Modelling the Fossil Fuel Signal in Trees Exposed to Elevated CO_2 in Cape Town

by

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ABSTRACT

Global CO_2 concentrations have been increasing at unprecedented rates since the Industrial Revolution. Urban areas have been a substantial source of CO_2 emissions globally. Fossil fuels have distinct isotopic signals which allow them to be traced into plants. Elevated CO_2 concentrations are known to affect plants' stomatal conductance and photosynthetic rate. These physiological responses lead to responses in other variables such as C_i/C_a. Models have been proposed to follow the fossil fuel signal from the atmosphere into plants. The model used in this study, relates C_i/C_a values to isotopic discrimination. Discrimination therefore is an integration of the effect of the $\delta^{13}C$ of the source and the physiological responses of the plant on the $\delta^{13}C$ of the plant. This study aimed to answer the following questions: (1) Is there a significant roadside build-up of CO_2 in Cape Town and can this be attributed to fossil fuel emissions? (2) Can $\delta^{13}C$ be used as a tracer of fossil fuel carbon in plants? (3) What are the implications of the results on the use of isotope records to trace increases in carbon emissions?

In order to determine the source air signal a Keeling Plot was created. The C_i/C_a of plants was measured on high traffic and low traffic days. A build up of roadside CO_2 was detected on high traffic days and was attributed to anthropogenic fossil fuel sources. The Keeling plot (R^2 = 0.98) indicated the source's isotopic signature resembled that of fossil fuels (-26.078‰). The modelled and measured isotopic signals of the plants did not agree strongly in the direction and magnitude of change between high and low traffic days. The trends observed were explained by the physiological responses of stomatal conductance and photosynthetic rates. These responses influenced the C_i/C_a values which influenced the modelled discrimination and $\delta^{13}C$.

This model has been employed in analysing tree-ring records as well as predicting future climate scenarios' influence of plants. The validity of using this model for these applications was discussed in conjunction with an expanded version of the model.

Global Change and CO_2 in Urban Ecosystems

Studies of atmospheric carbon dioxide (CO_2) concentrations have shown that prior to the industrial revolution the average global concentration was approximately 280ppm (Morgan et al. 2007) however by the end of 2010 they had risen to 390ppm (Conway & Tans 2011). It has been estimated that the earth has not experienced such high CO_2 concentrations in over 650 000 years and potentially in 25 million years (Royer 2006). Further to this the atmospheric CO_2 concentration is expected to nearly double during this century (Long et al. 2004).

The attribution of this global increase in atmospheric CO_2 concentration has predominantly been to the increase in anthropogenic green house gas emissions. Estimates suggest that since pre-industrial times human activities which produce green house gases have increased globally and between 1970 and 2004 these activities increased by 70% (Intergovernmental Panel on Climate Change (IPCC) 2007). Of the anthropogenic green house gas emissions that have been observed, CO_2 is the most prevalent. In 2004, CO_2 emissions represented approximately 77% of all green house gas emissions of which most originated from fossil fuel burning (IPCC 2007).

It is known that atmospheric CO_2 is generally well mixed (Jacobson 2010) however, urban areas tend to experience higher and more spatially variable CO_2 concentrations (Grimmond et al. 2002). This is considered to be a result of higher CO_2 emissions from localised fossil fuel burning (Idso et al. 2001) from both fixed and mobile sources (Grimmond et al. 2002). An intensive two-week study that measured atmospheric CO_2 concentrations along major roads around Phoenix, Arizona, found the highest weekday CO_2 concentration of 650ppm and the lowest concentration of 471ppm. When compared to the average concentration in the surrounding rural areas (369ppm) these values were 76% and 28% higher, respectively. The average weekday concentration was 43% higher than the surrounding rural area average and the average weekend concentration was 38% higher (Idso et al. 2001).

Carbon has two naturally occurring stable isotopes, ^{12}C and ^{13}C, of which ^{12}C is much more abundant (98.8%) with only 1.1% of carbon being ^{13}C (Farquhar et al. 1989). Fossil fuel derived CO_2 is naturally depleted in ^{13}C compared with atmospheric CO_2 (Keeling et al.

1979). The global increase in fossil fuel burning is therefore diluting the atmosphere of ^{13}C (Keeling et al. 1979). If atmospheric $\delta^{13}C$ values are decreasing this would suggest that the isotopic signature of plants taking up that CO_2 should reflect the decreasing trend. Numerous tree-ring data sets provide evidence for this gradual decreasing trend in $\delta^{13}C$ since the beginning of the 19th century (Treydte et al. 2009). However one should be careful when attributing the trend in $\delta^{13}C$ to increasing fossil fuels in the atmosphere. The predicted $\delta^{13}C_{plant}$ can be a reflection of physiological responses such as stomatal conductance, altered C:N allocation to carboxylation and leaf structure (Seibt et al. 2008).

In terms of fossil fuel signatures, depending on the source area, different fossil fuels often have distinguishable signatures (Pataki 2003a) (See Table 1). It is therefore possible to identify carbon sources, if the anthropogenic fossil fuel signals are known (Bush et al. 2007). The isotopic composition of fossil fuels however, varies geographically (Andres et al. 2000) and for this reason measurements of local emissions are required for each study area (Pataki et al. 2005).

