

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

Thesis submitted in accordance with the requirements of the University of  
Adelaide for an Honours Degree in Geology.

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November 2014



## 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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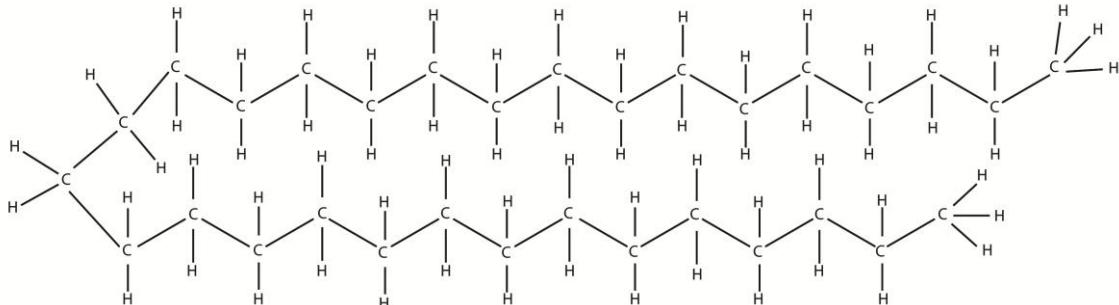
## INTRODUCTION

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:

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

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.

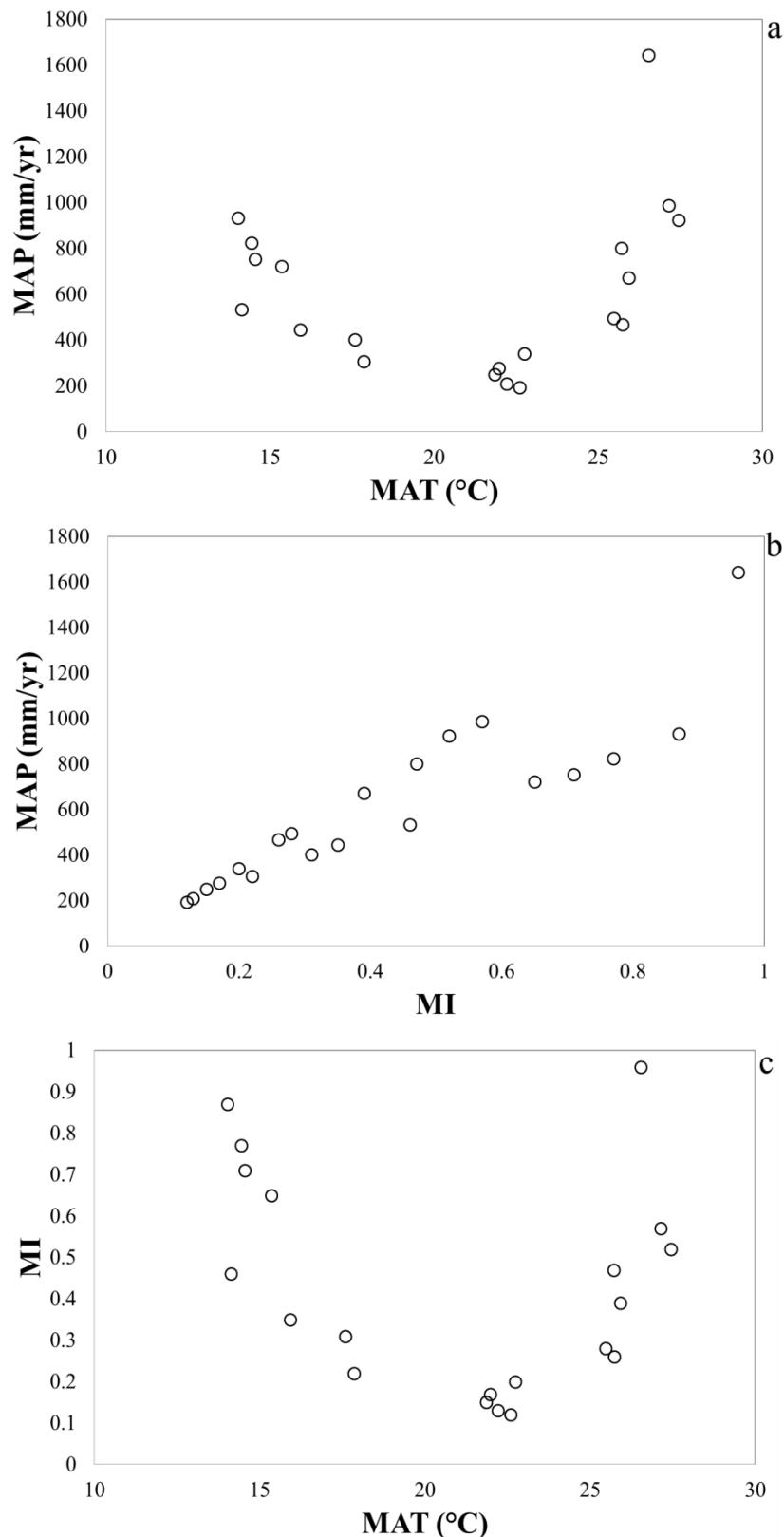
### **Climate and ecological setting**

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.



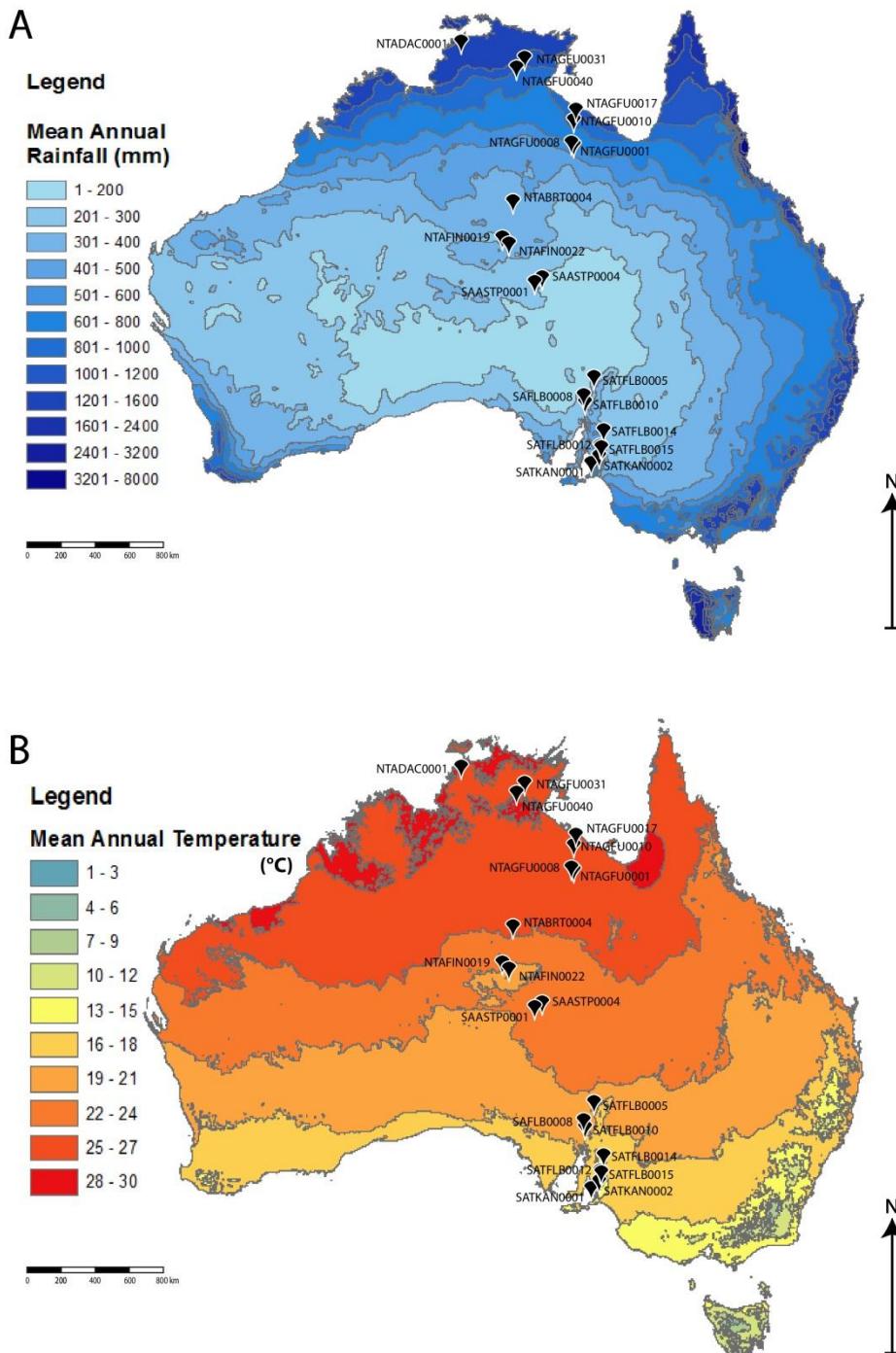
**Figure 2:** (a) Mean annual precipitation (MAP) versus mean annual temperature (MAT), (b) MAP versus moisture index (MI), and (c) MI versus MAT of selected sites.

## 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.

SITE	Bioregion	MAP (mm/yr)	MAT (°C)	MI (annual) (dimensionless)	MI - lowest quarter mean (dimensionless)	Aridity index - month max (dimensionless)	Radiation - highest period (MJ/m <sup>2</sup> /day)	Precipitation driest month (mm)	Vapour Pressure Deficit - month max (kPa)	Presence of Cryptogams
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 was 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  $\geq$ 50mg. 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

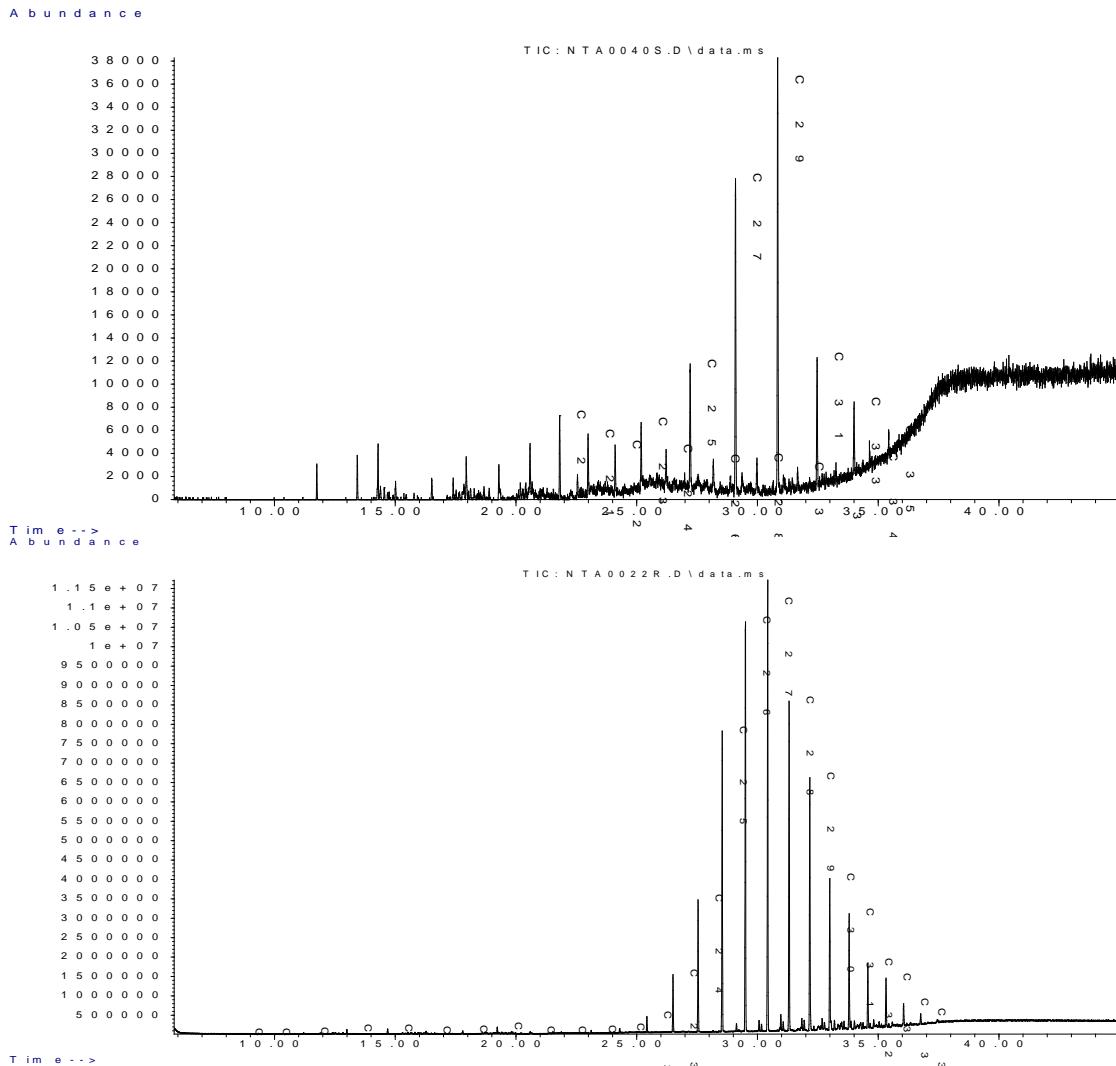
The polar and non-polar fractions of both the plant and the soil TLEs were separated by eluting them with, firstly, 4ml optima grade hexane to collect the non-polar, aliphatic hydrocarbon fraction, followed by 4ml 1:1 DCM:MeOH eluent to collect the polar fraction, through a silica gel glass short column. 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 100µL 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 0.25µm (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 1µL 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**).

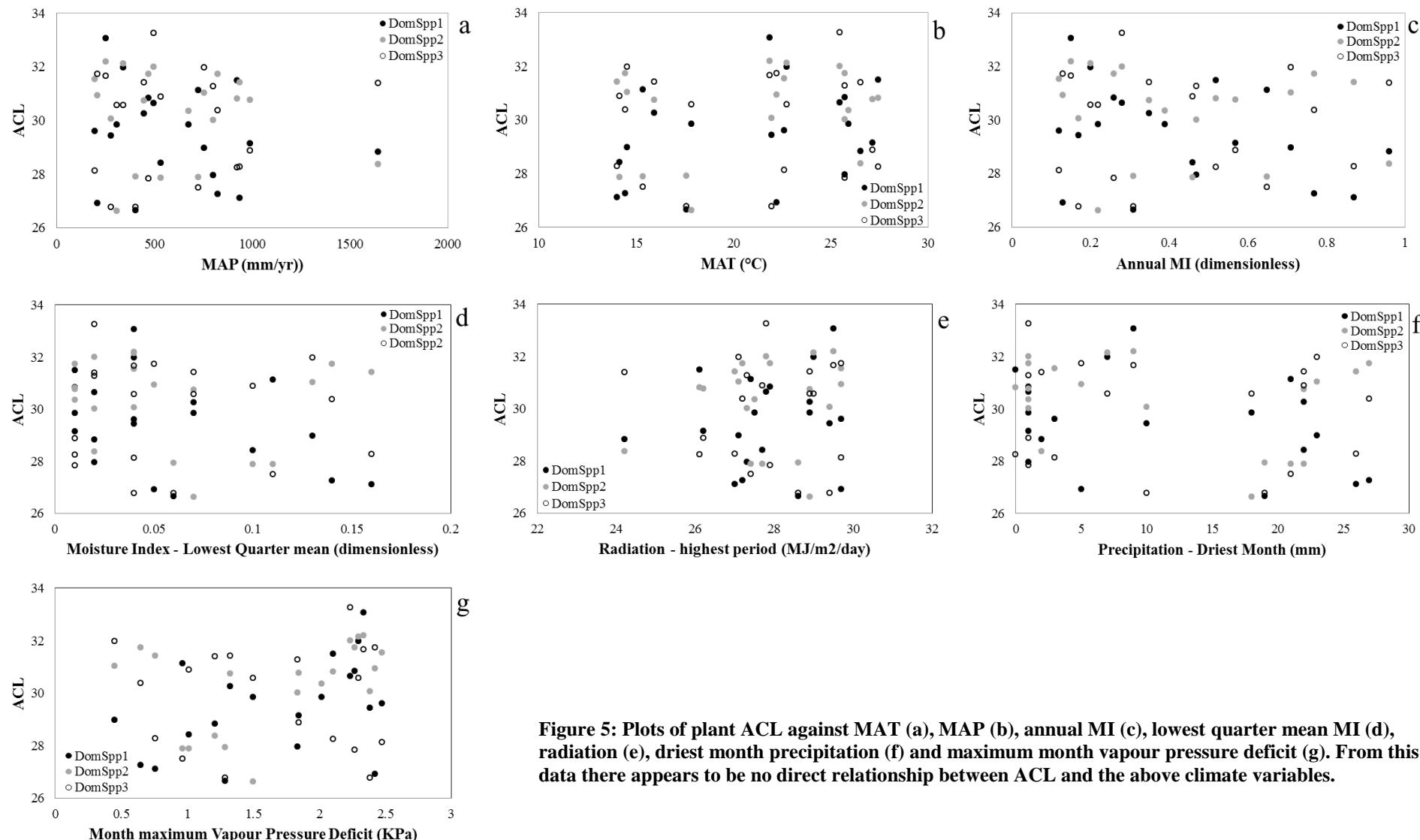
**Table 11: ACL for plant and soil samples. The total plant cover (%) is the sum of the top 3 dominant plants % cover. Soil samples used for further analysis were determined based on their carbon preference index (CPI>1.5). The predicted soil ACL is a weighted average of the ACL of the top three dominant 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 polypylus</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 dochotoma</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 pachyarthon</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 tectifica</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>Enchytraea 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 polypylus</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>Enchytraea tomentosa var. tomentosa</i>	6.1	26.8	83.2	2.00	26.8	28.4
SATFLB0012	<i>Allocasuarina muelleriana subsp. Muelleriana</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>Rutidosis helichrysoidea subsp. Helichrysoidea</i>	18.5	31.6	<i>Sida pubulifera</i>	11.7	28.1	55.8	1.26	29.9	28.0
NTADAC0001	<i>Eucalyptus tetrodonta</i>	N/A	28.8	<i>Eucalyptus miniatia</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.

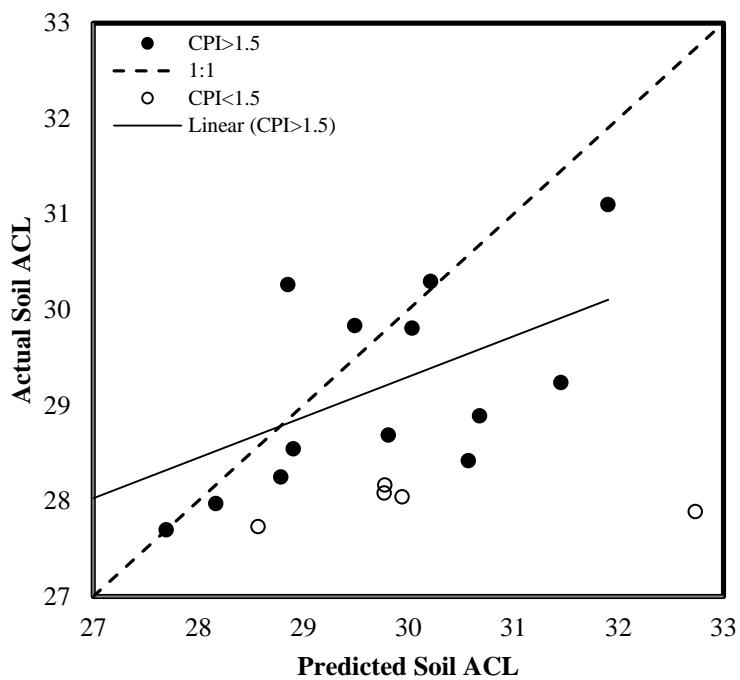
**Table 12: Results of least squares regression analysis for the plant ACL**

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 ( $p<0.05$ ).**

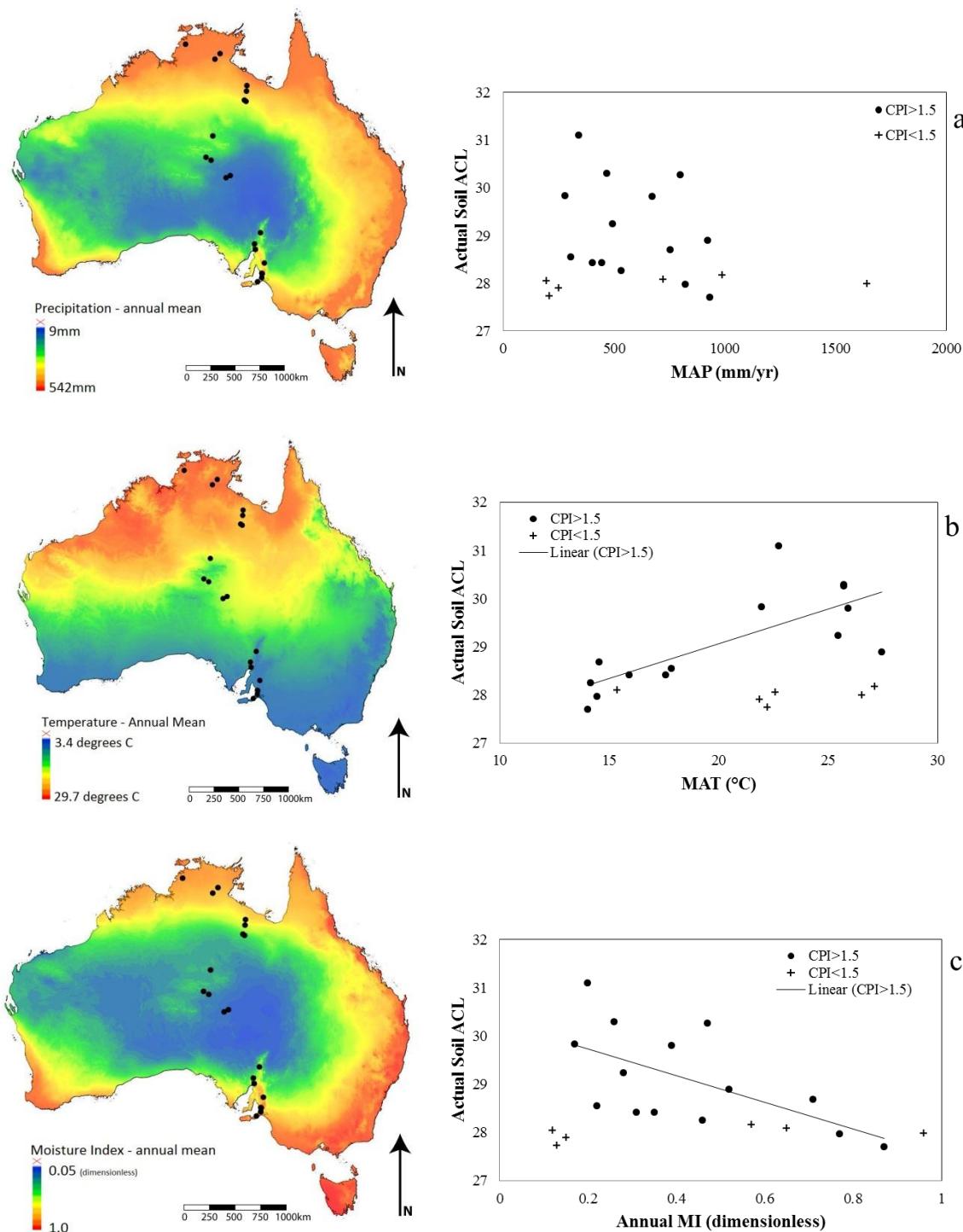
Climate Variable	r <sup>2</sup>	P-value	Equation
MAP	0.03	0.43	
MAT	0.19	0.06	
Annual MI	0.12	0.14	
<b>Lowest quarter mean MI</b>	<b>0.22</b>	<b>0.04</b>	<b>y=-9.87x + 29.33</b>
Radiation – highest period	0.01	0.62	
Precipitation – driest month	0.18	0.06	
VPD – month max	0.18	0.06	

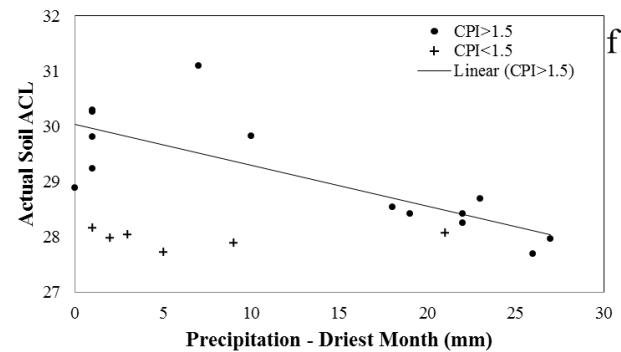
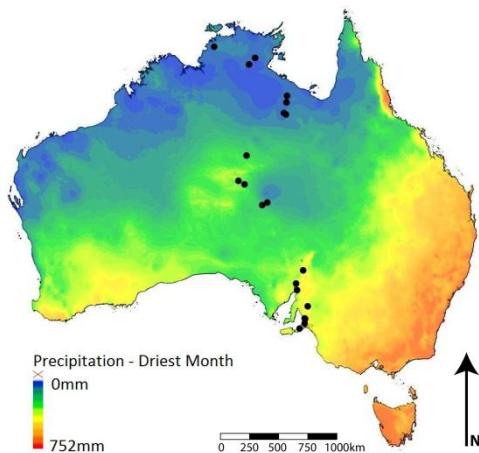
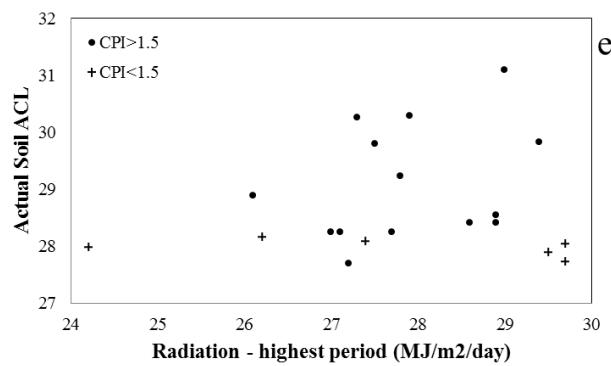
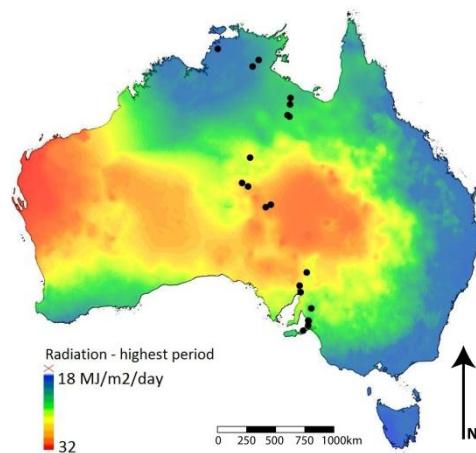
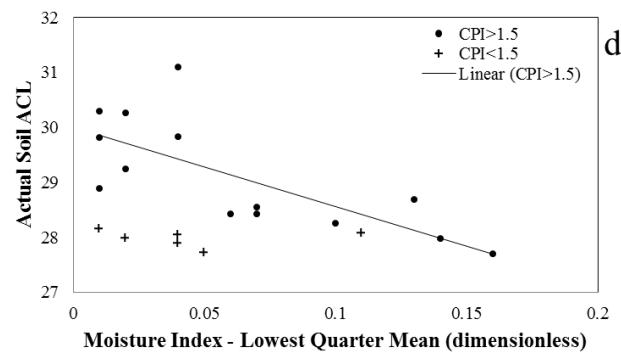
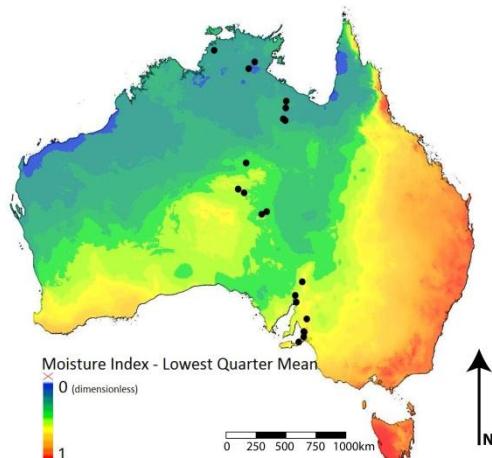
**Table 14: Results of least squares regression analysis for the actual soil ACL for soils with a CPI>1.5. Rows in bold indicate variables with statistical significance ( $p<0.05$ ).**

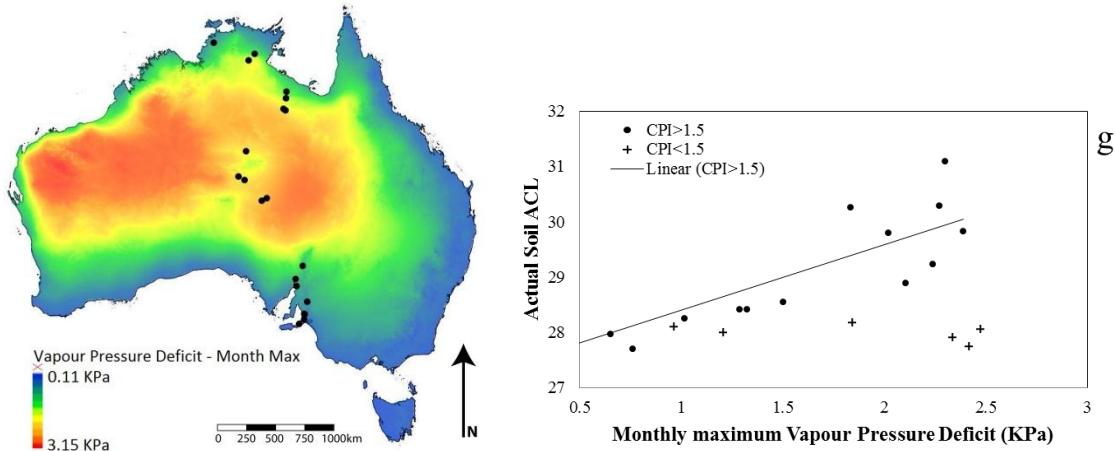
Climate Variable	r <sup>2</sup>	P-value	Equation
MAP	0.12	0.23	
<b>MAT</b>	<b>0.56</b>	<b>0.002</b>	<b>y=0.14x + 26.17</b>
<b>Annual MI</b>	<b>0.37</b>	<b>0.021</b>	<b>y=-2.77 + 30.28</b>
<b>Lowest quarter mean MI</b>	<b>0.54</b>	<b>0.003</b>	<b>y=-14.42x + 30.01</b>
Radiation - highest period	0.08	0.33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

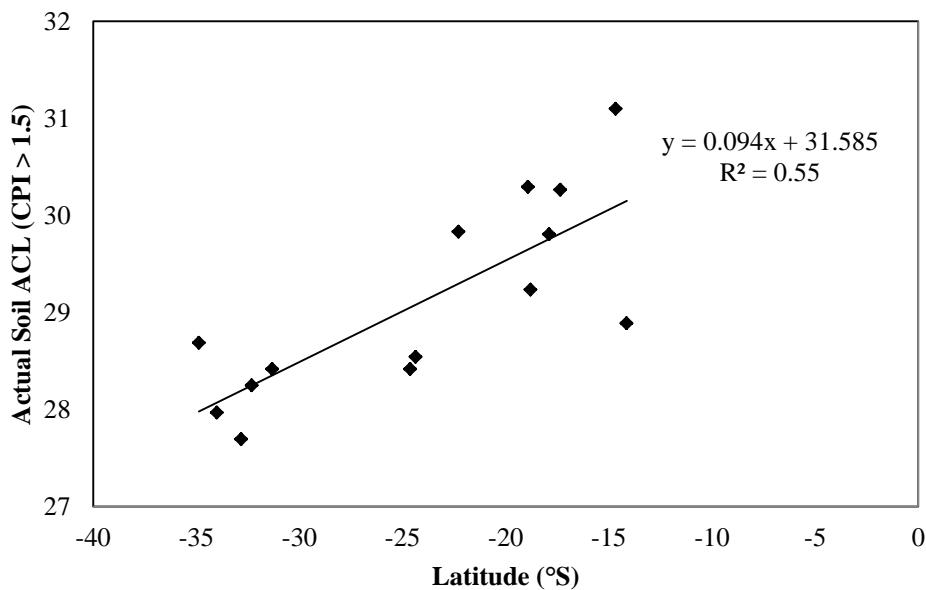




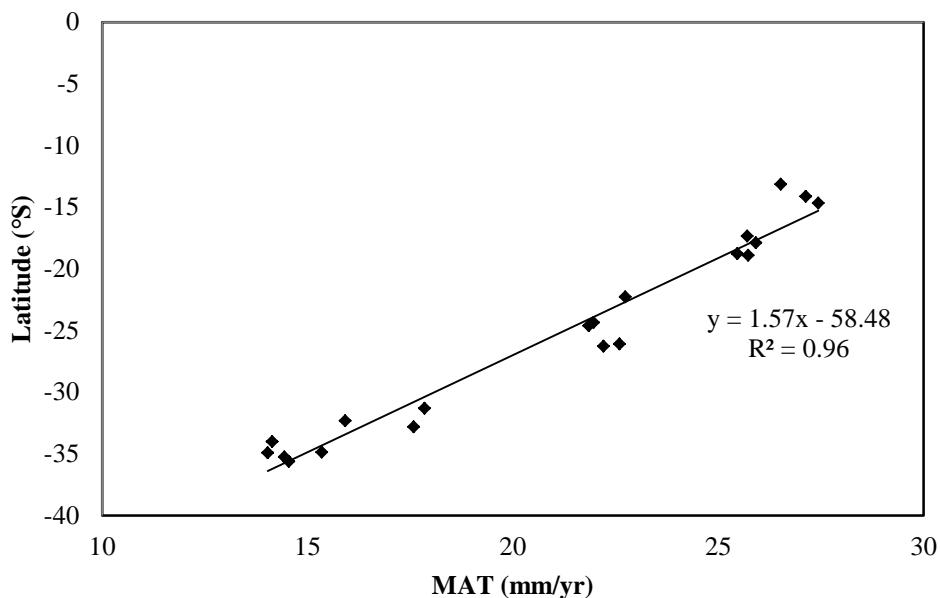


**Figure 7: Plots demonstrating the relationship between actual soil ACL and MAT (a), MAP (b), annual MI (c), lowest quarter mean MI (d), highest period radiation (e), driest month precipitation (f) and vapour pressure deficit (g). Maps of the location of sites (black dots) with respect to the various 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.

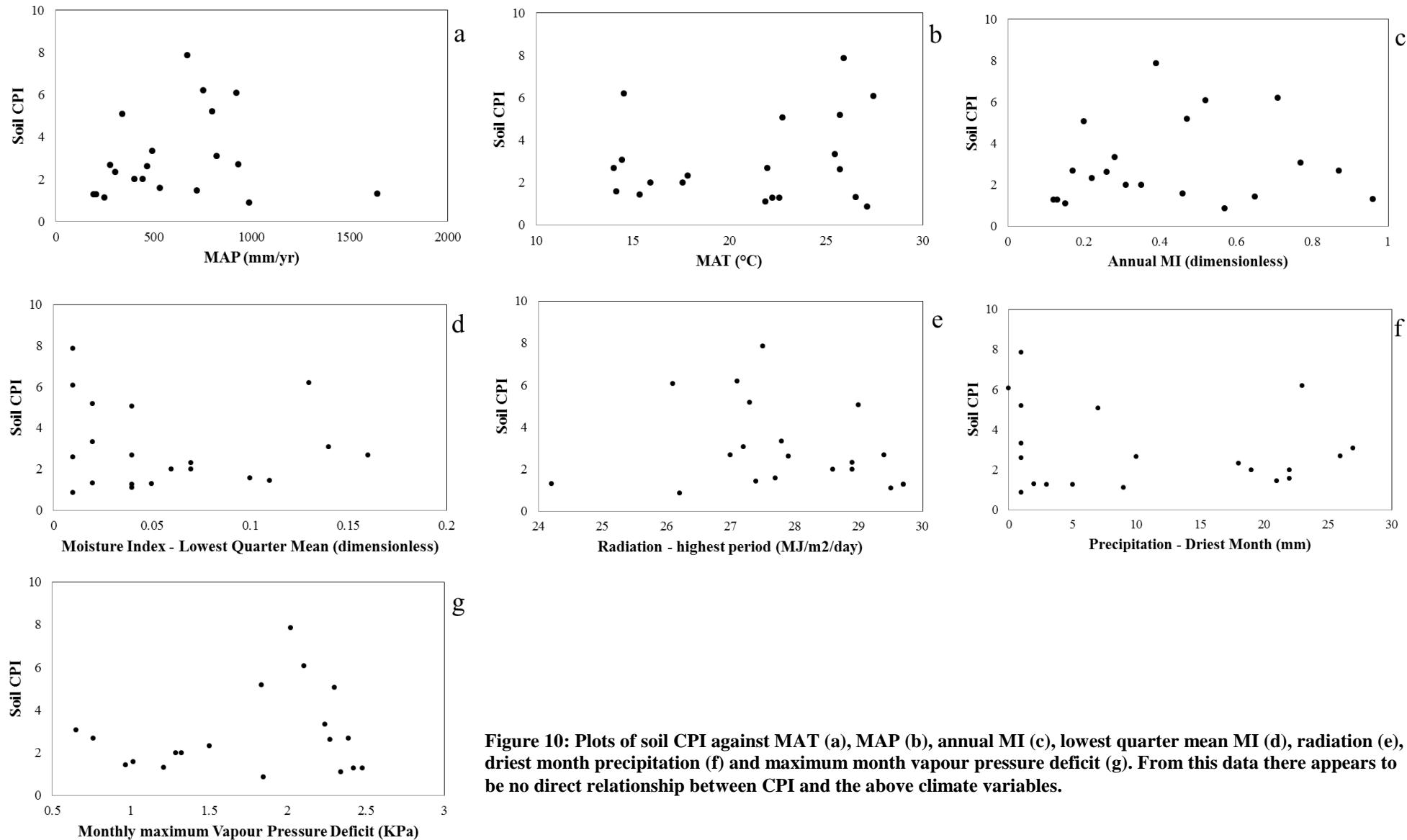


**Figure 8: Plot of actual soil ACL (CPI>1.5) with respect to latitude.**



**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 <sup>2</sup>	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
Latitude	$r^2=0.69$ Terrestrial and marine sediments from Italy (Leider et al. 2013)	$r^2=0.55$
MAT	$r^2=0.65$ 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		$r^2=0.54$
VPD	$r^2=0.45$ Soils from the east coast of the US (Tipple and Pagani 2013)	$r^2=0.63$
Aridity	$r^2=0.35$ 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.

