8.96361079</b>			<b>CPI</b>	<b>6.026106594</b>		



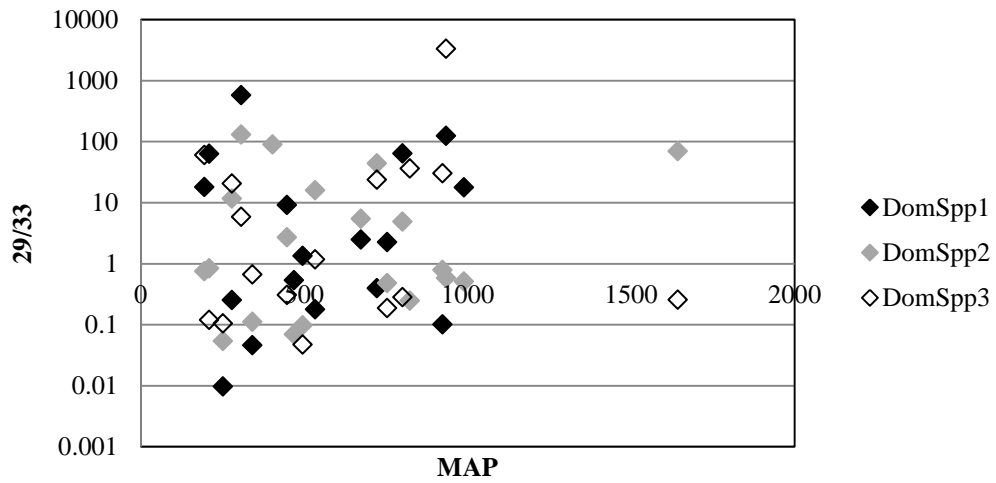


### MAP v 27/31 ratio



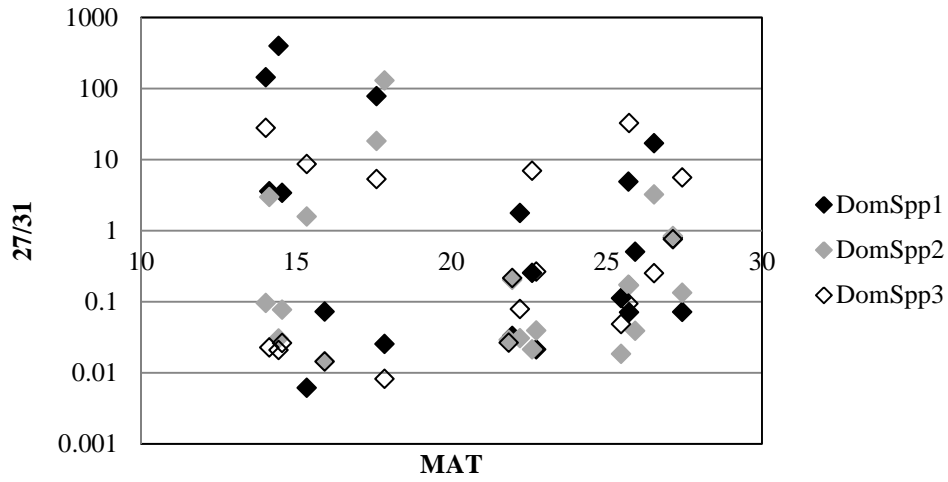
(a)

### MAP v 29/31 ratio



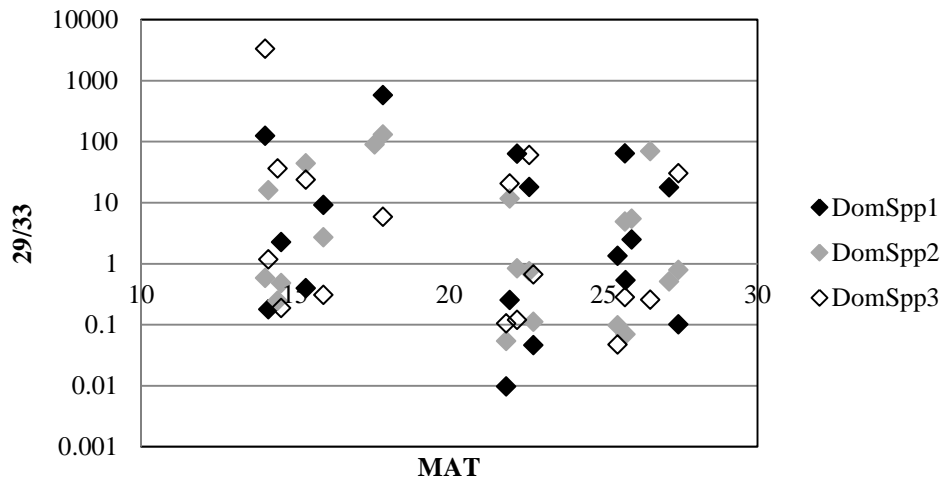
(b)

### MAT v 27/31 ratio



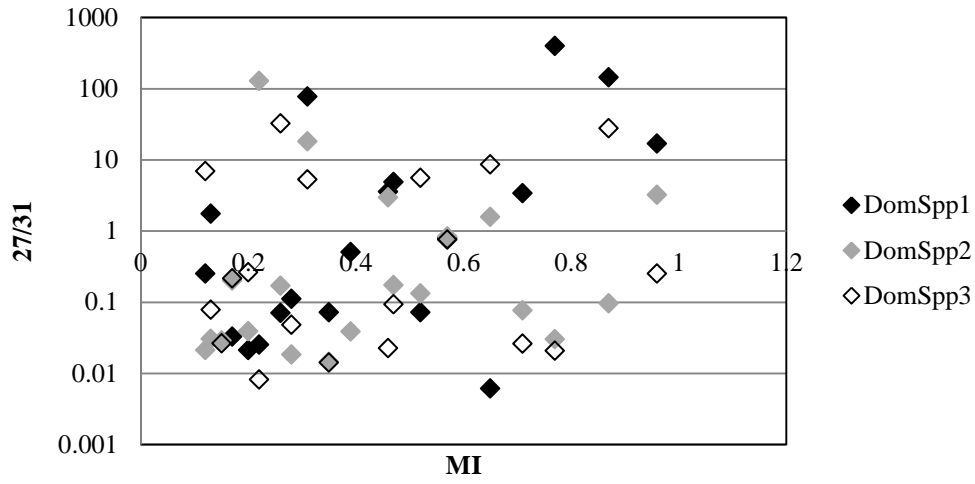
(c)

### MAT v 29/33 ratio



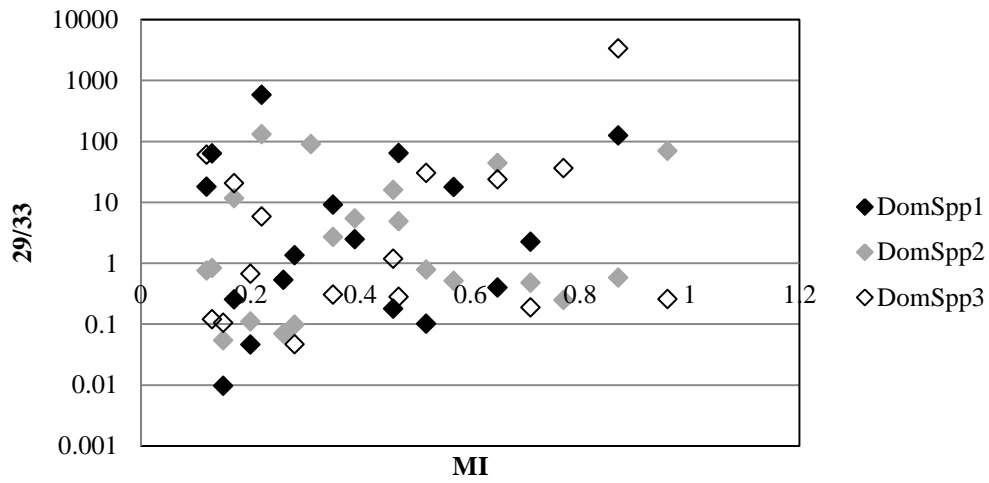
(d)