Elevated CO_2 and Plants

In recent years the results of all free air CO_2-enrichment (FACE) experiments have been brought together in meta-analyses (e.g. Long et al. 2004; Ainsworth & Long 2005; Ainsworth & Rogers 2007). These meta-analyses have shown that the direct effects of increased CO_2 concentrations on plants manifested in the response of the photosynthetic rate and stomatal conductance. All other responses were an indirect response as they were the result of the photosynthetic and/or stomatal responses (Drake et al. 1997; Long et al. 2004). Although the effect of elevated CO_2 concentration on photosynthetic rate was well characterised, experimental results did not always match the theory (Ainsworth & Long 2005). Stomatal conductance typically responded to elevated CO_2 concentration by decreasing however, the magnitude of the effect was variable and subject to environmental feedbacks (Morgan et al. 2004). Long et al. (2004) noted that secondary responses to elevated CO_2 concentrations included: increased leaf non-structural carbohydrates and therefore increased C:N ratio, improved water status, decreased leaf Rubisco (ribulose-1,5-biphosphate carboxylase-oxygenase) activity, stomatal density and root:shoot mass ratio.

In the short term, decreased stomatal conductance was the result of decreased stomatal aperture size with the increase in CO_2 concentration. In the long term however the decrease in stomatal conductance was related to a change in stomatal density and stomatal index (percentage of epidermal cells that are guard cells) as well as stomatal aperture (Ainsworth & Rogers 2007).

Increased CO_2 concentrations have been shown to increase the rate and efficiency of photosynthetic assimilation. This has been attributed to two responses: (1) currently Rubisco is substrate limited therefore under increased CO_2 concentrations the carboxylation rate would increase, (2) elevated CO_2 concentrations would competitively inhibit the oxygenation reaction of Rubisco thereby reducing CO_2 loss and energy costs (Ainsworth & Rogers 2007). The increase in carbon fixation is thought to cause an increase in ATP demand for the regeneration of ribulose-1,5-biphosphate (RubP) rather than the reaction being Rubsico limited. This regulation of photosynthesis may be more important in the short term while decreased Rubsico activity is likely to be more important in the long term since it has been correlated with decreased Rubsico content in the leaves (Moore et al. 1999).

Saurer et al. (2004) produced three scenarios of the response of gas exchange parameters to changing atmospheric CO_2 concentrations based on Eq. 1 and 2. Scenario 1 kept C_i constant with increasing CO_2. This would cause C_i/C_a (ratio of intercellular CO_2 concentration to ambient CO_2 concentration) and discrimination to decrease while the $\delta^{13}C_{plant}$ increased. Scenario 2 was based on a constant C_i/C_a and discrimination. A constant C_i/C_a is a reflection of proportional regulation of photosynthetic assimilation and stomatal conductance. This would cause $\delta^{13}C_{plant}$ to decrease in parallel with $\delta^{13}C_{atmosphere}$. The third scenario has C_i increasing by the same amount as C_a (i.e. C_a-C_i = constant). The $\delta^{13}C_{plant}$ would decrease while the discrimination increased. This indicates a relatively weak response in stomatal conductance.

The results of this study found that 96% of the trees' response was an intermediate between keeping C_i constant and increasing it at the same rate as C_a. On average the responses were best represented by scenario 2 (i.e. constant C_i/C_a). Saurer et al. (2004) explained that keeping C_i/C_a constant could be achieved by concurrently reducing stomatal

conductance and photosynthetic activity as atmospheric CO_2 concentrations increased. Meta-analyses by Long et al. (2004) and Ainsworth & Long (2005) both noted that C_i/C_a remained unchanged during FACE experiments over the previous 15 years.

With these considerations in mind, this study aimed to answer the following questions: (1) Is there a significant roadside build-up of CO_2 in Cape Town and can this be attributed to fossil fuel emissions? (2) Can $\delta^{13}C$ be used as a tracer of fossil fuel carbon in plants? (3) What are the implications of the results on the use of isotope records to trace increases in carbon emissions?

The Role of Isotopes in Tracing Fossil Fuels in the Atmosphere and Plants

With the build up of CO_2 in urban areas the question arises of whether a fossil fuel signal can be found in the atmosphere, and whether that signal is manifesting itself in the plants being exposed to pulses of high CO_2 concentrations. If the plants take up fossil fuel derived CO_2 and atmospheric CO_2 indiscriminately then the plant's isotopic signature should reflect the increase in fossil fuel derived CO_2 in the atmosphere, by being more negative than expected. This observation has been made in tree-ring trends (Treydte et al. 2009). However it is important to take the physiological responses to changes in CO_2 into consideration as they have an influence on the fractionation of the isotopes (Dawson et al. 2002).

Table 1: Various measured isotopic signatures of petrol, diesel, natural gas, mixed fossil fuels and atmospheric carbon. (*Uncertainty ≤1‰).

Petrol Exhaust (‰)	Diesel Exhaust (‰)	Natural Gas Combustion (‰)	Source Area	Citation
-24.4 ± 0.2			Pasadena, California	Affek & Eiler 2006
-28.4 ± 0.31	-28.5 ± 0.04	-37.1 ± 0.20	Salt Lake City, Utah	Bush et al. 2007
-26.0 ± 0.2		-40.2 ± 0.5	Pasadena, California	Newman et al. 2008
-27.9 ± 0.1		-37.1 ± 0.2	Salt Lake City, Utah	Pataki et al. 2005
-29.1 ± 0.1	-29.8 ± 0.2	-39.1 ± 1.1	France	Widory 2006
-26.5*		-44*		Andres et al. (2000)
-27.9 ± 0.1	-28.6 ± 0.1	-37.3 ± 0.7	Salt Lake City, USA	Bush et al. unpublished from Pataki et al. (2005)
-28.7 ± 0.2	-28.9 ± 0.1	-39.2 ± 0.5	Paris, France	Widory and Javoy (2003)
-27.2 ± 0.1			Dallas, USA	Clarke-Thorne & Yapp (2003)

In order to trace an isotopic signature into a plant the discrimination must be determined (Farquhar et al. 1989). Discrimination is defined as:

$$\Delta = \frac{\delta_{Air} - \delta_{Plant}}{1 + \delta_{Plant}} \quad (Eq. 1)$$