## ACKNOWLEDGMENTS

I would like to thank TERN for making this project possible by supplying samples, data, advice and support. Thanks are also given to my supervisor, Cesca McInerney for her ongoing support, encouragement and enthusiasm for this study. I would also like to thank Kristine Nielson and Tony Hall for their assistance with various lab methods, along with Katie Howard for her logistical advice and support. Special thanks to Stefan Caddy-Retalic and Robert Klaebe also for their valuable reviews and comments.

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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 and 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, plants 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            • Licence level: 1  
constraints        • 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 ⇒ 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

[CSIRO Ecosystem Sciences](#)

organization:

Organisation

role:

Metadata date: 2010-08

Reference date:	2008-02
Resource constraints:	<ul style="list-style-type: none"><li>• Licence level: 1</li><li>• Licence info:</li></ul>
Licence notes:	Permission to re-distribute ANUCLIM outputs should be obtained from Prof. Michael Hutchinson - <a href="http://fennerschool.anu.edu.au/publications/software/">http://fennerschool.anu.edu.au/publications/software/</a>
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:	<a href="http://fennerschool.anu.edu.au/publications/software/">http://fennerschool.anu.edu.au/publications/software/</a>
View in spatial portal :	<a href="#">Click to view this layer</a>

### Moisture Index - annual mean (Bio28)

Description:	Moisture Index - annual mean (Bio28)
Short Name:	bioclim_bio28
Metadata contact organization:	<a href="#">CSIRO Ecosystem Sciences</a>
Organisation role:	
Metadata date:	2010-08
Reference date:	2008-02
Resource constraints:	<ul style="list-style-type: none"><li>• Licence level: 1</li><li>• Licence info:</li></ul>
Licence notes:	Permission to re-distribute ANUCLIM outputs should be obtained from Prof. Michael Hutchinson - <a href="http://fennerschool.anu.edu.au/publications/software/">http://fennerschool.anu.edu.au/publications/software/</a>
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	<a href="http://fennerschool.anu.edu.au/publications/software/">http://fennerschool.anu.edu.au/publications/software/</a>

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:	<ul style="list-style-type: none"><li>• Licence level: 1</li><li>• Licence info:</li></ul>
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 ⇒ Precipitation
Units:	dimensionless
Data language:	eng
Scope:	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:	<a href="http://spatial.ala.org.au/geonetwork/srv/en/metadata.show?uuid=057e11df-fc1c-4d20-ad54-19dc0345e969">http://spatial.ala.org.au/geonetwork/srv/en/metadata.show?uuid=057e11df-fc1c-4d20-ad54-19dc0345e969</a>
View in spatial portal :	<a href="#">Click to view this layer</a>

### Radiation - highest period (Bio21)

Description:	Radiation - highest period (Bio21)
Short Name:	bioclim_bio21
Metadata contact organization:	<a href="#">CSIRO Ecosystem Sciences</a>
Organisation role:	
Metadata date:	2010-08
Reference date:	2008-02
Resource constraints:	<ul style="list-style-type: none"><li>• Licence level: 1</li><li>• Licence info:</li></ul>
Licence notes:	Permission to re-distribute ANUCLIM outputs should be obtained from Prof. Michael Hutchinson - <a href="http://fennerschool.anu.edu.au/publications/software/">http://fennerschool.anu.edu.au/publications/software/</a>
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://fennerschool.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 [WorldClim](#)

organization:

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:

(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:** Sian Howard

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

Hi Sian,

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

<http://www.csiro.au/Organisation-Structure/Flagships.aspx>

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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](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 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 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

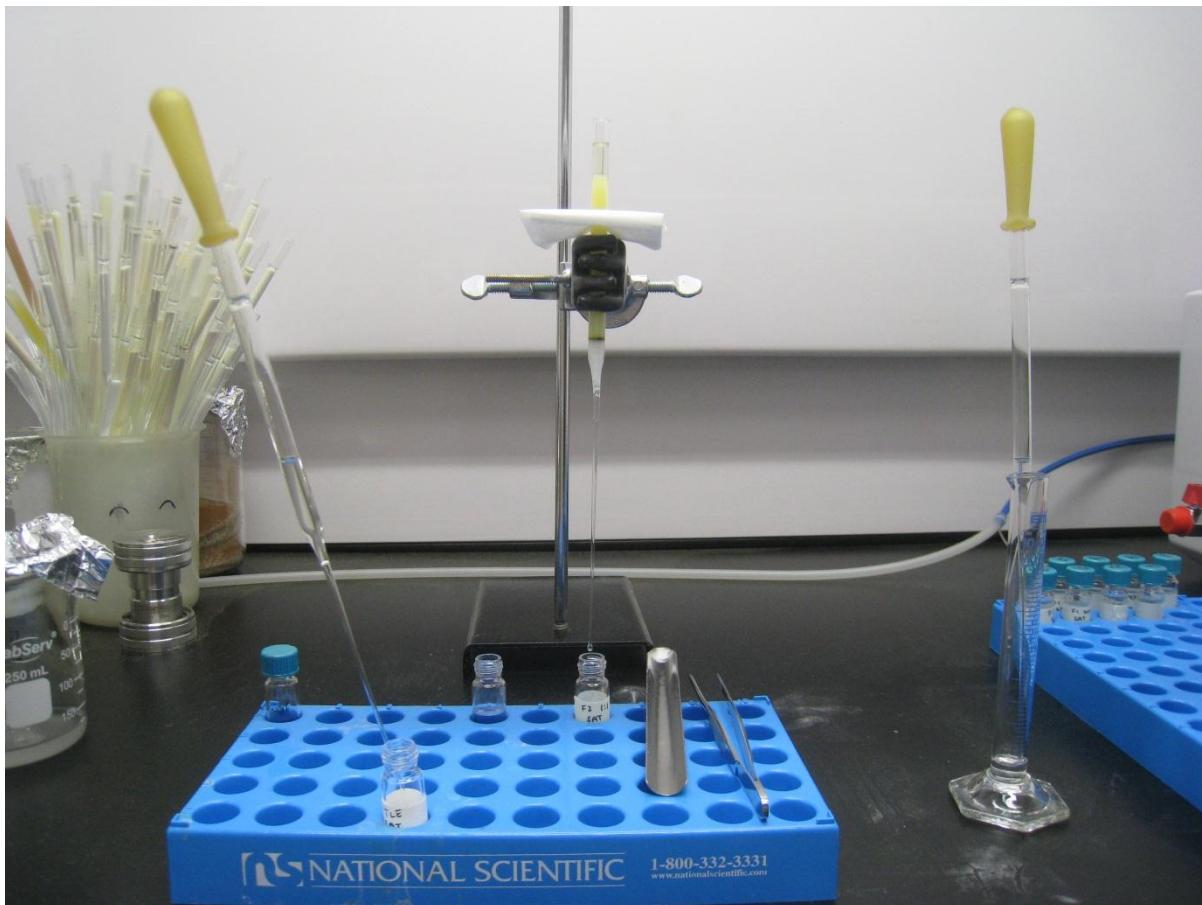
rinsed with 5ml of solvent solution a total of three times. The rinse volume was set to 60%, with a purge time of 120 seconds.

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, and 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 in to 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 0.25μm (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 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 0.25μm (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

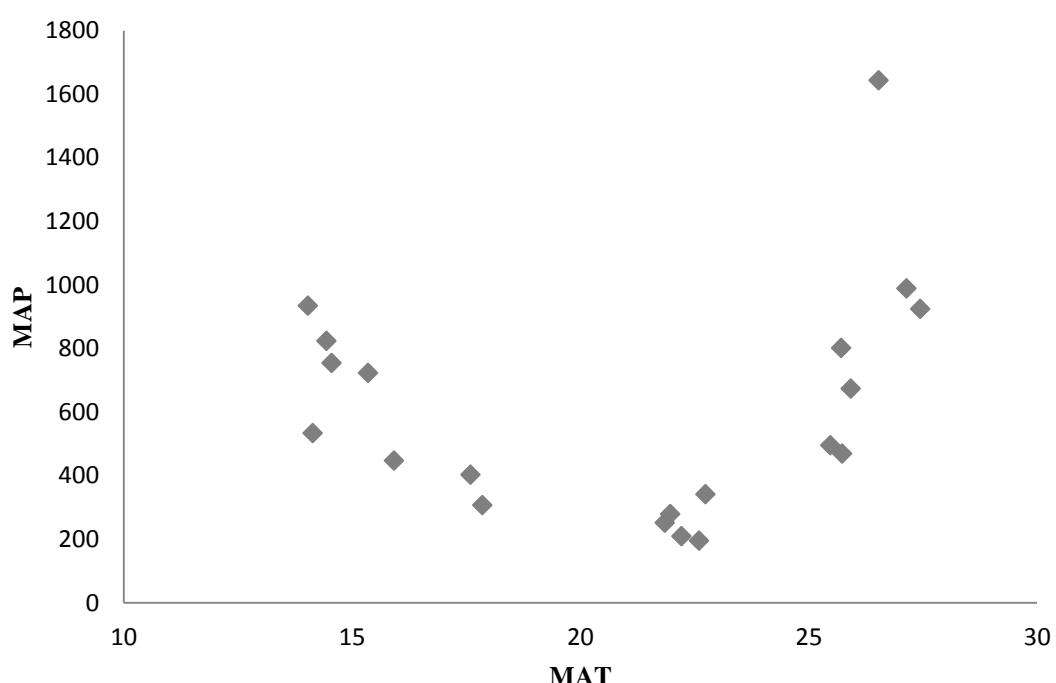
Using Turbomass software:

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	<i>Enneapogon polyphyllus</i>	Tussock Grass	NTA 001525	0.124	50	13.3
NTAGFU0008	Gulf fall and uplands	<i>Aristida contorta</i>	Tussock Grass	NTA 002011	0.135	50	19.5
NTAGFU0010	Gulf fall and uplands	<i>Eucalyptus leucophloia</i>	Tree Mallee	NTA 002140	0.188	50.6	36.4
NTAGFU0017	Gulf fall and uplands	<i>Chrysopogon fallax</i>	Tussock Grass	NTA 002610	0.166	50.6	10.4
NTAGFU0031	Gulf fall and uplands	<i>Schizachyrium pachyarthron</i>	Tussock Grass	NTA 003588	0.063	23.5	28.3
NTAGFU0040	Gulf fall and uplands	<i>Heteropogon contorus</i>	Tussock Grass	NTA 003995	0.091	22.8	15.9
NTABRT0004	Burt plain	<i>Aristida holathera</i>	Tussock Grass	NTA 001318	0.17	51	24.4
NTAFIN0019	Finke	<i>Acacia estrophiolata</i>	Tree/Palm	NTA 000784	0.123	50	19.2
NTAFIN0022	Finke	<i>Enneapogon polyphyllus</i>	Tussock Grass	NTA 000962	0.118	51.6	15
SATFLB0005	Flinders lofty block	<i>Eucalyptus flindersii</i>	Tree Mallee	SAT 000286	0.196	52	18.8
SATFLB0008	Flinders lofty block	<i>Cassinia laevis</i>	Shrub	SAT 000419	0.105	50.2	23.7
SATFLB0010	Flinders lofty block	<i>Rhagodia paradoxa</i>	Chenopod	SAT 000552	0.13	51.3	10.1
SATFLB0012	Flinders lofty block	<i>Hibbertia crinita</i>	Shrub	SAT 000657	0.112	51.2	15.5
SATFLB0014	Flinders lofty block	<i>Xanthorrhoea quadrangulata</i>	Shrub	SAT 000791	0.208	51.6	18.5
SATFLB0015	Flinders lofty block	<i>Lepidosperma semiteres</i>	Sedge	SAT 000860	0.123	51.4	8.5
SATKAN0001	Kanmantoo	<i>Lepidosperma semiteres</i>	Sedge	SAT 000167	0.218	50.5	11.3
SATKAN0002	Kanmantoo	<i>Lepidosperma semiteres</i>	Sedge	SAT 000218	0.16	50.1	9.2
SAASTP0001	Stony plains	<i>Eragrostis setifolia</i>	Tussock Grass	SAA 000294	0.136	50.6	12.8
SAASTP0004	Stony plains	<i>Rutidosis helichrysoides</i> subsp. <i>Helichrysoides</i>	Forb	SAA 000016	0.017	5.8	18.5
NTADAC0001	Darwin Coastal	<i>Eucalyptus miniata</i>		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>Enchytraea 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>Enchytraea tomentosa var. 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 involucrata</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 var. tenuis</i>	Shrub	SAA 000338	0.186	51.4	8.5
SAASTP0004	Stony plains	<i>Sida pubulifera</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**

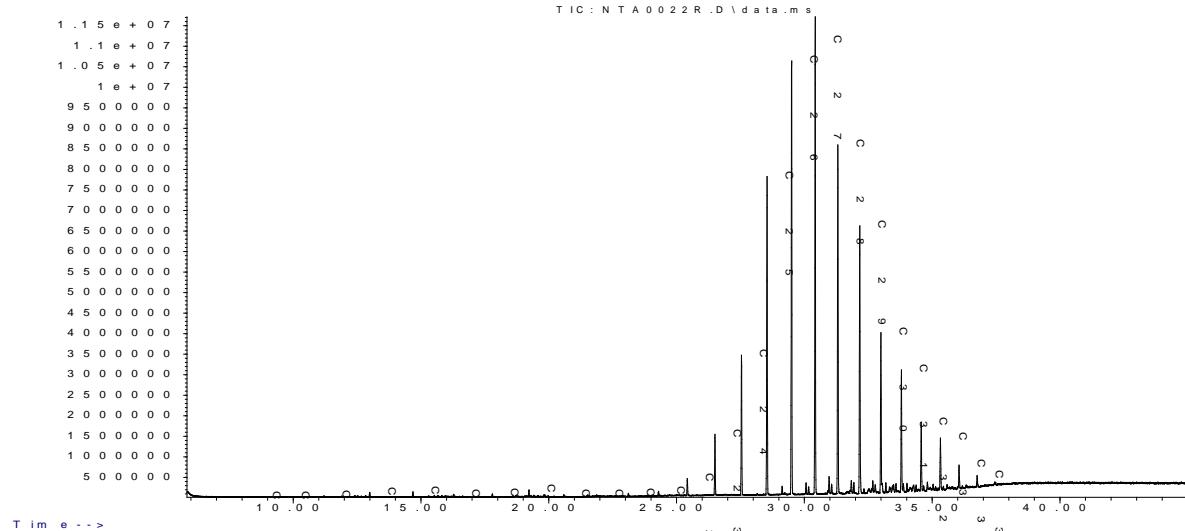
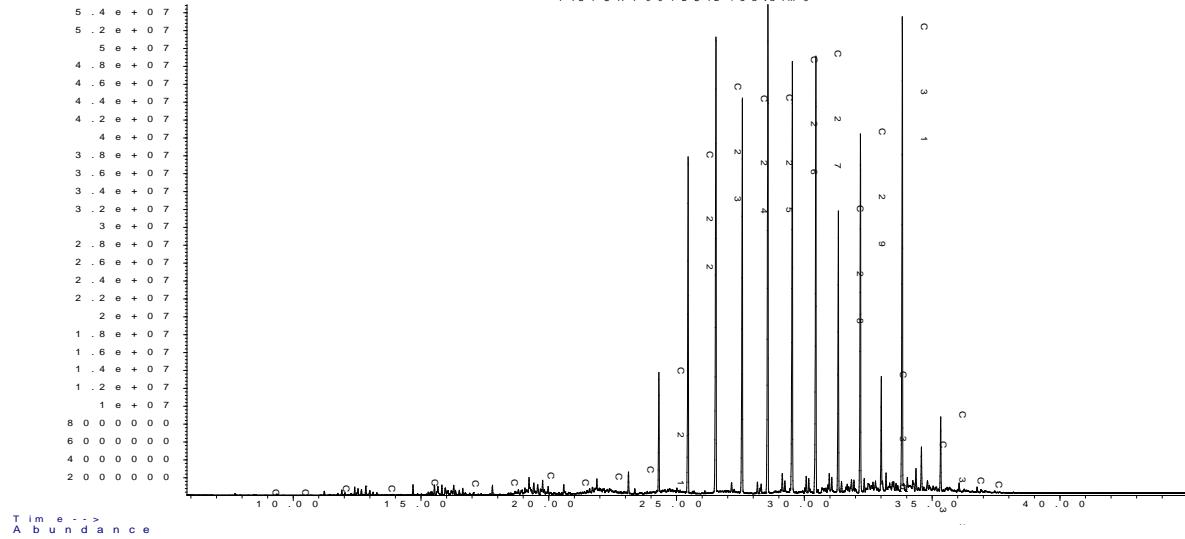
Site	Bioregion	Amount subsampled for ASE (g)
NTAGFU0001	Gulf fall and uplands	13.222
NTAGFU0008	Gulf fall and uplands	18.709
NTAGFU0010	Gulf fall and uplands	8.257
NTAGFU0017	Gulf fall and uplands	16.513
NTAGFU0031	Gulf fall and uplands	14.521
NTAGFU0040	Gulf fall and uplands	8.625
NTABRT0004	Burt plain	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

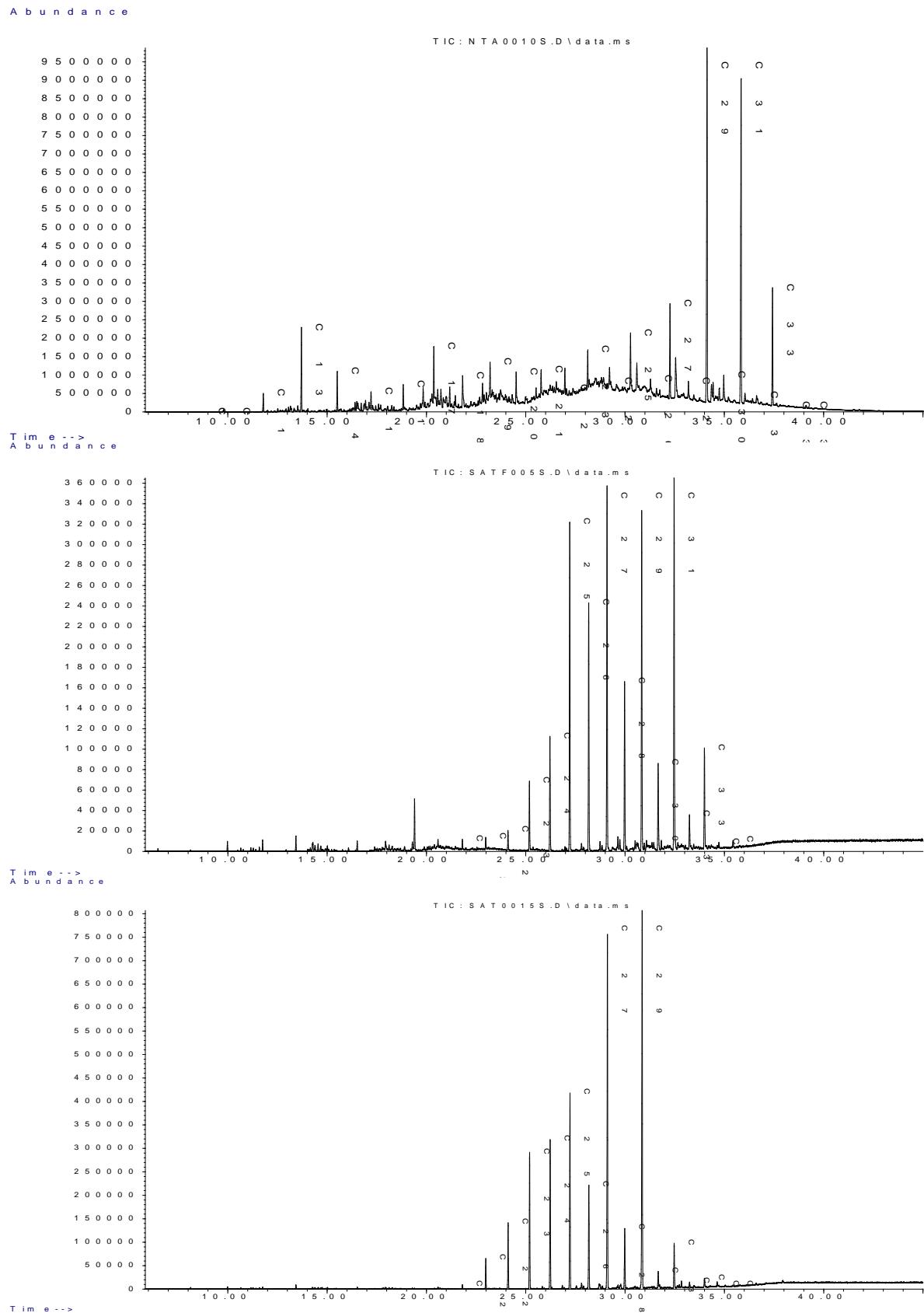
## APPENDIX B: ADDITIONAL DATA

### Appendix B – Additional Data

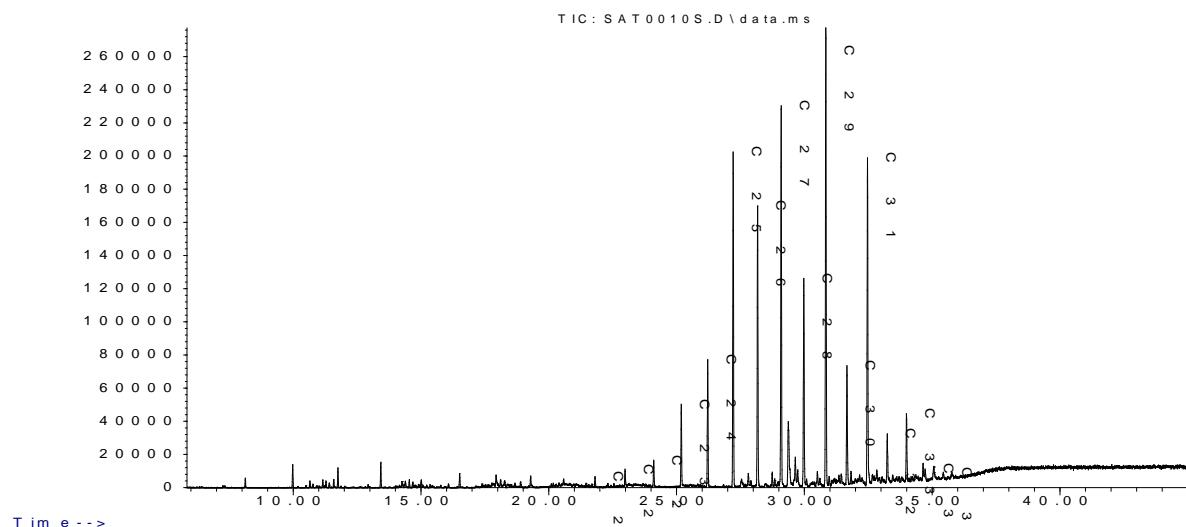
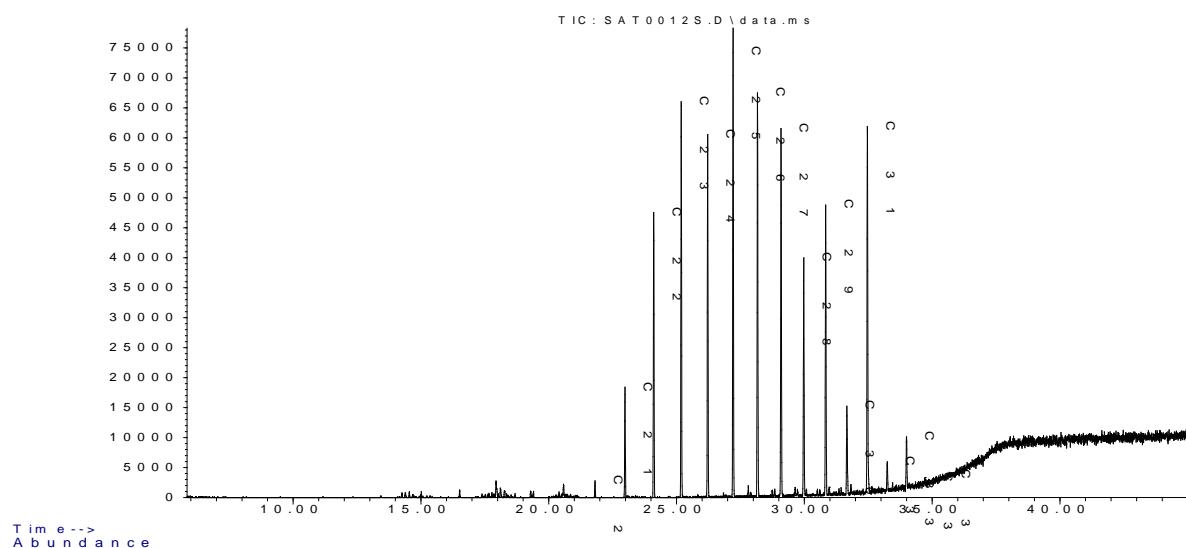
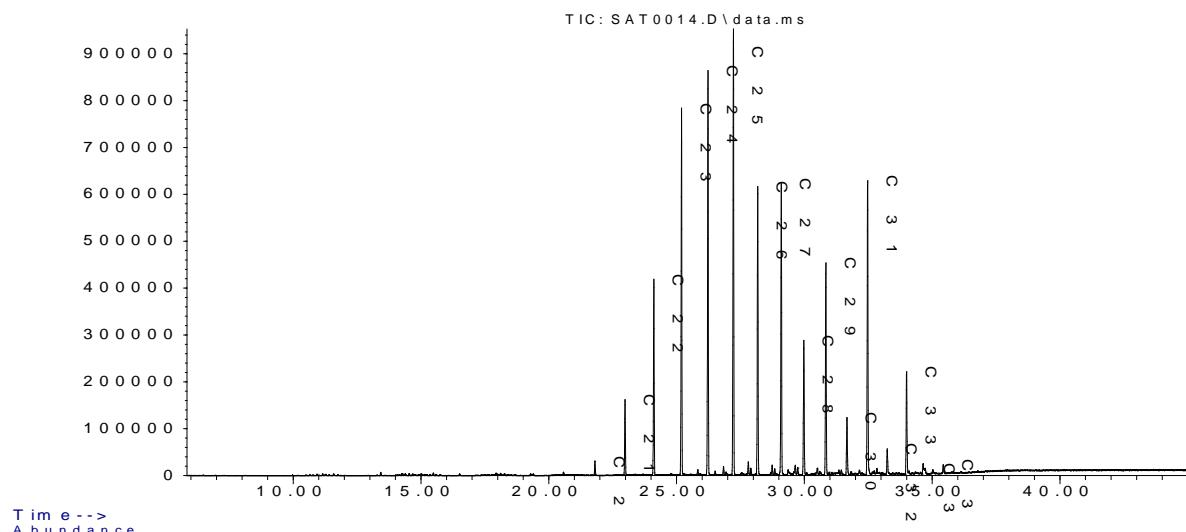
**Figure 1:** Below figures – chromatograms for GCMS results for soils and plants

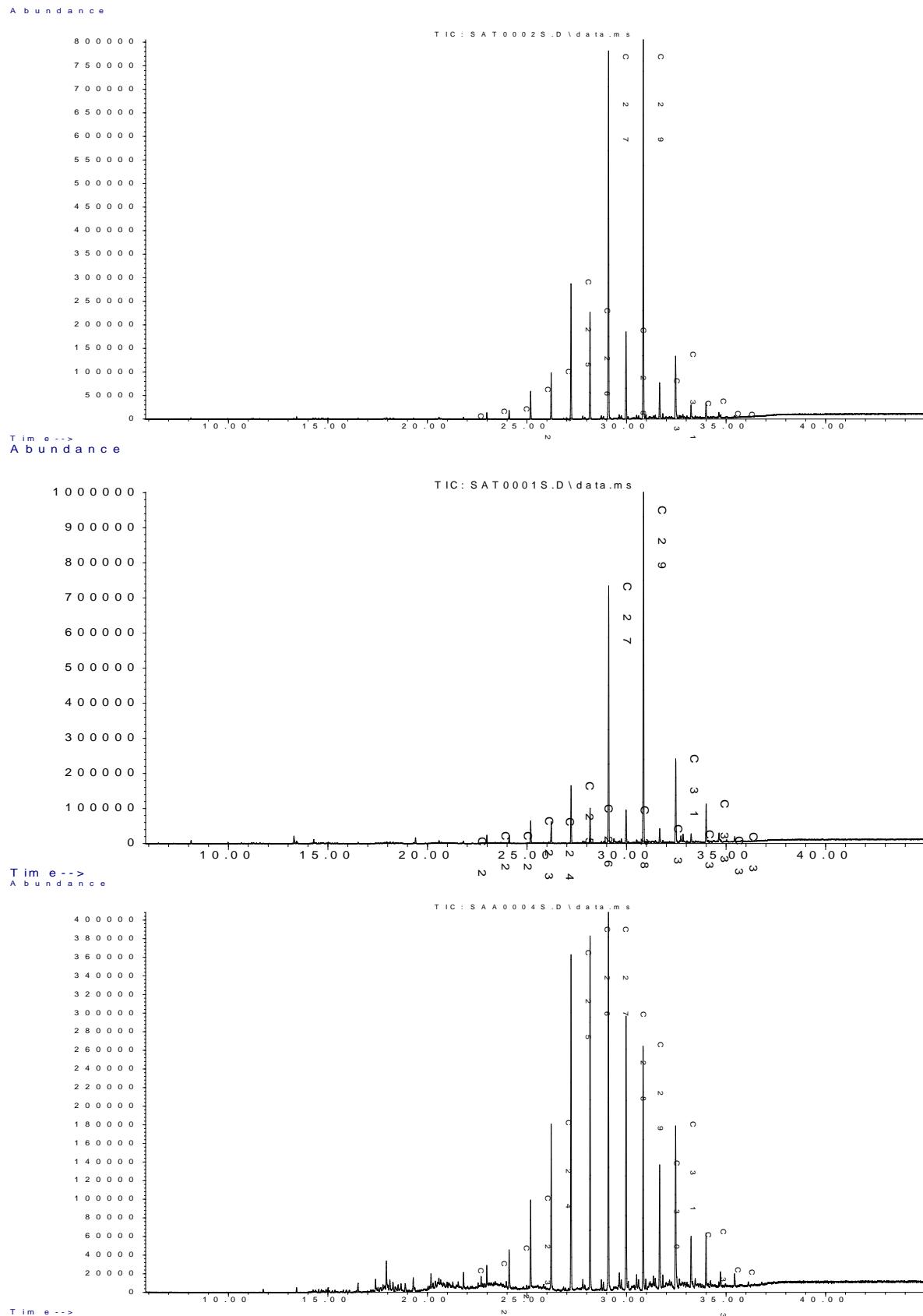
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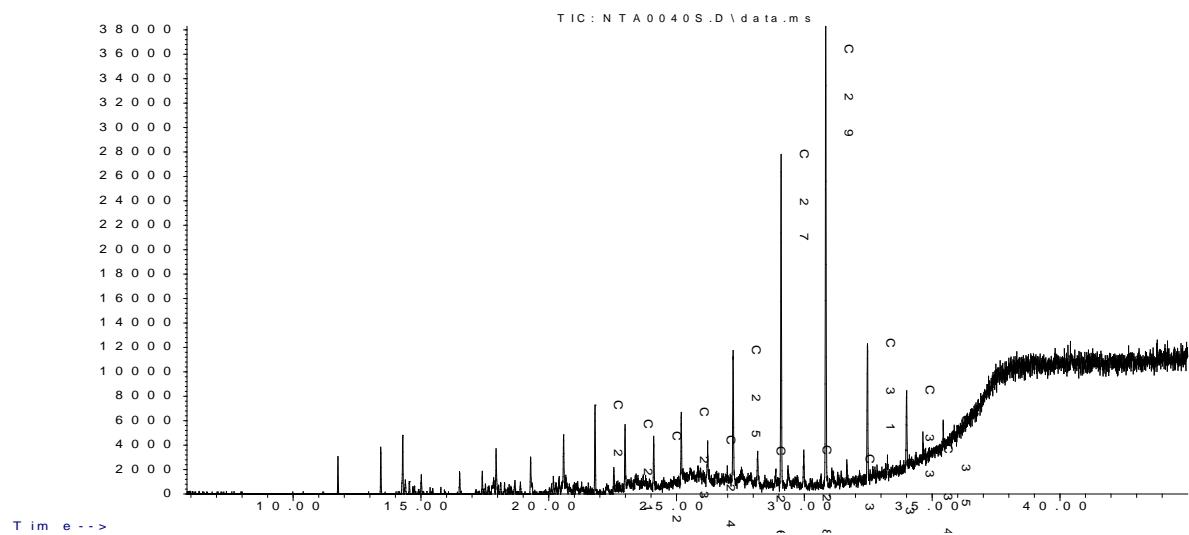
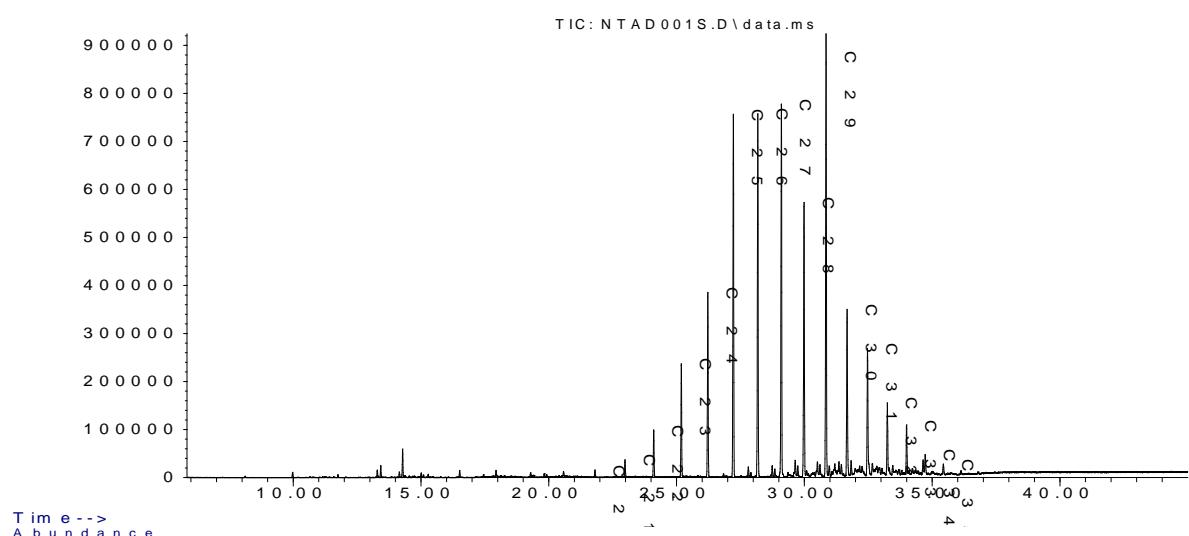
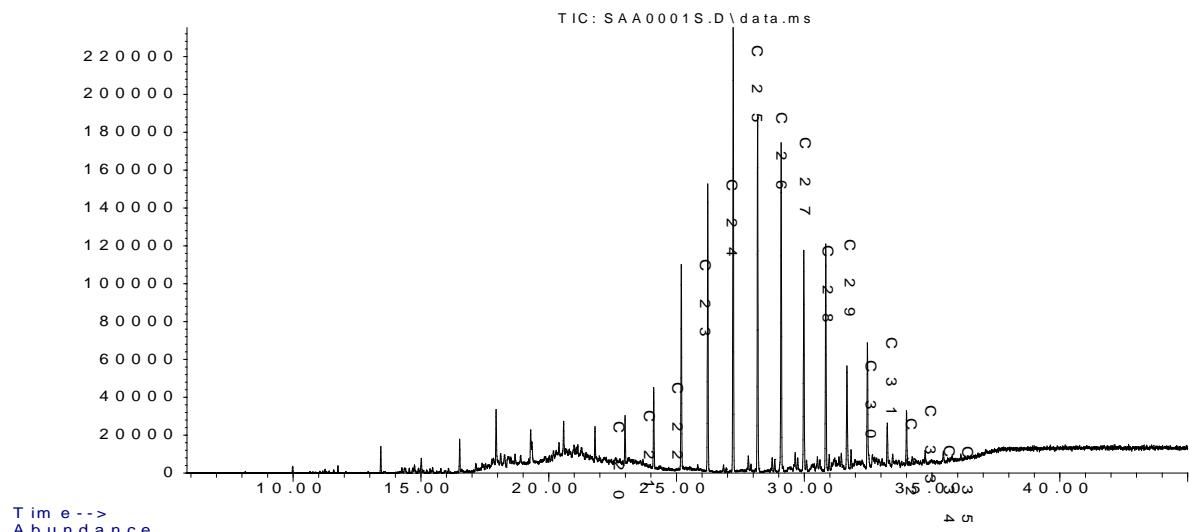