### MI v 27/31 ratio



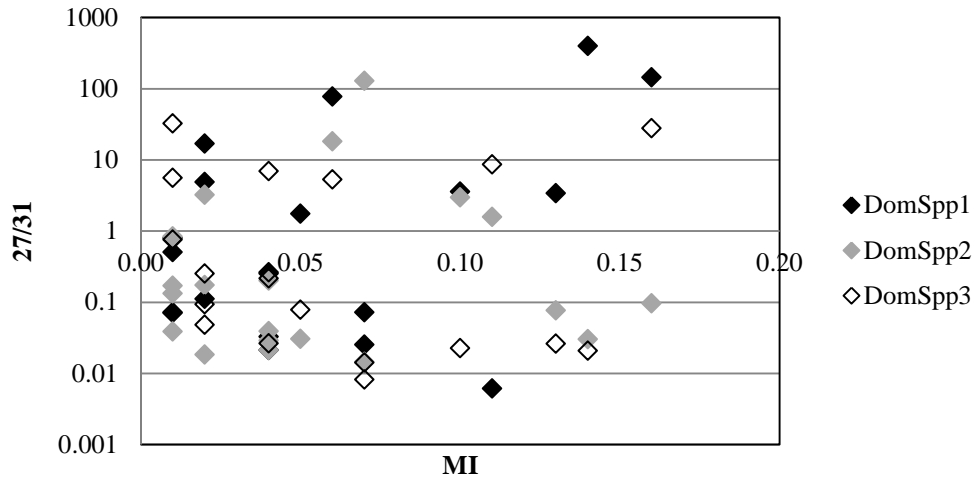
(e)

### MI v 29/33 ratio



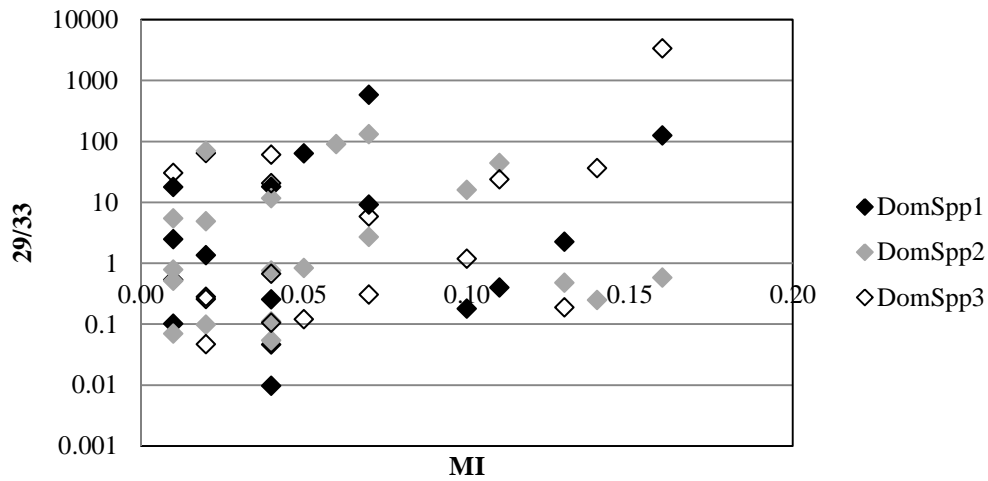
(f)

### MI - lowest quarter mean v 27/31 ratio



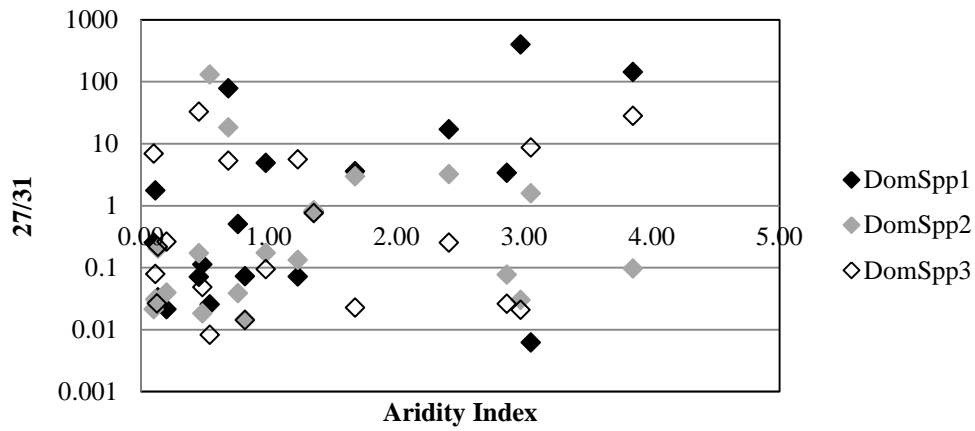
(g)

### MI - lowest quarter mean v 29/33 ratio



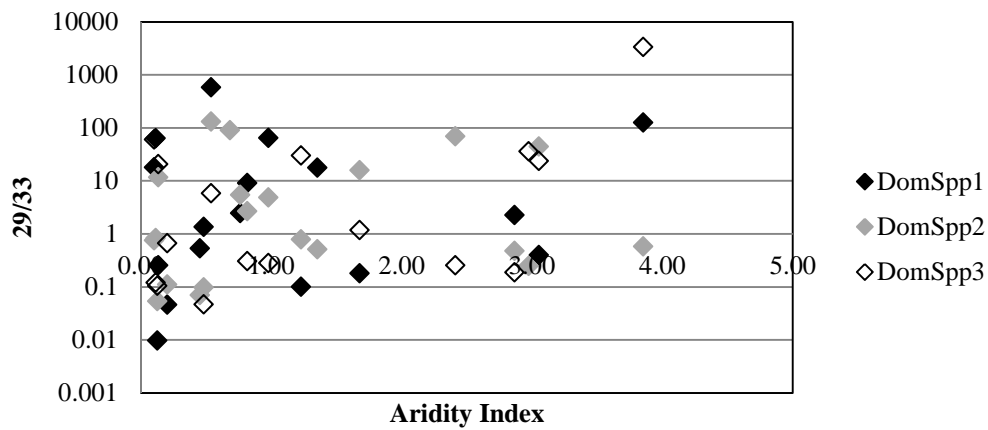
(h)

### Aridity Index - month max v 27/31 ratio



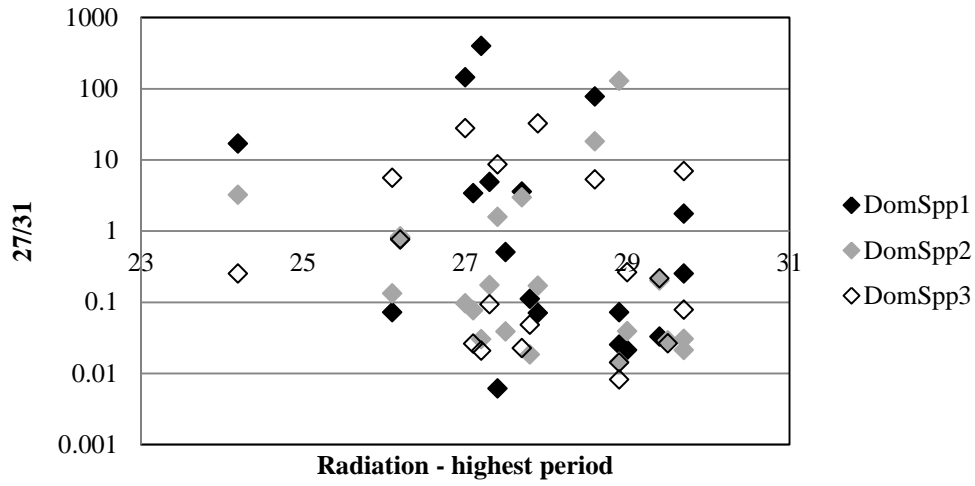
(i)