This gives an indication of how much the plant is changing the isotopic signature of the air when it is laid down as tissue. Discrimination can be modelled as a function of C_i/C_a:

$$\Delta = a + (b - a)\frac{c_i}{c_a} \quad (Eq. 2)$$

where *a* is the fractionation that occurs due to diffusion in air (constant used = 4.4‰) and *b* is the net fractionation that is caused by carboxylation (constant used = 27‰) (Farquhar et al. 1989). The constants *a* and *b* may vary between species. The value estimated for *a* in various studies was consistently 4.4‰ (Jahren et al. 2008; Seibt et al. 2008; Treydte et al. 2009) while the value for *b* varied slightly: 27-28‰ (Treydte et al. 2009), 26.4-28.2‰ (Jahren et al. 2008) and 27-29‰ (Lloyd & Farquhar 1994). Regardless of these variable results it has been generally accepted that the values of *a* and *b* are 4.4‰ and 27‰ respectively. Ehleringer & Cerling (1995) explained that C_i/C_a represents the balance between CO_2 diffusion into the leaf, which is controlled by stomatal conductance (g_s), and CO_2 assimilation into the plant tissue, which is controlled by the photosynthetic rate (A). For this reason C_i/C_a can be used as a proxy for the physiological influences on the isotopic composition of a plant. The constant fractionation processes represented by *a* and *b* in Eq. 2 result in the carbon isotope composition being a representation of the assimilation-weighted intercellular CO_2 concentration during the lifetime of the tissue (Farquhar et al. 1989).

Implications for Interpreting Current and Palaeo-isotope Records

Feng (1998) noted that using the relationship between C_i/C_a and discrimination (Eq. 2) to reconstruct past CO_2 concentrations may not be as simple as measuring carbon isotopes of tree rings and applying the model. This is because C_i/C_a has been found to vary in direction and magnitude over different CO_2 concentrations among different species. They suggested that this complexity was a result of the difficulty of separating climate effects and effects of atmospheric CO_2 concentrations on tree growth. Ehleringer & Cerling (1995) found that the

C_i/C_a of *Prosopis alba* decreased over the last 100 years while the C_i remained constant while Polley et al. (1993) found that C_i/C_a remained constant in oats and mustard but increased by 0.03 in wheat. These studies both calculated the C_i/C_a values from measured $\delta^{13}C$. This leaves the question of whether this model provides an accurate indication of C_i/C_a values over time or whether it is over-estimating the amount of change in C_i/C_a.

Materials And Methods

In order determine whether a significant roadside build-up of CO_2 occurs in Cape Town we compared conditions on a high traffic day and a low traffic day at two roadside study sites (Figs. 1-3). High traffic days were defined as weekdays with heavy, slow-moving traffic passing the study site. Low traffic days were defined as weekends or public holidays with light, fast-moving traffic passing the study site. This comparison allowed us to determine the major source of CO_2 during CO_2 build-up and follow the CO_2 isotopic signal into the plants.

Study Sites

Gas exchange sites were chosen based on the presence of high traffic levels and a vegetated median with individuals of the species being used in the study (Figs. 1-2). The species chosen for the study chosen were deep rooted, healthy with signs of recent growth and accessible to measure. Data was collected over four days with two days spent at each of the two study sites, one day was a high traffic day and the other was a low traffic day.

Figure 1: Study site at Paradise road. Two *Podocarpus falcatus* individuals: PF1 & PF2 were sampled (Image modified from Google Earth, 2010).

Figure 2: Study site at the intersection of the N2 & M3. Three *Erythrina lysistemon* individuals: B1, B2, B3 and one *Pinus pinea* individual of which three separate branches were sampled: P1, P2, P3 (Image modified from Google Earth, 2010).

Figure 3: Study sites relative positions. N2 Highway and Paradise Road – gas exchange study sites. UCT, Paradise Road and Wynberg – bulk leaf tissue sample sites (Image modified from Google Earth, 2010).

Gas Exchange and Isotopic Measurements

Gas exchange measurements and isotopic analyses were performed on three species, namely *Podocarpus falcatus, Pinus pinea* and *Erythrina lysistemon*. In order to ensure that the plants were not water stressed during the study a Scholender bomb was used to determine the xylem pressure potential. The *P. falcatus* was present at the Paradise Road (Fig. 1) study site while the other two species, *P. pinea* and *E. lysistemon* were present at the N2 highway study site (Fig. 2).

Gas exchange measurements were performed over four days; two days of low traffic (Paradise Road: 09/08/2011 & N2: 27/08/2011) and two days of heavy traffic (Paradise Road: 10/08/2011 & N2: 29/08/2011). At Paradise Road two *P. falcatus* individuals were measured along the median of the road (Fig. 1) and at the N2 highway study site three *E. lysistemon* individuals were measured (Fig. 2). Three separate branches on one *P. pinea*

individual, at the N2 highway study site, were measured due to the lack of other accessible individuals. The leaves being measured were chosen on the basis on being north-facing and well-lit through the day. An infrared gas analyser (LI-6400, LI-COR Inc., Lincoln, NE, United States) (IRGA) was used for the gas exchange measurements. Each individual was measured on an hourly basis from sunrise to sunset. The *P. falcatus* leaves were measured using a Conifer Chamber while the *P. pinea* and *E. lysistemon* leaves were measured using a 2x3 Chamber. The Conifer Chamber allowed for the ambient light intensity to be used during measurements. The 2x3 Chamber was set to track the light intensity being measured by the Quantum Sensor. Atmospheric CO_2 concentrations were highly variable. In order to reduce the variability, a CO_2 mixer was introduced and gas exchange measurements were taken at a constant concentration of 400ppm. When the leaves were in the chamber the IRGA was given sufficient time to stabilize after which the data were logged and the leaves were removed from the chamber. The leaves were kept in the chamber for the shortest possible time, typically two minutes, to avoid physiological responses taking place. Between hourly gas exchange measurements, the IRGA was used to measure the ambient CO_2 concentration. The number of cars passing the study site over a five minute period was counted while the CO_2 concentration was being measured. In order to determine the leaf area of the *P. falcatus* and *P. pinea* the image editing program ImageJ (W. Rashband, National Institute of Health, USA) was used.