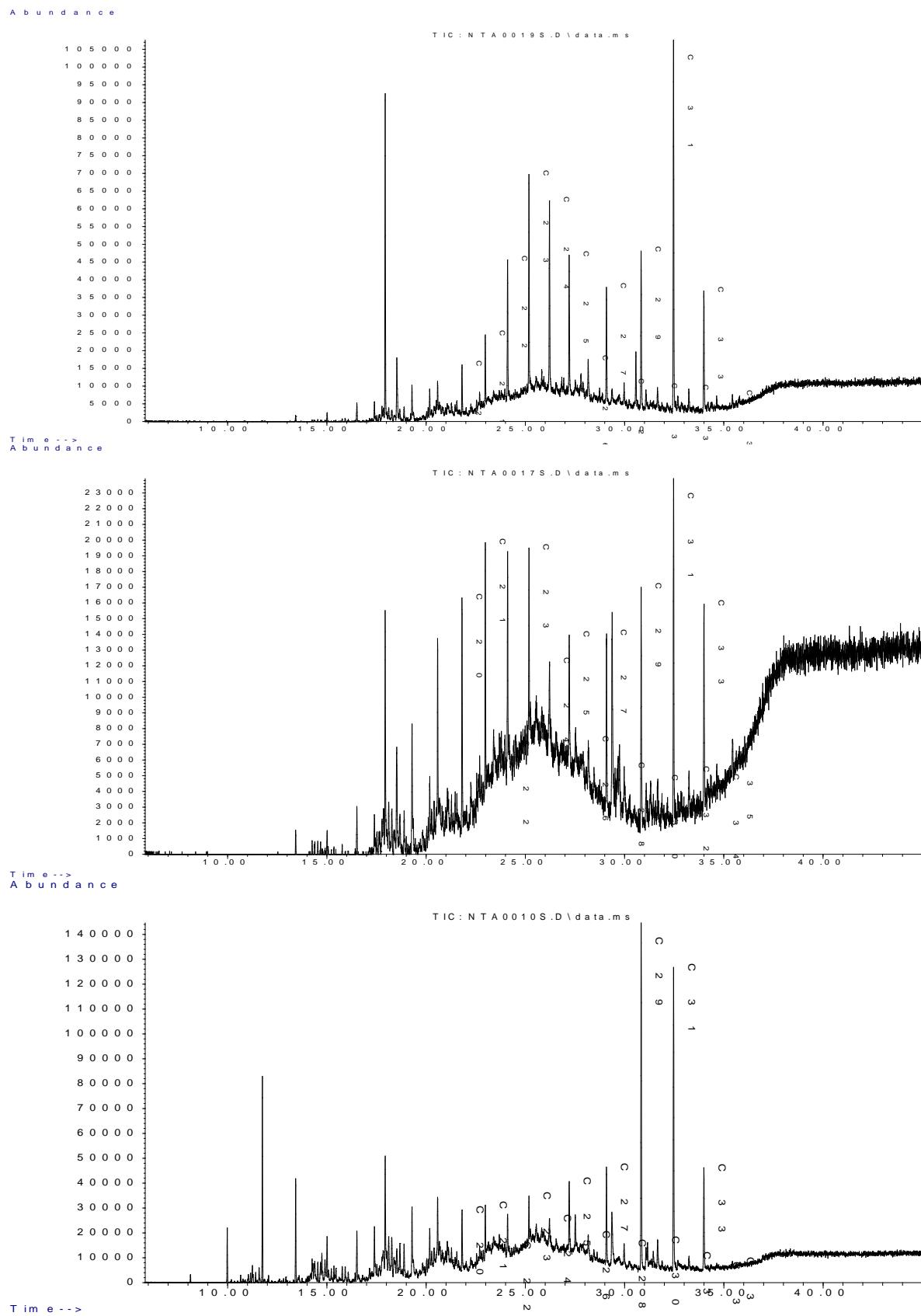
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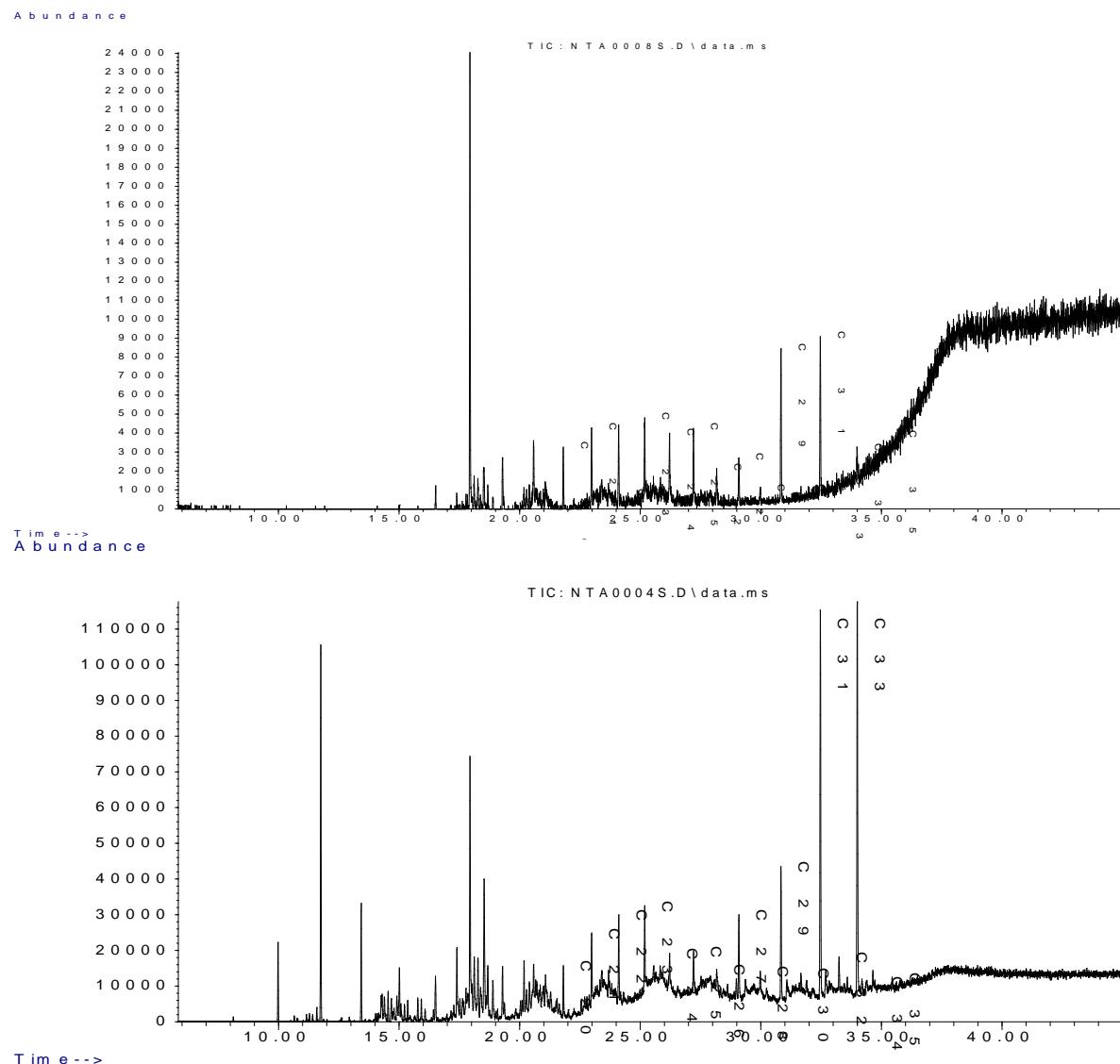




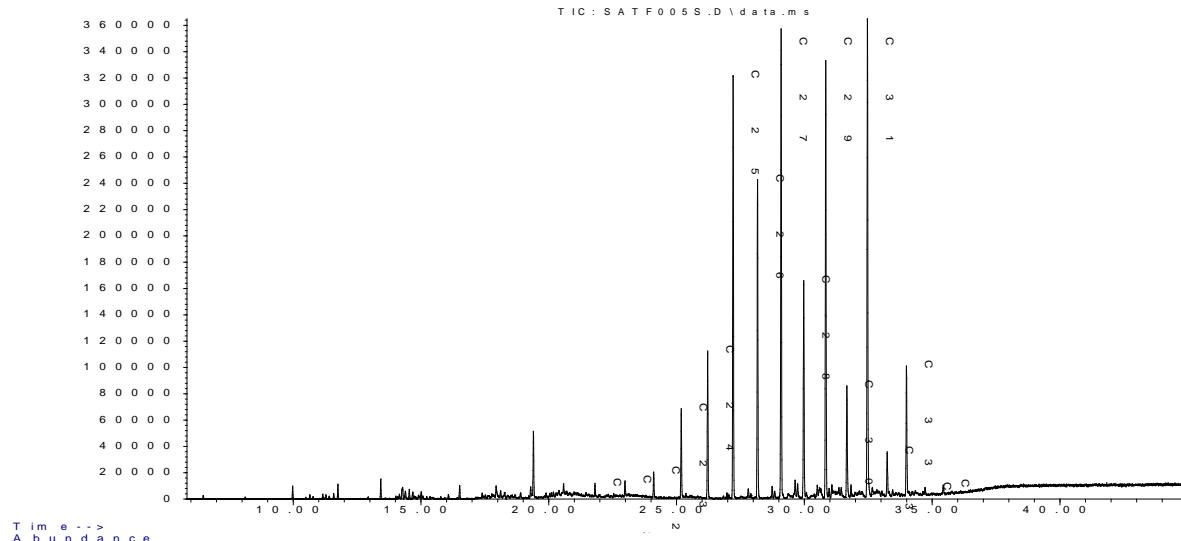
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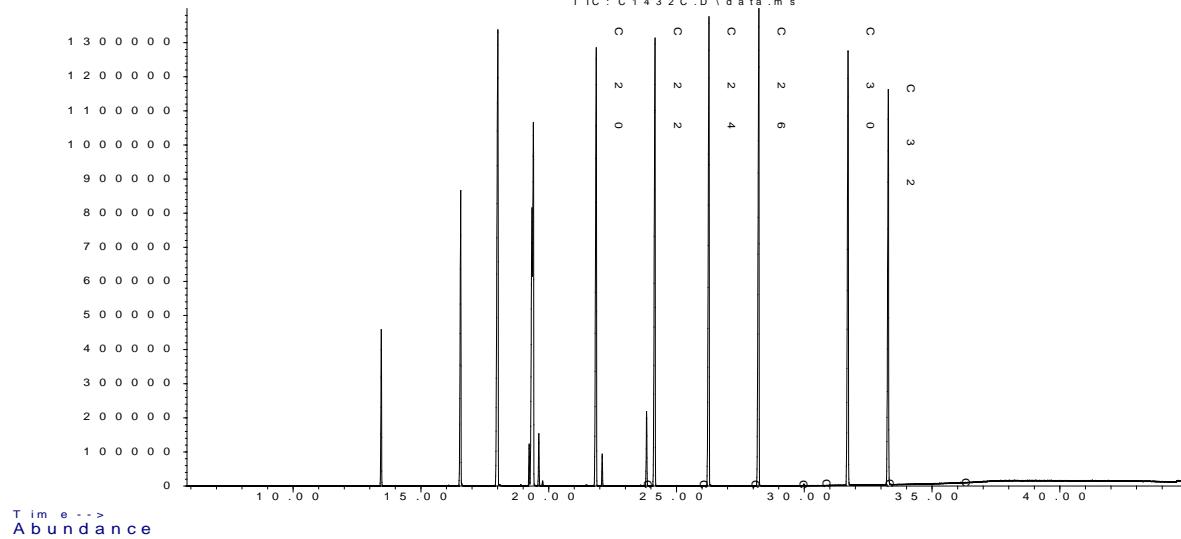




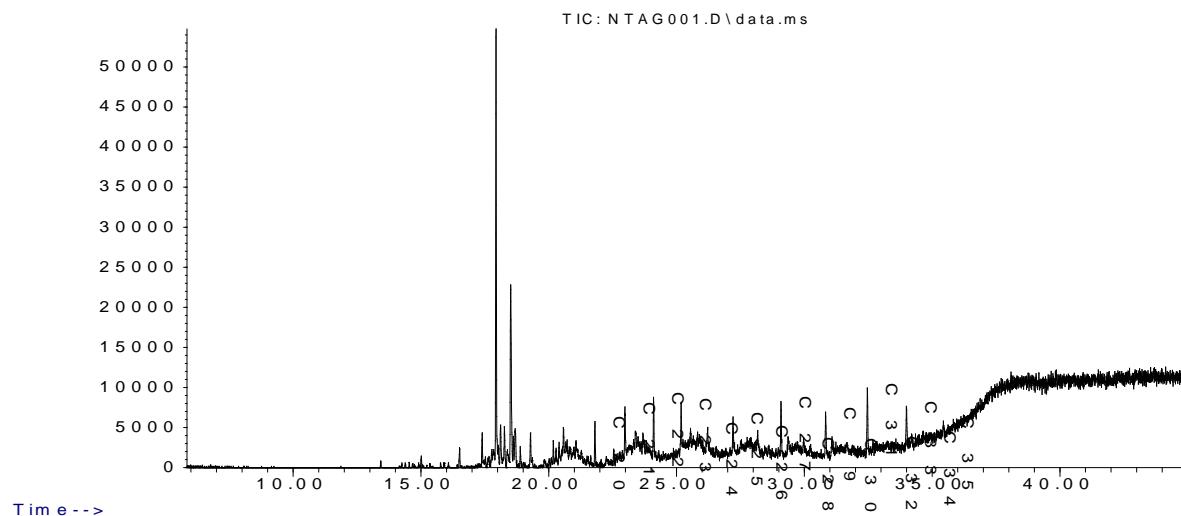
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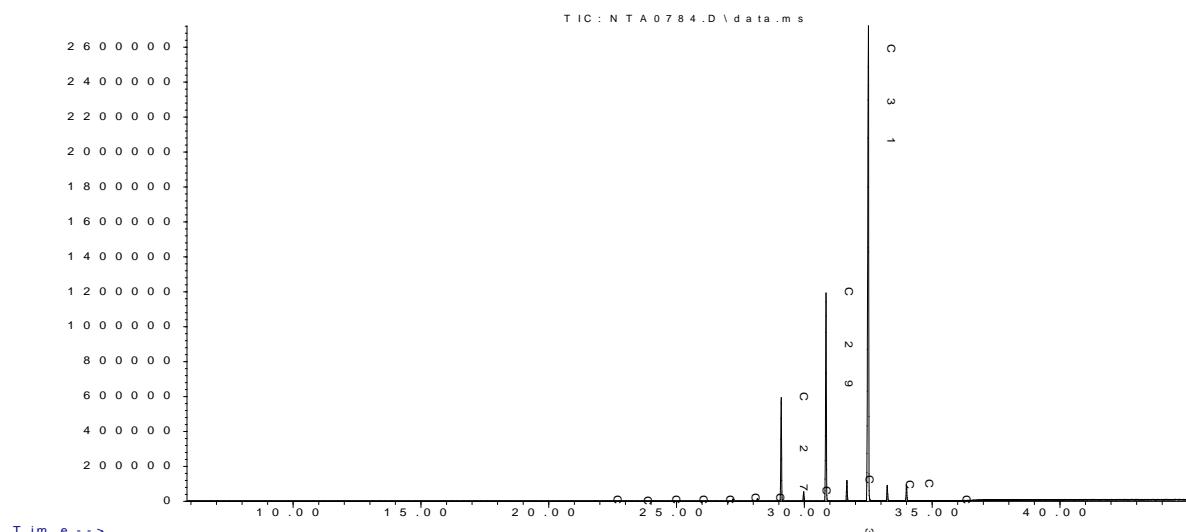
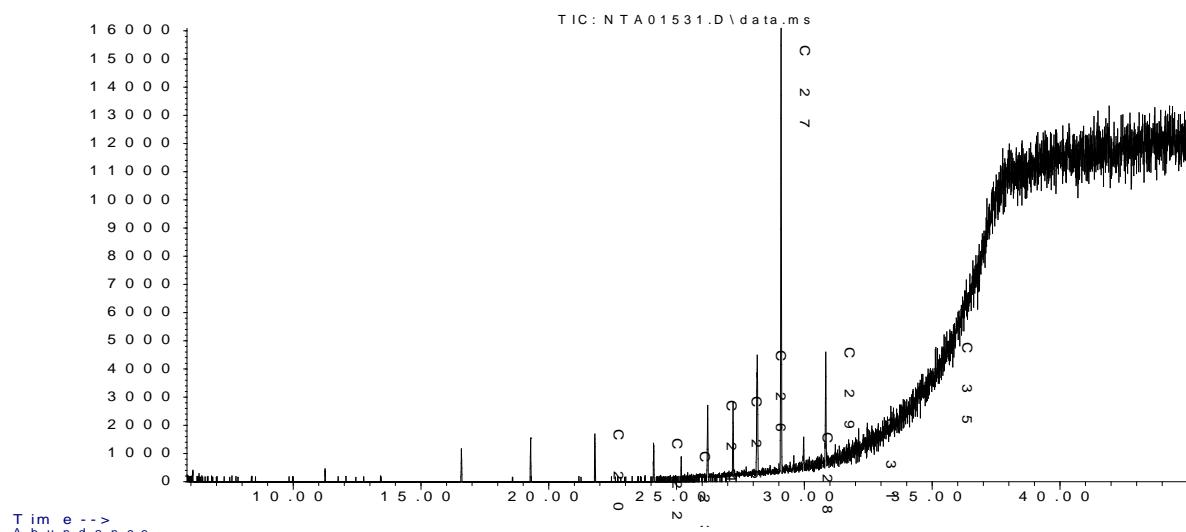
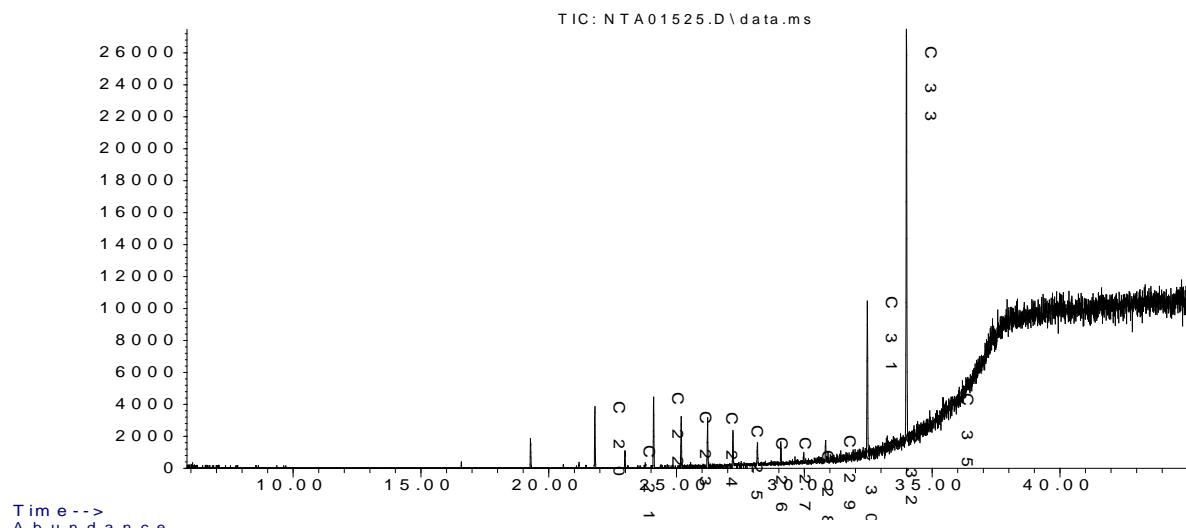
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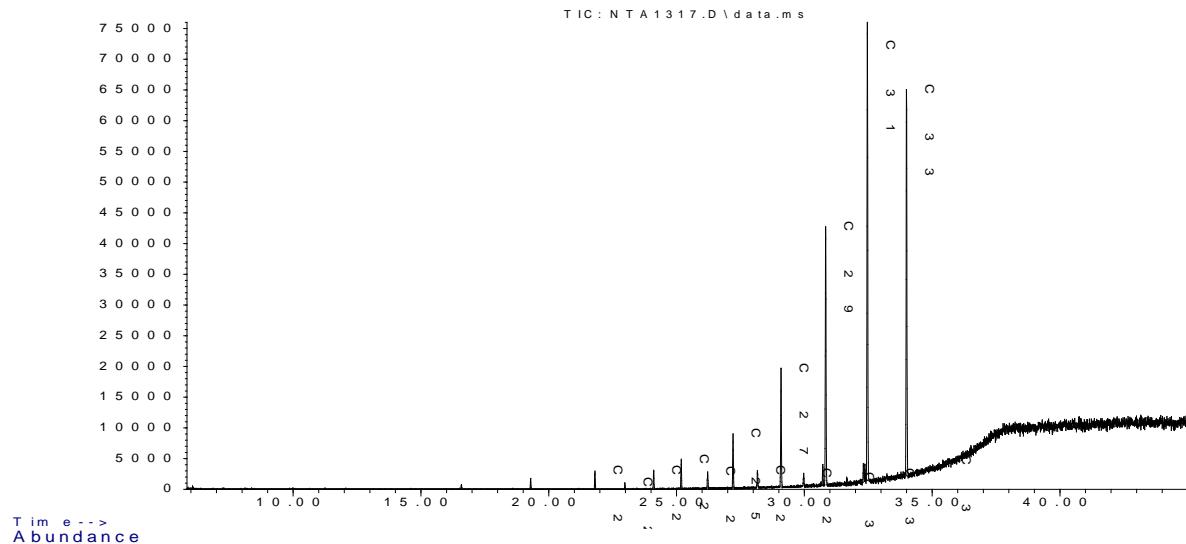
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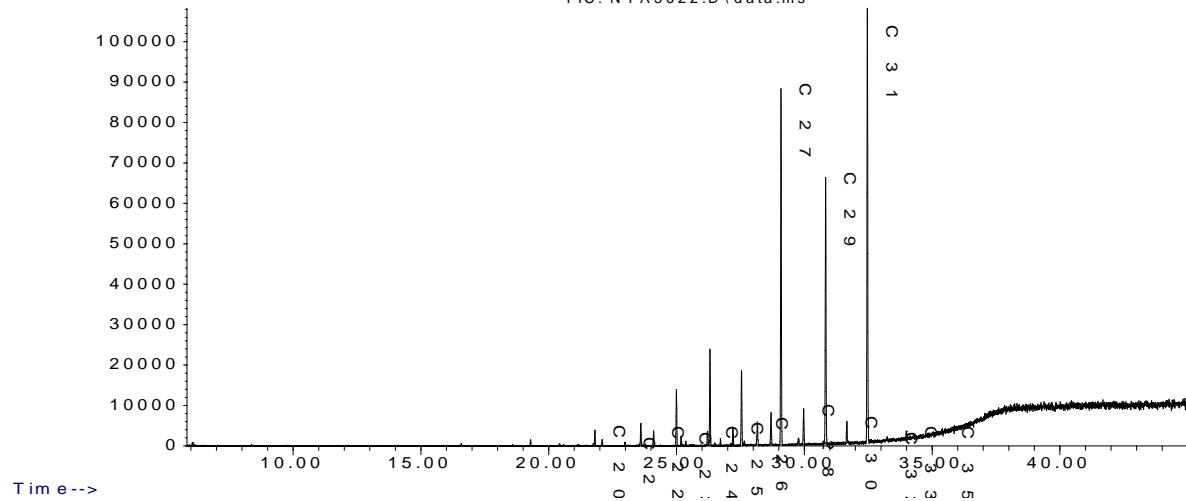
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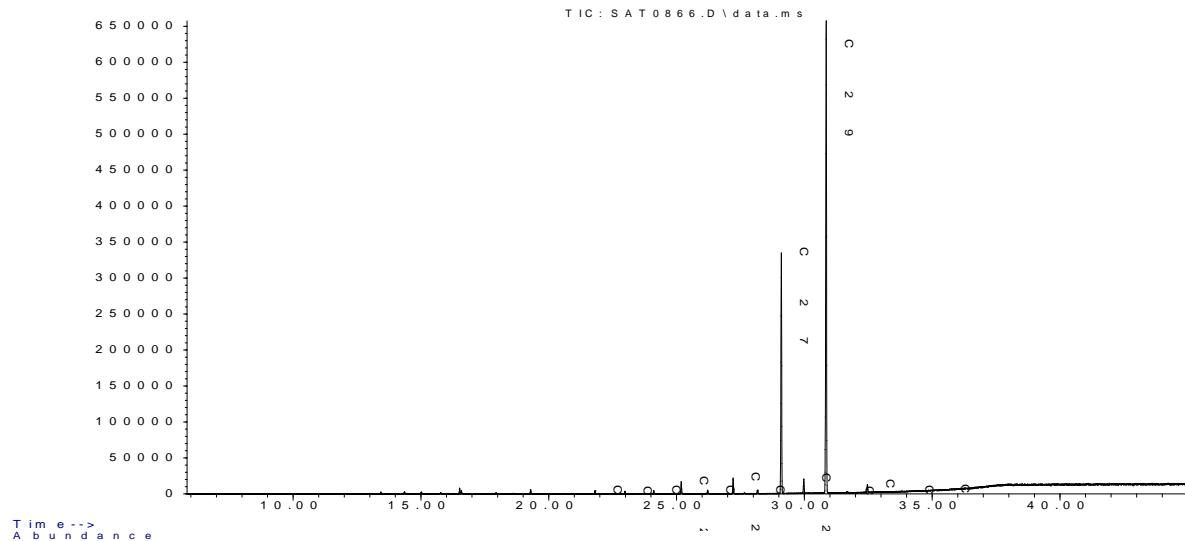
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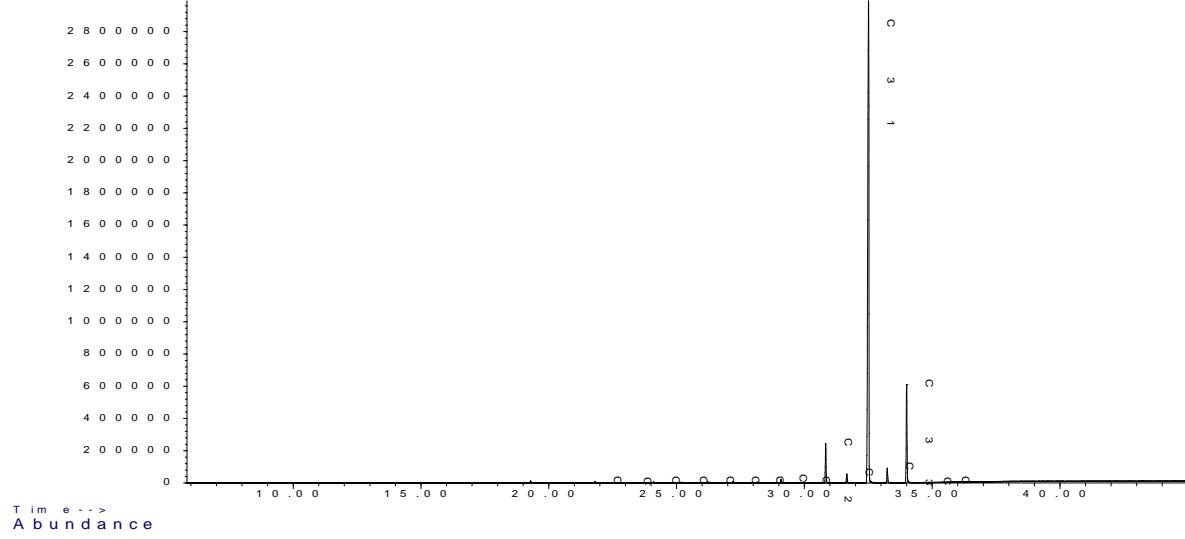
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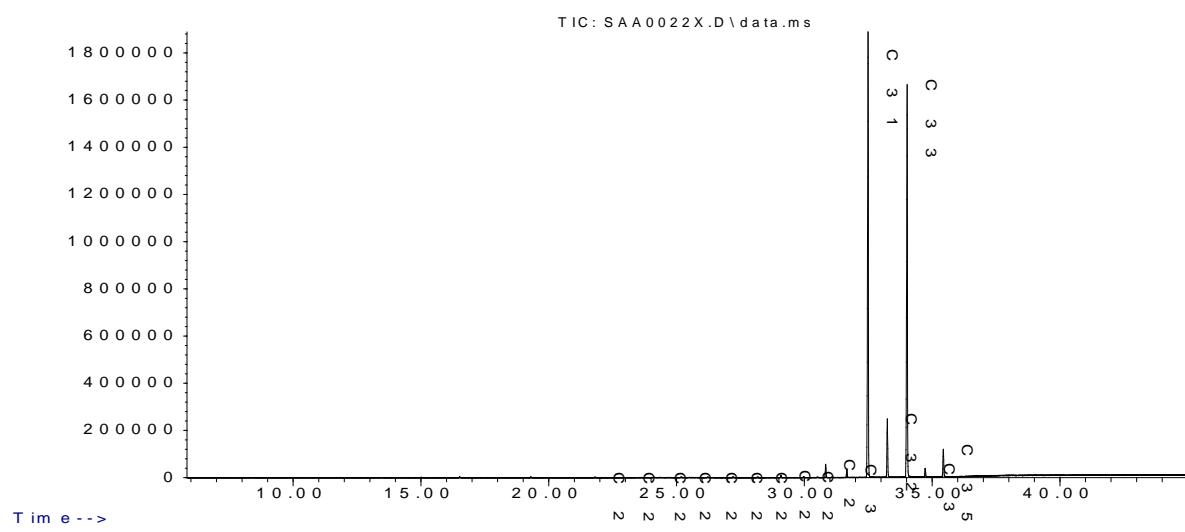


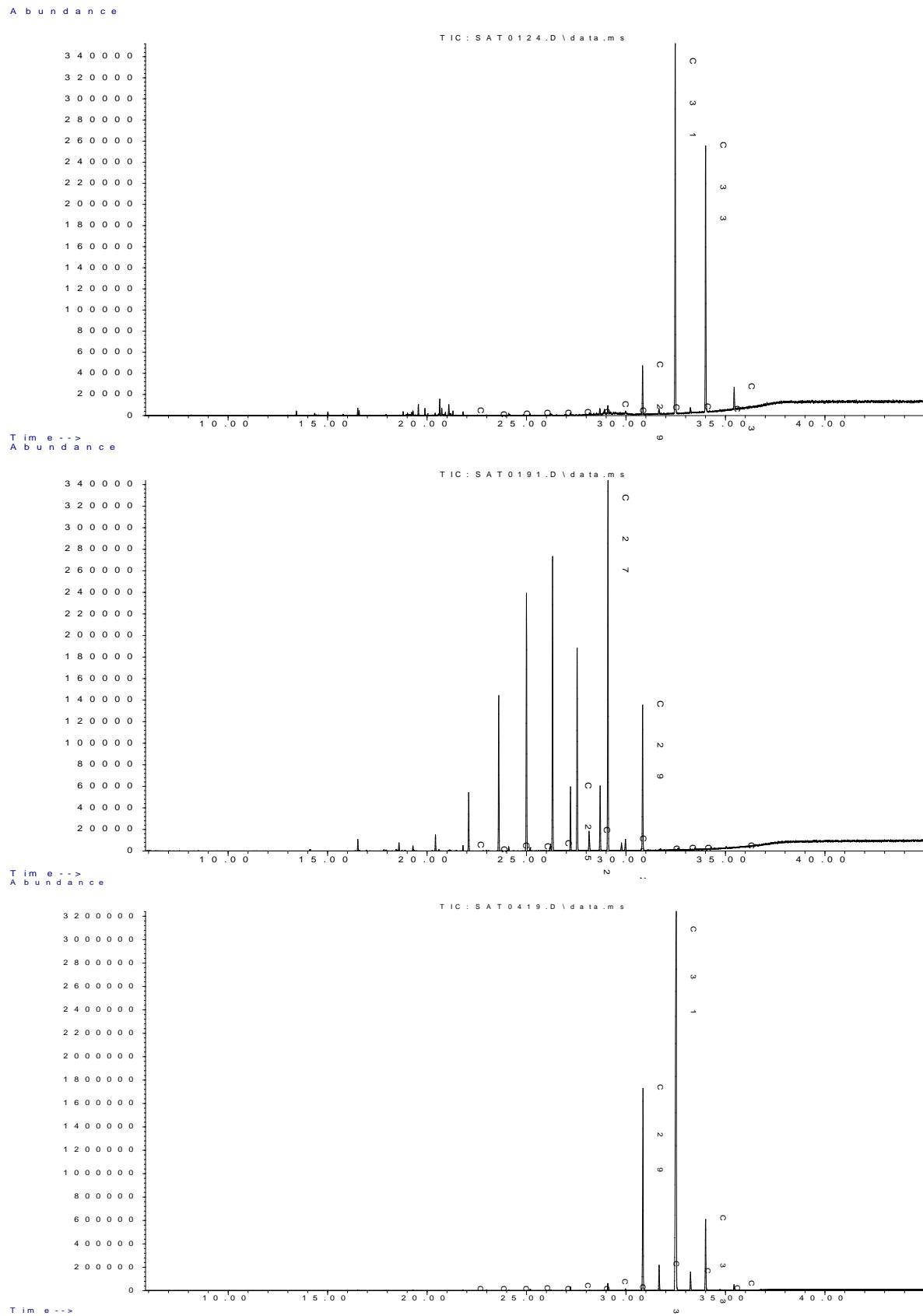
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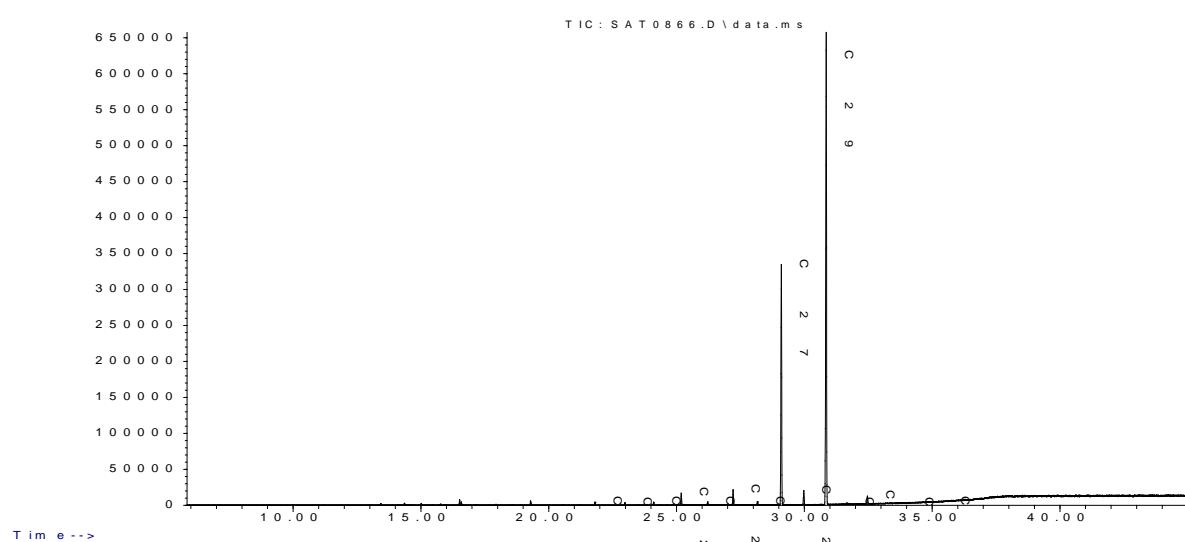
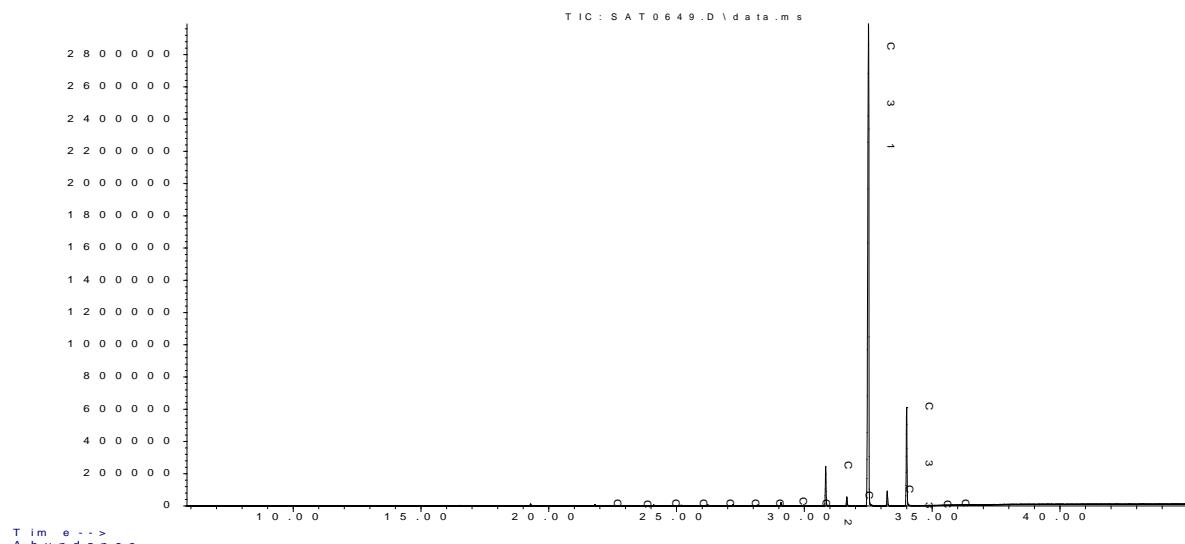
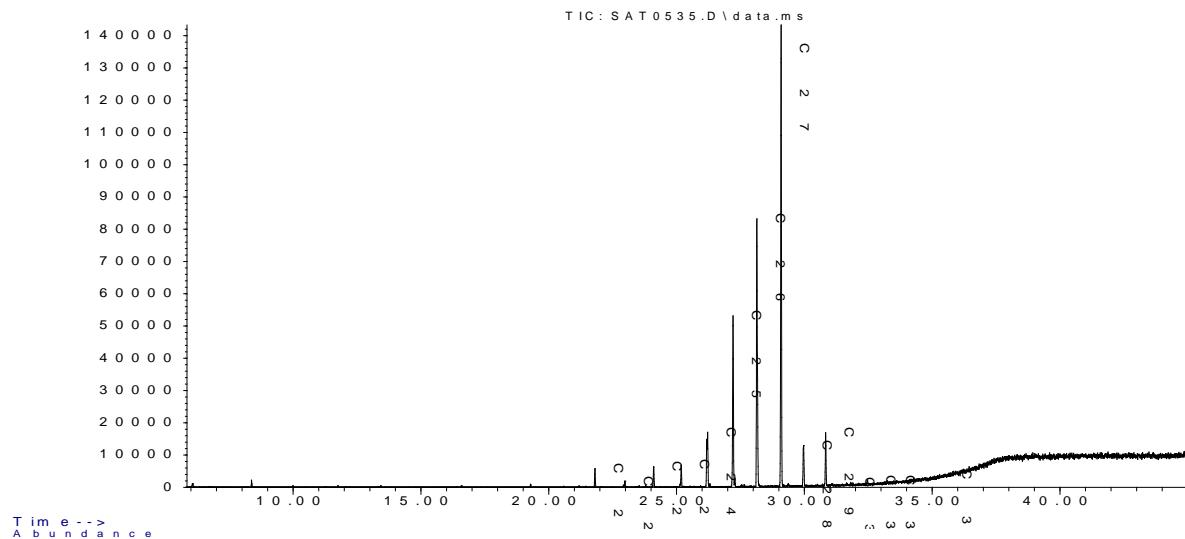
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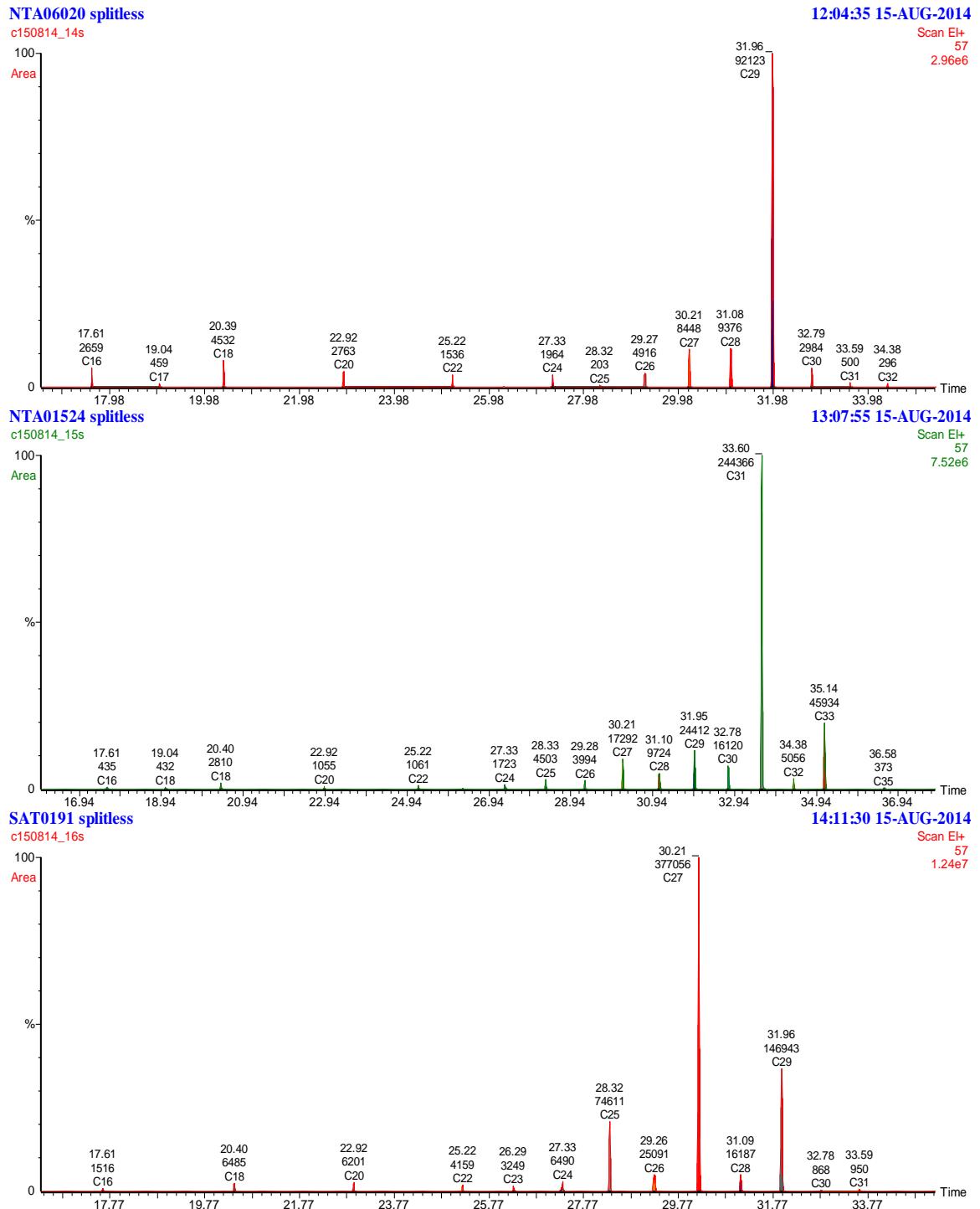
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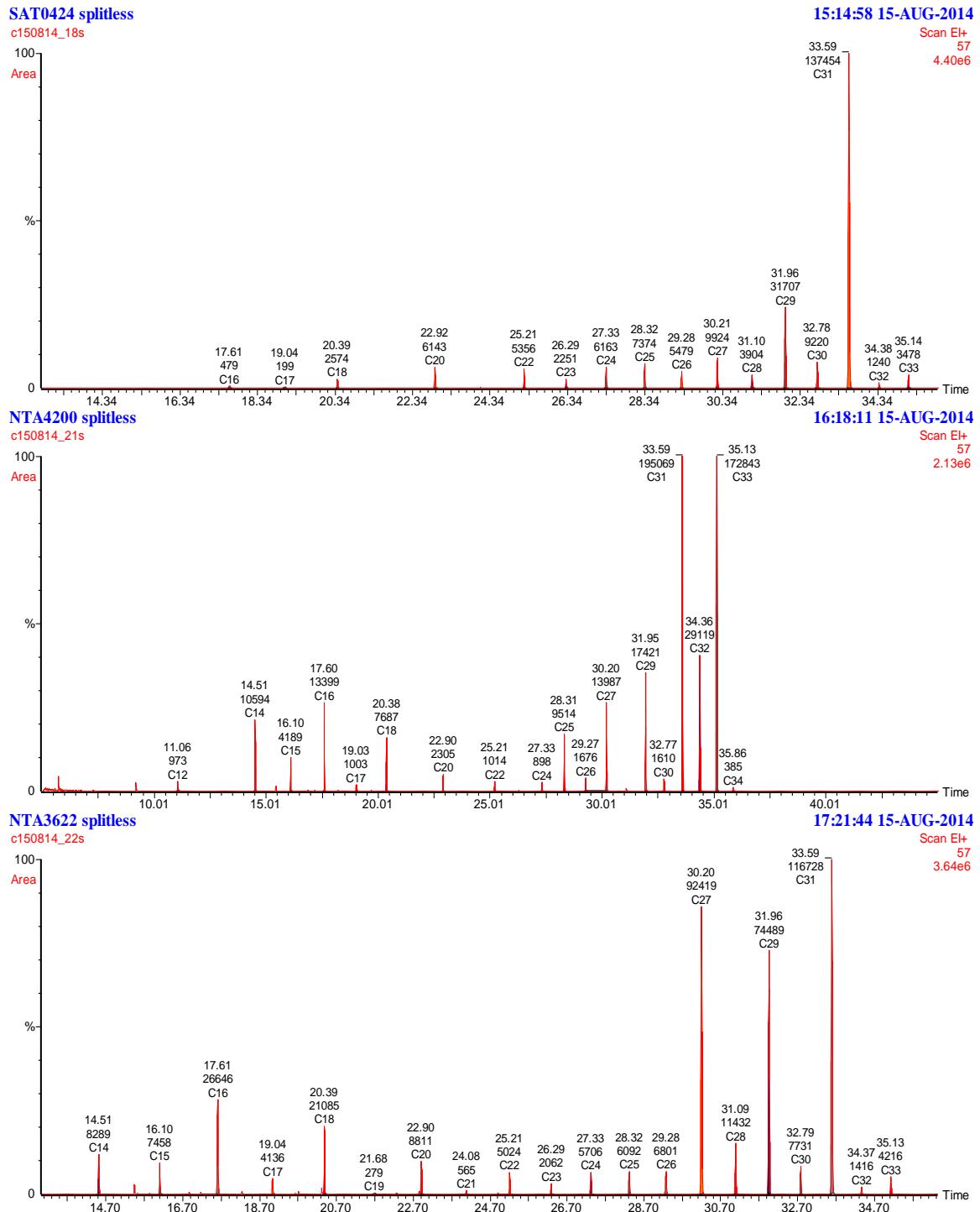