### Aridity Index - month max v 29/33 ratio



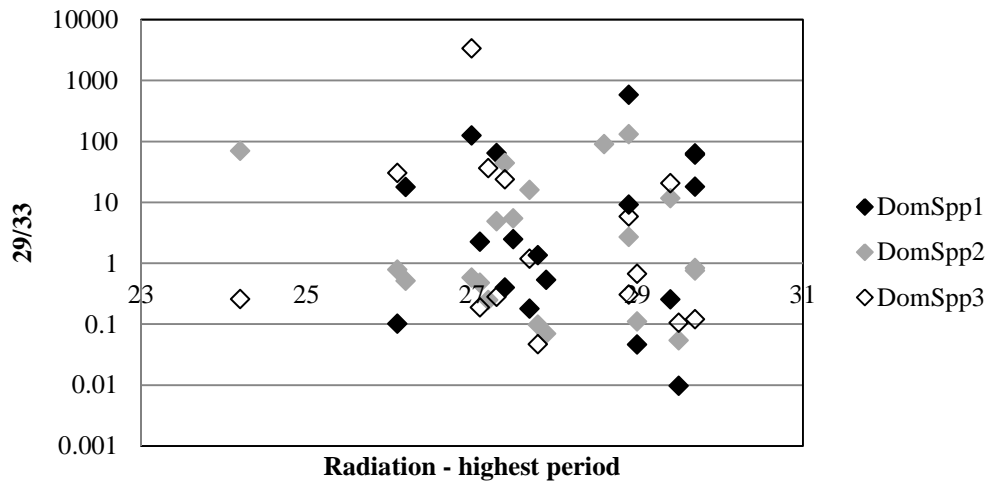
(j)

### Radiation - highest period v 27/31 ratio



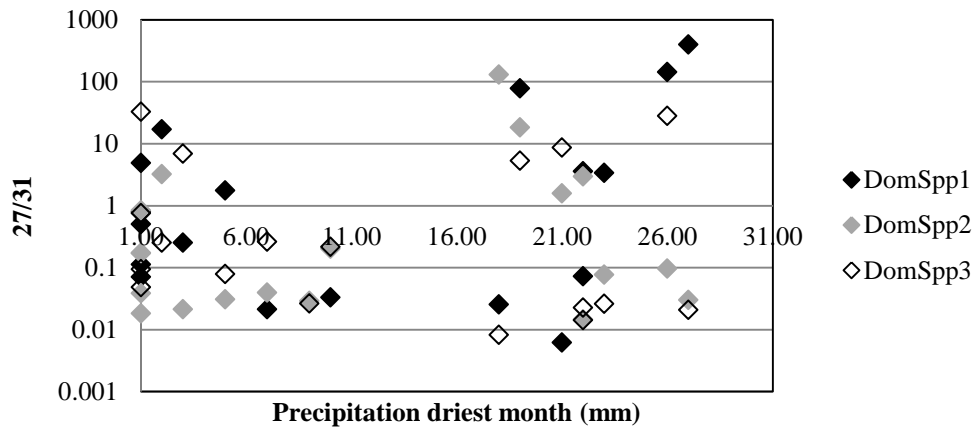
(k)

### Radiation - highest period v 29/33 ratio



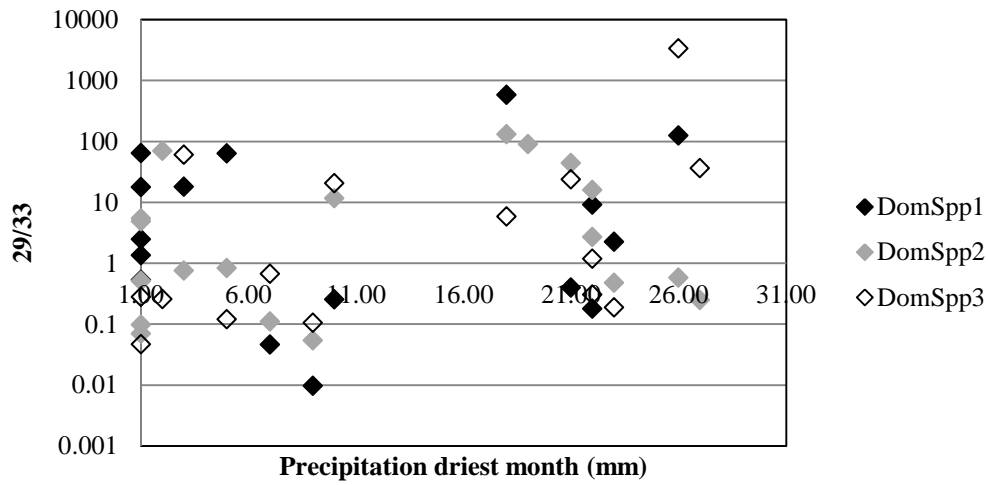
(l)

### Precipitation driest month v 27/31 ratio



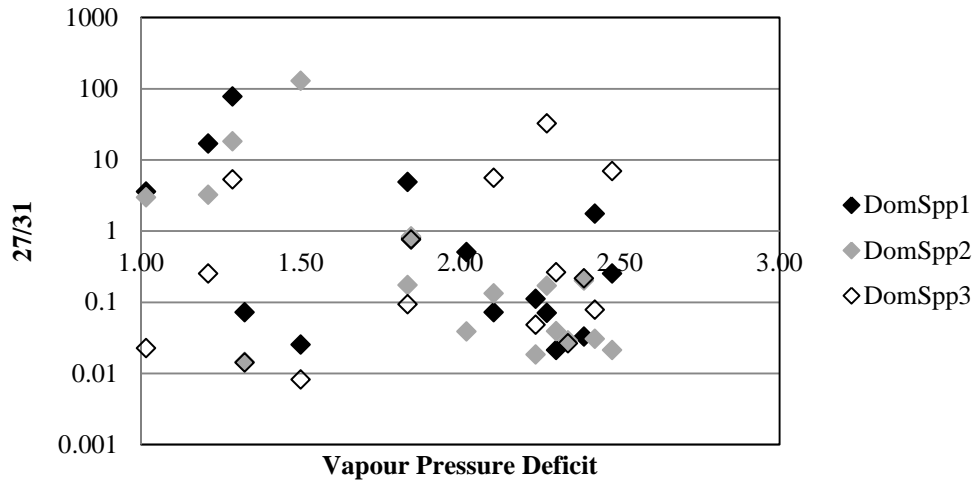
(m)

### Precipitation driest month v 29/33 ratio



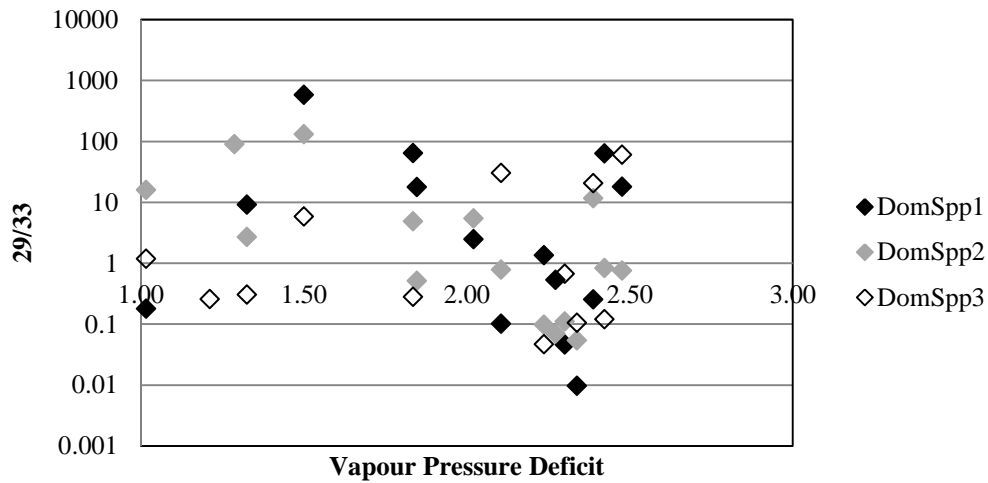
(n)