Leaves, other than the ones measured, were selected at the end of each day to be used for isotopic analysis. The leaves were placed in a microwave at high power for 60 seconds in order to stop all metabolic processes immediately. The leaves were then dried for a 24 hours at 80°C.

Leaf tissue was ground in a ball mill to a fine powder of which 150mg was used to extract soluble carbohydrates. The soluble carbohydrates were extracted from the leaves by following a procedure modified from that described by Brugnoli et al. (1988) and were subsequently isotopically analysed. 150mg of the ground leaves was placed in a 50ml centrifuge tube with 35ml of Milli-Q water. The tube was placed in a water bath and boiled for 30 minutes after which it was left to cool to room temperature. The samples were centrifuged at 1210*g* for 15 minutes. The supernatant was collected and passed through two resin columns. The first column was made up of Sephadex G-25 Medium (Pharmacia

Fine Chemicals Inc., Stockholm, Sweden) to remove the large organic molecules such as tannins and phenolics. The second was an ion-exchange column which contained Dowex Marathon MR-3 (Supelco Analytical, Sigma-Adrich Inc. St Louis, MO, USA). The samples were freeze-dried.

From the dried soluble carbohydrate samples, 2mg was weighed out into tin capsules with an accuracy of 1µg. The samples were combusted and the gases were passed to a Delta V Plus isotope ratio mass spectrometer (Thermo Scientific, Bremen Germany) (IRMS). In-house standards were used for correcting results. The in-house standards have been calibrated against the International Atomic Energy Agency standards and the carbon isotope values were expressed in terms of their value relative to Pee-Dee Belemnite.

Keeling Plot

Pataki et al. (2003b) explained that the Keeling Plot, developed by Keeling (1958, 1961) is based on conservation of mass where the atmospheric concentration of a gas is a reflection of the combination of background atmospheric concentration and variable amounts of gas added by other sources.

$$c_a = c_b + c_s \quad (Eq. 3)$$

where C_a is the atmospheric CO_2 concentration, C_b is the background CO_2 concentrations and C_s is the concentration of the additional source which raises the atmospheric CO_2 concentration above that of the background. Therefore given that the conservation of mass can be expressed as:

$$\delta^{13}C_a c_a = \delta^{13}C_b c_b + \delta^{13}C_s c_s \quad (Eq. 4)$$

where $\delta^{13}C$ is the isotopic signature of each CO_2 component. By combining Eq. 3 and 4 the following relationship is formed:

$$\delta^{13}C_a = c_b(\delta^{13}C_b - \delta^{13}C_s)\left(\frac{1}{c_a}\right) + \delta^{13}C_s \quad (Eq. 5)$$

This relationship allows one to determine the integrated isotopic signature of the CO_2 sources in the ecosystem ($\delta^{13}C_s$) (Pataki et al. 2003b).

To the author's knowledge this method has not been used to determine the isotopic signature of the CO_2 sources in Cape Town in order to trace the fossil fuel signal into plants. For this reason it was necessary to develop a method of determining both the isotopic signature and CO_2 concentration of an air sample.

Air samples were collected during early morning traffic on multiple days at the two study sites, Paradise Road and the N2 highway, as well as two 'clean air' sites, Signal Hill and Victoria Road, which experienced onshore breezes. Septum-capped vials were flushed with ambient air for approximately one minute. The collected air samples were placed in a temperature controlled sampler tray at 72°C. Once the samples had reached 72°C the gas from each vial was sampled and passed to a Thermo Finnigan Modell II Gasbench (Germany). The gas compounds were separated through a "Poraplot Q" GC column and passed to a Finnigan MAT 252 isotope ratio mass spectrometer (IRMS). The standards used were in-house standards that have been calibrated against IAEA standards and the carbon isotope values were expressed relative to Pee-Dee Belemnite. The isotopic composition of each sample was determined using the equation:

$$\delta^{13}C = 1000 \times \left(\frac{R_{Sample}}{R_{Standard}} - 1\right) \quad (Eq. 6)$$

where $\delta^{13}C$ is the isotopic signature of the material, R_{Sample} is the ^{12}C:^{13}C ratio of the sampled material and $R_{Standard}$ is the ^{12}C:^{13}C ratio of the standard.

The IRMS produced the $\delta^{13}C$ value of each sample as well as the maximum amplitude (mV) and maximum area of the peak ($mV.s^{-1}$). The maximum area of the peak provided a relative measure of CO_2 concentration. This was used to determine the CO_2 concentration of the sample. However, the relative measure needed to be calibrated into an absolute measure of concentration. This was achieved by creating CO_2 standards of known concentration using a LI-6400 IRGA. To create the standards atmospheric air was scrubbed with a CO_2 desiccant (soda lime) and a known concentration of CO_2 was introduced with a mixer. A new set of five standards was created each time the analysis was run with concentrations of: 350ppm, 450ppm, 550ppm, 650ppm and 750ppm. The area under the curve of the first injection (the maximum peak area) of each of the known standard sample concentrations was used to create the standard curve (Fig. 6). The CO_2 concentrations of the collected air

samples were then determined using the regression equation of the standard curve. A Keeling Plot was created with the $\delta^{13}C$ values and the inverse of the CO_2 concentrations ($1/[CO_2]$) of each sample (Fig. 7).