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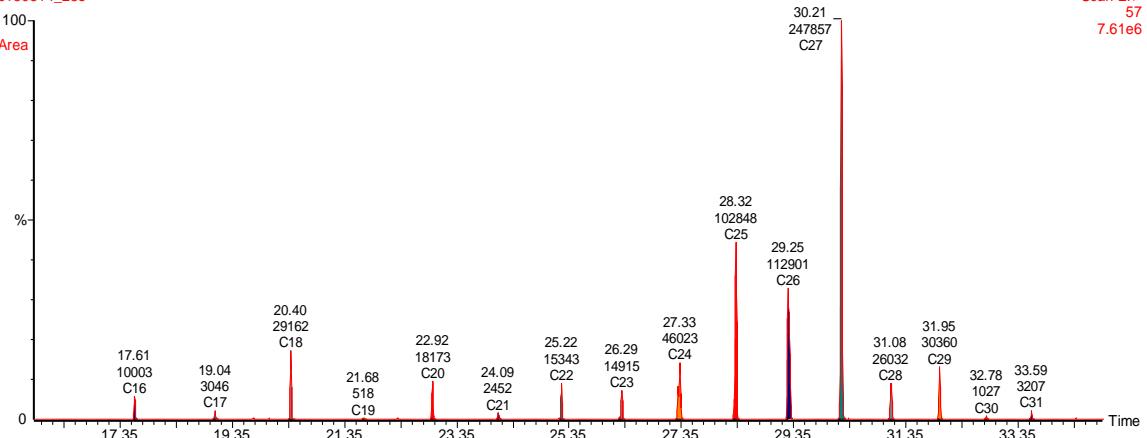






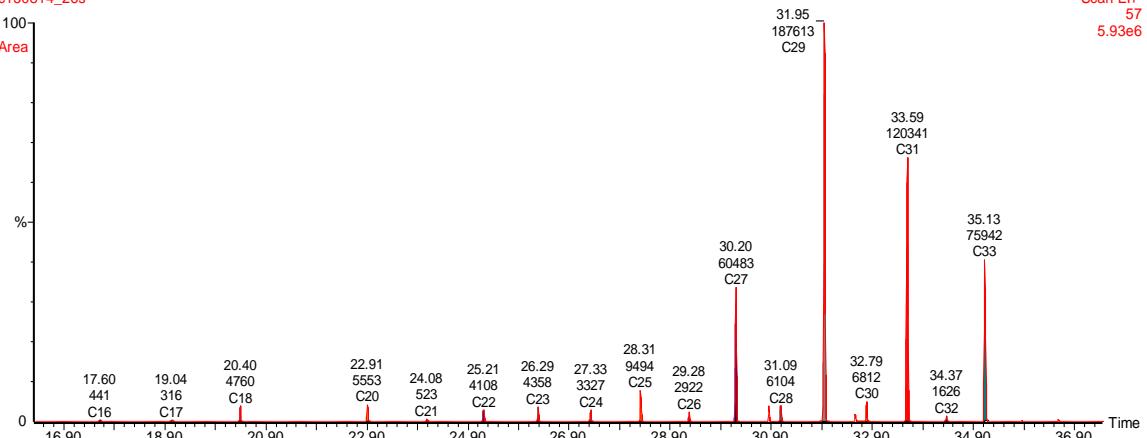
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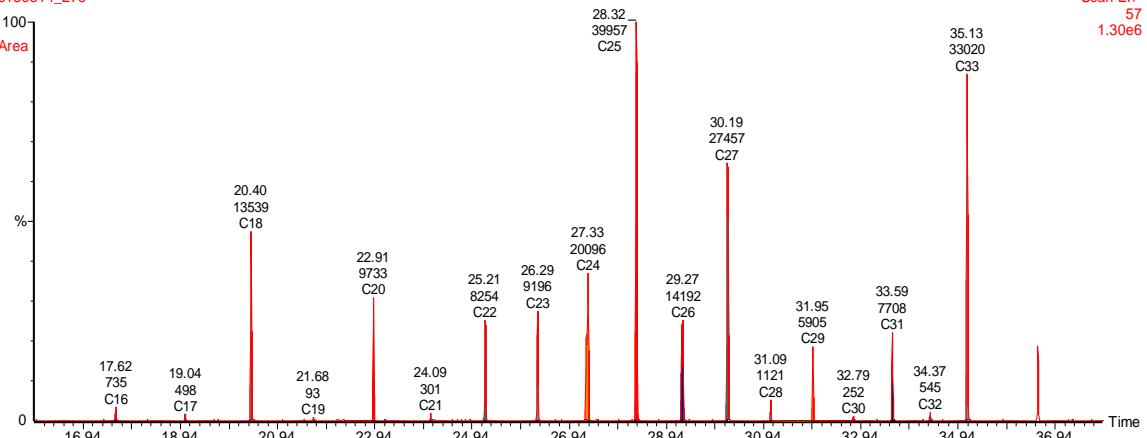
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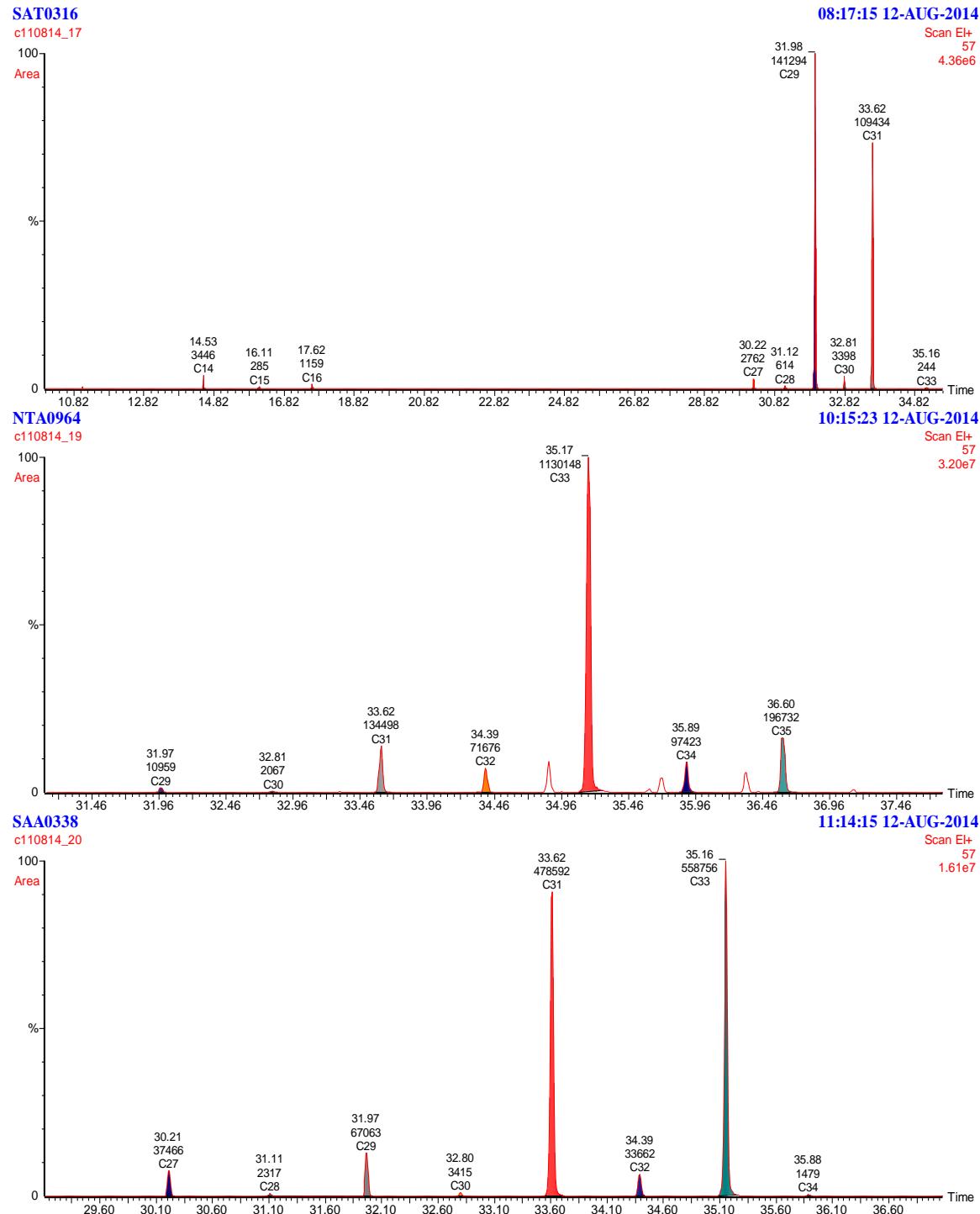
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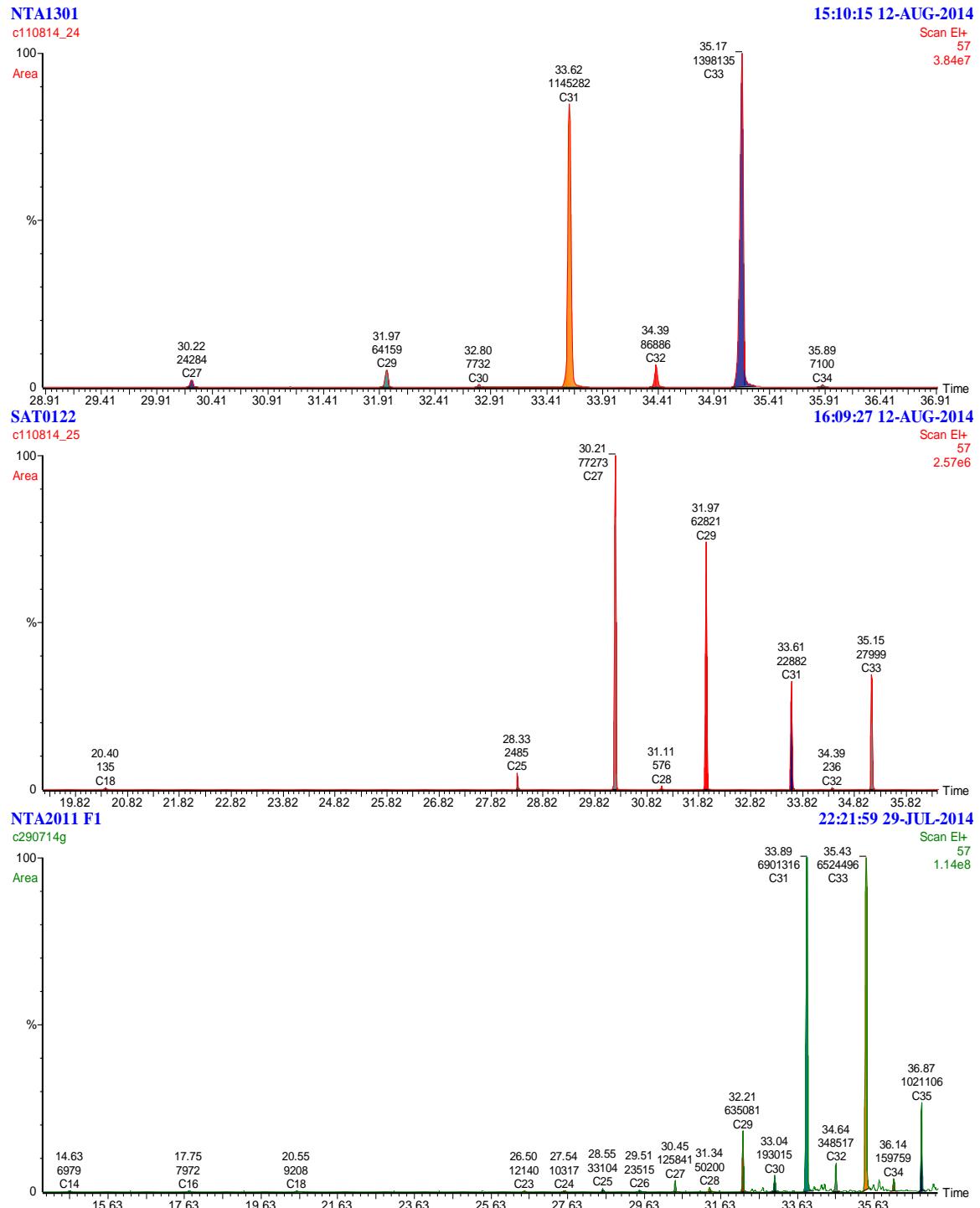


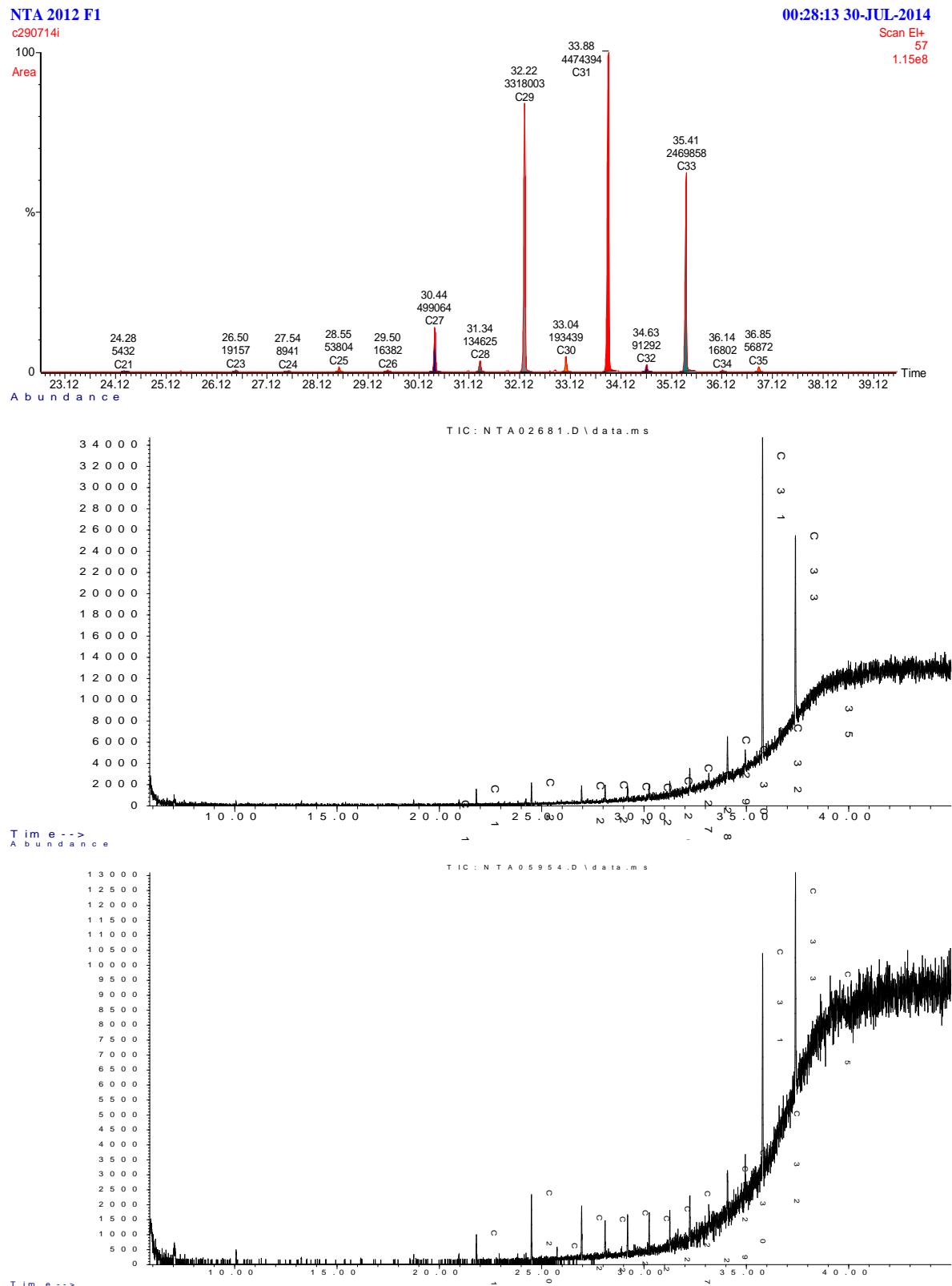
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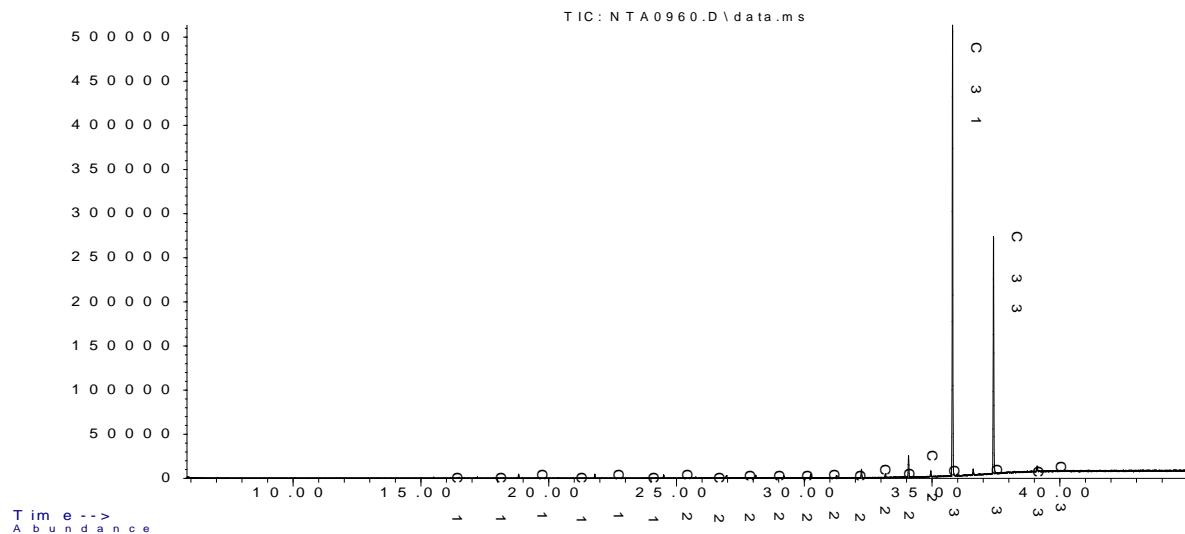




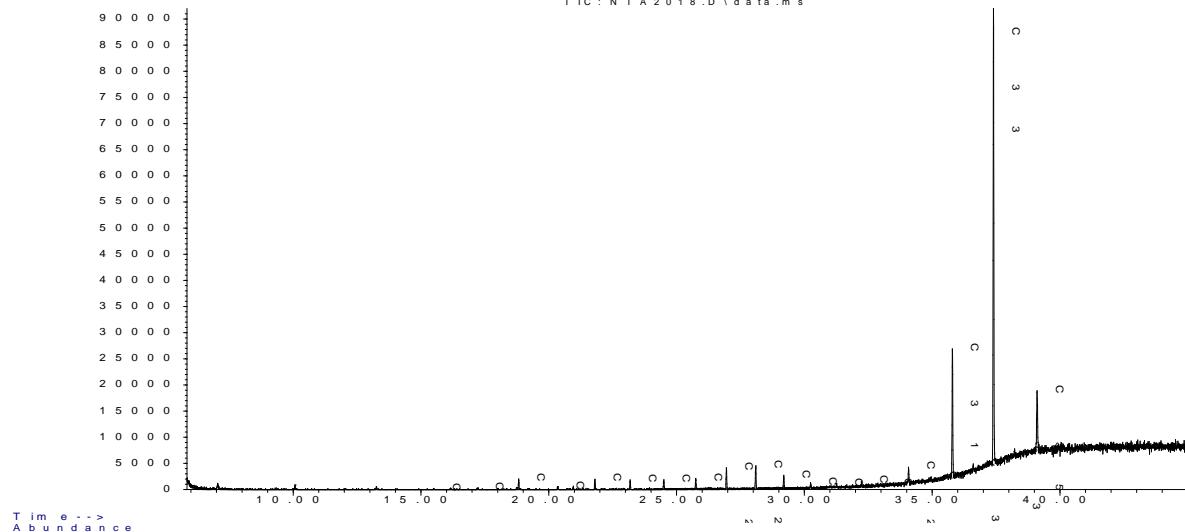




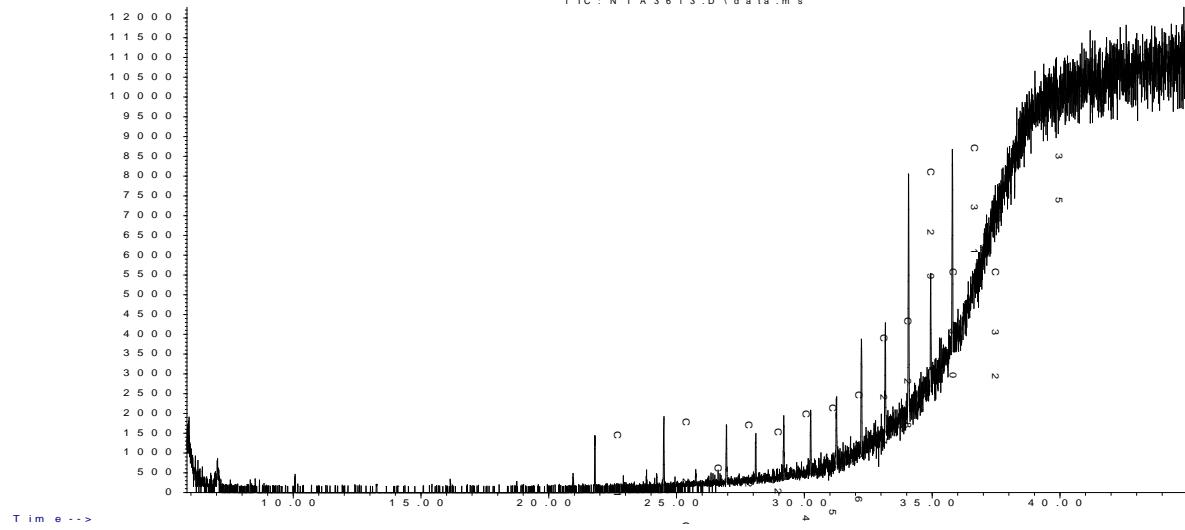
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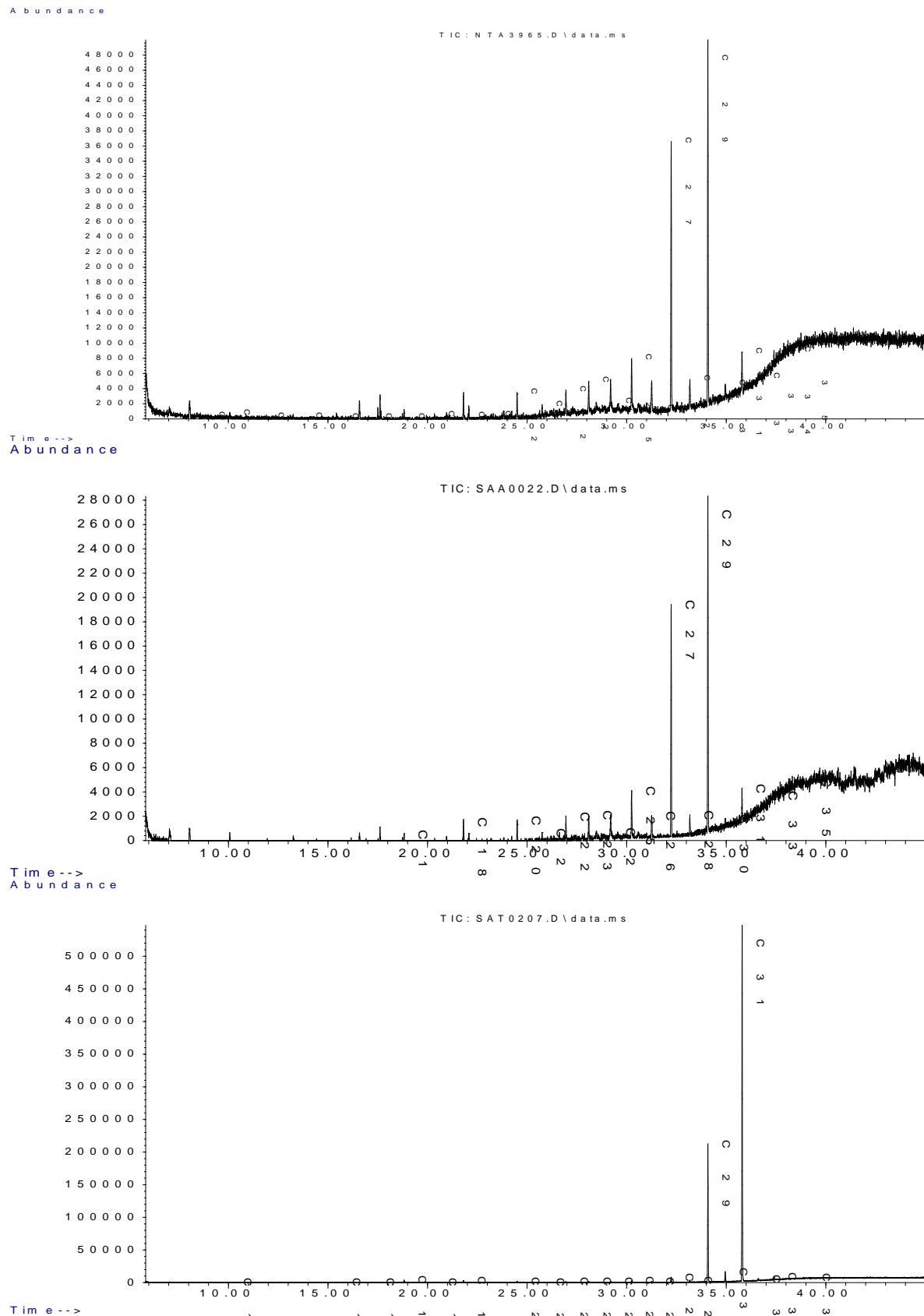


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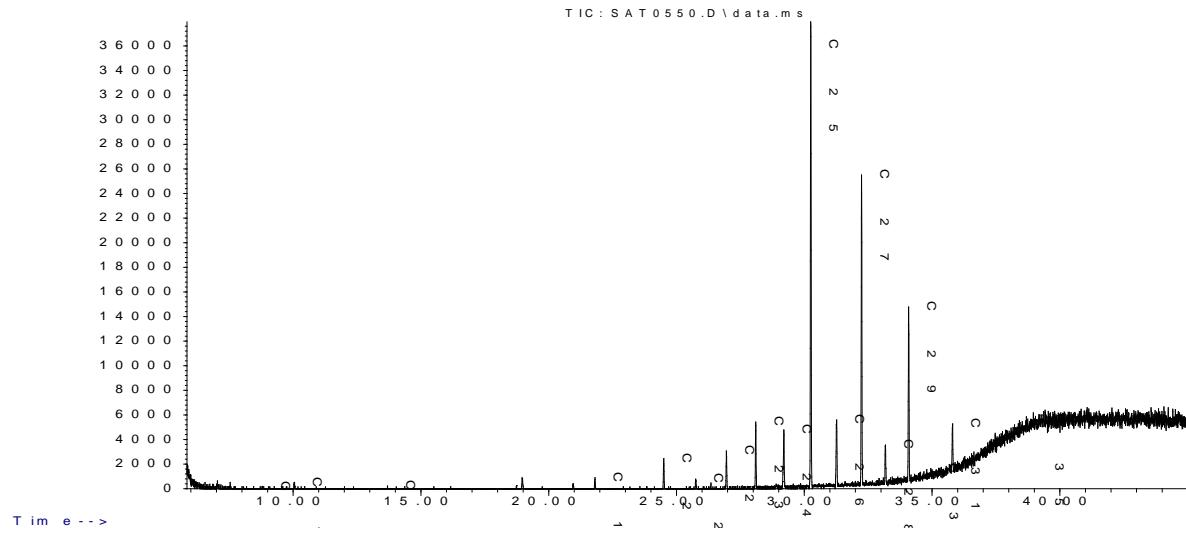
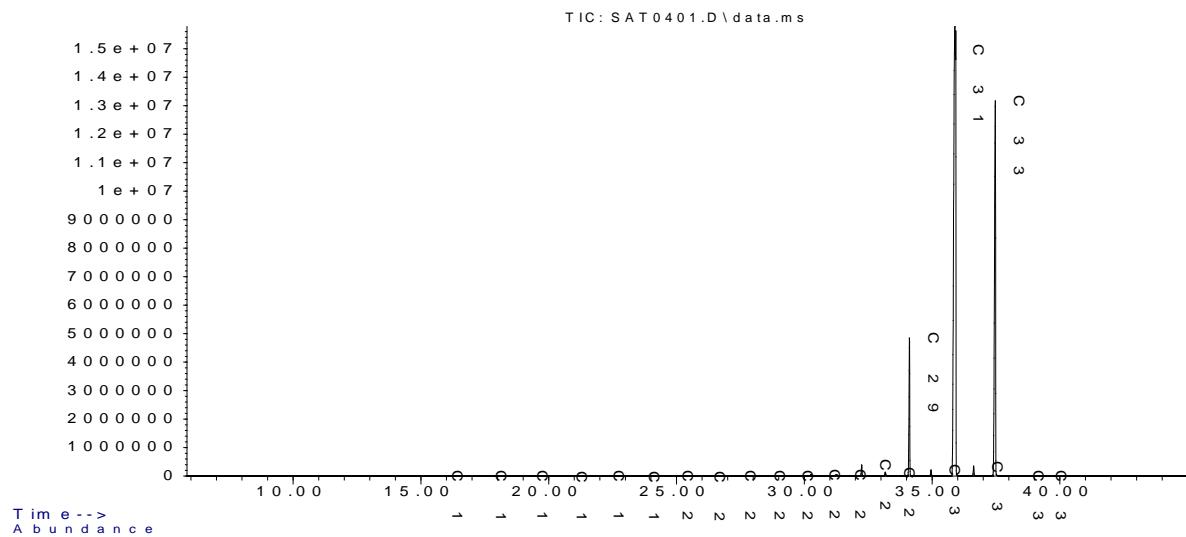
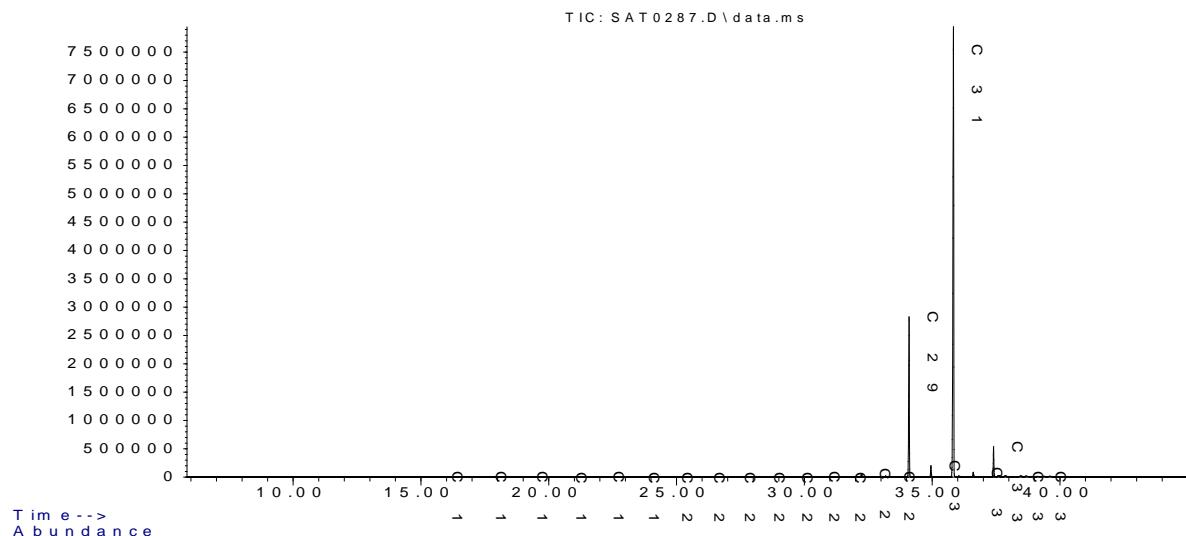