### Vapour Pressure Deficit v 27/31 ratio



(o)

### Vapour Pressure Deficit v 29/33 ratio

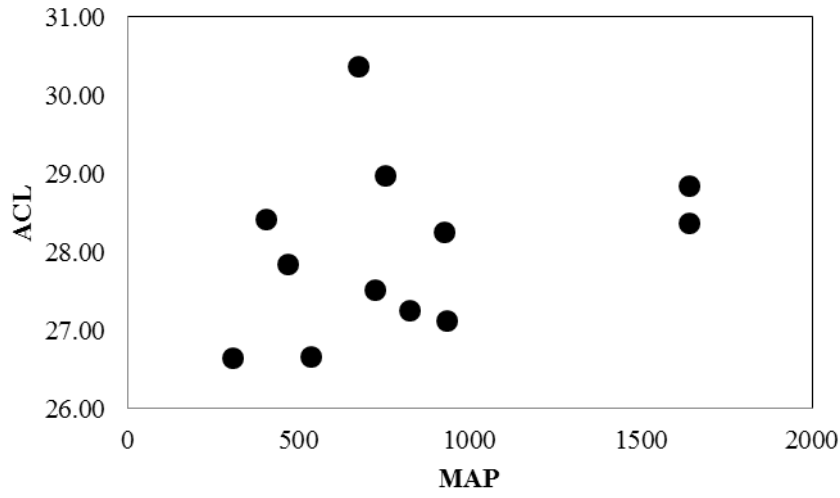


(p)

Figure 2: Plots (a)-(p) showing that there is no relationship between the plant 27/31 and 29/33 chain length ratios to the different climate variables MAP, MAT, annual MI, lowest quarter mean MI, aridity index, radiation, driest month precipitation and vapour pressure deficit.

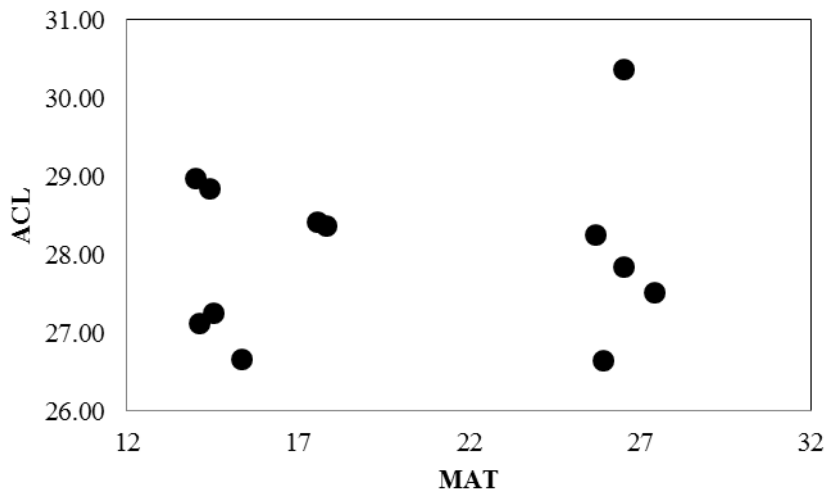


### Eucalyptus genus MAP v ACL



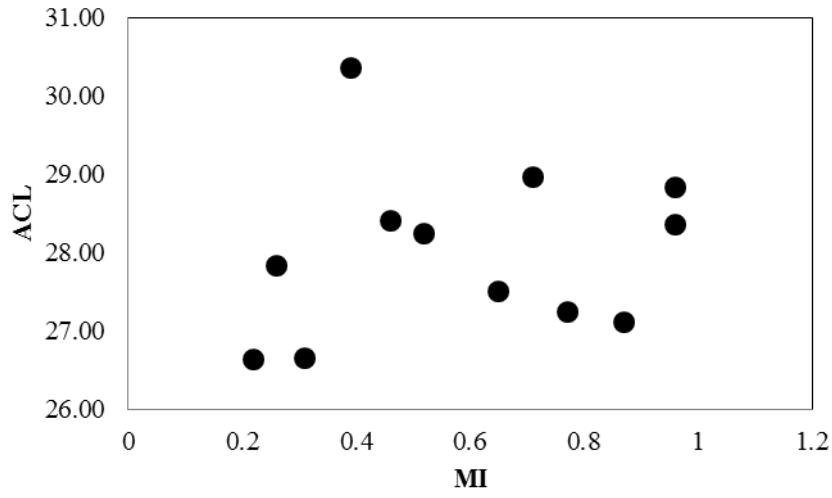
(a)

### Eucalyptus genus MAT v ACL



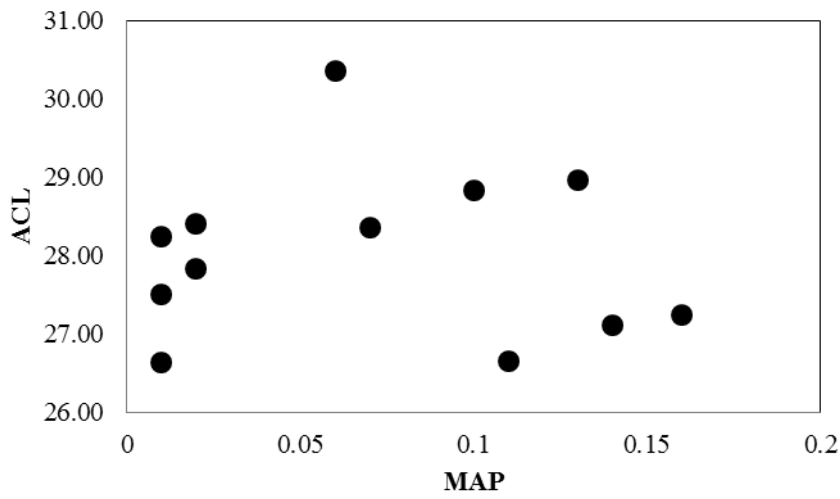
(b)

### Eucalyptus genus Ann MI v ACL



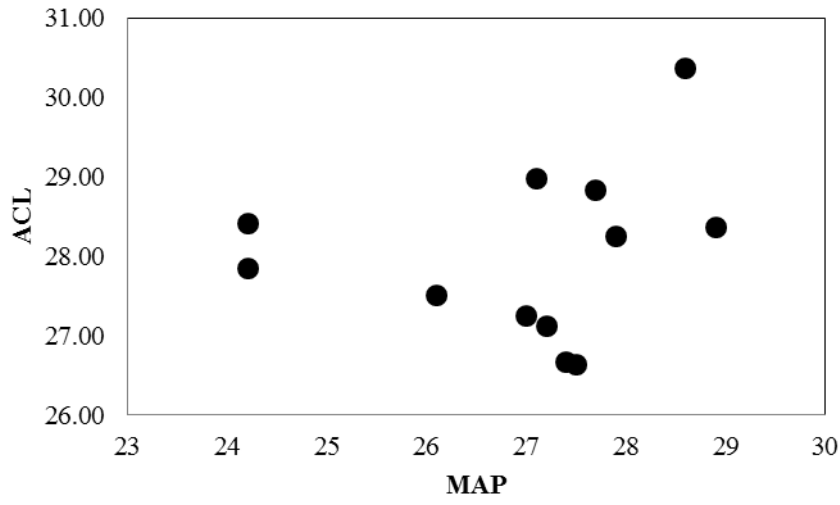
(c)