Samples of exhaust fumes from a petrol car and a diesel car were collected and isotopically analysed. This was done to support the source isotopic signature as being strongly influenced by fossil fuel emissions from petrol and diesel cars. Since the concentration of CO_2 was too high for the mass spectrometer to handle, a small sample of the collected air was analysed. This was achieved by using a 1ml syringe to puncture the septum of the vials and draw out 1ml of the exhaust fumes. The 1ml was then pushed into a helium flushed vial which diluted the exhaust fumes for isotopic analysis, by the above method, in the IRMS.

Models

The gas exchange data: intercellular CO_2 concentration (c_i), atmospheric CO_2 concentration (c_a) and stomatal conductance (g_s) were weighted by the amount of photosynthetic assimilation (A) experienced by each plant on each of the days. The values were weighted using the following equation:

$$X = \frac{\sum(X.A)}{\sum A} \quad (Eq. 7)$$

where X represents, C_i, C_a or g_s; and A is the average photosynthetic assimilation rate for each measurement period through the day.

From the photosynthetically weighted values a photosynthetically weighted C_i/C_a value was calculated for each plant on each day. This was then used to determine the discrimination of the plant using Eq. 2.

The $\delta^{13}C_{atmosphere}$ was determined by inputting the photosynthetically weighted C_a, for each plant on each day, into the Keeling Plot regression equation. The calculated discrimination and $\delta^{13}C_{atmosphere}$ were used to predict the $\delta^{13}C_{plant}$. The modelled $\delta^{13}C_{plant}$ and the measured isotopic signature of each plant ($\delta^{13}C_{soluble\ carbohydrates}$) were compared as well as the trends of changes in isotopic signatures between high and low traffic days.

Seibt et al. (2008) explained that the model proposed by Farquhar et al. (1989) does not directly include processes which the plant parameters may be sensitive to, such as internal conductance (g_i) and photorespiration. The model used in this study was the model proposed by Farquhar et al. (1989) which used C_i/C_a as a proxy for C_c/C_a (ratio of chloroplast to ambient CO_2 mole fraction) which is considered to be the true determining parameter of discrimination. The reason for using the simplified model was based on the fact that it is not possible to measure C_c/C_a from the same measurements as C_i/C_a, since internal conductance has been shown to differ substantially between species as well as functional types (De Lucia et al. 2003). For this reason however, applying the simplified model leads to potential problems in interpreting discrimination or $\delta^{13}C$ values and trends (Seibt et al. 2008).

The expanded equation for discrimination based on the CO_2 concentration at the site of carboxylation is defined as:

$$\Delta_C = a\frac{C_a - C_i}{C_a} + a_m\frac{C_i - C_c}{C_a} + b\frac{C_c}{C_a} - f\frac{\Gamma_*}{C_a} \quad (Eq. 8)$$

where a_m is internal (mesophyll) CO_2 transfer fractionation (1.8‰), b is fractionation during carboxylation (29‰), f represents fractionation during photorespiration (±8‰) and Γ_* is the CO_2 compensation point in the absence of dark respiration (Seibt et al. 2008).

If one uses Fick's Law: $A = g_i(C_i - C_c)$ to replace the C_c term, the equation can be modified to:

$$\Delta_C = a + (b - a)\frac{C_i}{C_a} - (b - a_m)\frac{A}{g_i C_a} - f\frac{\Gamma_*}{C_a} \quad (Eq. 9)$$

By replacing A with the ratio g_s/g_i (ratio of stomatal to internal conductance) and rearranging the equation to have C_i/C_a as the subject, it can be seen that this model corrects the C_i/C_a values for systematic changes in factors that affect discrimination such as internal conductance and photorespiration. The rearranged equation:

$$\frac{C_i}{C_a} = \frac{\Delta_c - a + (b - a_m)\frac{g_s}{1.6g_i} + f\frac{\Gamma_*}{C_a}}{b - a + (b - a_m)\frac{g_s}{1.6g_i}} \quad (Eq. 10)$$

where g_s is the stomatal conductance and g_i is the internal conductance. The simplified model only includes these variables indirectly, if at all.

Seibt et al. (2008) used the simplified model to determine how much of an influence excluding internal conductance and photorespiration had on the estimates of C_i/C_a. Their results (Table 4) as well as further calculations of the same scenarios using the expanded model (Table 5) are presented. The two models were used to calculate the discrimination and C_i/C_a for three different scenarios with varying internal conductance (g_i) values. The simple model implicitly includes g_i in the value of *b* (Eq. 2) while the expanded model accounted for the changes. All other variables were kept constant in order to determine the influence of g_i on the results.

Statistical Analyses

The normality of the data sets was evaluated using probability plots. When the modelled and measured isotopic values of all individuals were compared the data were normal therefore a Paired t-test was used to determine whether there was a significant difference between the two data sets. When the individual species were considered the data were not normally distributed and the sample sizes were small therefore the non-parametric Wilcoxon's Matched Pair's Test was used.

Results

Is there a significant roadside build-up of CO_2 in Cape Town and can it be attributed to fossil fuel emissions?

During gas exchange data collection the number of cars that passed the study site over a period of five minutes was counted on an hourly basis (Fig. 4). At both study sites the number of cars passing over a period of five minutes was significantly higher on the high traffic day compared to the low traffic day (Paradise Road: $t = -4.84$; $p < 0.001$; $df = 15$; N2 highway: $t = -4.37$; $p < 0.001$; $df = 16$).