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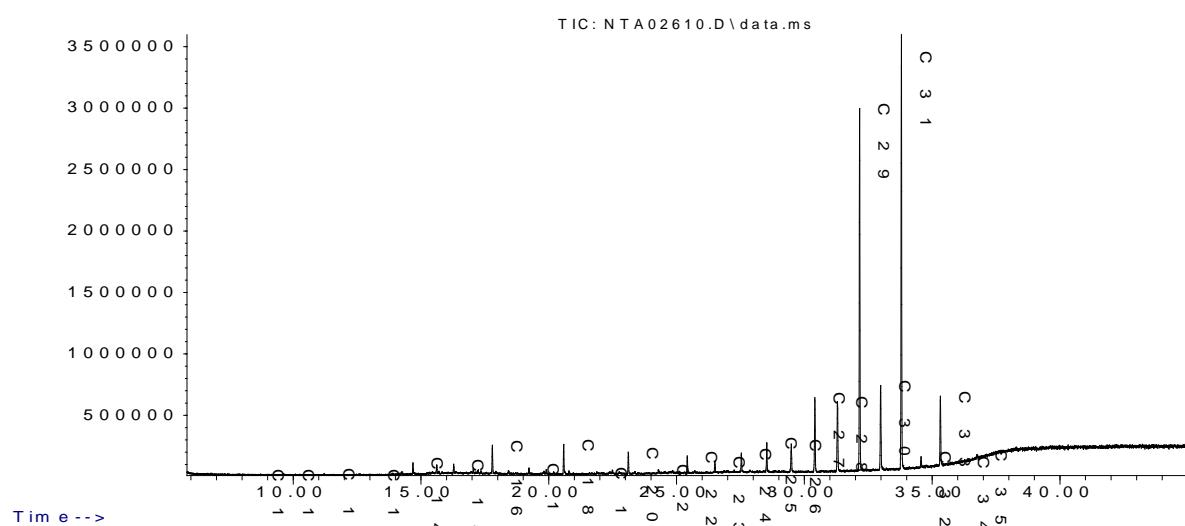
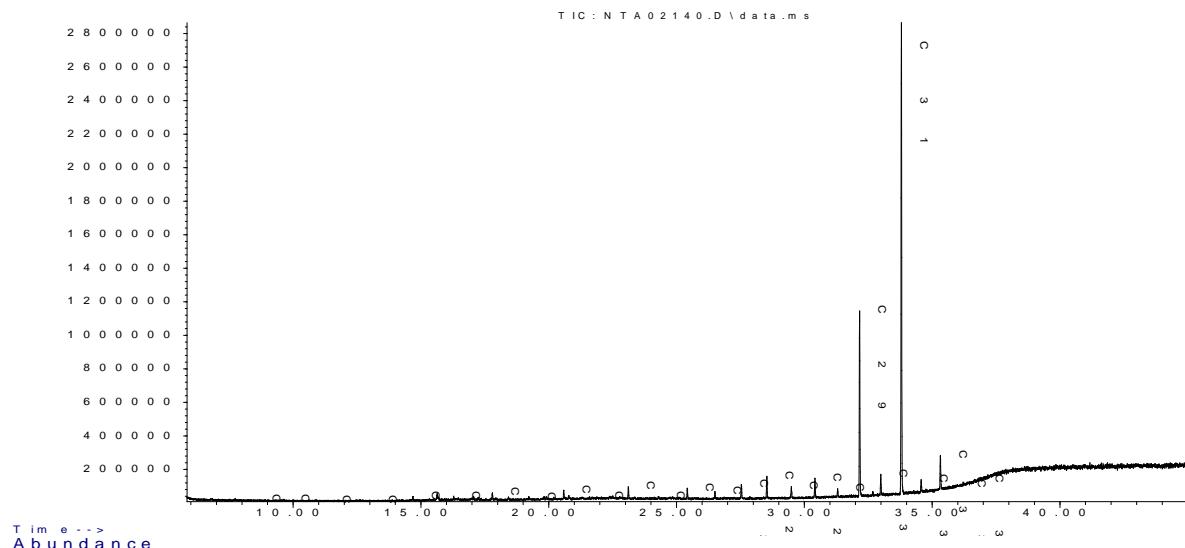
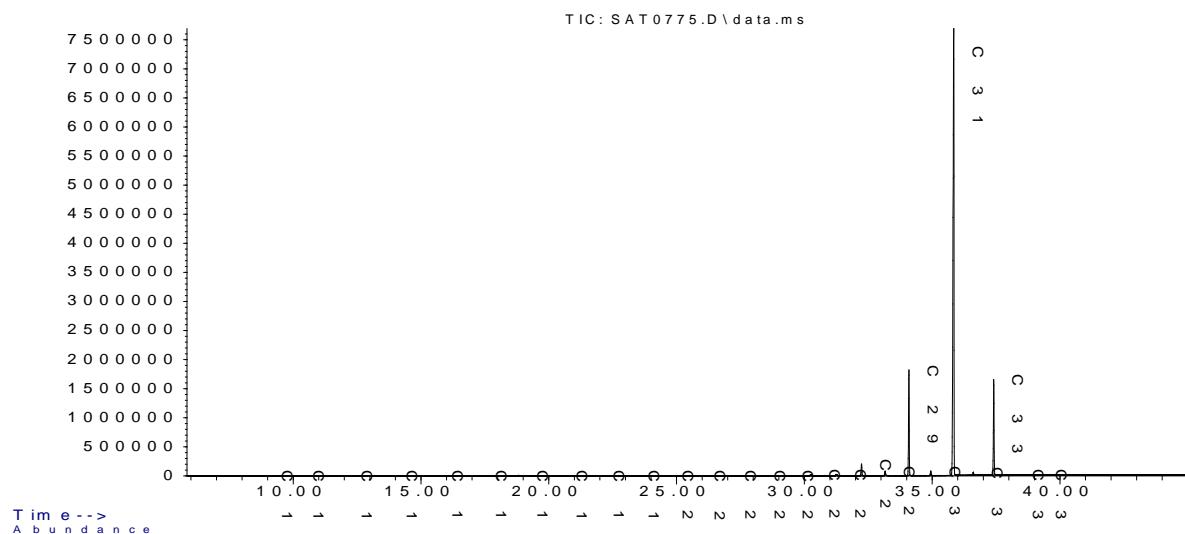




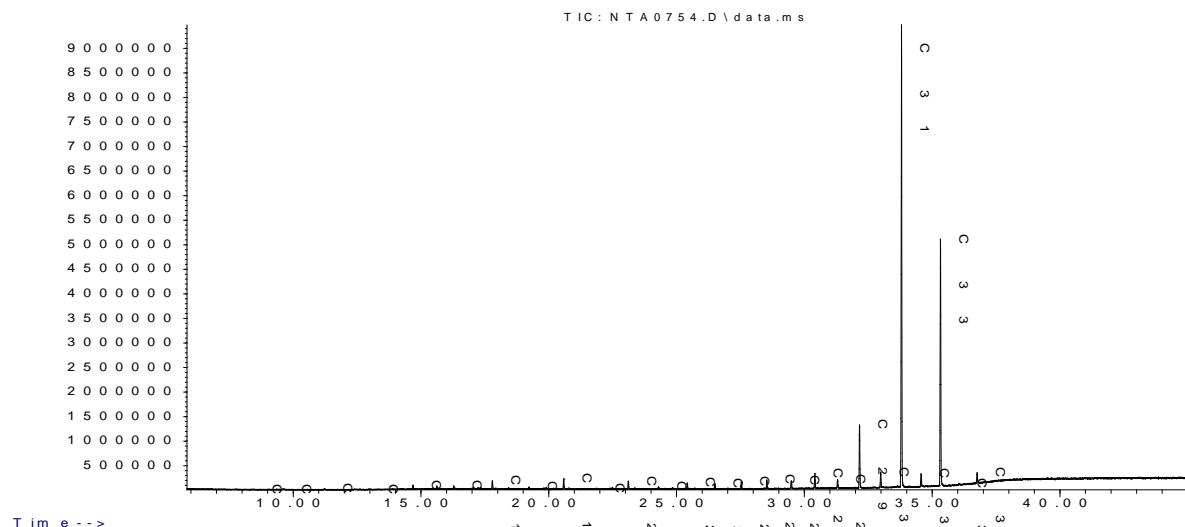
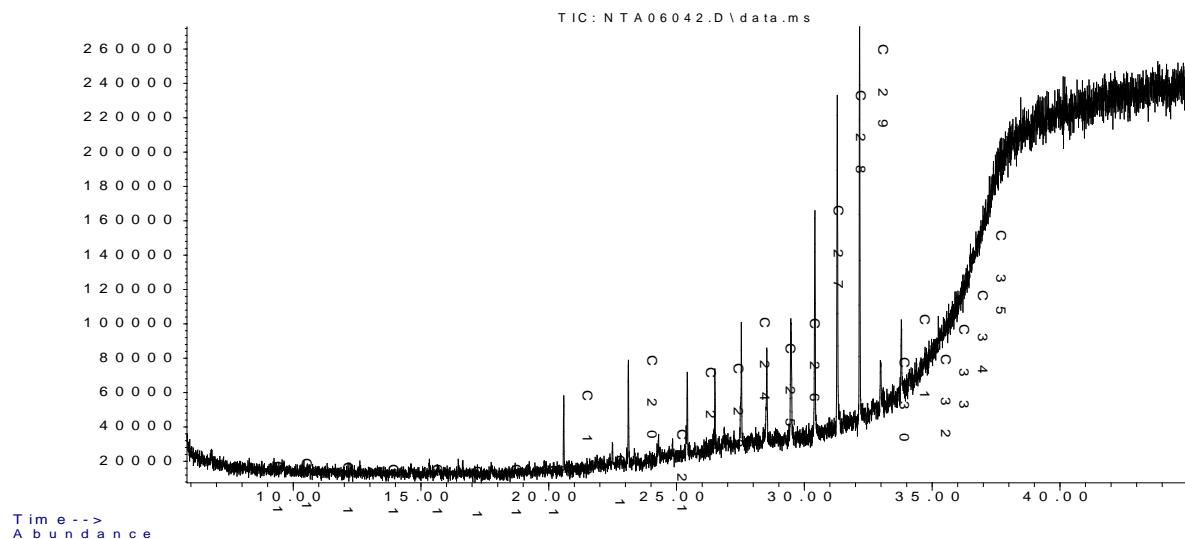
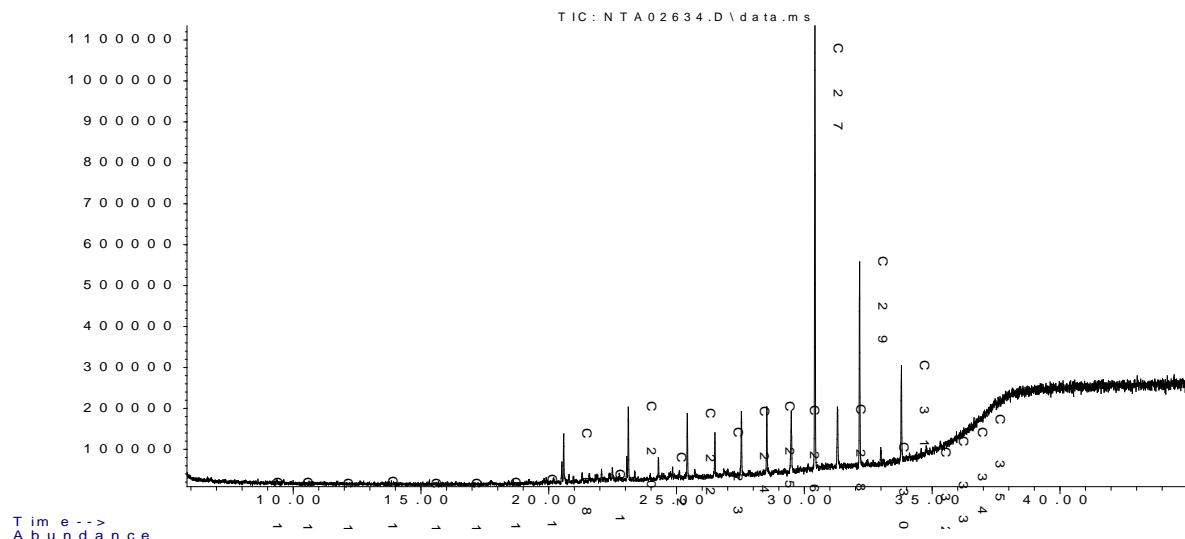
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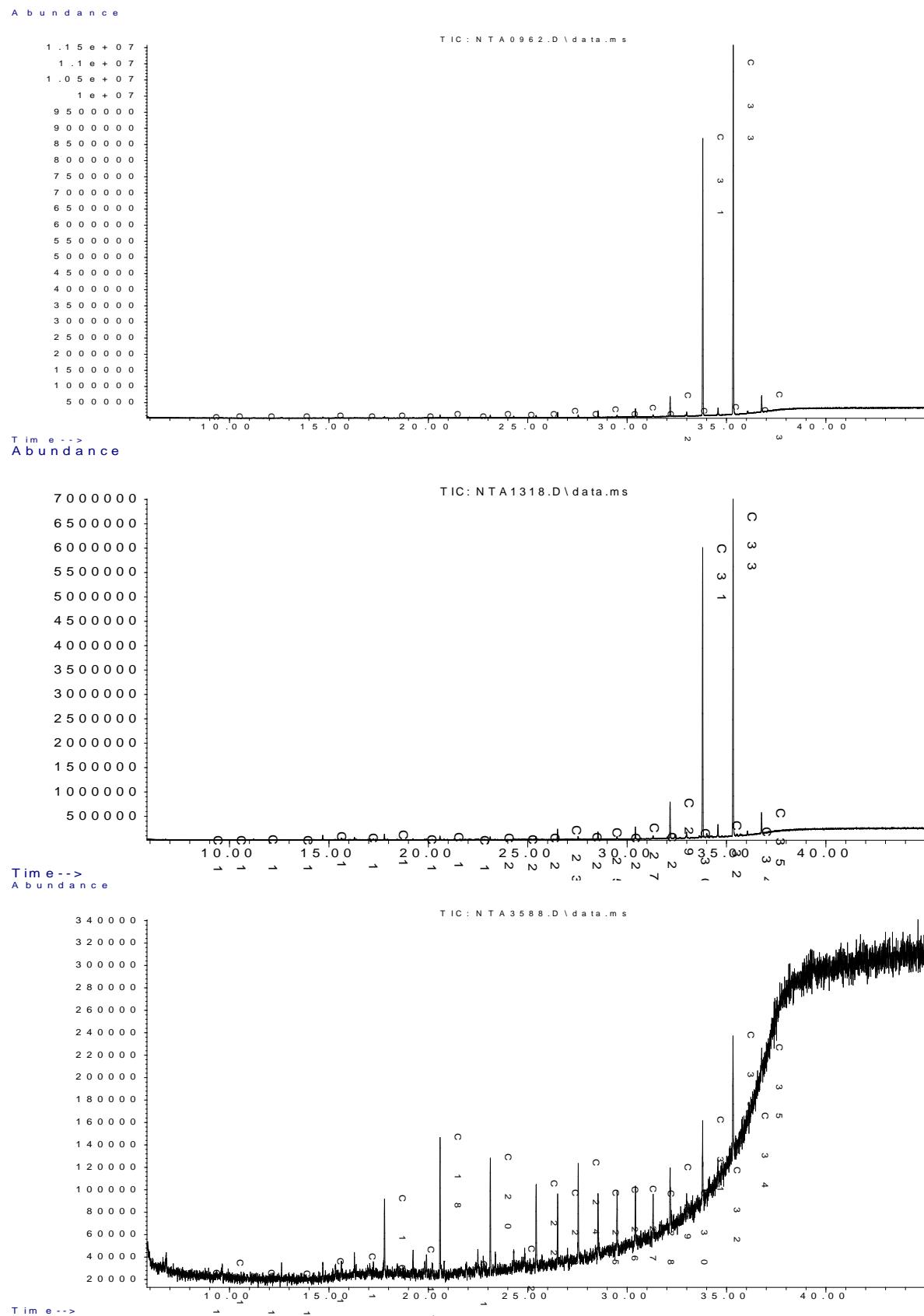


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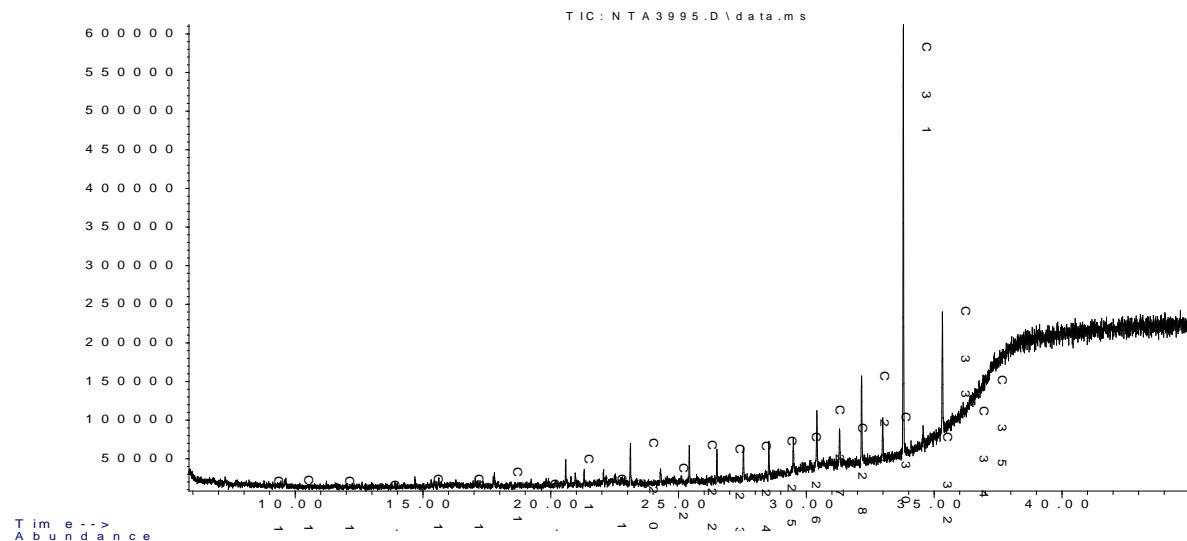


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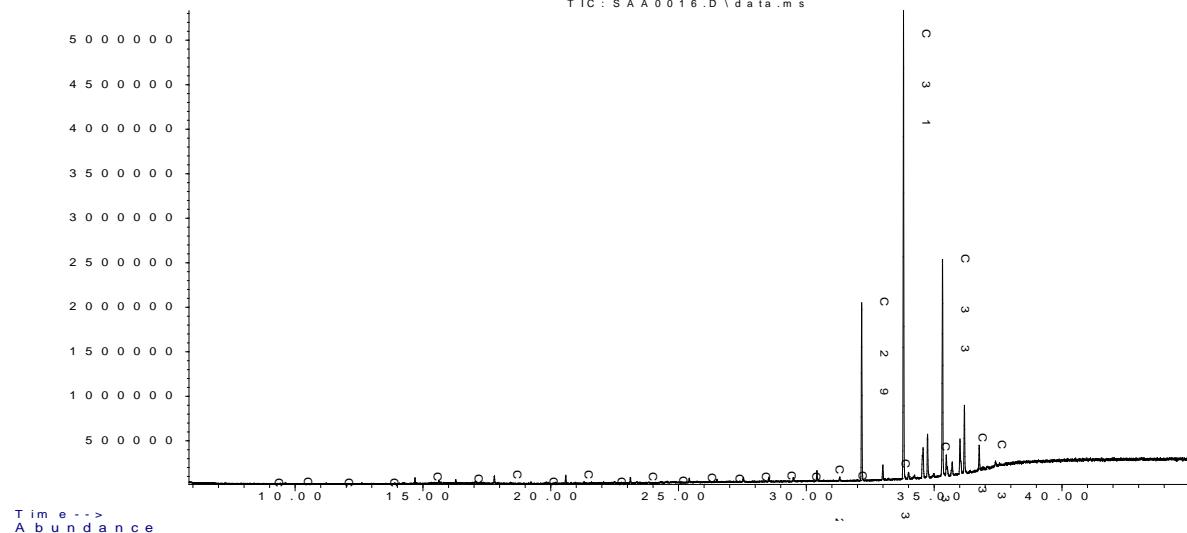




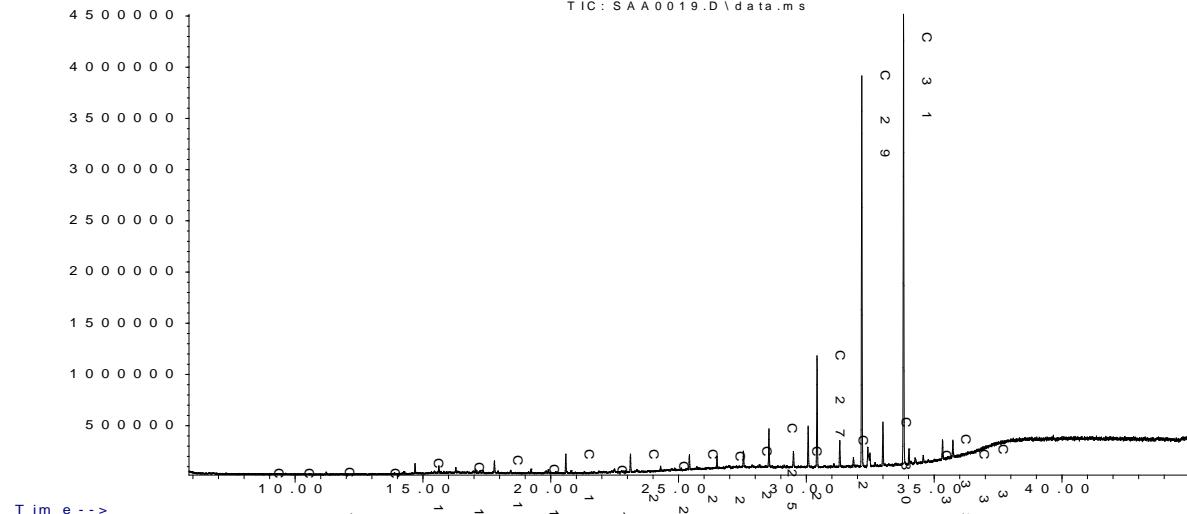
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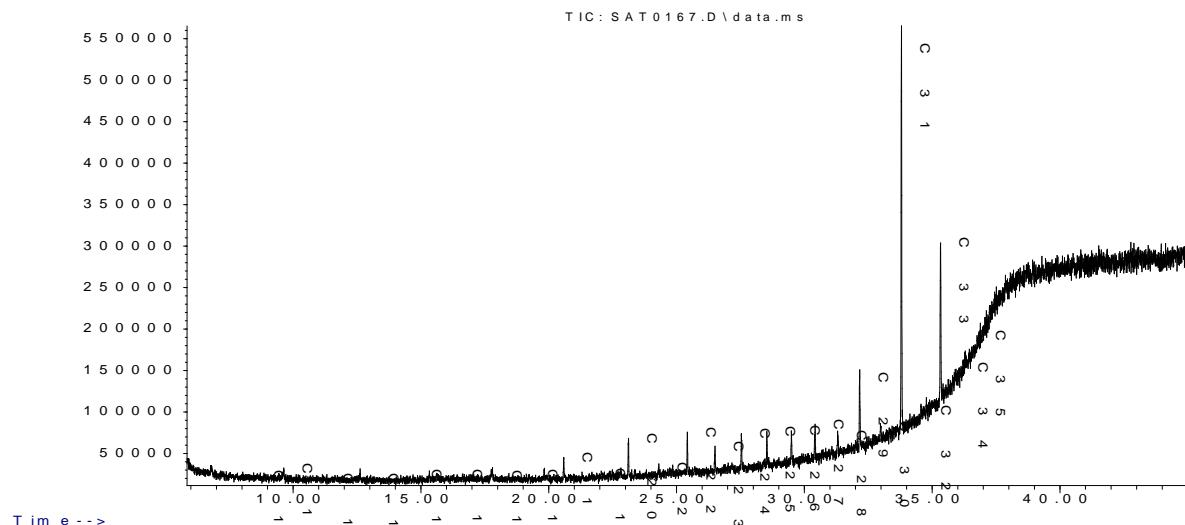
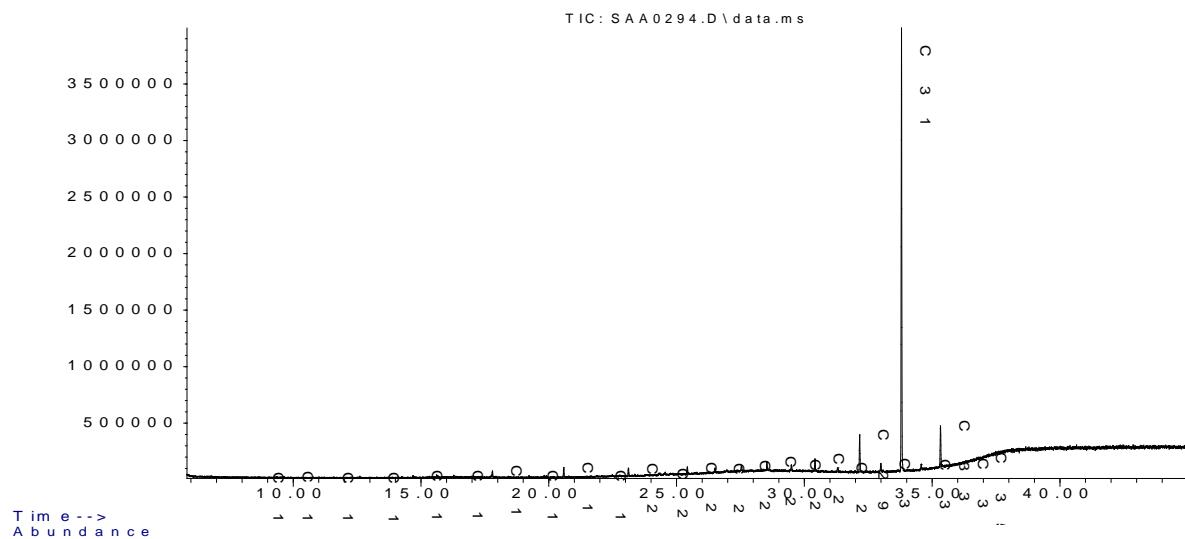
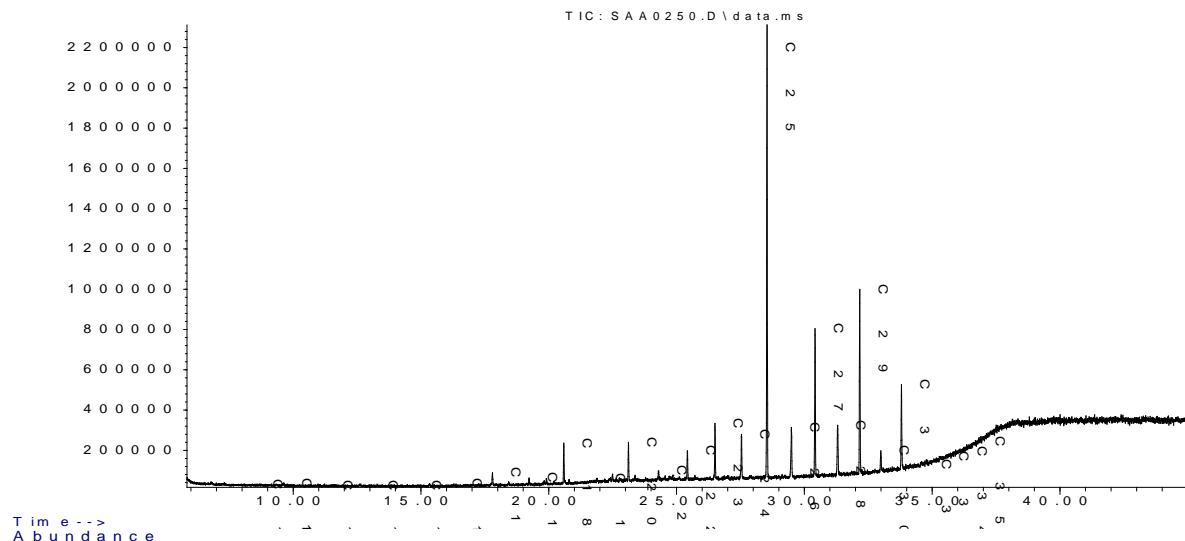
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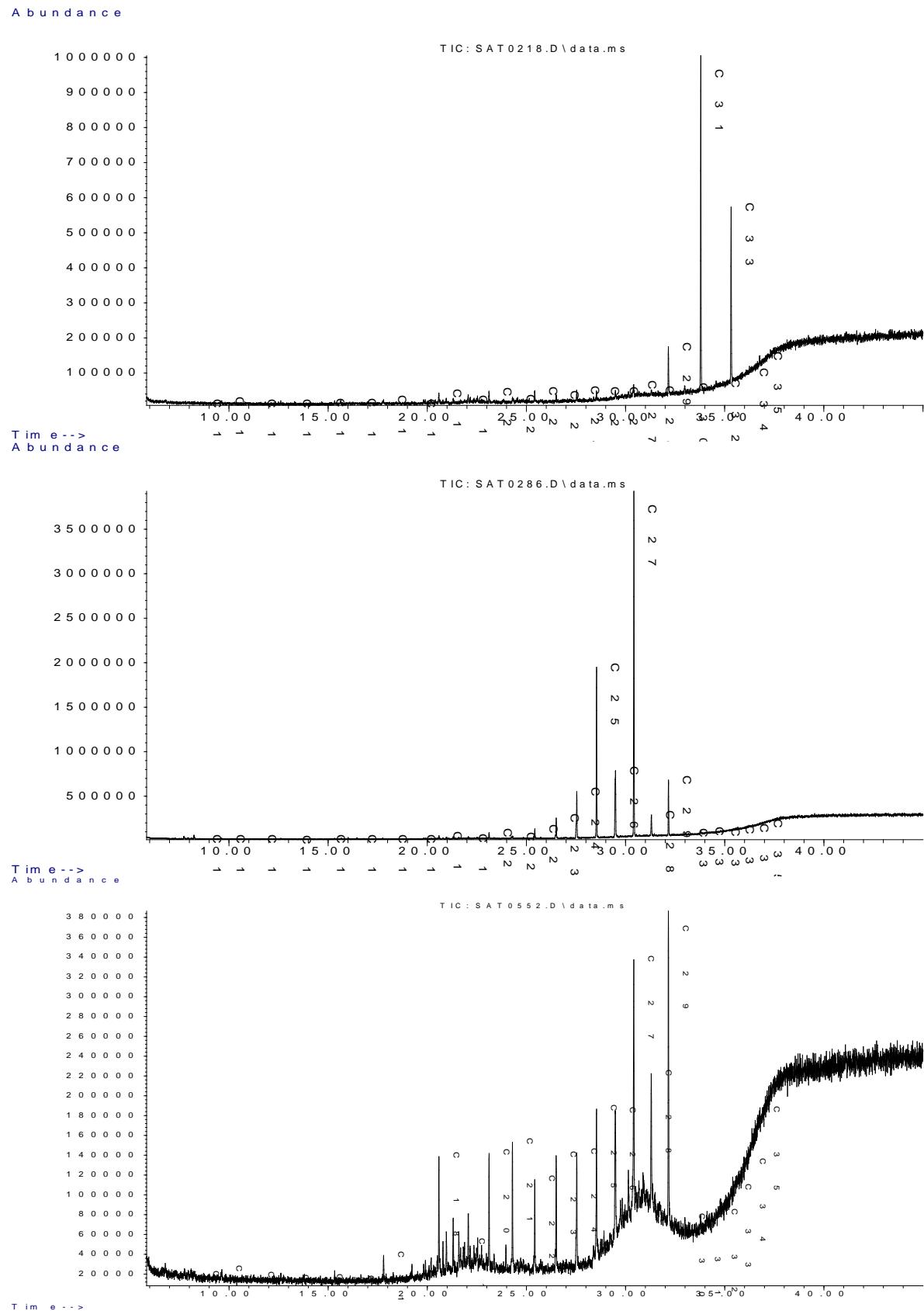


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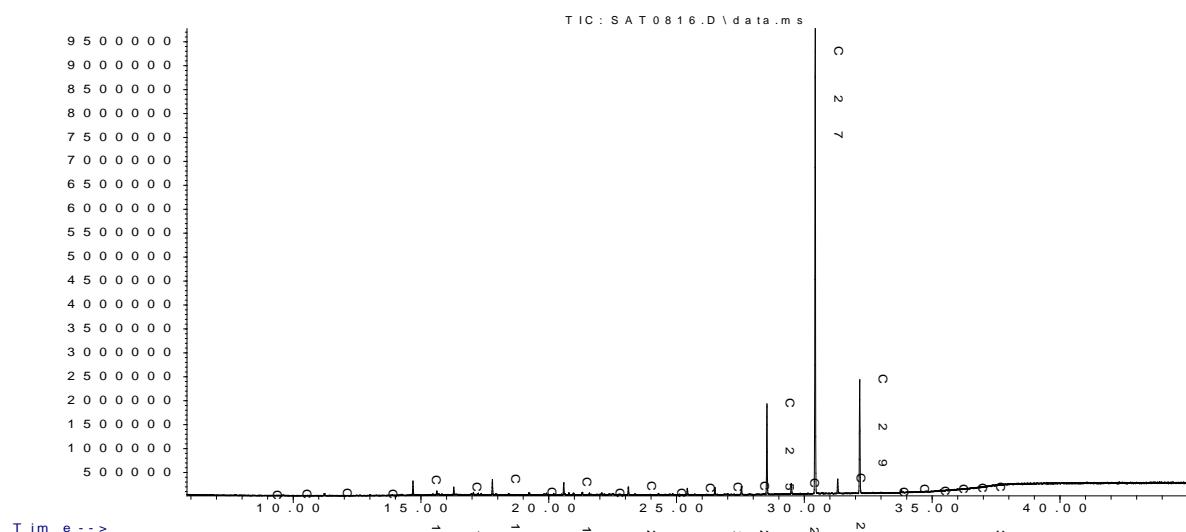
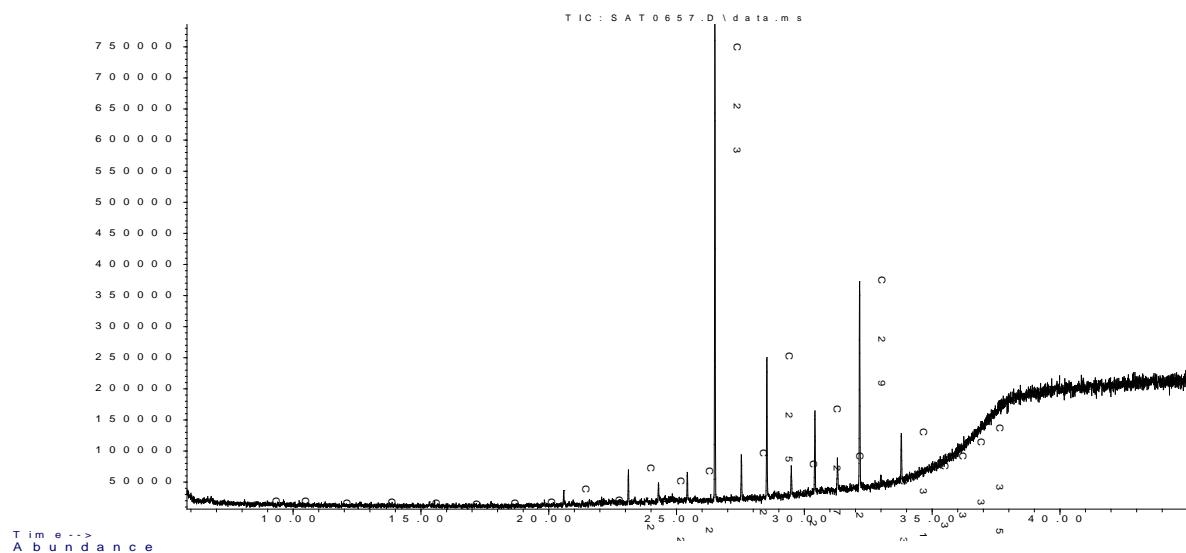
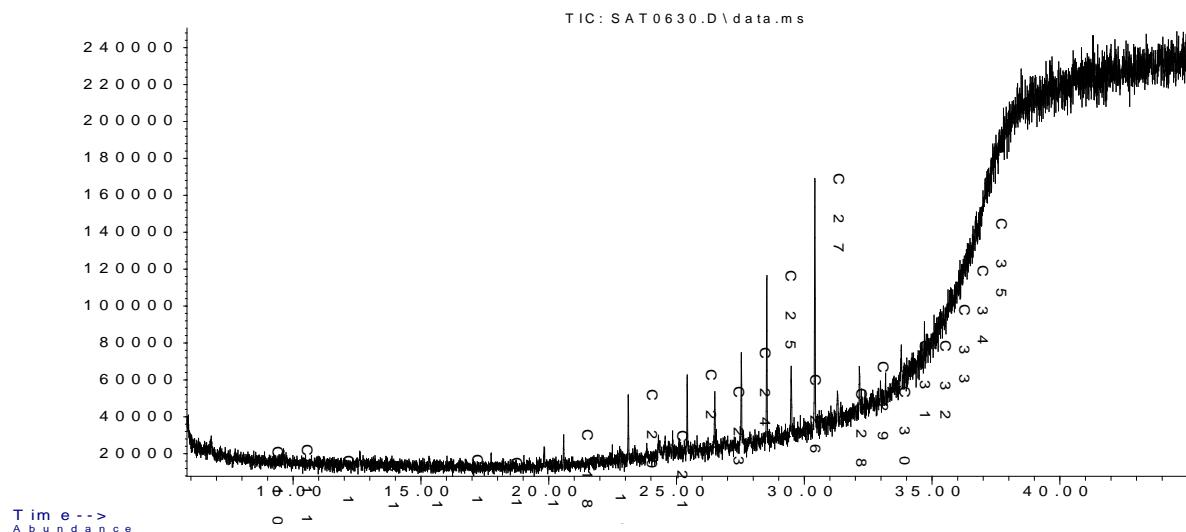