### Eucalyptus genus Low MI v ACL



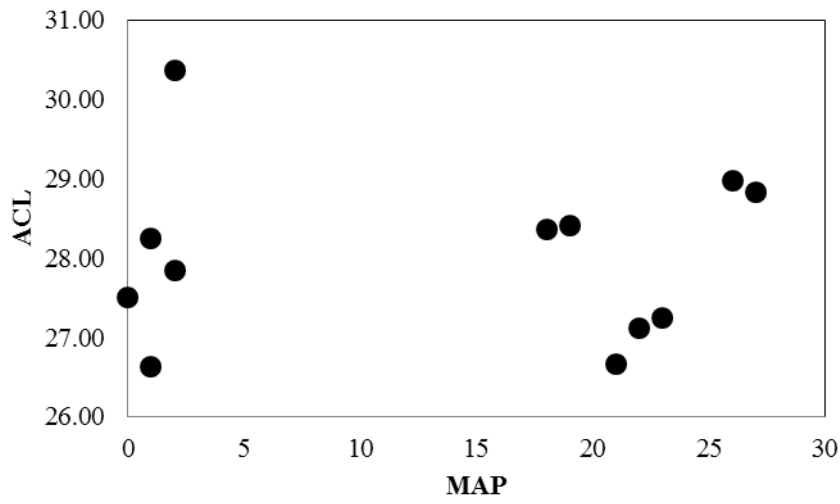
(d)

### Eucalyptus genus Radiation v ACL



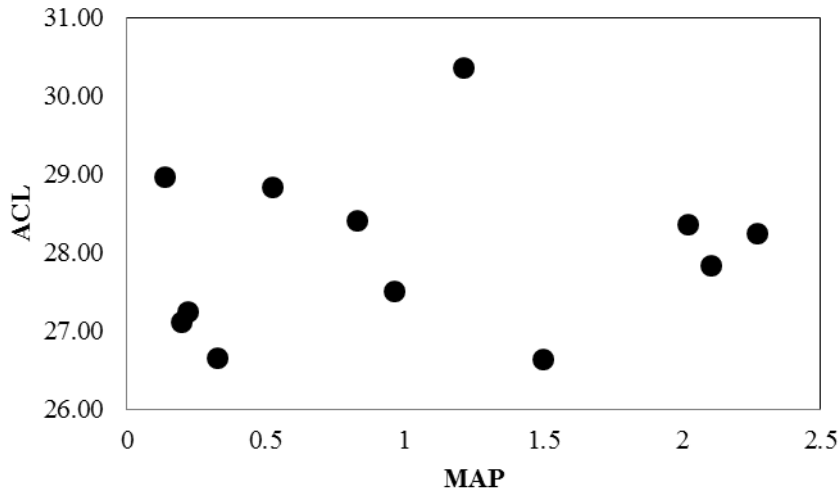
(e)

### Eucalyptus genus Low Precip v ACL



(f)

## Eucalyptus genus VPD v ACL

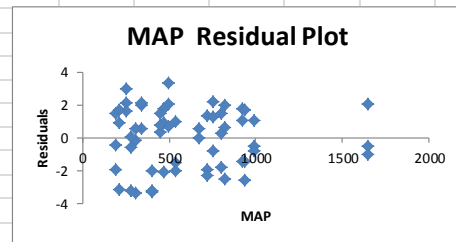


(g)

**Figure 2: Plots (a)-(p) showing that there is no relationship Eucalyptus genus ACL with the different climate variables MAP, MAT, annual MI, lowest quarter mean MI, aridity index, radiation, driest month precipitation and vapour pressure deficit.**

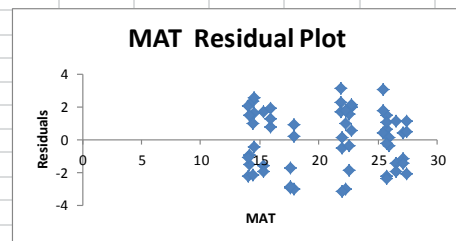
### Following Tables: Regression analyses for plants and soils

SUMMARY OUTPUT								
<b>Regression Statistics</b>								
Multiple F	0.093468							
R Square	0.008736							
Adjusted R	-0.00865							
Standard Error	1.813193							
Observations	59							
<b>ANOVA</b>								
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>			
Regression	1	1.651582	1.651582	0.502356	0.481355			
Residual	57	187.3971	3.287669					
Total	58	189.0487						
	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	30.17651	0.475957	63.40174	1.49E-54	29.22342	31.12959	29.22342	31.12959
MAP	-0.00048	0.000679	-0.70877	0.481355	-0.00184	0.000879	-0.00184	0.000879



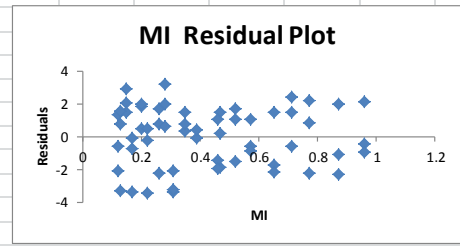
### Plant ACL v MAP

SUMMARY OUTPUT								
<b>Regression Statistics</b>								
Multiple F	0.198398							
R Square	0.039362							
Adjusted R	0.022508							
Standard Error	1.784964							
Observations	59							
<b>ANOVA</b>								
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>			
Regression	1	7.441289	7.441289	2.335551	0.131981			
Residual	57	181.6074	3.186095					
Total	58	189.0487						
	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	28.34801	1.03131	27.48737	1.41E-34	26.28285	30.41317	26.28285	30.41317
MAT	0.07356	0.048134	1.528251	0.131981	-0.02283	0.169947	-0.02283	0.169947



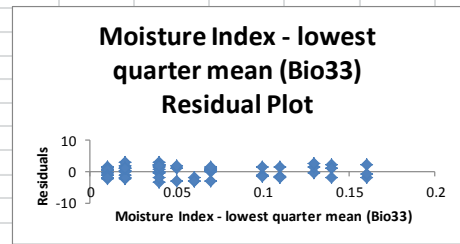
### Plant ACL v MAT

SUMMARY OUTPUT								
<b>Regression Statistics</b>								
Multiple F	0.151021							
R Square	0.022807							
Adjusted R	0.005664							
Standard Error	1.800278							
Observations	59							
<b>ANOVA</b>								
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>			
Regression	1	4.311717	4.311717	1.330366	0.253554			
Residual	57	184.737	3.241					
Total	58	189.0487						
	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	30.34331	0.462386	65.62326	2.15E-55	29.4174	31.26922	29.4174	31.26922
MI	-1.07253	0.929871	-1.15341	0.253554	-2.93456	0.789508	-2.93456	0.789508



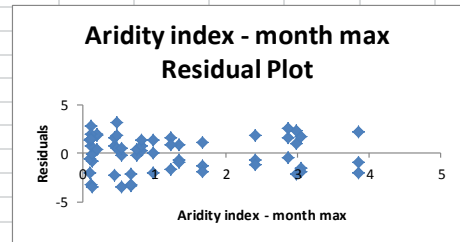
**Plant ACL v Annual MI**

SUMMARY OUTPUT								
<b>Regression Statistics</b>								
Multiple F	0.170011							
R Square	0.028904							
Adjusted R	0.011867							
Standard Error	1.794653							
Observations	59							
<b>ANOVA</b>								
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>			
Regression	1	5.46421	5.46421	1.696548	0.197978			
Residual	57	183.5845	3.220781					
Total	58	189.0487						
	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	30.2712	0.378354	80.00762	3.01E-60	29.51356	31.02884	29.51356	31.02884
Moisture	-6.64815	5.104084	-1.30252	0.197978	-16.8689	3.5726	-16.8689	3.5726