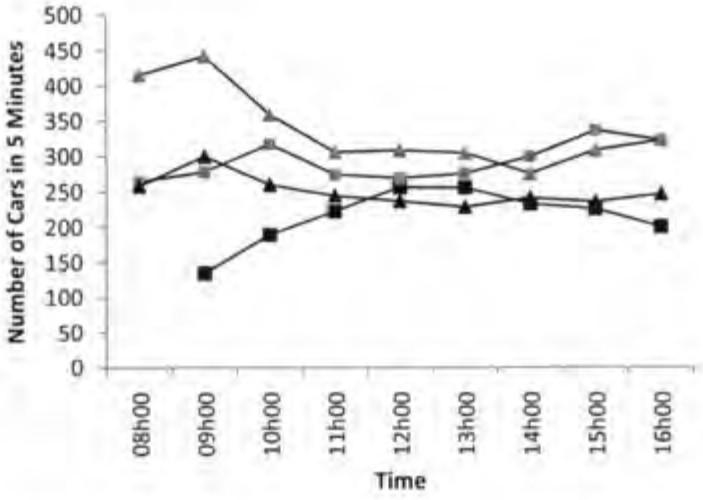

Figure 4: Number of cars passing the study sites over a period of five minutes. Squares – Paradise Road; Triangles – N2 Highway; Grey – High traffic; Black – Low traffic.

The atmospheric CO_2 concentrations followed the pattern of the traffic with higher CO_2 concentrations on days with higher traffic levels. The CO_2 concentrations were significantly higher on high traffic days compared to low traffic days ($t = 4.71$; $p < 0.01$; $df = 36$).

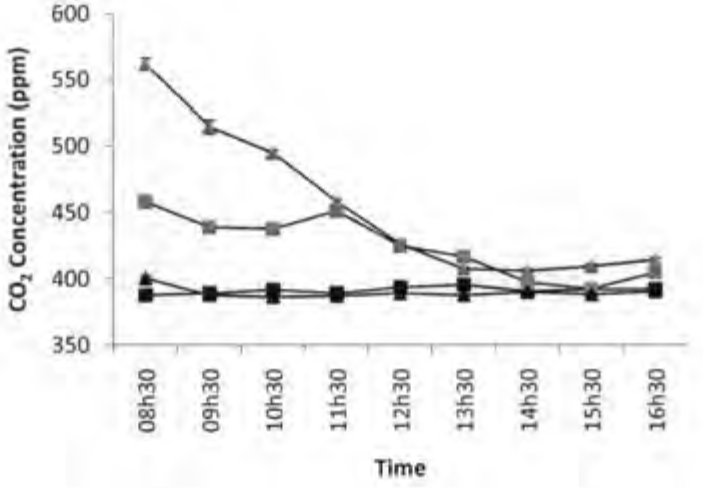

Figure 5: Atmospheric CO_2 concentration through the day during four sampling days. Squares – Paradise Road; Triangles – N2 Highway; Grey – High traffic; Black – Low traffic. Standard errors included as error bars.

The CO_2 concentration of the 23 atmospheric air samples, used in the Keeling Plot, were determined using the standard curves (Fig. 6). The calculated CO_2 concentrations ranged from 380ppm to 575ppm. The isotopic analysis of atmospheric air samples revealed a strong predictive power (R^2=0.98) between the two variables. The equation describing the relationship between $\delta^{13}C$ and $\frac{1}{[CO_2]}$ was y = 6835.3x – 26.078 (Fig. 7).

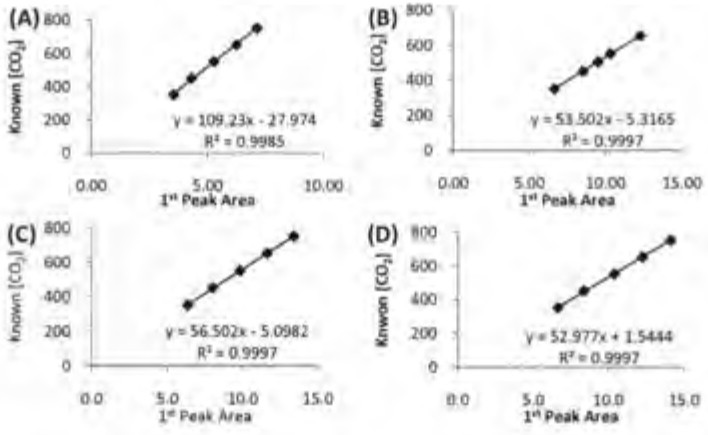

Figure 6: Standard Curves created for each sampling. (A) Initial standard curve created to determine whether peak area could be calibrated as a relative measure of CO_2 concentration. (B) Standard curve created for first collected samples. (C) Standard curve created for air samples collected at Paradise Road and Victoria Road. (D) Standard curve created for air samples collected at N2 highway.

The $\delta^{13}C$ of the source air, the Keeling Plot intercept, was identical to the $\delta^{13}C$ determined for the petrol sample ($\delta^{13}C_{source}$ = -26.08‰; $\delta^{13}C_{petrol}$ = -26.08‰). This indicated that petrol was strongly influencing the isotopic signal of the source air.