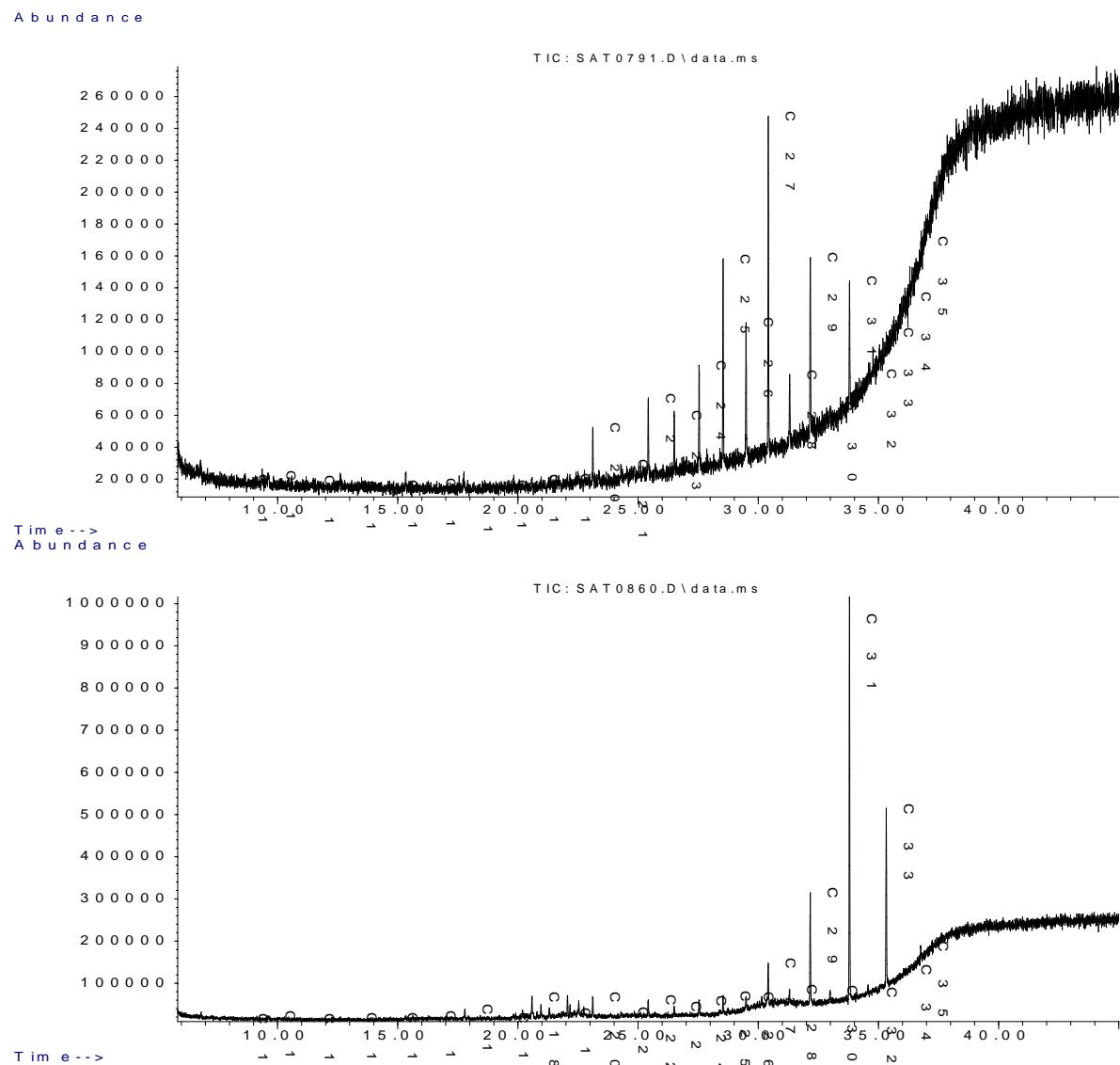
Abundance





A b u n d a n c e





**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			
NTAGFU001	Gulf fall and uplands	Aristida pruinosa	Tree Mallee	NTA 001524	Enneapogon polypylylus	Tussock Grass	NTA 001525	Eucalyptus pruinosa	Tree Mallee	NTA 001531	NTAGFU001			
		NAME	FOUND_RT	AREA	NAME	FOUND_RT	AREA	NAME	FOUND_RT	AREA	NAME			
		C16	17.610	434.566	C20	21.802	2726	C20	21.806	1299	C20			
		C18	19.043	432.281	C21	22.98	982	C21	0	0	C21			
		C18	20.395	2810.143	C22	24.101	3108	C22	24.105	1084	C22			
		C20	22.919	1054.922	C23	25.179	2341	C23	25.178	557	C23			
		C22	25.217	1060.543	C24	26.216	2273	C24	26.215	2077	C24			
		C24	27.326	1723.095	C25	27.205	1752	C25	27.209	1742	C25			
		C25	28.329	4502.880	C26	28.168	1208	C26	28.162	3010	C26			
		C26	29.282	3993.667	C27	29.079	1053	C27	29.089	9496	C27			
		C27	30.213	17291.709	C28	29.959	329	C28	29.974	874	C28			
		C28	31.100	9724.136	C29	30.828	1107	C29	30.838	2567	C29			
		C29	31.951	24412.240	C30	31.671	60	C30	0	0	C30			
		C30	32.780	16119.520	C31	32.462	6210	C31	32.461	292	C31			
		C31	33.602	244365.578	C32	33.236	282	C32	0	0	C32			
		C32	34.380	5055.872	C33	33.996	15930	C33	0	0	C33			
		C33	35.136	45933.559	C34	0	0	C34	0	0	C34			
		C35	36.583	372.683	C35	35.435	1301	C35	35.439	1271	C35			
			ACL	30.847	Average		ACL	31.736	Average		ACL	27.845	Average	
			C27/C31	30.73566			C27/C31	0.170	30.42007		C27/C31	32.521	27.11933	
			C29/C33	0.531	31.61187		C29/C33	0.069	32.7401		C29/C33	#DIV/0!	29	
			CPI	8.326748907			CPI	4.068112948			CPI	2.170262598		
												ACL	30.293	Average
												C27/C31	0.800	29.22241
												C29/C33	0.842	31.17104
												CPI	2.603871368	

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				
NTAGFU008	Gulf fall and uplands	Triodia pungens	Hummock grass	NTA 002012	Aristida contorta	Tussock Grass	NTA 002011	Fimbristylis dochotoma	Sedge	NTA 002018	NTAGFU008				
		NAME	FOUND_RT	AREA	NAME	FOUND_RT	AREA	NAME	TIME	PEAK AREA	NAME				
		C21	24.279	5432.154	C14	14.629	6979.258	C10	0	0	C20				
		C23	26.5	19156.979	C16	17.748	7971.676	C11	0	0	C21				
		C24	27.538	8940.808	C18	20.553	9207.618	C12	0	0	C22				
		C25	28.546	53803.813	C23	26.5	12139.624	C13	0	0	C23				
		C26	29.503	16381.983	C24	27.545	10316.734	C14	15.509	48	C24				
		C27	30.438	499063.969	C25	28.546	33103.785	C15	17.205	382	C25				
		C28	31.336	134624.813	C26	29.51	23514.857	C16	18.828	1415	C26				
		C29	32.22	3318002.75	C27	30.445	125840.734	C17	20.352	775	C27				
		C30	33.039	193438.5	C28	31.336	50200.406	C18	21.802	1616	C28				
		C31	33.879	4474394	C29	32.206	635080.75	C19	23.179	1464	C29				
		C32	34.631	91291.695	C30	33.039	193015.188	C20	24.503	1494	C30				
		C33	35.413	2469857.5	C31	33.886	6901315.5	C21	25.749	1499	C31				
		C34	36.136	16801.893	C32	34.639	348517.281	C22	26.953	2763	C32				
		C35	36.852	56871.539	C33	35.435	6524495.5	C23	28.1	3175	C33				
			ACL	30.65159259	Average	C34	36.136	159758.859	C24	29.194	1963	C34			
			C27/C31	0.111537779	30.59862	C35	36.867	1021105.625	C25	30.241	546	C35			
			C29/C33	1.343398455	30.70692		ACL	31.9947727	Average	C26	31.257	667	ACL		
			CPI	23.54476491			C27	32.231	628	C27	35.796	13042	29.235	Average	
							C28	33.178	180	C28	36.608	760	C27/C31	0.369	29.92144
							C29	34.074	2013	C29	37.403	42991	C29/C33	4.987	29.66815
							C30	34.958	420	C30	38.21	922	CPI	3.338507635	
							C31	35.796	13042	C31	39.105	32702			
							C32	36.608	760	C32					
							C33	37.403	42991	C33					
							C34	38.21	922	C34					
							C35	39.105	32702	C35					
								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	
NTAGFU0010	Gulf fall and uplands	<i>Triodia pungens</i>	Hummock grass	NTA 002136	Eucalyptus <i>Teucriophloia</i>	Tree Mallee	NTA 002140	N/A	N/A	N/A	NTAGFU0010	Gulf fall and uplands
NAME	FOUND_RT	AREA	NAME	TIME	PEAK AREA	NAME	FOUND_RT	AREA	NAME	FOUND_RT	AREA	
C16	17.603	441.499	C10	8.465	619	C20	21.812	15704	C20	21.812	15704	
C17	19.035	316.026	C11	9.606	879	C21	22.98	15651	C21	22.98	15651	
C18	20.395	4760.39	C12	11.219	1197	C22	24.174	1763	C22	24.174	1763	
C20	22.911	5553.133	C13	13.009	456	C23	25.179	12748	C23	25.179	12748	
C21	24.082	523.02	C14	14.69	13210	C24	26.21	7326	C24	26.21	7326	
C22	25.209	4107.61	C15	16.287	9739	C25	27.21	16007	C25	27.21	16007	
C23	26.293	4357.735	C16	17.794	20657	C26	28.163	4730	C26	28.163	4730	
C24	27.326	3327.128	C17	19.234	8997	C27	29.085	23477	C27	29.085	23477	
C25	28.315	9494.427	C18	20.585	29287	C28	29.975	6448	C28	29.975	6448	
C26	29.282	2922.196	C19	21.878	3427	C29	30.833	84321	C29	30.833	84321	
C27	30.198	60483.355	C20	23.114	29082	C30	31.666	8650	C30	31.666	8650	
C28	31.093	6104.42	C21	24.292	7822	C31	32.467	76169	C31	32.467	76169	
C29	31.951	187612.844	C22	25.428	30277	C32	33.242	2550	C32	33.242	2550	
C30	32.787	6811.742	C23	26.506	19808	C33	33.995	26914	C33	33.995	26914	
C31	33.594	120340.938	C24	27.538	38924	C34	34.739	380	C34	34.739	380	
C32	34.365	1625.671	C25	28.527	63746	C35	35.43	6520	C35	35.43	6520	
C33	35.129	75941.594	C26	29.49	32399							
		ACL 29.84936469 Average	C27	30.417	52380							
		C27/C31 0.50259996 29.66205	C28	31.302	20027							
		C29/C33 2.470488623 30.15258	C29	32.16	500833							
		CPI 18.41426136	C30	32.988	53383							
			C31	33.799	1357342							
			C32	34.574	36215							
			C33	35.318	91997							
			C34	36.061	1117							
			C35	36.752	18251							
		ACL 30.35877543 Average										
		C27/C31 0.038590127 30.85137										
		C29/C33 5.444014479 29.62073										
		CPI 9.885667932										

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	<i>Melaleuca viridiflora</i>	Shrub	NTA 002634	Chrysopogon fallax	Tussock Grass	NTA 002610	<i>Schizachyrium fragile</i>	Tussock Grass	NTA 002681	NTAGFU0017	Gulf fall and uplands			
		NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA		
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			ACL 30.263 Average
C27			30.414	526718	C27		30.409	288556	C27		32.236	1438			C27/C31 0.547 29.58504
C28			31.304	80270	C28		31.299	290281	C28		33.179	916			C29/C33 1.186 30.82941
C29			32.163	223566	C29		32.163	1366131	C29		34.069	2593			CPI 5.190847914
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			
		ACL	27.96220902	Average		ACL	30.01957606	Average		ACL	31.27762326	Average			
		C27/C31	4.878870682	27.6804		C27/C31	0.172892691	30.41037		C27/C31	0.093498049	30.65799			
		C29/C33	64.11413823	29.06143		C29/C33	4.842186786	29.68468		C29/C33	0.280870884	32.12288			
		CPI	3.188978131			CPI	4.409230192			CPI	5.995866216				

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					
NTAGFU0031	Gulf fall and uplands	<i>Metaleuca viridiflora</i>	Shrub	NTA 003622	<i>Schizachyrium pachyarthron</i>	Tussock Grass	NTA 003588	<i>Petalostigma banksii</i>	Shrub	NTA 003613	NTAGFU0031	Gulf fall and uplands				
		NAME	FOUND_RT	AREA	NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA			
		C14	14.512	8289.107	C10	8.453	676	C10	0	0	C20	21.796	2167679			
		C15	16.105	7458.247	C11	9.631	216	C11	0	0	C21	22.864	159538			
		C16	17.61	2645.686	C12	11.223	1770	C12	0	0	C22	24.094	1917995			
		C17	19.035	4135.528	C13	13.008	500	C13	0	0	C23	25.058	117571			
		C18	20.388	21085.092	C14	14.688	10030	C14	0	0	C24	26.204	863067			
		C19	21.682	278.873	C15	16.291	9085	C15	0	0	C25	27.194	1604077			
		C20	22.904	8810.646	C16	17.793	36206	C16	0	0	C26	28.157	926753			
		C21	24.082	565.309	C17	19.233	11006	C17	0	0	C27	29.188	7197			
		C22	25.209	5023.824	C18	20.589	60295	C18	21.812	1043	C28	29.974	481740			
		C23	26.286	2061.55	C19	21.887	4828	C19	0	0	C29	30.822	990027			
		C24	27.326	5705.745	C20	23.112	49690	C20	24.492	1554	C30	31.654	222979			
		C25	28.322	6092.261	C21	24.29	10720	C21	25.754	217	C31	32.45	700874			
		C26	29.282	6800.876	C22	25.421	38911	C22	26.958	1238	C32	33.236	63661			
		C27	30.198	92419.172	C23	26.51	28746	C23	28.1	927	C33	33.974	344928			
		C28	31.093	11432.354	C24	27.542	35010	C24	29.194	1211	C34	35.79	24189			
		C29	31.958	74488.508	C25	28.531	29591	C25	30.246	1297	C35	35.79	87600			
		C30	32.787	7730.771	C26	29.489	26119	C26	31.262	1268		ACL	28.164	Average		
		C31	33.587	116728.352	C27	30.411	26441	C27	32.23	2103		C27/C31	0.010	30.95934		
		C32	34.372	1416.048	C28	31.306	15746	C28	33.168	1560		C29/C33	2.870	30.03353		
		C33	35.129	4215.513	C29	32.164	24480	C29	34.079	3649		CPI	0.863980274			
					C30	32.986	7523	C30	34.942	1315						
					C31	33.798	31709	C31	35.791	2800						
					C32	34.636	973	C32	36.607	245						
					C33	35.327	48024	C33	0	0						
					C34	36.049	2049	C34	0	0						
					C35	36.766	46701	C35	39.099	416						
						ACL	30.76120341	Average		ACL	28.8735509	Average				
						C27/C31	0.833864203	29.18119		C27/C31	0.7510171429	29.28432				
						C29/C33	0.509745127	31.64945		C29/C33		29				
						CPI	1.723262699			CPI	1.622422115					

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		
		Acacia dimidiata	Shrub	NTA 004200	Heteropogon contortus	Tussock Grass	NTA 003995	Eucalyptus tecitifica	Tree/Palm	NTA 003965	NTAGFU0040	Gulf fall and uplands	
		NAME	FOUND RT	AREA	NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA
		C12	11.058	972.519	C10	8.456	809	C10	8.825	452	C20	21.813	5205
		C14	14.512	10593.617	C11	9.618	477	C11	10.076	292	C21	22.98	3473
		C15	16.097	4189.289	C12	11.226	1205	C12	11.793	113	C22	24.106	2592
		C16	17.603	13398.804	C13	13.006	2654	C13	13.694	49	C23	25.189	3586
		C17	19.028	1003.426	C14	14.692	7294	C14	15.516	48	C24	26.221	2059
		C18	20.381	7687.274	C15	16.294	1793	C15	17.212	111	C25	27.21	6652
		C20	22.904	2305.004	C16	17.791	9783	C16	18.851	99	C26	28.168	1395
		C22	25.209	1014.159	C17	19.262	571	C17	20.359	562	C27	29.09	15059
		C24	27.326	898.318	C18	20.581	16276	C18	21.866	333	C28	29.98	1824
		C25	28.315	9513.63	C19	21.885	2731	C19	23.196	347	C29	30.838	22981
		C26	29.275	1676.128	C20	23.115	23707	C20	24.5	2630	C30	31.66	1629
		C27	30.198	13986.568	C21	24.293	9146	C21	25.756	1398	C31	32.472	7598
		C29	31.951	17421.111	C22	25.419	20680	C22	26.95	2351	C32	33.231	632
		C30	32.773	1609.915	C23	26.498	18237	C23	28.097	2811	C33	34.001	4122
		C31	33.587	195069.422	C24	27.539	21291	C24	29.259	404	C34	34.723	288
		C32	34.358	29118.508	C25	28.534	20169	C25	30.254	3953	C35	35.43	3058
		C33	35.129	172842.672	C26	29.487	21663	C26	31.348	339			ACL 28.887 Average
		C34	35.863	384.847	C27	30.408	33870	C27	32.238	19696	C27/C31	1.982	28.3416
					C28	31.299	23837	C28	33.175	2968	C29/C33	5.575	29.60835
					C29	32.162	57290	C29	34.075	26519	CPI	6.071935886	
					C30	32.99	20564	C30	34.955	1390			
					C31	33.791	255852	C31	35.787	3548			
					C32	34.628	848	C32	36.683	200			
					C33	35.319	72946	C33	37.395	882			
					C34	36.042	1638	C34	38.227	113			
					C35	36.759	33086	C35	39.091	557			
							ACL 30.8038135 Average						
							C27/C31 0.13238122 30.53238						
							C29/C33 0.785375483 31.24043						
							CPI 4.338361035						
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		
		Acacia aptaneura	Shrub	NTA 001301	Aristida holathera	Tussock Grass	NTA 001318	Triodia schinzii	Hummock Grass	NTA 001317	NTABRT0004	Burt plain	
		NAME	FOUND RT	AREA	NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA
		C27	30.220	24283.799	C10	8.459	1134	C20	21.808	2018	C20	21.807	8433
		C29	31.973	64158.770	C11	9.626	1218	C21	22.98	671	C21	22.98	12094
		C30	32.802	7732.443	C12	11.218	11943	C22	24.106	2117	C22	24.111	14548
		C31	33.616	1145281.750	C13	12.992	4969	C23	25.184	2988	C23	25.184	14042
		C32	34.394	86886.180	C14	14.684	47497	C24	26.216	2096	C24	26.215	6724
		C33	35.165	1398135.000	C15	16.286	28571	C25	27.211	5346	C25	27.215	7389
		C34	35.892	7100.010	C16	17.788	59740	C26	28.163	2151	C26	28.173	3424
					C17	19.223	9466	C27	29.09	10999	C27	29.09	13580
					C18	20.584	39884	C28	29.975	1759	C28	29.974	5433
					C19	21.877	1044	C29	30.833	23599	C29	30.833	20706
					C20	23.113	26861	C30	31.655	680	C30	31.665	2393
					C21	24.296	9067	C31	32.467	41928	C31	32.466	62809
					C22	25.427	25432	C32	33.237	343	C32	33.241	4856
					C23	26.5	99747	C33	33.996	35478	C33	33.995	63085
					C24	27.531	29107	C34	0	0	C34	34.712	417
					C25	28.531	73507	C35	35.435	478	C35	35.445	7715
					C26	29.495	24386						ACL 31.097 Average
					C27	30.416	109111						C27/C31 0.2162330662 30.16874
					C28	31.301	24867						C29/C33 0.665172783 31.40215
					C29	32.16	350802						CPI 5.076742955
					C30	32.992	58911						
					C31	33.798	2786083						
					C32	34.568	114054						
					C33	35.332	3177061						
					C34	36.055	44970						
					C35	36.761	905869						
							ACL 32.13456049 Average						
							C27/C31 0.039162868 30.84925						
							C29/C33 0.110417143 32.60225						
							CPI 21.92743432						

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		
NTAFIN0019	Finke	Cenchrus ciliaris	Tussock Grass	NTA 000754	Acacia estrophiolata	Tree/Palm	NTA 000784	Enchytraea tomentosa	Tussock Grass	NTA 000761	NTAFIN0019	Finke	
<b>NAME</b> <b>TIME</b> <b>PEAK AREA</b>													
C10		8.462	831		C20	21.802	1161	C20	21.785	364482	C20	21.809	9251
C11		9.619	871		C21	22.985	99	C21	22.874	4443	C21	22.977	12448
C12		11.221	8372		C22	24.095	852	C22	24.089	270476	C22	24.102	25007
C13		13.001	4124		C23	25.179	634	C23	25.052	1962	C23	25.181	39052
C14		14.687	46390		C24	26.205	878	C24	26.199	210696	C24	26.218	33634
C15		16.278	28870		C25	27.205	7866	C25	27.188	219670	C25	27.207	23952
C16		17.791	87119		C26	28.163	8576	C26	28.152	135009	C26	28.165	6788
C17		19.226	25679		C27	29.095	330778	C27	29.183	3582	C27	29.087	18983
C18		20.587	100796		C28	29.975	31603	C28	29.958	32150	C28	29.977	4041
C19		21.875	8705		C29	30.849	637049	C29	30.822	80787	C29	30.835	27758
C20		23.116	73160		C30	31.666	70294	C30	31.665	7835	C30	31.657	4325
C21		24.294	17462		C31	32.509	1644877	C31	32.45	16704	C31	32.463	65116
C22		25.419	56893		C32	33.242	54584	C32	33.225	2997	C32	33.233	3648
C23		26.503	45775		C33	33.996	54912	C33	33.968	3927	C33	33.987	21692
C24		27.54	61564		C34	35.79	2973	C34	35.736	206	C34	35.736	206
C25		28.529	71834		C35	35.451	1716	C35	35.806	13563	C35	35.432	9254
C26		29.487	73490			ACL	30.0558345 Average		ACL	26.76677616 Average		ACL	29.832 Average
C27		30.409	138675			C27/C31	0.201095887 30.33029		C27/C31	0.214439655 30.2937		C27/C31	0.292 30.09711
C28		31.299	80855			C29/C33	11.60127112 29.31743		C29/C33	20.57219251 29.18542		C29/C33	1.280 30.75466
C29		32.157	595789			CPI	16.0505525		CPI	0.506897375		CPI	2.671045345
C30		32.99	142674										
C31		33.801	4225591										
C32		34.566	127665										
C33		35.325	2349530										
C34		36.042	13091										
C35		36.759	458649										
						ACL	29.427285943 Average						
						C27/C31	0.032817895 30.8729						
						C29/C33	0.25357795 32.19087						
						CPI	13.78066976						

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
NTAFIN0022	Finke	<i>Eremophila freelingii</i>	Shrub	NTA 000964	<i>Enneapogon polypylylus</i>	Tussock Grass	NTA 000962	<i>Aristida contorta</i>	Tussock Grass	NTA 000960	NTAFIN0022
		NAME	FOUND RT	AREA	NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA	NAME
		C29	31.973	10959.372	C10	8.454	826	C10	0	0	C10
		C30	32.809	20671.08	C11	9.627	959	C11	0	0	C11
		C31	33.616	134497.719	C12	11.229	5573	C12	0	0	C12
		C32	34.394	71675.898	C13	13.004	1349	C13	0	0	C13
		C33	35.165	1130147.625	C14	14.689	14430	C14	15.5	1146	C14
		C34	35.892	97422.758	C15	16.286	10036	C15	17.212	1316	C15
		C35	36.605	196731.594	C16	17.794	22991	C16	18.825	3320	C16
			ACL	33.05476348 Average	C17	19.218	5325	C17	20.364	675	C17
			C29/C33	0.009697292 32.96158	C18	20.59	44141	C18	21.804	3278	C18
			CPI	8.027133937	C19	21.888	4582	C19	23.186	213	C19
					C20	23.119	37356	C20	24.495	2421	C20
					C21	24.297	22804	C21	25.752	832	C21
					C22	25.427	34056	C22	26.95	2034	C22
					C23	26.506	74393	C23	28.097	2331	C23
					C24	27.537	31732	C24	29.196	1890	C24
					C25	28.537	89773	C25	30.249	2877	C25
					C26	29.495	26315	C26	31.264	1872	C26
					C27	30.417	117873	C27	32.233	5500	C27
					C28	31.307	27137	C28	33.17	2861	C28
					C29	32.165	280538	C29	34.081	12307	C29
					C30	32.998	54941	C30	34.95	4102	C30
					C31	33.809	4068718	C31	35.798	209798	C31
					C32	34.579	109802	C32	36.605	3971	C32
					C33	35.338	5211515	C33	37.4	117241	C33
					C34	36.055	38207	C34	38.222	902	C34
					C35	36.767	1016099	C35	39.112	18255	C35
			ACL	32.19766320 Average			ACL	31.65568482 Average		ACL	27.88749423 Average
			C27/C31	0.02897055 30.88738			C27/C31	0.026215693 30.89782		C27/C31	3.610377664 27.86762
			C29/C33	0.053830412 32.79568			C29/C33	0.10497181 32.62		C29/C33	4.709051717 29.70064
			CPI	32.16195878			CPI	20.39459505		CPI	3.105192068

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. <i>arShrub</i>		SAT 000316	<i>Eucalyptus flindersii</i>	Tree Mallee	SAT 000286	<i>Chrysocelphalum semipap</i> Forb		SAT 000287	SATFLB0005	
		NAME	FOUND RT	AREA	NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA	NAME	
		C14	14.526	3445.533	C10	8.467	420	C10	0	0	C20	
		C15	16.112	285.478	C11	9.651	315	C11	0	0	C21	
		C16	17.624	1158.865	C12	11.232	567	C12	0	0	C22	
		C27	30.22	2762.435	C13	12.986	424	C13	0	0	C23	
		C28	31.122	614.363	C14	14.698	311	C14	15.499	2615	C24	
		C29	31.98	141293.922	C15	16.289	283	C15	17.205	2382	C25	
		C30	32.809	3397.923	C16	17.823	369	C16	18.818	4309	C26	
		C31	33.616	109433.781	C17	19.258	629	C17	20.357	603	C27	
		C33	35.158	244.056	C18	20.582	16343	C18	21.813	2161	C28	
			ACL	29.84465918	Average	C19	21.875	3435	C19	23.184	203	C29
			C27/C31	0.025242982	30.90151	C20	23.116	32413	C20	24.498	1590	C30
			C29/C33	578.5405792	29.0069	C21	24.294	12486	C21	25.75	634	C31
			CPI	63.23930896		C22	25.425	49453	C22	26.949	1145	C32
						C23	26.504	105446	C23	28.1	1427	C33
						C24	27.54	268200	C24	29.195	1189	C34
						C25	28.535	909930	C25	30.247	3672	C35
						C26	29.493	449406	C26	31.257	1590	ACL
						C27	30.42	1799995	C27	32.236	29766	28.54328554
						C28	31.305	114210	C28	33.174	9778	Average
						C29	32.163	285938	C29	34.095	1249753	C27/C31
						C30	33.001	7861	C30	34.948	93881	0.976977912
						C31	33.802	13975	C31	35.833	3636459	29.02329
						C32	34.608	582	C32	36.608	42122	C29/C33
						C33	35.325	2190	C33	37.404	214231	3.113707926
						C34	36.048	673	C34	38.221	3305	29.97236
						C35	36.755	9518	C35	39.105	15636	CPI
							ACL	26.63502128	Average			
							C27/C31	128.8010733	27.03082			
							C29/C33	130.5652968	29.0304			
							CPI	3.513621636				

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
		C16	17.61	478.964	C20	21.806	2282	C10	0	0	C20
		C17	19.035	199.162	C21	22.979	1450	C11	0	0	C21
		C18	20.388	2573.548	C22	24.11	1696	C12	0	0	C22
		C20	22.919	6143.172	C23	25.178	6669	C13	0	0	C23
		C22	25.209	5356.313	C24	26.22	3155	C14	15.5	4650	C24
		C23	26.293	2250.673	C25	27.209	18274	C15	17.207	4113	C25
		C24	27.326	6162.694	C26	28.167	2326	C16	18.83	9045	C26
		C25	28.322	7374.059	C27	29.083	34297	C17	20.353	1956	C27
		C26	29.282	5478.503	C28	29.973	8873	C18	21.809	7210	C28
		C27	30.205	9924.186	C29	30.853	938087	C19	23.191	598	C29
		C28	31.1	3904.074	C30	31.664	130169	C20	24.505	4718	C30
		C29	31.958	31706.822	C31	32.518	2377226	C21	25.756	1288	C31
		C30	32.78	9219.813	C32	33.24	93439	C22	26.95	3897	C32
		C31	33.594	13745.375	C33	33.999	349549	C23	28.096	5161	C33
		C32	34.38	1239.889	C34	34.727	4937	C24	29.201	8648	C34
		C33	35.136	3478.314	C35	35.434	116321	C25	30.253	28572	C35
		ACL 30.2608204 Average			ACL 30.74995161 Average			ACL 31.26286021 Average			
		C27/C31	0.07199855	30.73065	C27/C31	0.01442732	30.94311	C27/C31	0.0593284903	29.5105	
		C29/C33	9.115572085	29.39543	C29/C33	2.683706719	30.08586	C29/C33	6.104089834	29.5630	
		CPI	6.128206254		CPI	15.4663321		CPI	1.924778829		
		CPI 31.41418288 Average			CPI 30.94394 Average			CPI 30.94394 Average			
		C27/C31	0.0041213432	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	TrePalm	SAT 000535	Rhagodia paradoxa	Chenopod	SAT 000552	Enchytraea tomentosa var.	Chenopod	SAT 000550	SATFLB0010				
		NAME	FOUND RT	AREA	NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA	NAME				
		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	29.492	76341	C26	31.265	4135		ACL	28.41799761	Average
			C27/C31	77.28750059	27.05109	C27	30.414	107719	C27	32.244	14271		C27/C31	1.158845608	28.85284
			C29/C33	-	29	C28	31.299	53958	C28	33.176	2177		C29/C33	6.341945095	29.54481
			CPI	1.988879452		C29	32.157	135063	C29	34.081	8961		CPI	1.997683671	
						C30	32.979	5244	C30	34.951	401				
						C31	33.785	5950	C31	35.799	2692				
						C32	34.623	377	C32	0	0				
						C33	35.309	1512	C33	0	0				
						C34	36.042	549	C34	0	0				
						C35	36.754	19705	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	
SATFLB0012	Flinders lofty block	Allocasuarina muelleriana	Shrub	SAT 000649	Hibbertia crinita	Shrub	SAT 000657	Eucalyptus fasciculosa	Tree Mallee	SAT 000630	SATFLB0012	
		NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA	NAME	
		C20	21.806	6472	C10	8.457	756	C10	8.459	900	C20	
		C21	22.974	930	C11	9.603	1346	C11	9.611	794	C21	
		C22	24.105	4336	C12	11.221	349	C12	11.223	652	C22	
		C23	25.183	2655	C13	12.986	423	C13	12.993	339	C23	
		C24	26.209	3898	C14	14.718	481	C14	14.705	420	C24	
		C25	27.209	4871	C15	16.3	253	C15	16.281	208	C25	
		C26	28.168	3121	C16	17.792	380	C16	17.815	173	C26	
		C27	29.089	12424	C17	19.242	837	C17	19.254	444	C27	
		C28	29.979	3083	C18	20.582	11772	C18	20.579	7582	C28	
		C29	30.832	139072	C19	21.881	2757	C19	21.877	1067	C29	
		C30	31.66	31651	C20	23.116	22020	C20	23.113	16371	C30	
		C31	32.508	2029176	C21	24.289	13409	C21	24.296	4870	C31	
		C32	33.241	56559	C22	25.425	24365	C22	25.416	19899	C32	
		C33	34	351808	C23	26.498	355444	C23	26.495	14542	C33	
		C34	34.728	143	C24	27.53	29525	C24	27.537	25140	C34	
		C35	35.429	2546	C25	28.535	106894	C25	28.537	39722	C35	
			ACL	31.14045215	Average	C26	29.488	23809	C26	29.495	14744	ACL
			C27/C31	0.006122682	30.97566	C27	30.414	56710	C27	30.411	61653	C27/C31
			C29/C33	0.395306531	31.86675	C28	31.299	20408	C28	31.306	9936	C29/C33
			CPI	24.72730103		C29	32.158	153049	C29	32.155	11046	CPI
						C30	33.022	859	C30	33.003	796	
						C31	33.791	36043	C31	33.793	7152	
						C32	34.629	874	C32	34.594	526	
						C33	35.325	3471	C33	35.322	466	
						C34	36.043	780	C34	36.044	460	
						C35	36.777	8265	C35	36.777	11549	
						ACL	27.88748518	Average	ACL	27.50493966	Average	
						C27/C31	1.573398441	28.55436	C27/C31	8.620385906	27.41578	
						C29/C33	44.09363296	29.0887	C29/C33	23.70386266	29.16192	
						CPI	7.179964222		CPI	1.99701999		

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
SATFLB0014	Flinders lofty block	Eucalyptus odorata	Tree Mallee	SAT 000746	Xanthorrhoea quadrangulata	Shrub	SAT 000791	Allocasuarina verticillata	Shrub	SAT 000775	SATFLB0014
		NAME	FOUND RT	AREA	NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA	NAME
		C16	17.617	735.08	C10	8.472	918	C10	8.828	100	C20
		C17	19.035	497.925	C11	9.634	222	C11	10.048	236	C21
		C18	20.395	13539.137	C12	11.231	485	C12	11.948	96	C22
		C19	21.682	93.445	C13	12.98	778	C13	13.702	61	C23
		C20	22.911	9733.062	C14	14.707	406	C14	15.493	3168	C24
		C21	24.09	301.117	C15	16.294	229	C15	17.205	3307	C25
		C22	25.209	8253.909	C16	17.838	274	C16	18.822	6189	C26
		C23	26.293	9196.23	C17	19.257	427	C17	20.356	1256	C27
		C24	27.326	20096.285	C18	20.597	3542	C18	21.807	3664	C28
		C25	28.322	39957.383	C19	21.885	468	C19	23.183	400	C29
		C26	29.275	14191.534	C20	23.11	19167	C20	24.503	2561	C30
		C27	30.191	27456.541	C21	24.288	5206	C21	25.749	955	C31
		C28	31.093	1121.091	C22	25.424	22250	C22	26.948	2323	C32
		C29	31.951	5905.064	C23	26.508	18163	C23	28.089	2410	C33
		C30	32.787	252.312	C24	27.539	28597	C24	29.194	4411	C34
		C31	33.594	7707.731	C25	28.534	53835	C25	30.251	12374	C35
		C32	34.372	545.024	C26	29.492	37415	C26	31.257	13637	ACL
		C33	35.129	33019.504	C27	30.414	97943	C27	32.236	93724	28.24878787
					C28	31.309	21619	C28	33.162	30427	Average
					C29	32.162	46907	C29	34.089	766889	C27/C31
					C30	32.995	4390	C30	34.948	31305	C29/C33
					C31	33.791	33093	C31	35.843	4160270	CPI
					C32	34.618	869	C32	36.607	24460	
					C33	35.319	2951	C33	37.408	651395	
					C34	36.052	479	C34	38.22	911	
					C35	36.759	9724	C35	39.11	5116	
						ACL	27.87548118	ACL	30.88406135	ACL	
						Average		Average	Average		
						C27/C31	2.959628925	C27/C31	0.022528346	C27/C31	
						C29/C33	15.89528973	C29/C33	1.177302558	C29/C33	
						CPI	2.251853069	CPI	52.98881108	CPI	

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		
		NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA	NAME		
		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				ACL	27.69524885	Average
		C27	30.424	4430991	C27	30.41	42859				C27/C31	27.77689181	27.139
		C28	31.304	137263	C28	31.3	16611				C29/C33	3336.920792	29.0012
		C29	32.168	1107100	C29	32.164	114433				CPI	25.38156237	
		C30	32.984	9531	C30	32.986	11791				CPI	2.688643185	
		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						
			ACL	27.11742235	Average		ACL	31.41396481	Average				
			C27/C31	143.0736519	27.02776		C27/C31	0.095821195	30.65023				
			C29/C33	124.7015093	29.03182		C29/C33	0.579762791	31.53203				
			CPI	16.98600949			CPI	10.6877974					

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 involucrata	Shrub	SAT 000124	SATKAN001	
		NAME	FOUND_RT	AREA	NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA	NAME	
		C18	20.395	135.159	C10	8.517	186	C20	21.813	2558	C20	
		C25	28.329	2485.209	C11	9.627	234	C21	22.98	663	C21	
		C27	30.213	77272.953	C12	11.229	1586	C22	24.137	571	C22	
		C28	31.107	576.377	C13	13.009	1071	C23	25.174	888	C23	
		C29	31.965	62820.504	C14	14.69	3558	C24	26.216	1682	C24	
		C31	33.609	22881.779	C15	16.292	3069	C25	27.211	1596	C25	
		C32	34.394	235.546	C16	17.842	572	C26	28.163	1787	C26	
		C33	35.151	27998.75	C17	19.224	3413	C27	29.09	4690	C27	
			ACL	28.96522169	Average	C18	20.59	12309	C28	29.975	2001	C28
			C27/C31	3.377051802	27.91386	C19	21.878	2121	C29	30.834	25476	C29
			C29/C33	2.243689593	30.23316	C20	23.114	23752	C30	31.656	2996	C30
			CPI	238.272835		C21	24.297	5532	C31	32.477	180401	C31
						C22	25.423	20384	C32	33.237	2827	C32
						C23	26.501	16103	C33	34.001	136195	C33
						C24	27.538	20466	C34	34.713	185	C34
						C25	28.527	18302	C35	35.436	49734	C35
						C26	29.496	15358		ACL	31.98479246	Average
						C27	30.423	17070		C27/C31	0.025997639	30.89864
						C28	31.313	12197		C29/C33	0.187055325	32.36968
						C29	32.155	44421		CPI	31.07681135	
						C30	32.993	7610				ACL
						C31	33.799	222951				28.6866561
						C32	34.621	549				Average
						C33	35.328	93157				C27/C31
						C34	36.056	1254				28.22714495
						C35	36.752	24634				C29/C33
							ACL	31.04260287	Average			
							C27/C31	0.076563309	30.71552			
							C29/C33	0.476840173	31.70849			
							CPI	5.488280346				