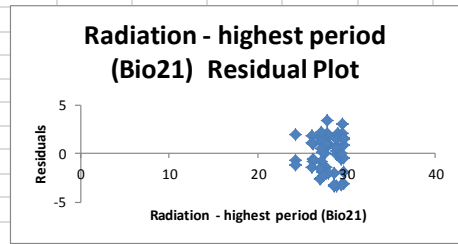
**Plant ACL v lowest quarter mean MI**

SUMMARY OUTPUT								
<b>Regression Statistics</b>								
Multiple F	0.177663							
R Square	0.031564							
Adjusted R	0.014574							
Standard Error	1.792194							
Observations	59							
<b>ANOVA</b>								
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>			
Regression	1	5.96713	5.96713	1.857786	0.178241			
Residual	57	183.0816	3.211958					
Total	58	189.0487						
	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	30.23002	0.345031	87.6154	1.77E-62	29.53911	30.92094	29.53911	30.92094
Aridity inc	-0.27741	0.203529	-1.36301	0.178241	-0.68497	0.130148	-0.68497	0.130148



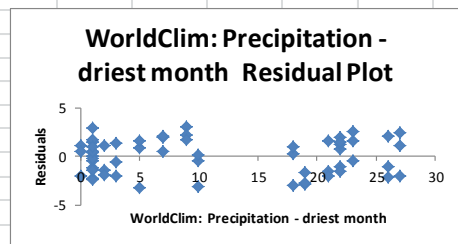
**Plant ACL v aridity index month max**

SUMMARY OUTPUT								
<b>Regression Statistics</b>								
Multiple F	0.08117							
R Square	0.006589							
Adjusted R	-0.01084							
Standard Error	1.815156							
Observations	59							
<b>ANOVA</b>								
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>			
Regression	1	1.245574	1.245574	0.378043	0.541099			
Residual	57	187.8032	3.294792					
Total	58	189.0487						
	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	26.95145	4.774675	5.644667	5.45E-07	17.39033	36.51258	17.39033	36.51258
Radiation	0.105241	0.171165	0.614852	0.541099	-0.23751	0.447993	-0.23751	0.447993



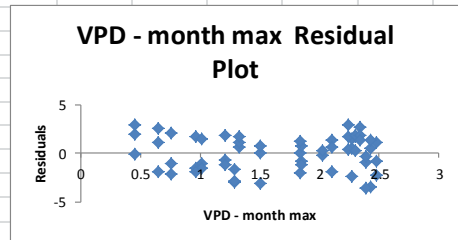
**Plant ACL v radiation highest period**

SUMMARY OUTPUT								
<b>Regression Statistics</b>								
Multiple F	0.215792							
R Square	0.046566							
Adjusted R	0.029839							
Standard Error	1.778258							
Observations	59							
<b>ANOVA</b>								
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>			
Regression	1	8.80328	8.80328	2.783909	0.1007			
Residual	57	180.2454	3.162201					
Total	58	189.0487						
	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	30.32409	0.351145	86.35772	4.01E-62	29.62094	31.02725	29.62094	31.02725
WorldClim	-0.03962	0.023746	-1.66851	0.1007	-0.08717	0.00793	-0.08717	0.00793



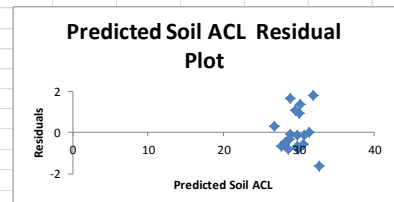
**Plant ACL v precipitation – driest month**

SUMMARY OUTPUT								
<b>Regression Statistics</b>								
Multiple F	0.240763							
R Square	0.057967							
Adjusted R	0.04144							
Standard Error	1.767594							
Observations	59							
<b>ANOVA</b>								
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>			
Regression	1	10.95854	10.95854	3.507417	0.066226			
Residual	57	178.0902	3.124389					
Total	58	189.0487						
	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	28.78363	0.630797	45.63058	1.44E-46	27.52048	30.04678	27.52048	30.04678
VPD - mor	0.660739	0.352806	1.87281	0.066226	-0.04574	1.36722	-0.04574	1.36722



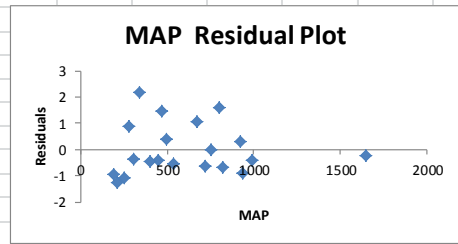
**Plant ACL v vapour pressure deficit month max**

SUMMARY OUTPUT								
<b>Regression Statistics</b>								
Multiple R	0.33832985							
R Square	0.11444462							
Adjusted R Square	0.059176489							
Standard Error	0.969467409							
Observations	19							
<b>ANOVA</b>								
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>			
Regression	1	2.003961	2.003961	2.132174946	0.16247			
Residual	17	15.97774	0.939867					
Total	18	17.9817						
	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	21.93751098	4.708313	4.659314	0.000224815	12.00384	31.87118	12.00384	31.87118
Predicted Soil ACL	0.231271592	0.158384	1.460197	0.162470209	-0.10289	0.565432	-0.10289	0.565432



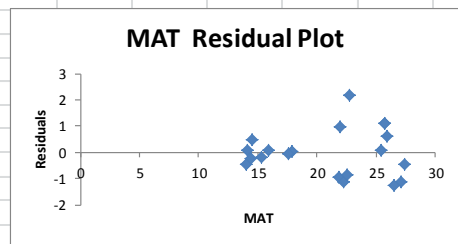
**Predicted v Actual Soil ACL**

SUMMARY OUTPUT								
<b>Regression Statistics</b>								
Multiple R	0.185174							
R Square	0.034289							
Adjusted R Square	-0.01936							
Standard Error	0.999454							
Observations	20							
<b>ANOVA</b>								
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>			
Regression	1	0.638423	0.638423	0.639121	0.434453			
Residual	18	17.98035	0.998908					
Total	19	18.61877						
	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	29.07987	0.453985	64.05468	1.08E-22	28.12608	30.03366	28.12608	30.03366
MAP	-0.00052	0.000648	-0.79945	0.434453	-0.00188	0.000844	-0.00188	0.000844



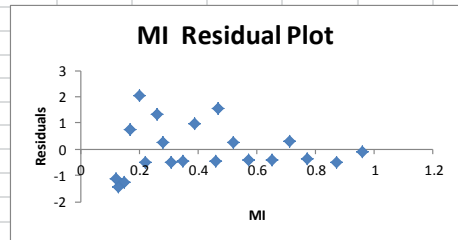
### All Soils ACL v MAP

SUMMARY OUTPUT								
<b>Regression Statistics</b>								
Multiple R	0.434091							
R Square	0.188435							
Adjusted R Square	0.143348							
Standard Error	0.916222							
Observations	20							
<b>ANOVA</b>								
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>			
Regression	1	3.508429	3.508429	4.179372	0.055829			
Residual	18	15.11034	0.839463					
Total	19	18.61877						
	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	26.9468	0.91217	29.54143	1.05E-16	25.0304	28.8632	25.0304	28.8632
MAT	0.0867	0.04241	2.044351	0.055829	-0.0024	0.1758	-0.0024	0.1758