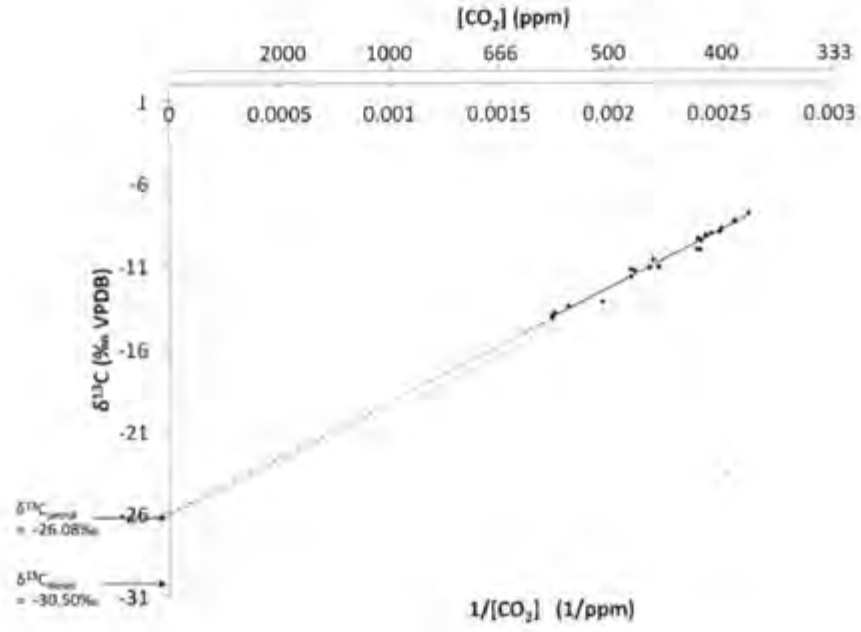

Figure 7: Keeling Plot of atmospheric air samples from Paradise Road, N2 Highway, Signal Hill and Victoria Road. Extended dashed line indicates intercept of regression which represents the isotopic signature of the source CO_2 (-26.078‰). Isotopic signatures of petrol and diesel are indicated on the graph. CO_2 concentration (ppm) is indicated on the upper axis.

The Keeling plot was used to determine the isotopic signal of the average atmospheric CO_2 values. The $\delta^{13}C$ of the atmospheric air was significantly more negative on high traffic days ($t = -14.19$; $p < 0.01$; $df = 14$) indicating that there was significantly more fossil fuel present in the atmosphere on high traffic days.

Can $\delta^{13}C_{plant}$ be used as a tracer of fossil fuel emissions in Cape Town?

In order to follow the isotopic signal of fossil fuel from the atmosphere into the plant the discrimination equation (Eq. 2) was used in conjunction with the C_i/C_a value and the soluble carbohydrate isotopic signal ($\delta^{13}C_{soluble\ carbohydrate}$) of each plant (Figs. 9-11). The C_i/C_a values on the high traffic days were significantly lower than those on low traffic days ($t = -3.42$; $p = 0.006$; $df = 7$). The discrimination equation, using those C_i/C_a values, resulted in an average discrimination value for plants on high traffic and high CO_2 days of 15.5‰ and 17.0‰ for plants on low traffic and low CO_2 days (Table 3). To ensure the gas exchange measurements and isotopic signals of the trees were not influenced by water stress the xylem pressure potential was measured. On average the xylem pressure potential was 0.3 MPa for all the individuals which indicated the plants were not water stressed.

For all three species both the average photosynthetic assimilation rate (A) and average photosynthetically weighted stomatal conductance (g_s) were lower on the high traffic, high CO_2 days (Figs. 9-11) and the overall trend was significant (A: t = -6.05, p = 0.0005, df = 7; gs: t = -4.56, p = 0.003, df = 7). The percentage change in g_s was consistently greater than that in A from low to high traffic days (Table 2).

The gas exchange measurements for the *P. falcatus* individuals all followed the expected trends. C_i/C_a decreased at the beginning of the day, stabilised at a low value and increased towards the end. A and g_s, as expected, both increased at the beginning of the day, stabilised at a high value and decreased towards the end. The *P. pinea* individuals had unexpectedly high starting values for g_s which generally decreased through the day. A of *P. pinea* showed a good trend of increasing values in the morning, relatively constant high values during the day and decreasing later in the day. The C_i/C_a values experienced the expected trend although the values during the day were not as stable as expected for P2-Low. *E. lysistemon* in general had good shapes for the A and g_s curves except for B1-Low and B2-Low at the end of the day when there was an unexpected sharp increase which led to a sharp decrease in C_i/C_a. Although noisy the C_i/C_a values for the high day seemed to represent good values. A and g_s curves for the high day remained within a small range of values for B1-High and B2-High while B3-High displayed the expected diurnal shape. The 14h00 data point for B3-High was removed as the data point was unrealistic and likely to have been influenced by shading during measurements.

Of the three measured species, *P. falcatus* had the most consistent gas exchange measurements. The *P. pinea* measurements were also relatively consistent except for the decreasing trend in g_s values. These individuals experienced more noise within the gas exchange data. *E. lysistemon* values remained constant through the day with a few high and low points.

When comparing the predicted $\delta^{13}C_{plant}$ and measured $\delta^{13}C_{soluble\ carbohydrates}$ for each of the plants an error range of 1‰ was applied. This was a conservative estimate based on estimates of potential error in fieldwork, laboratory error and machine error (±0.2‰) (calculated from working standards).

The change in isotopic signature of *P. falcatus*, between high and low traffic days, predicted by the model was -0.8‰, indicating a more negative $\delta^{13}C$ on the high traffic day. The measured trend for species was 0.2‰ between the two days, indicating a more negative $\delta^{13}C$ on the low traffic day (Table 3). Both of these values were within the error limits associated with our measurements. The predicted change for *P. pinea* was -1.6‰, while the measured change was 1.0‰. The model did not agree with the direction of the trend in the measured values (Table 3). For the measurements of *E. lysistemon* the model predicted no change (0.0‰). The actual isotopic signatures indicated that the species (-0.3‰) experienced a more negative signature on the high traffic day (Table 3). These values were both within the error limits associated with the measurements.