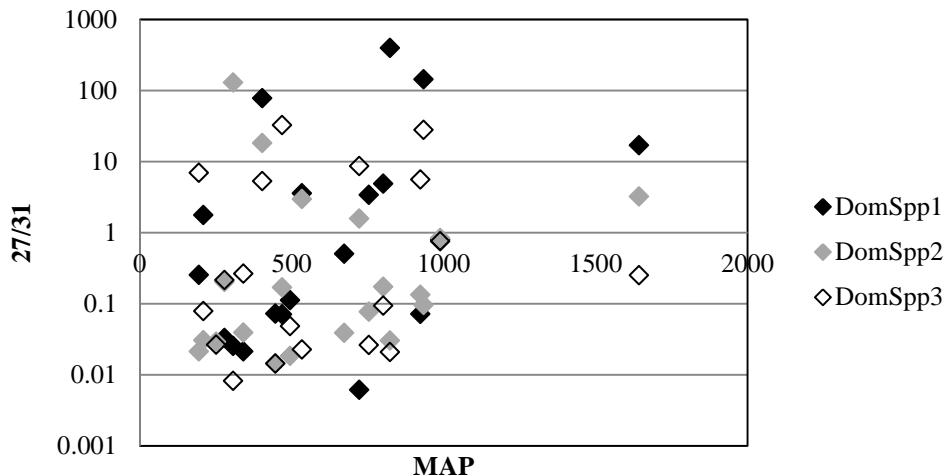
Site	Bioregion	Dominant Spp 1	Growth form	Genetic Voucher No	Dominant Spp 2	Growth form	Genetic Voucher No	Dominant Spp 3	Growth form	Genetic Voucher No	Soil				
		SATKAN002	Kanmantoo	Eucalyptus obliqua	Tree/Palm	SAT 000191	Lepidosperma semiteres	Sedge	SAT 000218	Hakea rostrata	Shrub	SAT 000207	SATKAN002	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	3.07369167	
						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	92929	C35	39.104	1412				
							ACL	31.72818969	Average						
							C27/C31	0.03015424	30.88344						
							C29/C33	0.24673096	32.20839						
							CPI	13.21275322							
										ACL	30.37851226	Average			
										C27/C31	0.020673386	30.91898			
										C29/C33	36.11638251	29.10777			
										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		
		<i>Maireana aphylla</i>	Chenopod	SAA 000250	<i>Eragrostis setifolia</i>	Tussock Grass	SAA 000294	<i>Acacia aneura var. tenuis</i>	Shrub	SAA 000338	SAASTP0001 Stony plains		
		NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA	NAME	FOUND_RT	AREA	NAME	FOUND_RT	AREA
		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		ACL	31.72995869 Average	C28	29.982	63607
		C19	21.891	11707	C19	21.879	3458		C27/C31	0.078283546 30.7096	C29	30.84	61038
		C20	23.116	84853	C20	23.115	37934		C29/C33	0.120021672 32.57136	C30	31.667	32517
		C21	24.289	25598	C21	24.298	10965		CPI	27.93741929	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		ACL	27.72981228 Average			
		C27	30.419	357702	C27	30.413	55108		C27/C31	2.353492804 28.19279			
		C28	31.304	126898	C28	31.303	17886		C29/C33	3.891240597 29.81779			
		C29	32.168	425130	C29	32.162	147976		CPI	1.281449146			
		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						
			ACL	26.91911334 Average		ACL	30.94142066 Average						
			C27/C31	1.753690476 28.4526		C27/C31	0.030549179 30.88143						
			C29/C33	63.43330349 29.06208		C29/C33	0.83227501 31.18308						
			CPI	4.560441882		CPI	13.856489						

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
		Malvastrum americanum va	Forb	SAA 000019	Rutidosis helichrysoidea subs	Forb	SAA 000016	Sida fulbillera	Forb	SAA 000022	SAASTP004 Stony plains
		NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA	NAME
		C10	8.446	384	C10	8.479	673	C10	0	0	C20
		C11	9.635	917	C11	9.609	1741	C11	0	0	C21
		C12	11.216	11775	C12	11.217	3017	C12	0	0	C22
		C13	12.996	2338	C13	12.997	3652	C13	0	0	C23
		C14	14.692	46846	C14	14.688	35594	C14	0	0	C24
		C15	16.284	27882	C15	16.279	20433	C15	0	0	C25
		C16	17.792	62889	C16	17.798	46197	C16	18.818	458	C26
		C17	19.226	23866	C17	19.227	8432	C17	0	0	C27
		C18	20.588	93883	C18	20.583	40806	C18	21.797	1498	C28
		C19	21.881	10092	C19	21.876	3891	C19	0	0	C29
		C20	23.121	84877	C20	23.112	30028	C20	24.498	1381	C30
		C21	24.299	20628	C21	24.3	6430	C21	25.755	524	C31
		C22	25.425	69453	C22	25.415	22929	C22	26.954	1430	C32
		C23	26.504	62511	C23	26.499	14050	C23	28.09	1763	C33
		C24	27.54	73483	C24	27.541	20405	C24	29.252	511	C34
		C25	28.535	178626	C25	28.53	22813	C25	30.252	3295	C35
		C26	29.498	77015	C26	29.494	19091	C26	31.252	1908	
		C27	30.42	512838	C27	30.41	51425	C27	32.236	10689	
		C28	31.31	117003	C28	31.3	19133	C28	33.173	1185	
		C29	32.168	1740962	C29	32.164	929720	C29	34.079	15287	
		C30	32.996	202182	C30	32.996	79294	C30	34.943	501	
		C31	33.802	2032081	C31	33.802	2415093	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	
			ACL	29.60649581	Average		ACL	31.55066528	Average		ACL
			C27/C31	0.251370845	30.19394		C27/C31	0.021257967	30.81674		C27/C31
			C29/C33	17.88006573	29.21186		C29/C33	0.749862484	31.28589		C29/C33
			CPI	8.05723832			CPI	8.96361079			CPI

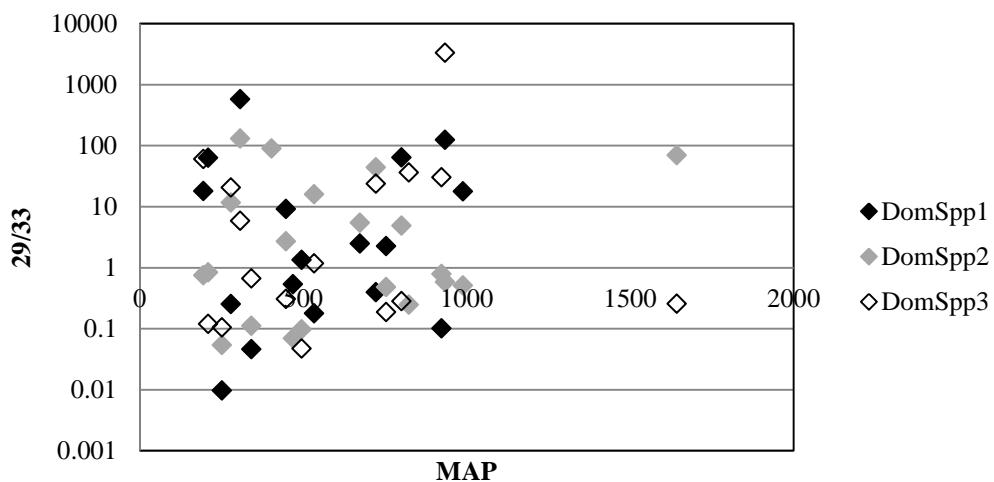
Site	Bioregion	Dominant Spp 1	Growth form	Genetic Voucher No	Dominant Spp 2	Growth form	Genetic Voucher No	Dominant Spp 3	Growth form	Genetic Voucher No	Soil
NTADAC0001	Darwin Coastal	Eucalyptus tetrodonta		NTA 006020	Eucalyptus miniatia		NTA 006042	Sorghum plumosum		NTA 005954	NTADAC0001
		NAME	FOUND RT	AREA	NAME	TIME	PEAK AREA	NAME	TIME	PEAK AREA	NAME
		C16	17.61	2659.282	C10	8.477	571	C10	0	0	C20
		C17	19.043	458.763	C11	9.603	1284	C11	0	0	C21
		C18	20.388	4531.807	C12	11.226	247	C12	0	0	C22
		C20	22.919	2763.422	C13	13.006	335	C13	0	0	C23
		C22	25.217	1536.286	C14	14.713	166	C14	0	0	C24
		C24	27.333	1964.098	C15	16.283	184	C15	0	0	C25
		C25	28.322	203.287	C16	17.817	265	C16	0	0	C26
		C26	29.275	4915.825	C17	19.273	599	C17	0	0	C27
		C27	30.205	8447.58	C18	20.582	21021	C18	21.805	1027	C28
		C28	31.078	9375.743	C19	21.87	2504	C19	0	0	C29
		C29	31.958	92122.781	C20	23.116	25755	C20	24.496	1554	C30
		C30	32.787	2983.688	C21	24.294	4858	C21	25.747	259	C31
		C31	33.594	499.75	C22	25.414	19893	C22	26.962	1342	C32
		C32	34.38	295.635	C23	26.498	16722	C23	28.093	922	C33
				ACL 28.83501286 Average	C24	27.534	25157	C24	29.203	970	C34
				C27/C31 16.90361181 27.22342	C25	28.529	27567	C25	30.244	889	C35
				C29/C33 - 29	C26	29.487	41353	C26	31.26	822	
				CPI 4.806230188	C27	30.414	62952	C27	32.239	1035	
					C28	31.288	62516	C28	33.182	182	
					C29	32.163	107823	C29	34.072	1126	
					C30	32.99	13129	C30	34.946	733	
					C31	33.796	19531	C31	35.794	4120	
					C32	34.602	499	C32	36.611	213	
					C33	35.33	1555	C33	37.401	4411	
					C34	36.063	654	C34	0	0	
					C35	36.77	7759	C35	39.098	2103	
					ACL 28.36468196 Average			ACL 31.40251388 Average			
					C27/C31 3.223183657 27.94715			C27/C31 0.251213592 30.1969			
					C29/C33 69.33954984 29.05687			C29/C33 0.255270914 32.18656			
					CPI 1.48564347			CPI 3.210699202			

### MAP v 27/31 ratio



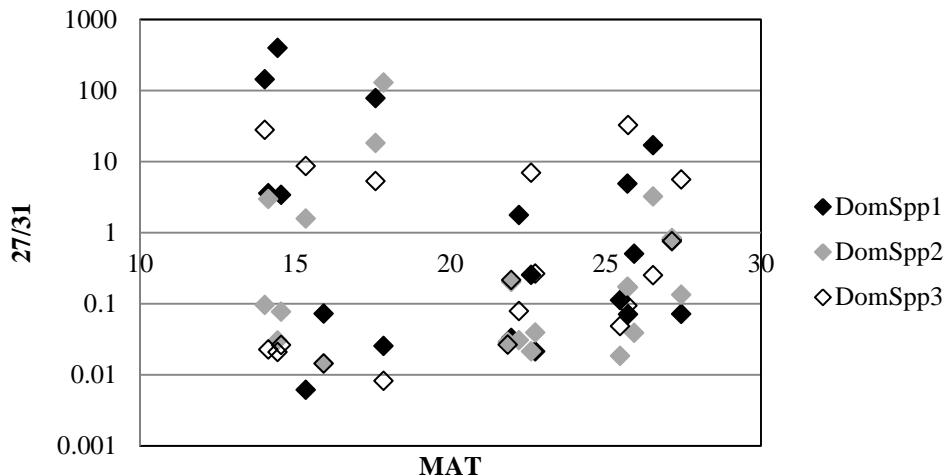
(a)

### MAP v 29/31 ratio



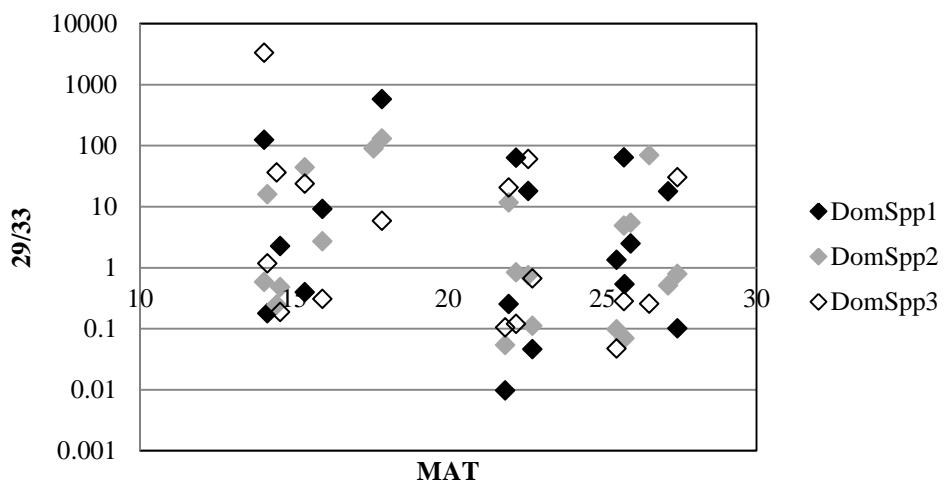
(b)

### MAT v 27/31 ratio



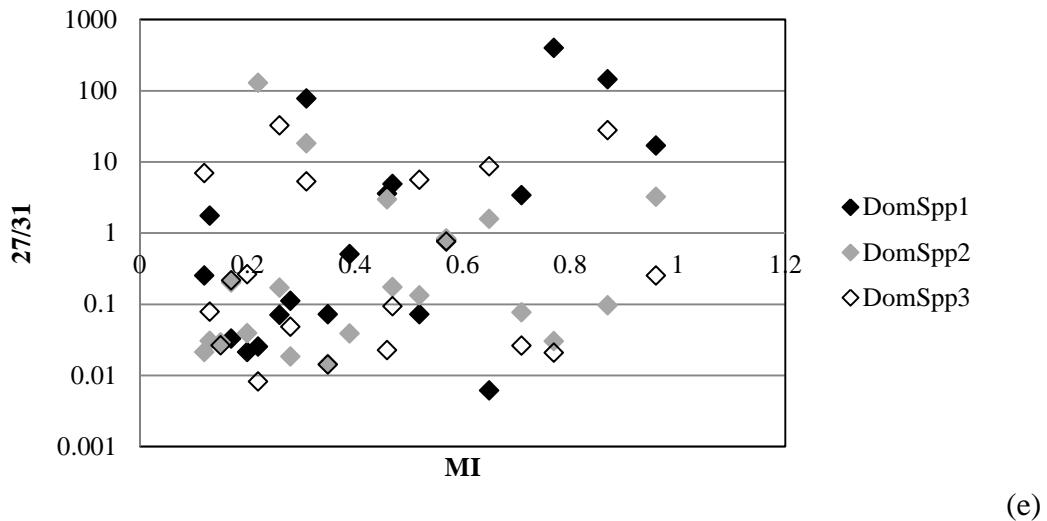
(c)

### MAT v 29/33 ratio

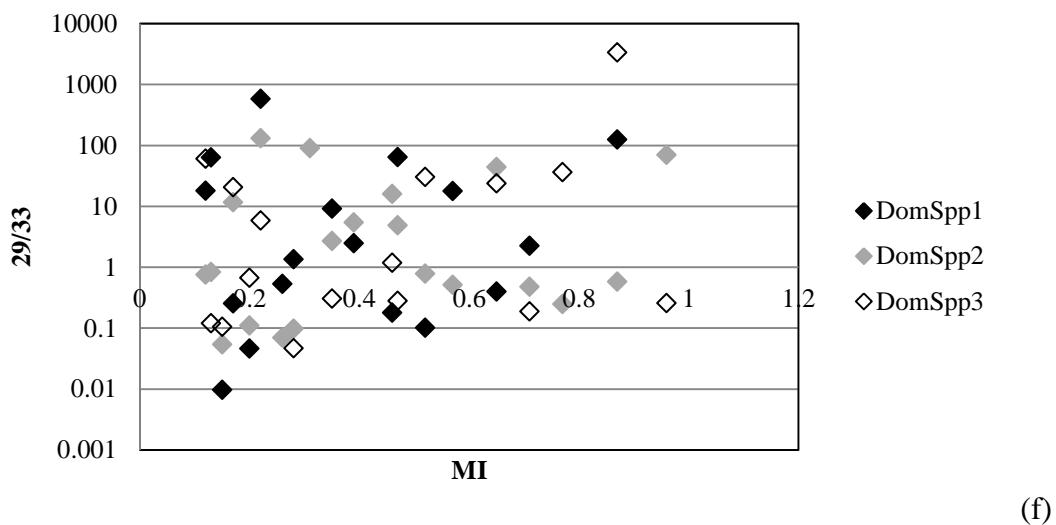


(d)

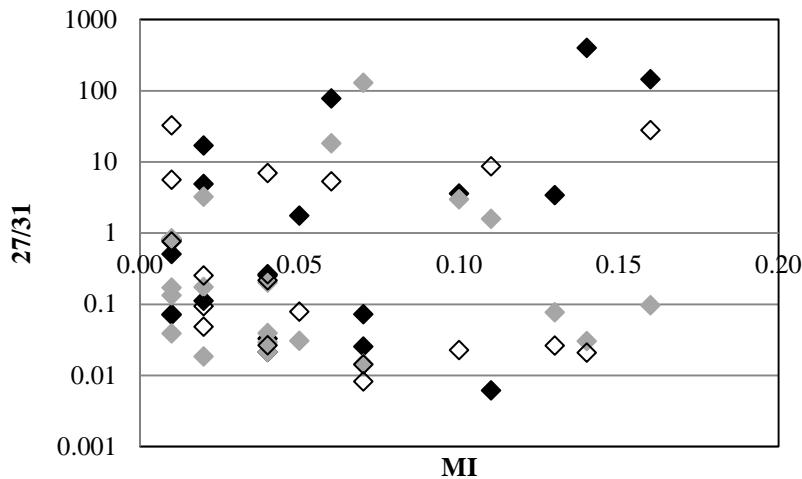
### MI v 27/31 ratio



### MI v 29/33 ratio

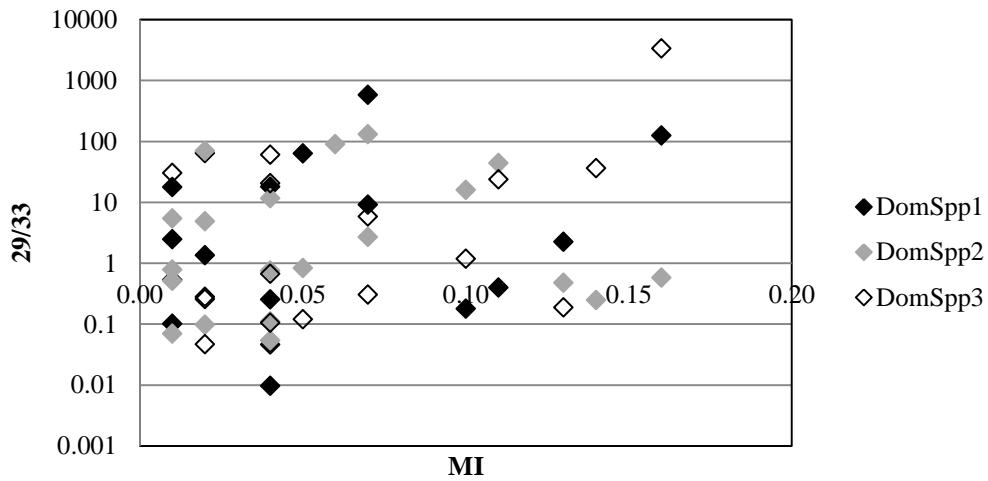


### MI - lowest quarter mean v 27/31 ratio



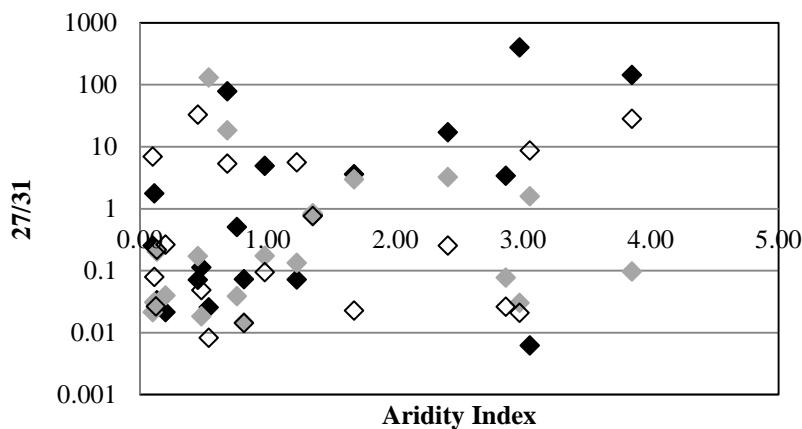
(g)

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



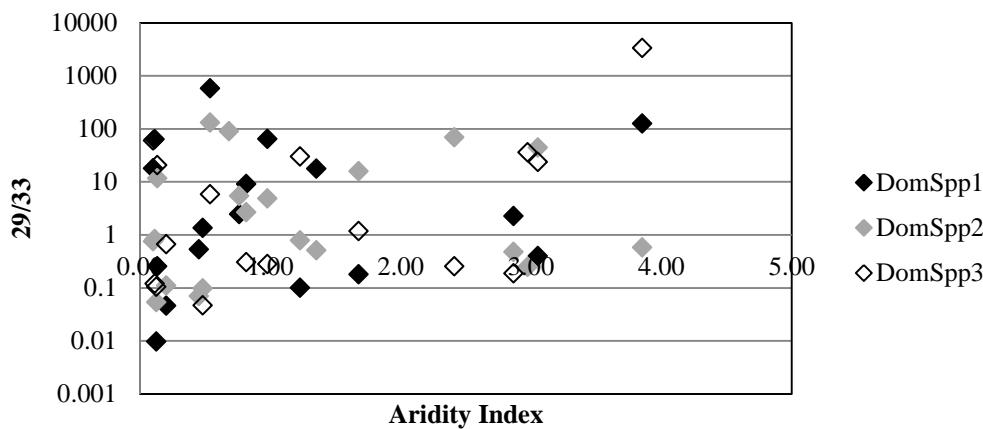
(h)

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



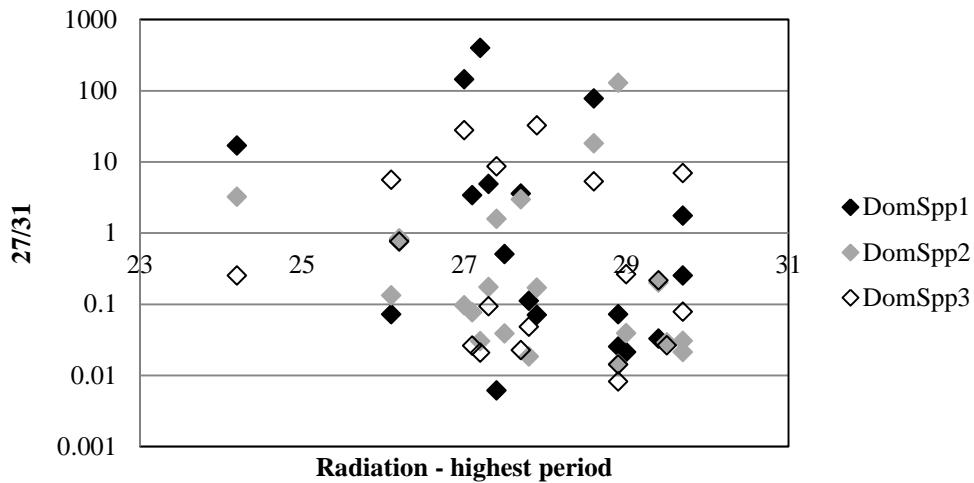
(i)

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



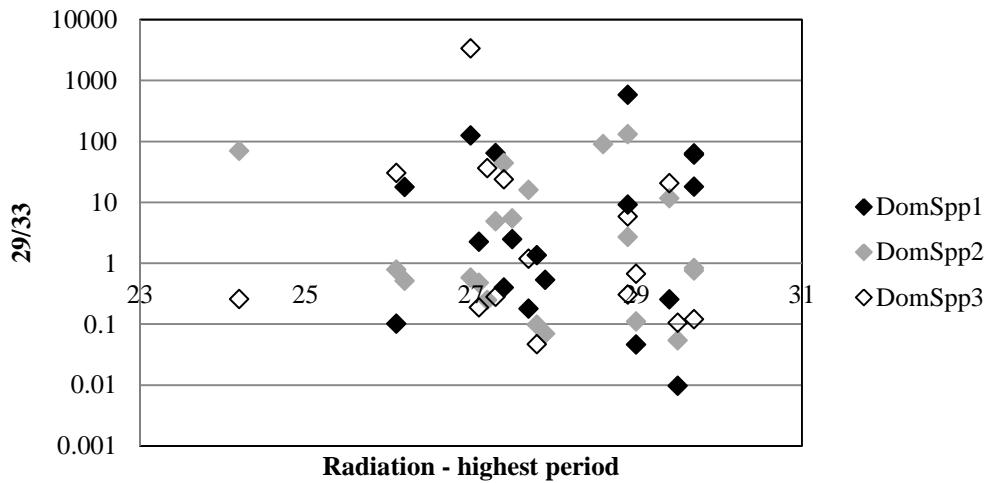
(j)

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



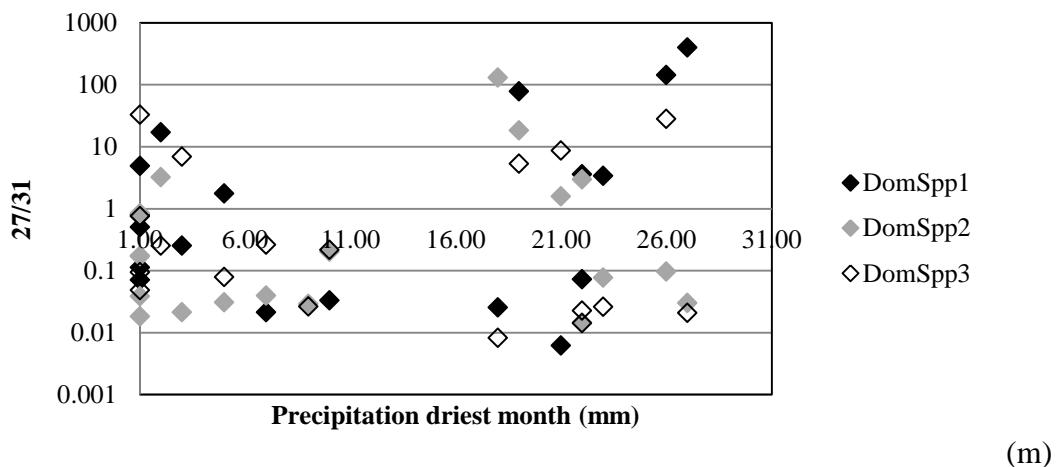
(k)

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

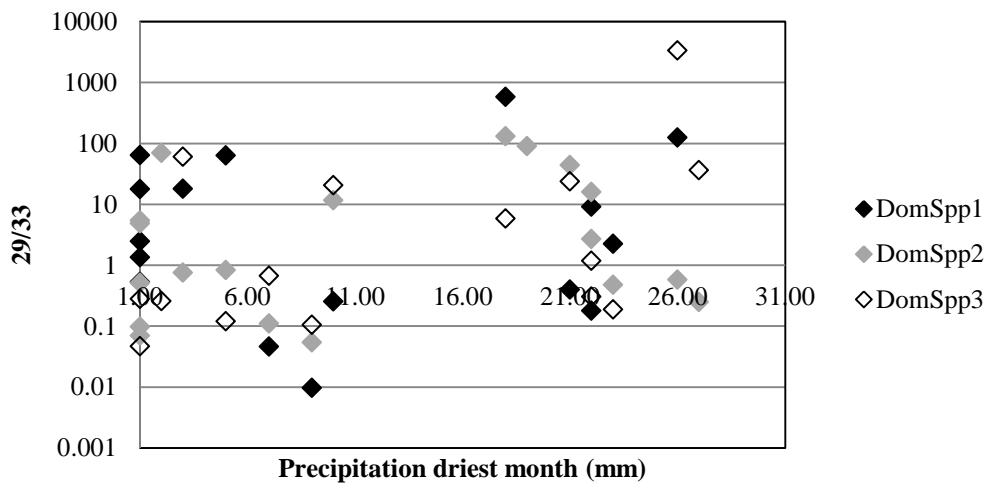


(l)

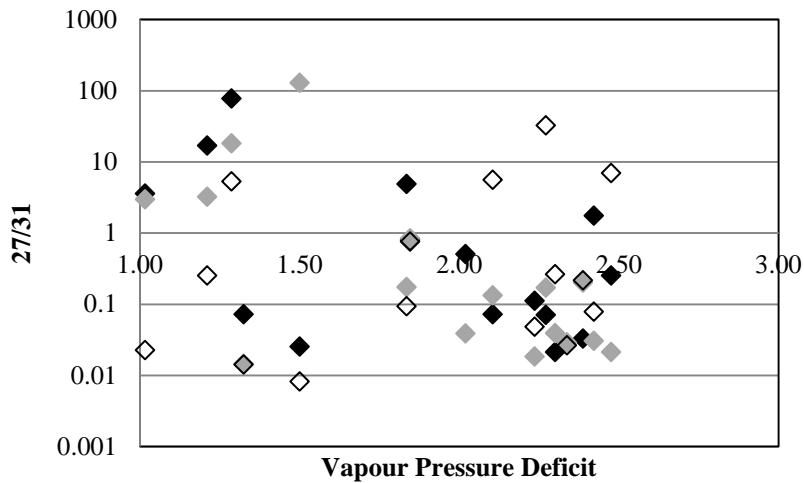
### Precipitation driest month v 27/31 ratio



### Precipitation driest month v 29/33 ratio

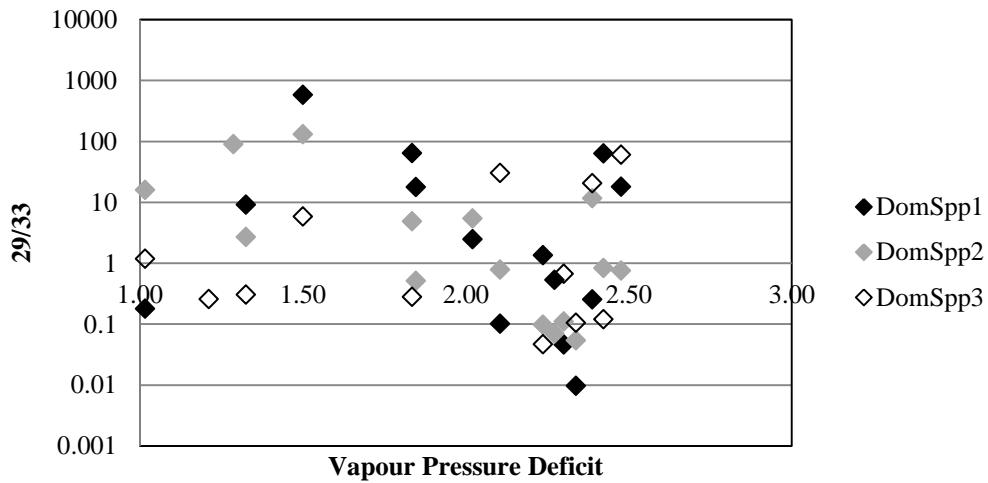


### Vapour Pressure Deficit v 27/31 ratio



(o)

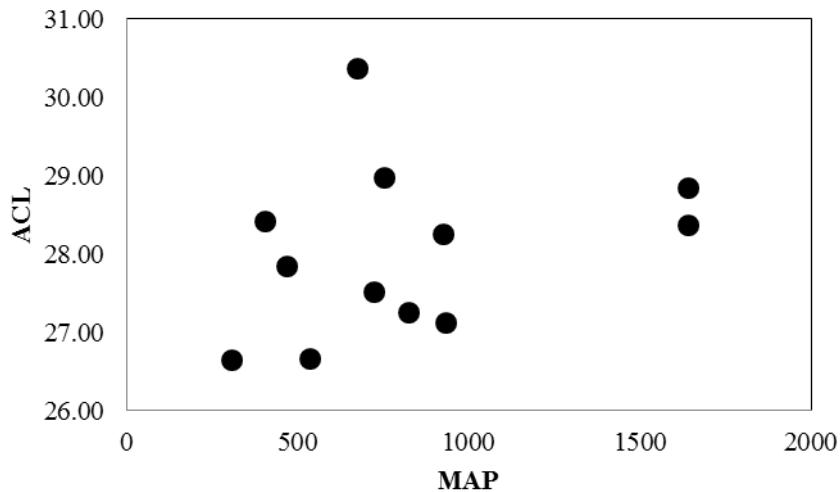
### Vapour Pressure Deficit v 29/33 ratio



(p)

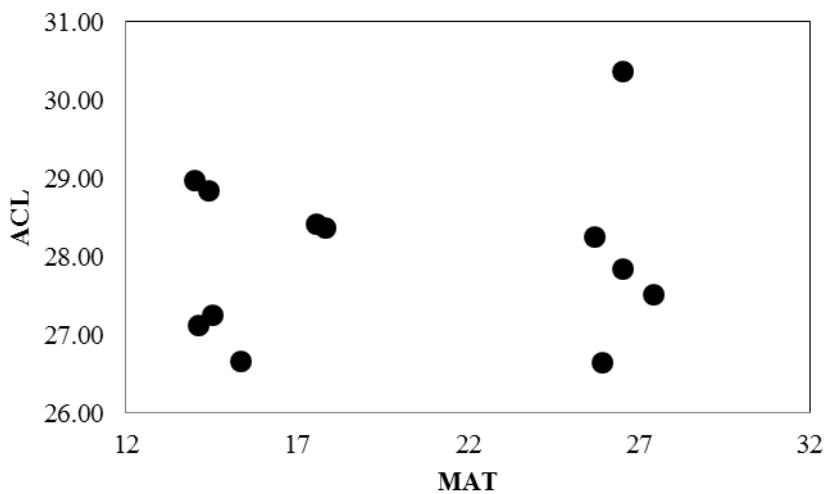
Figure 2: Plots (a)-(p) showing that there is no relationship between the plant 27/31 and 29/33 chain length ratios to the different climate variables MAP, MAT, annual MI, lowest quarter mean MI, aridity index, radiation, driest month precipitation and vapour pressure deficit.

### Eucalyptus genus MAP v ACL



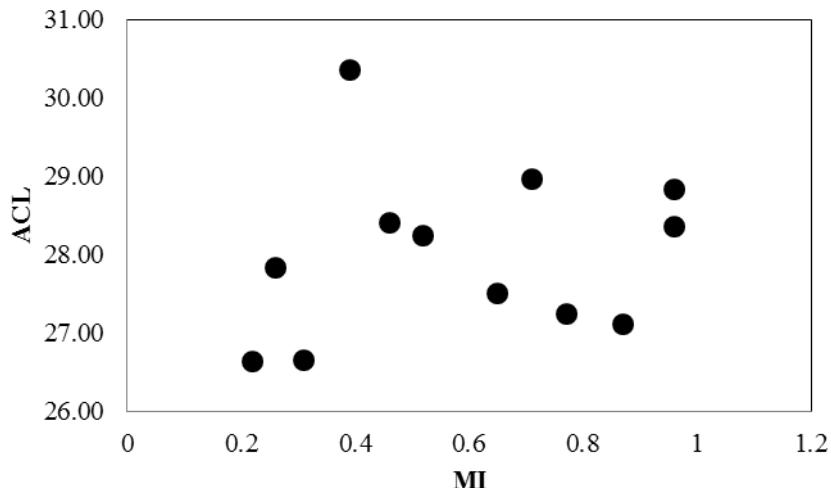
(a)

### Eucalyptus genus MAT v ACL



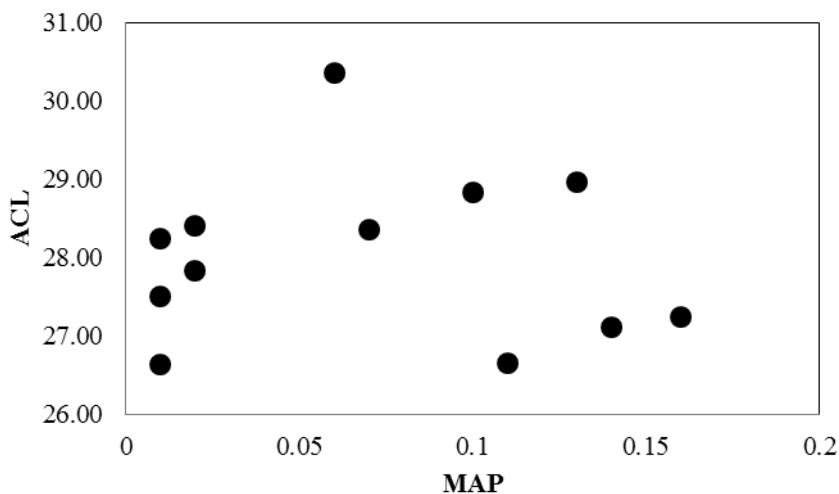
(b)

### Eucalyptus genus Ann MI v ACL



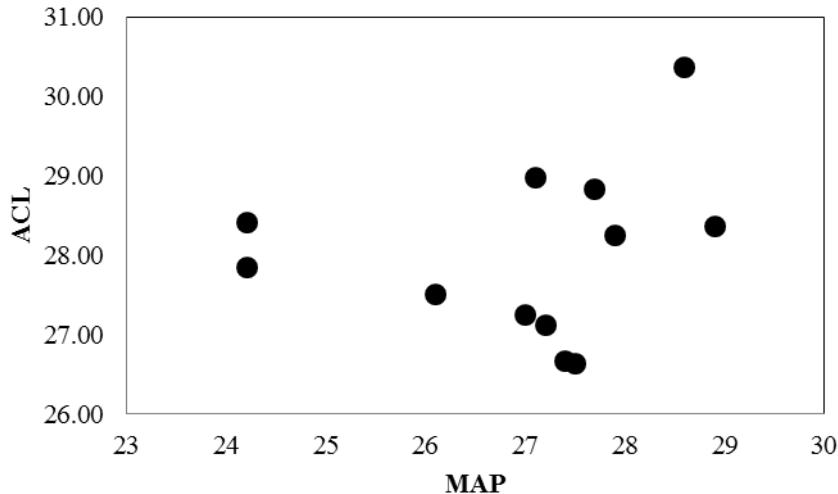
(c)

### Eucalyptus genus Low MI v ACL



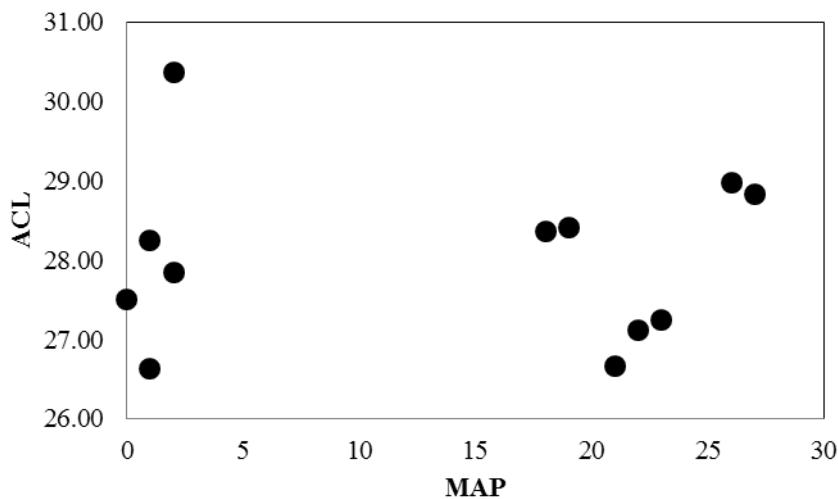
(d)

**Eucalyptus genus Radiation v ACL**



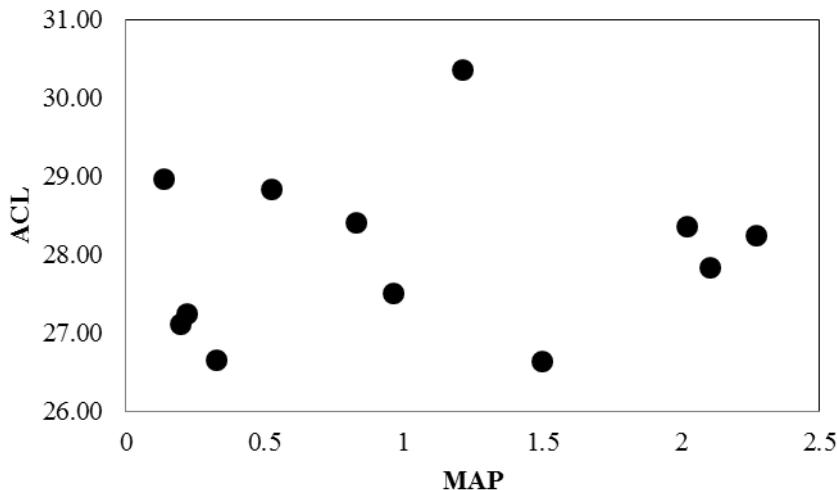
(e)

**Eucalyptus genus Low Precip v ACL**



(f)

## Eucalyptus genus VPD v ACL

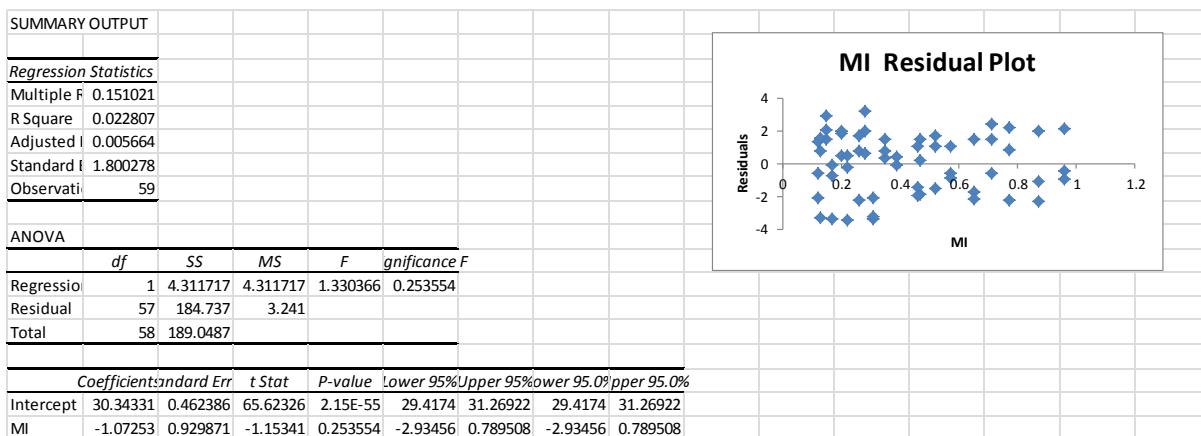


(g)

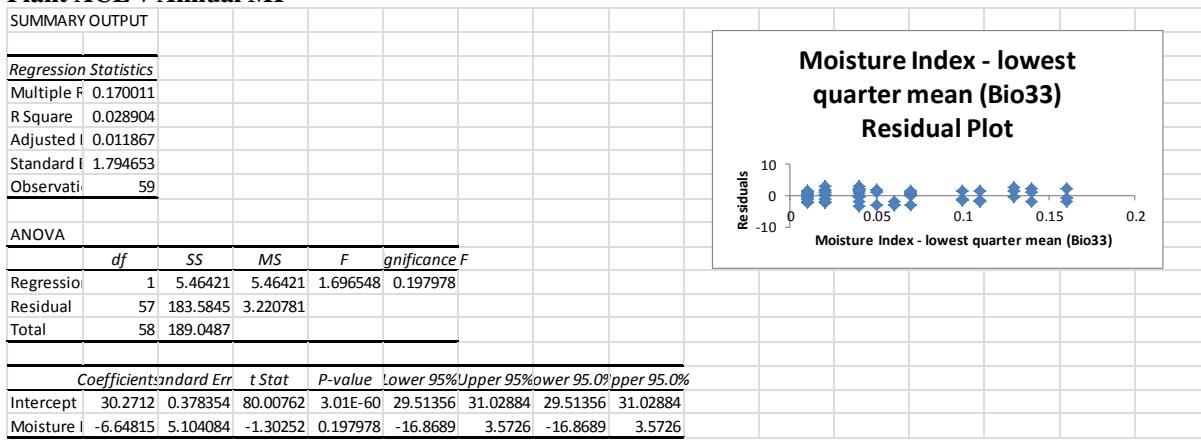
Figure 2: Plots (a)-(p) showing that there is no relationship Eucalyptus genus ACL with the different climate variables MAP, MAT, annual MI, lowest quarter mean MI, aridity index, radiation, driest month precipitation and vapour pressure deficit.