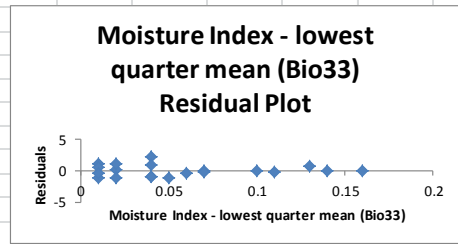
### All Soils ACL v MAT

SUMMARY OUTPUT								
<b>Regression Statistics</b>								
Multiple R	0.340886							
R Square	0.116203							
Adjusted R Square	0.067103							
Standard Error	0.956127							
Observations	20							
<b>ANOVA</b>								
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>			
Regression	1	2.163557	2.163557	2.366668	0.141345			
Residual	18	16.45521	0.914178					
Total	19	18.61877						
	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	29.32706	0.423896	69.18456	2.7E-23	28.43648	30.21763	28.43648	30.21763
MI	-1.31566	0.855213	-1.5384	0.141345	-3.11239	0.481078	-3.11239	0.481078



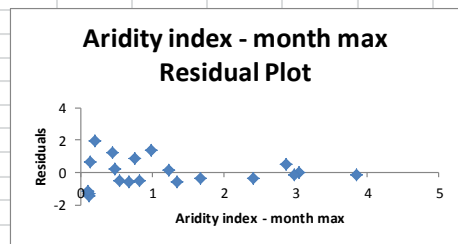
### All Soils ACL v Annual MI

SUMMARY OUTPUT								
<b>Regression Statistics</b>								
Multiple R	0.46878							
R Square	0.219754							
Adjusted R Square	0.176407							
Standard Error	0.898369							
Observations	20							
<b>ANOVA</b>								
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>			
Regression	1	4.091555	4.091555	5.069657	0.037077			
Residual	18	14.52721	0.807067					
Total	19	18.61877						
	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	29.33165	0.322372	90.98695	1.98E-25	28.65437	30.00893	28.65437	30.00893
Moisture	-9.87299	4.384899	-2.25159	0.037077	-19.0853	-0.66066	-19.0853	-0.66066



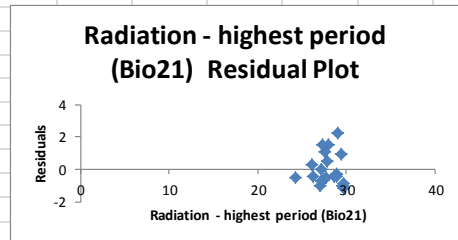
**All Soils ACL v lowest quarter mean MI**

SUMMARY OUTPUT								
<b>Regression Statistics</b>								
Multiple R	0.425022							
R Square	0.180644							
Adjusted R Square	0.135124							
Standard Error	0.92061							
Observations	20							
<b>ANOVA</b>								
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>			
Regression	1	3.36336	3.36336	3.96846	0.061752			
Residual	18	15.25541	0.847523					
Total	19	18.61877						
	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	29.21085	0.304471	95.93967	7.63E-26	28.57118	29.85052	28.57118	29.85052
Aridity inc	-0.36019	0.18081	-1.9921	0.061752	-0.74006	0.019676	-0.74006	0.019676



**All Soils ACL v Aridity Index month max**

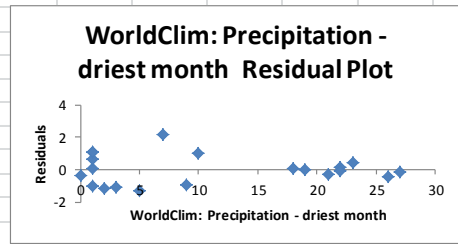
SUMMARY OUTPUT								
<b>Regression Statistics</b>								
Multiple R	0.118481							
R Square	0.014038							
Adjusted R Square	-0.04074							
Standard Error	1.009879							
Observations	20							
<b>ANOVA</b>								
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>			
Regression	1	0.261368	0.261368	0.256279	0.618832			
Residual	18	18.3574	1.019856					
Total	19	18.61877						
	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	26.43938	4.597383	5.750964	1.88E-05	16.78064	36.09813	16.78064	36.09813
Radiation	0.083453	0.164848	0.50624	0.618832	-0.26288	0.429785	-0.26288	0.429785



**All Soils ACL v radiation highest period**

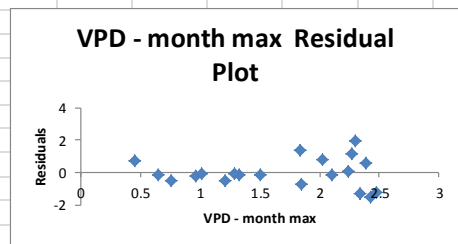


SUMMARY OUTPUT								
<b>Regression Statistics</b>								
Multiple R	0.428826							
R Square	0.183891							
Adjusted R Square	0.138552							
Standard Error	0.918784							
Observations	20							
<b>ANOVA</b>								
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>			
Regression	1	3.423831	3.423831	4.055888	0.059213			
Residual	18	15.19494	0.844163					
Total	19	18.61877						
	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	29.22842	0.308865	94.63159	9.76E-26	28.57952	29.87733	28.57952	29.87733
WorldClim	-0.04242	0.021062	-2.01392	0.059213	-0.08667	0.001832	-0.08667	0.001832



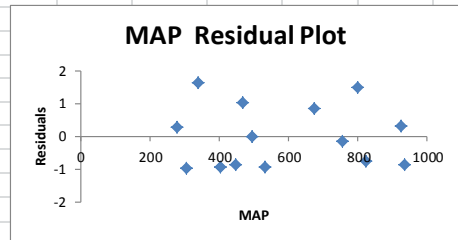
**All Soils ACL v precipitation driest month**

SUMMARY OUTPUT								
<b>Regression Statistics</b>								
Multiple R	0.430006							
R Square	0.184905							
Adjusted R Square	0.139622							
Standard Error	0.918213							
Observations	20							
<b>ANOVA</b>								
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>			
Regression	1	3.442712	3.442712	4.083328	0.058441			
Residual	18	15.17606	0.843114					
Total	19	18.61877						
	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	27.69496	0.567463	48.80491	1.4E-20	26.50276	28.88715	26.50276	28.88715
VPD - month	0.639868	0.316653	2.020725	0.058441	-0.02539	1.305131	-0.02539	1.305131



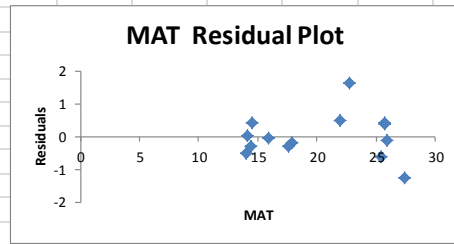
**All Soils ACL v vapour pressure deficit month max**

SUMMARY OUTPUT								
<b>Regression Statistics</b>								
Multiple R	0.343359							
R Square	0.117896							
Adjusted R Square	0.044387							
Standard Error	0.986836							
Observations	14							
<b>ANOVA</b>								
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>			
Regression	1	1.561882	1.561882	1.603831	0.229392			
Residual	12	11.68614	0.973845					
Total	13	13.24802						
	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	29.98308	0.745928	40.19567	3.63E-14	28.35784	31.60832	28.35784	31.60832
MAP	-0.00151	0.001194	-1.26642	0.229392	-0.00411	0.001089	-0.00411	0.001089