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

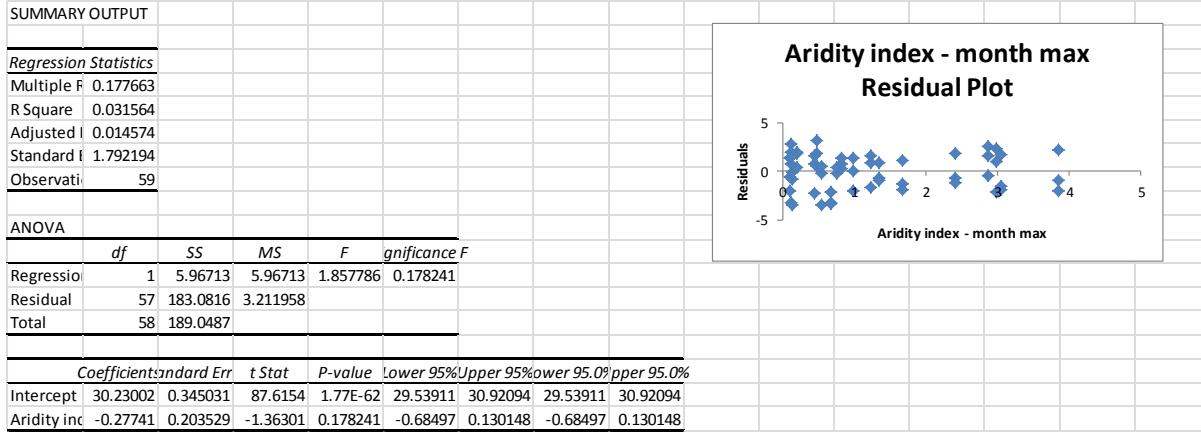
SUMMARY OUTPUT								
<i>Regression Statistics</i>								
Multiple R	0.093468							
R Square	0.008736							
Adjusted R	-0.00865							
Standard E	1.813193							
Observati	59							
ANOVA								
	df	SS	MS	F	Significance F			
Regression	1	1.651582	1.651582	0.502356	0.481355			
Residual	57	187.3971	3.287669					
Total	58	189.0487						
Coefficients Standard Err t Stat P-value Lower 95% Upper 95% Lower 95% Upper 95%								
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								
<i>Regression Statistics</i>								
Multiple R	0.198398							
R Square	0.039362							
Adjusted R	0.022508							
Standard E	1.784964							
Observati	59							
ANOVA								
	df	SS	MS	F	Significance F			
Regression	1	7.441289	7.441289	2.335551	0.131981			
Residual	57	181.6074	3.186095					
Total	58	189.0487						
Coefficients Standard Err t Stat P-value Lower 95% Upper 95% Lower 95% Upper 95%								
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								
<b>MAP Residual Plot</b>								
<b>MAT Residual Plot</b>								



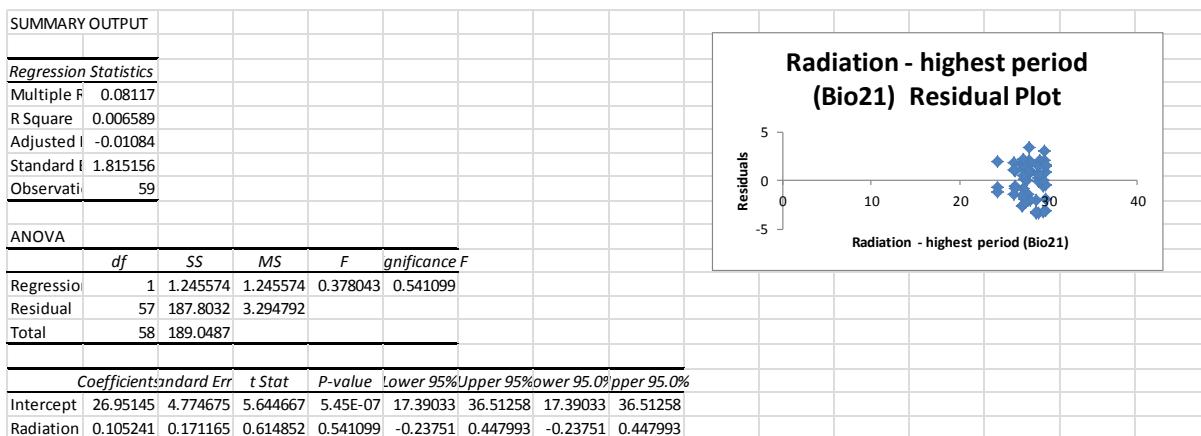
### Plant ACL v Annual MI



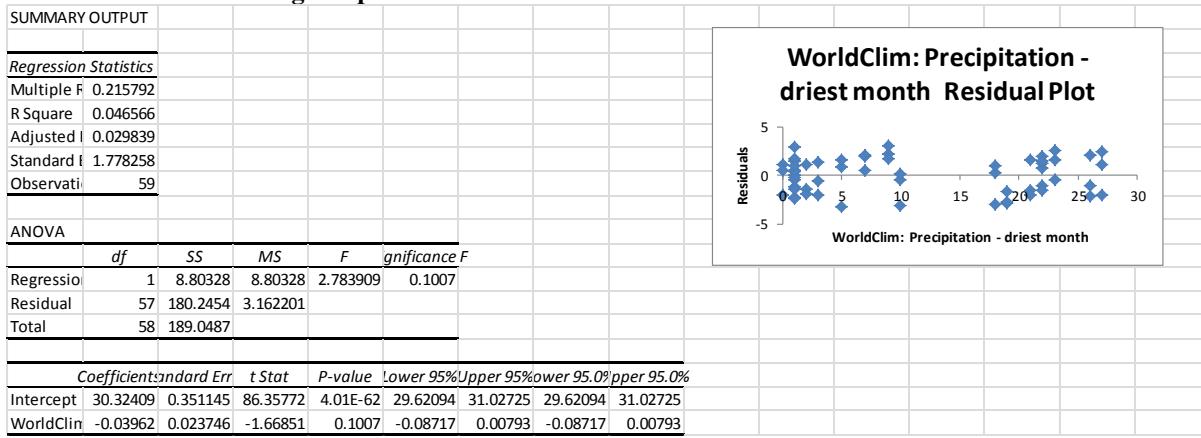
### Plant ACL v lowest quarter mean MI



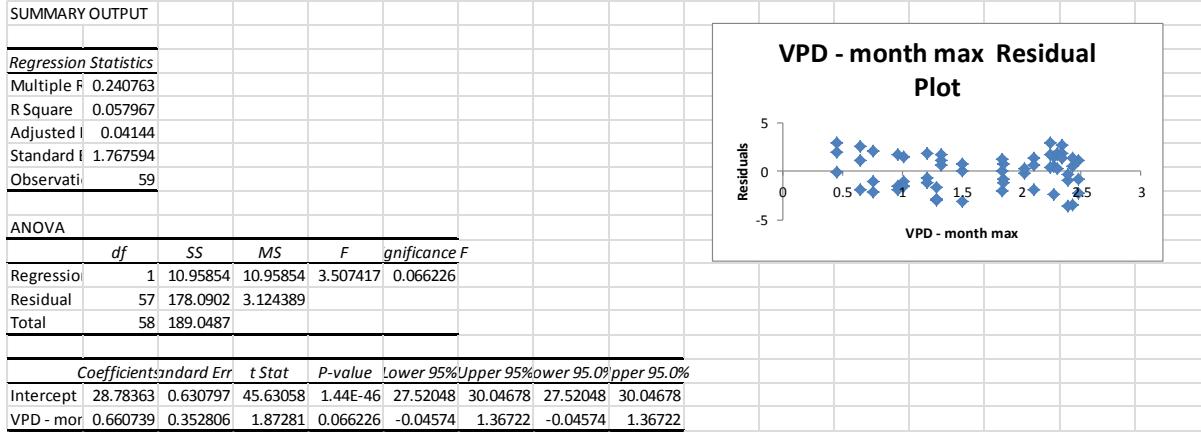
### Plant ACL v aridity index month max



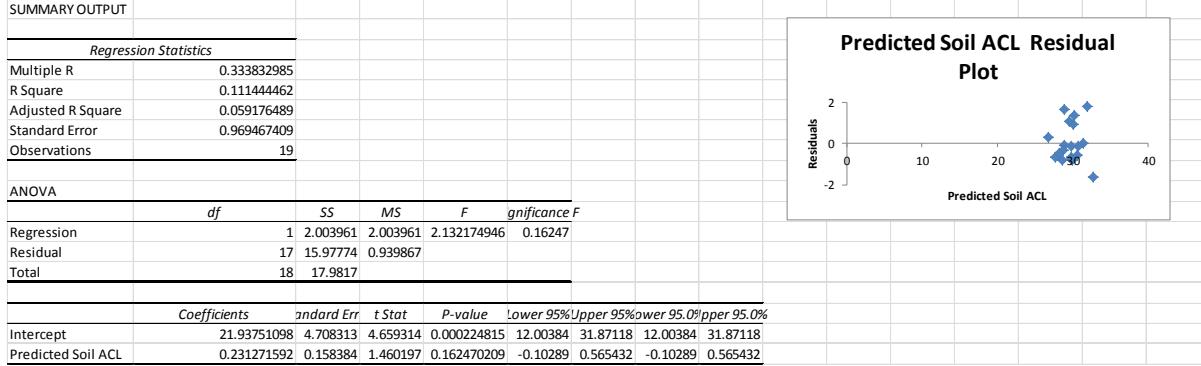
### Plant ACL v radiation highest period



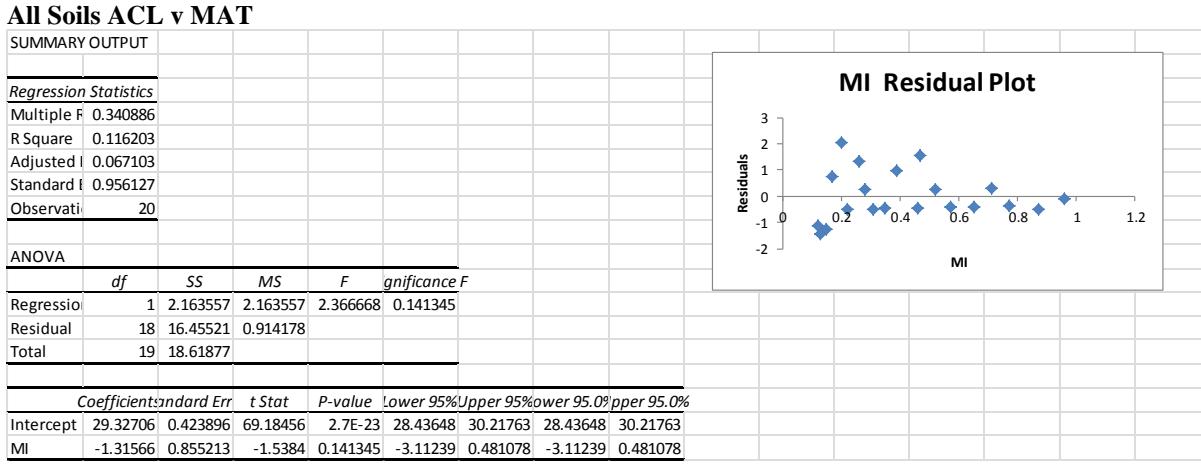
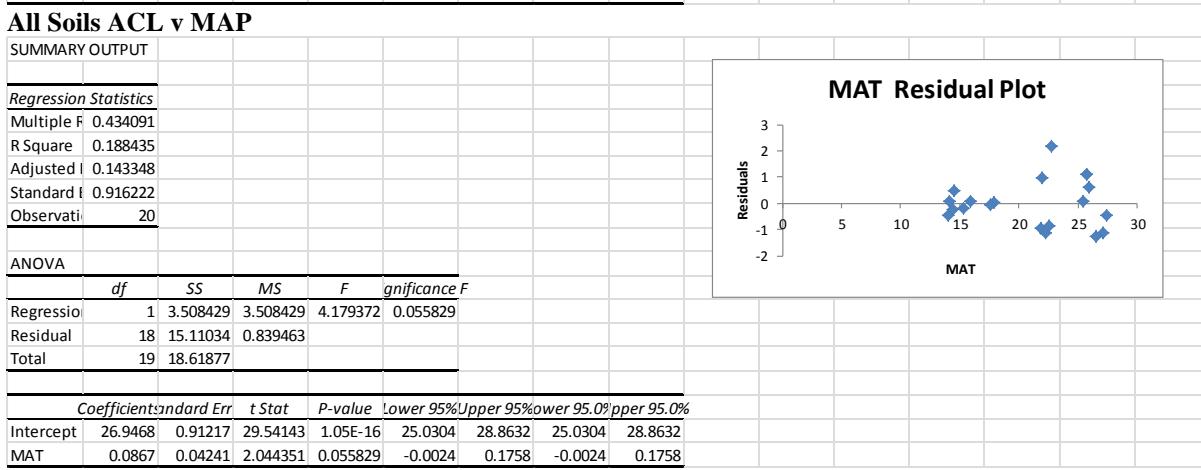
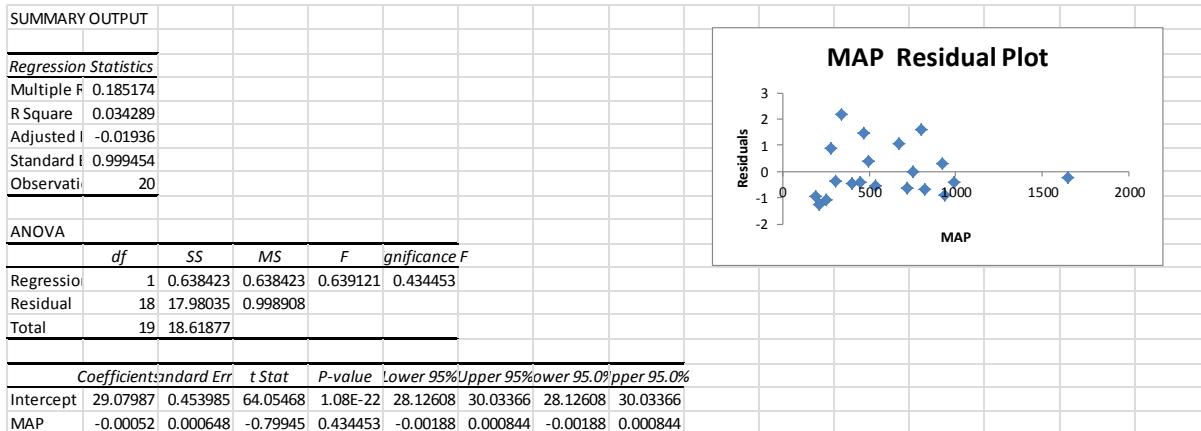
### Plant ACL v precipitation – driest month



### Plant ACL v vapour pressure deficit month max



### Predicted v Actual Soil ACL



### All Soils ACL v Annual MI

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

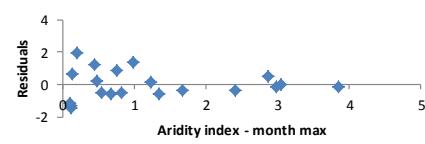
Moisture Index - lowest quarter mean (Bio33)  
Residual Plot



### All Soils ACL v lowest quarter mean MI

SUMMARY OUTPUT								
<i>Regression Statistics</i>								
Multiple R	0.425022							
R Square	0.180644							
Adjusted R	0.135124							
Standard Error	0.92061							
Observations	20							
ANOVA								
	df	SS	MS	F	Significance F			
Regression	1	3.36336	3.36336	3.96846	0.061752			
Residual	18	15.25541	0.847523					
Total	19	18.61877						
Coefficients Standard Err t Stat P-value Lower 95% Upper 95% Lower 95% Upper 95%								
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

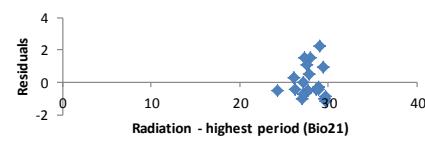
Aridity index - month max  
Residual Plot



### All Soils ACL v Aridity Index month max

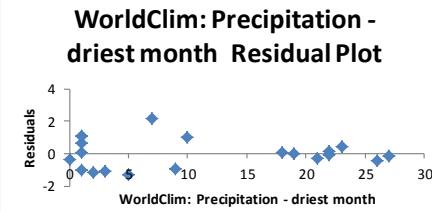
SUMMARY OUTPUT								
<i>Regression Statistics</i>								
Multiple R	0.118481							
R Square	0.014038							
Adjusted R	-0.04074							
Standard Error	1.009879							
Observations	20							
ANOVA								
	df	SS	MS	F	Significance F			
Regression	1	0.261368	0.261368	0.256279	0.618832			
Residual	18	18.3574	1.019856					
Total	19	18.61877						
Coefficients Standard Err t Stat P-value Lower 95% Upper 95% Lower 95% Upper 95%								
Intercept	26.43938	4.597383	5.750964	1.88E-05	16.78064	36.09813	16.78064	36.09813
Radiation	0.083453	0.164948	0.50624	0.618832	-0.26288	0.429785	-0.26288	0.429785

Radiation - highest period (Bio21) Residual Plot



### All Soils ACL v radiation highest period

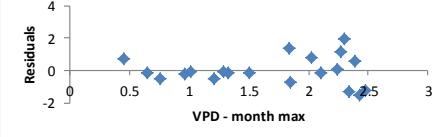
SUMMARY OUTPUT								
Regression Statistics								
Multiple R	0.428826							
R Square	0.183891							
Adjusted R	0.138552							
Standard Error	0.918784							
Observations	20							
ANOVA								
	df	SS	MS	F	Significance F			
Regression	1	3.423831	3.423831	4.055888	0.059213			
Residual	18	15.19494	0.844163					
Total	19	18.61877						
Coefficients Standard Error t Stat P-value Lower 95% Upper 95% Lower 95.0% Upper 95.0%								
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								
Regression Statistics								
Multiple R	0.430006							
R Square	0.184905							
Adjusted R	0.139622							
Standard Error	0.918213							
Observations	20							
ANOVA								
	df	SS	MS	F	Significance F			
Regression	1	3.442712	3.442712	4.083328	0.058441			
Residual	18	15.17606	0.843114					
Total	19	18.61877						
Coefficients Standard Error t Stat P-value Lower 95% Upper 95% Lower 95.0% Upper 95.0%								
Intercept	27.69496	0.567463	48.80491	1.4E-20	26.50276	28.88715	26.50276	28.88715
VPD - mor	0.639868	0.316653	2.020725	0.058441	-0.02539	1.305131	-0.02539	1.305131

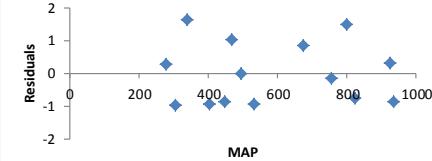
### VPD - month max Residual Plot



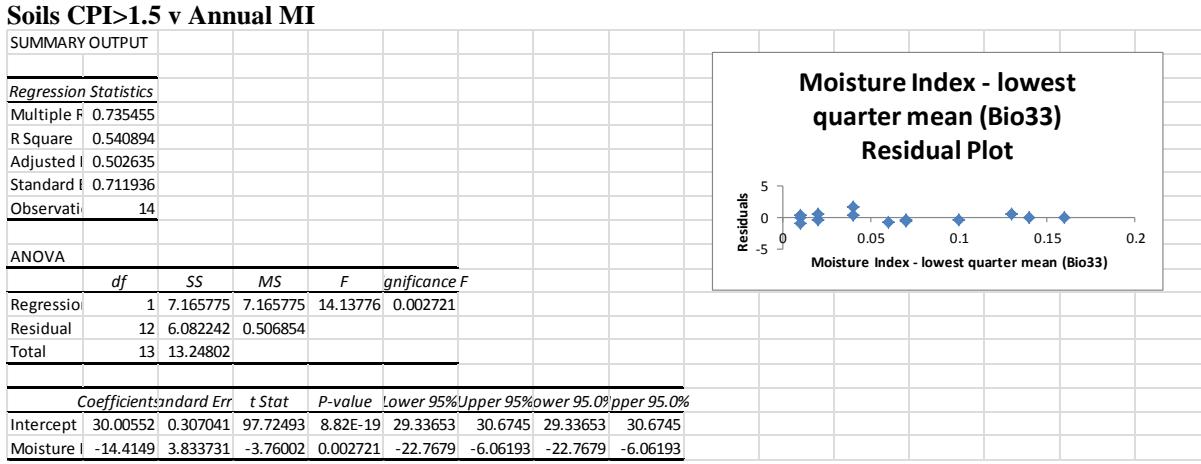
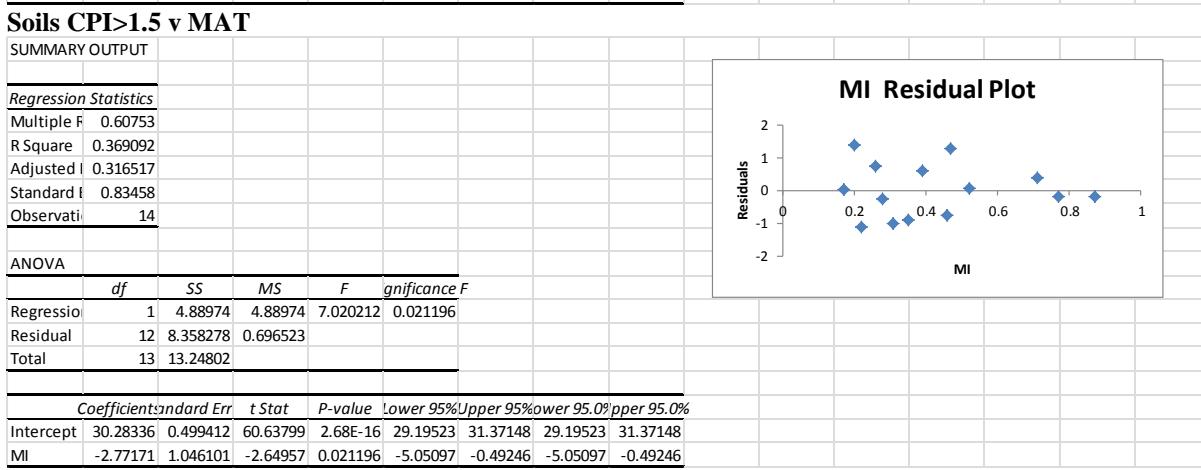
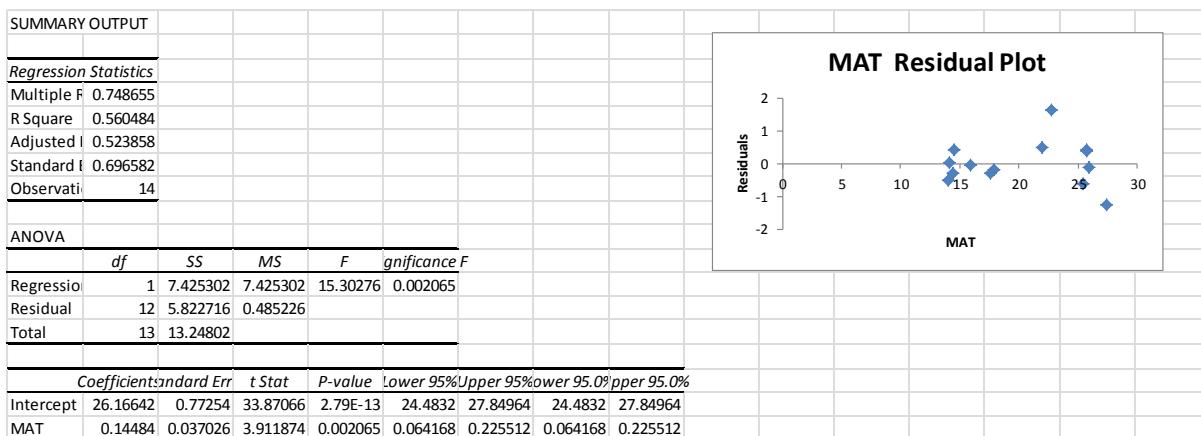
### All Soils ACL v vapour pressure deficit month max

SUMMARY OUTPUT								
Regression Statistics								
Multiple R	0.343359							
R Square	0.117896							
Adjusted R	0.044387							
Standard Error	0.986836							
Observations	14							
ANOVA								
	df	SS	MS	F	Significance F			
Regression	1	1.561882	1.561882	1.603831	0.229392			
Residual	12	11.68614	0.973845					
Total	13	13.24802						
Coefficients Standard Error t Stat P-value Lower 95% Upper 95% Lower 95.0% Upper 95.0%								
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

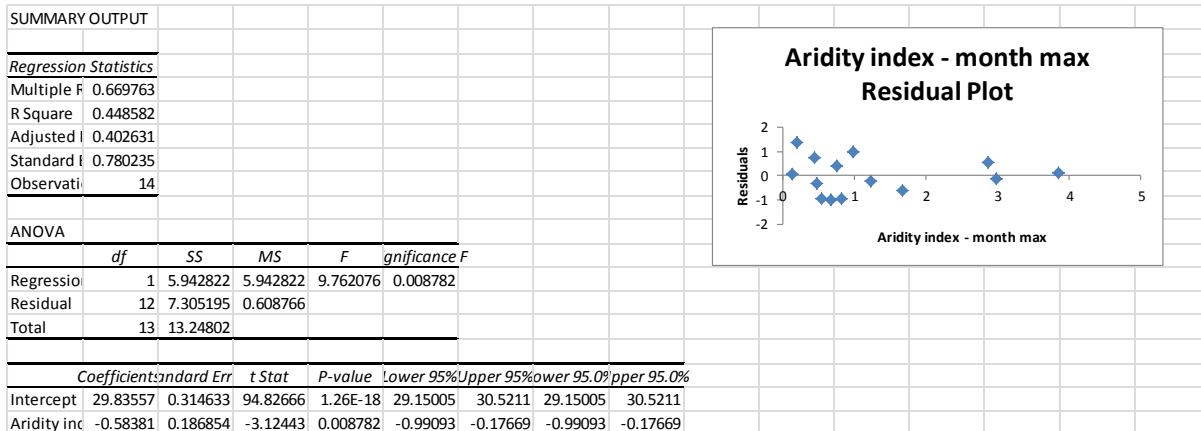
### MAP Residual Plot



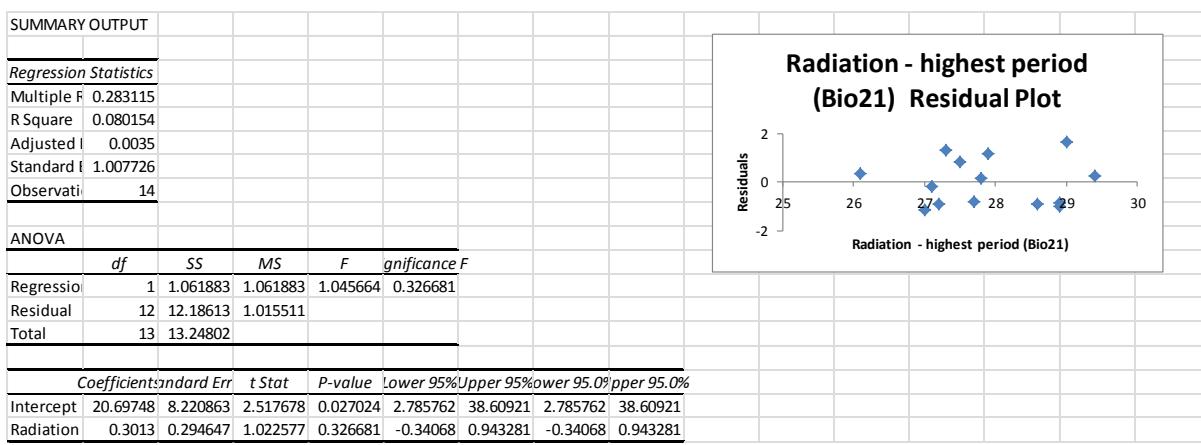
### Soils CPI>1.5 v MAP



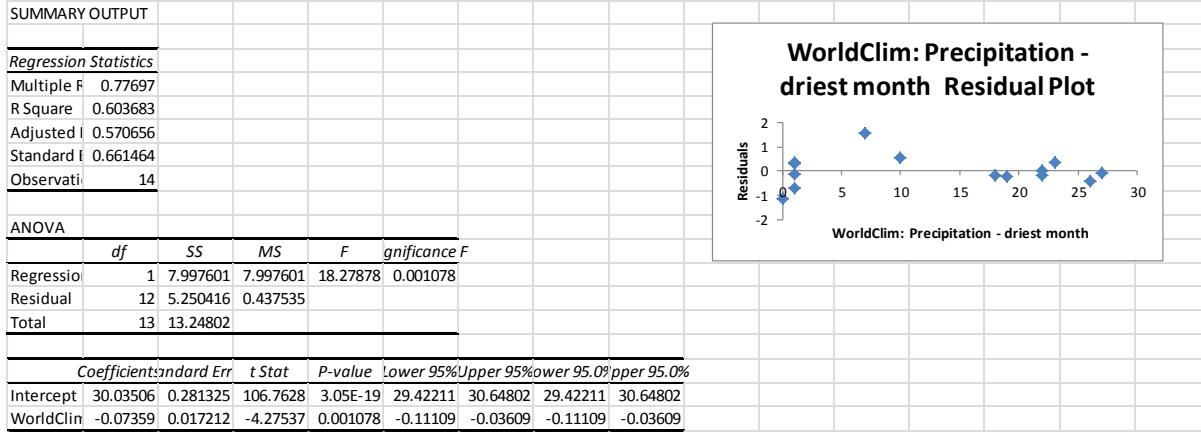
### Soils CPI>1.5 v lowest quarter mean MI



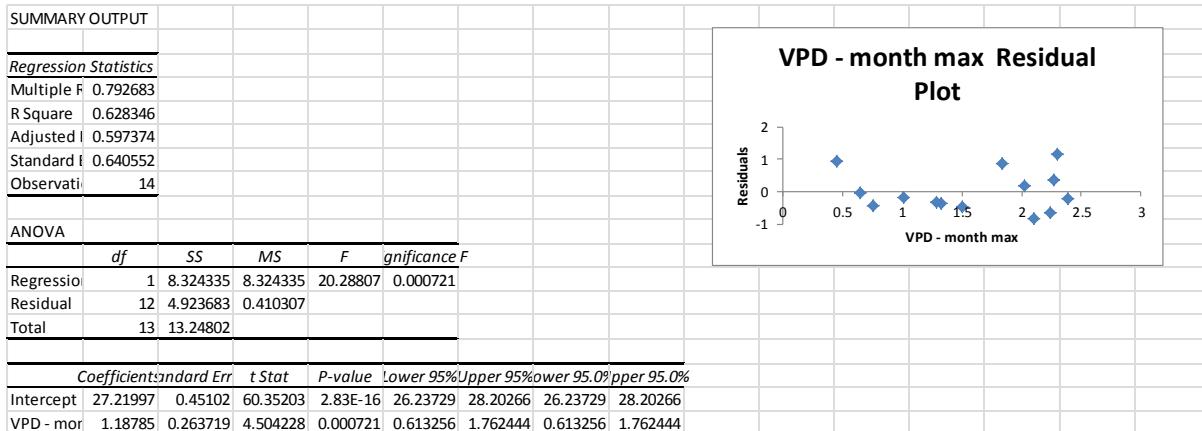
### Soils CPI>1.5 v aridity index month max



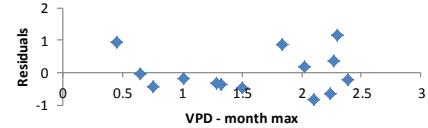
### Soils CPI>1.5 v radiation highest period



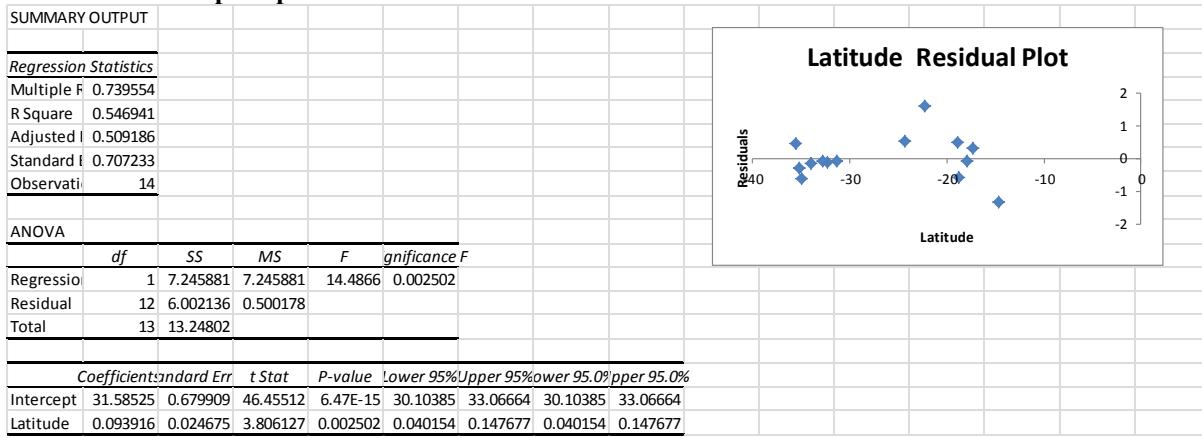
### Soils CPI>1.5 v precipitation driest month



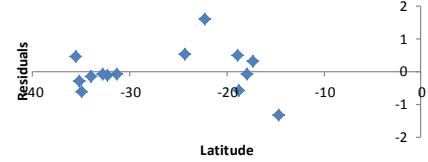
**VPD - month max Residual Plot**



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



**Latitude Residual Plot**



### Soils CPI>1.5 v latitude