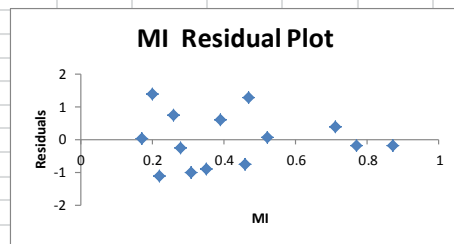
**Soils CPI>1.5 v MAP**

SUMMARY OUTPUT								
<b>Regression Statistics</b>								
Multiple R	0.748655							
R Square	0.560484							
Adjusted R Square	0.523858							
Standard Error	0.696582							
Observations	14							
<b>ANOVA</b>								
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>			
Regression	1	7.425302	7.425302	15.30276	0.002065			
Residual	12	5.822716	0.485226					
Total	13	13.24802						
	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	26.16642	0.77254	33.87066	2.79E-13	24.4832	27.84964	24.4832	27.84964
MAT	0.14484	0.037026	3.911874	0.002065	0.064168	0.225512	0.064168	0.225512



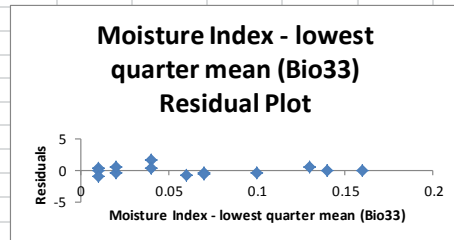
**Soils CPI>1.5 v MAT**

SUMMARY OUTPUT								
<b>Regression Statistics</b>								
Multiple R	0.60753							
R Square	0.369092							
Adjusted R Square	0.316517							
Standard Error	0.83458							
Observations	14							
<b>ANOVA</b>								
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>			
Regression	1	4.88974	4.88974	7.020212	0.021196			
Residual	12	8.358278	0.696523					
Total	13	13.24802						
	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	30.28336	0.499412	60.63799	2.68E-16	29.19523	31.37148	29.19523	31.37148
MI	-2.77171	1.046101	-2.64957	0.021196	-5.05097	-0.49246	-5.05097	-0.49246



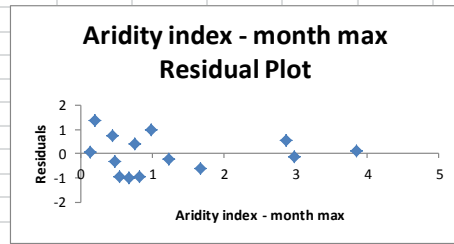
**Soils CPI>1.5 v Annual MI**

SUMMARY OUTPUT								
<b>Regression Statistics</b>								
Multiple R	0.735455							
R Square	0.540894							
Adjusted R Square	0.502635							
Standard Error	0.711936							
Observations	14							
<b>ANOVA</b>								
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>			
Regression	1	7.165775	7.165775	14.13776	0.002721			
Residual	12	6.082242	0.506854					
Total	13	13.24802						
	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	30.00552	0.307041	97.72493	8.82E-19	29.33653	30.6745	29.33653	30.6745
Moisture Index - lowest quarter mean (Bio33)	-14.4149	3.833731	-3.76002	0.002721	-22.7679	-6.06193	-22.7679	-6.06193



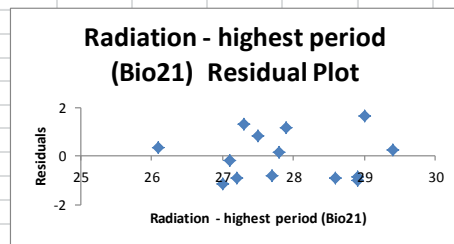
**Soils CPI>1.5 v lowest quarter mean MI**

SUMMARY OUTPUT								
<b>Regression Statistics</b>								
Multiple R	0.669763							
R Square	0.448582							
Adjusted R Square	0.402631							
Standard Error	0.780235							
Observations	14							
<b>ANOVA</b>								
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>			
Regression	1	5.942822	5.942822	9.762076	0.008782			
Residual	12	7.305195	0.608766					
Total	13	13.24802						
	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	29.83557	0.314633	94.82666	1.26E-18	29.15005	30.5211	29.15005	30.5211
Aridity inc	-0.58381	0.186854	-3.12443	0.008782	-0.99093	-0.17669	-0.99093	-0.17669



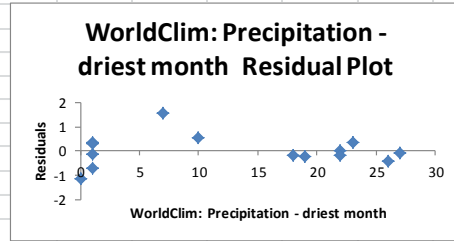
**Soils CPI>1.5 v aridity index month max**

SUMMARY OUTPUT								
<b>Regression Statistics</b>								
Multiple R	0.283115							
R Square	0.080154							
Adjusted R Square	0.0035							
Standard Error	1.007726							
Observations	14							
<b>ANOVA</b>								
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>			
Regression	1	1.061883	1.061883	1.045664	0.326681			
Residual	12	12.18613	1.015511					
Total	13	13.24802						
	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	20.69748	8.220863	2.517678	0.027024	2.785762	38.60921	2.785762	38.60921
Radiation	0.3013	0.294647	1.022577	0.326681	-0.34068	0.943281	-0.34068	0.943281



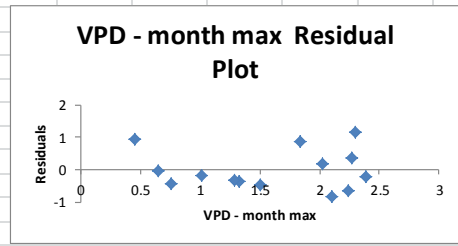
**Soils CPI>1.5 v radiation highest period**

SUMMARY OUTPUT								
<b>Regression Statistics</b>								
Multiple R	0.77697							
R Square	0.603683							
Adjusted R Square	0.570656							
Standard Error	0.661464							
Observations	14							
<b>ANOVA</b>								
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>			
Regression	1	7.997601	7.997601	18.27878	0.001078			
Residual	12	5.250416	0.437535					
Total	13	13.24802						
	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	30.03506	0.281325	106.7628	3.05E-19	29.42211	30.64802	29.42211	30.64802
WorldClim	-0.07359	0.017212	-4.27537	0.001078	-0.11109	-0.03609	-0.11109	-0.03609



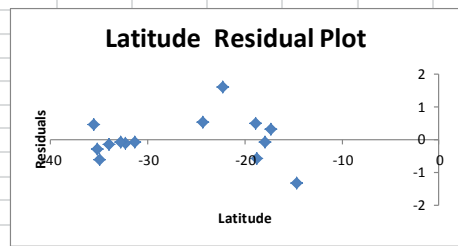
**Soils CPI>1.5 v precipitation driest month**

SUMMARY OUTPUT								
<b>Regression Statistics</b>								
Multiple R	0.792683							
R Square	0.628346							
Adjusted R Square	0.597374							
Standard Error	0.640552							
Observations	14							
<b>ANOVA</b>								
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>			
Regression	1	8.324335	8.324335	20.28807	0.000721			
Residual	12	4.923683	0.410307					
Total	13	13.24802						
	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	27.21997	0.45102	60.35203	2.83E-16	26.23729	28.20266	26.23729	28.20266
VPD - month	1.18785	0.263719	4.504228	0.000721	0.613256	1.762444	0.613256	1.762444



**Soils CPI>1.5 v vapour pressure deficit month max**

SUMMARY OUTPUT								
<b>Regression Statistics</b>								
Multiple R	0.739554							
R Square	0.546941							
Adjusted R Square	0.509186							
Standard Error	0.707233							
Observations	14							
<b>ANOVA</b>								
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>			
Regression	1	7.245881	7.245881	14.4866	0.002502			
Residual	12	6.002136	0.500178					
Total	13	13.24802						
	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 95.0%</i>	<i>Upper 95.0%</i>
Intercept	31.58525	0.679909	46.45512	6.47E-15	30.10385	33.06664	30.10385	33.06664
Latitude	0.093916	0.024675	3.806127	0.002502	0.040154	0.147677	0.040154	0.147677



**Soils CPI>1.5 v latitude**