Although the trends were visible in the data very few were statistically significant. When the modelled and measured values of all the individuals were compared there was no significant difference between the values ($t = 2.05$, $p = 0.06$, $df = 15$) (Fig. 8). And this was reflected when each of the species was considered (PF: $T = 0$, $p = 0.068$, $N = 4$; P: $T = 0$, $p = 0.60$, $N = 6$; B: $T = 0$, $p = 0.35$, $N = 6$).

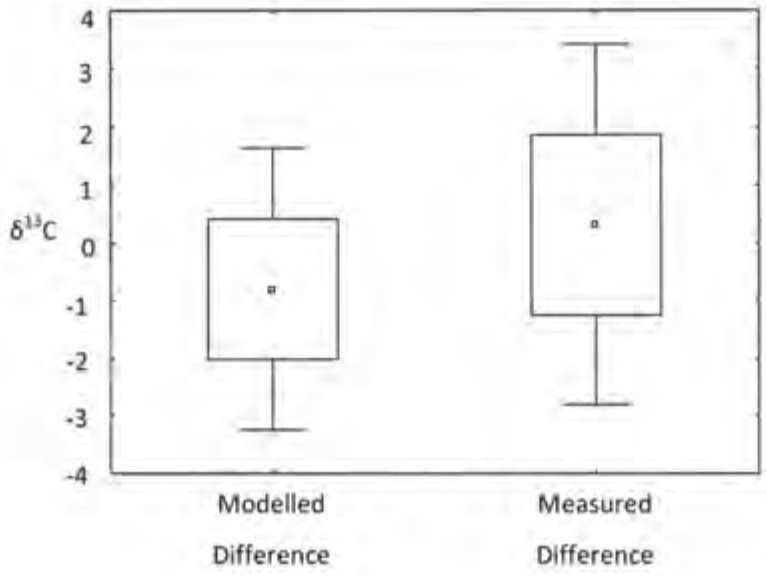

Figure 8: Mean change in isotopic signature between high and low days predicted by the model and measured through isotopic analysis. Central square = mean; box = mean ± standard deviation; whisker = mean ± 2 standard deviations.

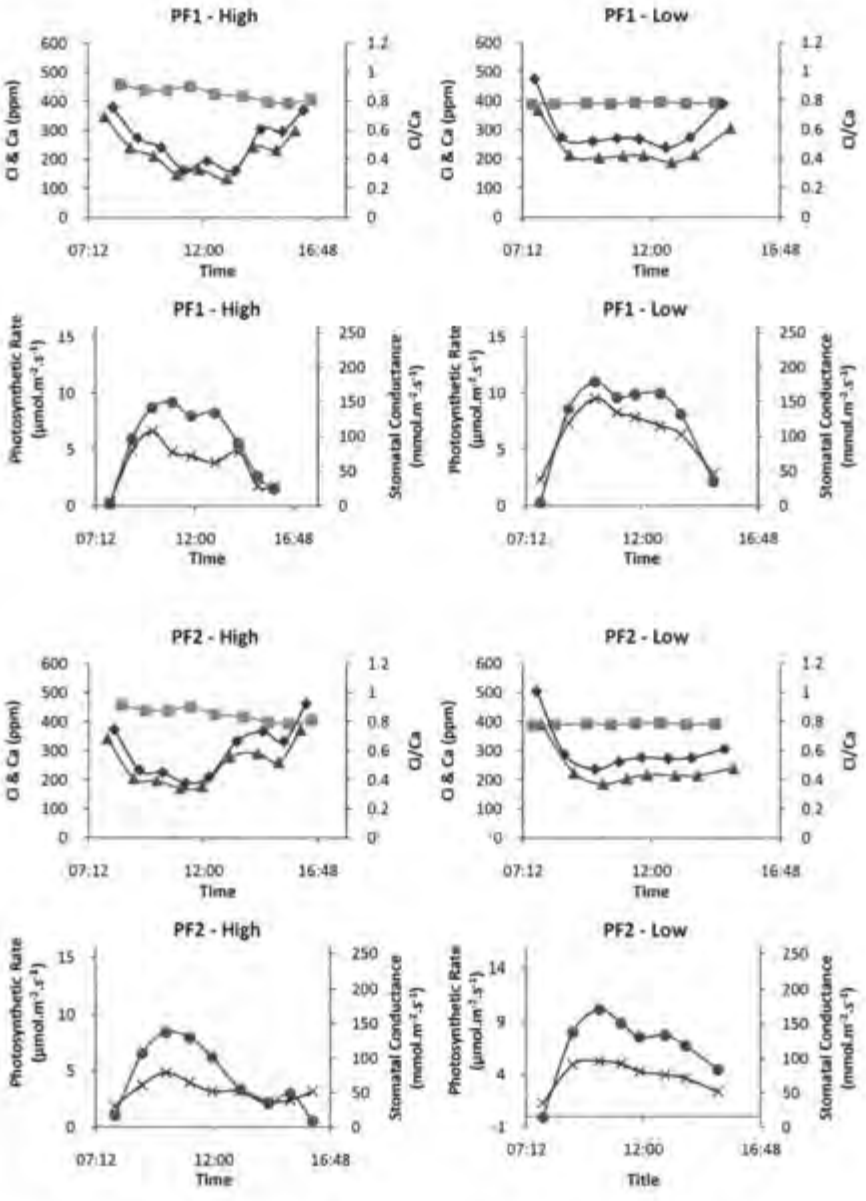

Figure 9: Diurnal trends in C_i, C_a, C_i/C_a, Photosynthetic Assimilation (A) and Stomatal Conductance (g_s) for two *P. falcatus* individuals (PF1 & PF2) on a high traffic day and a low traffic day. Squares – C_a; Triangles – C_i; Diamonds - C_i/C_a; Circles – A and Crosses – g_s.

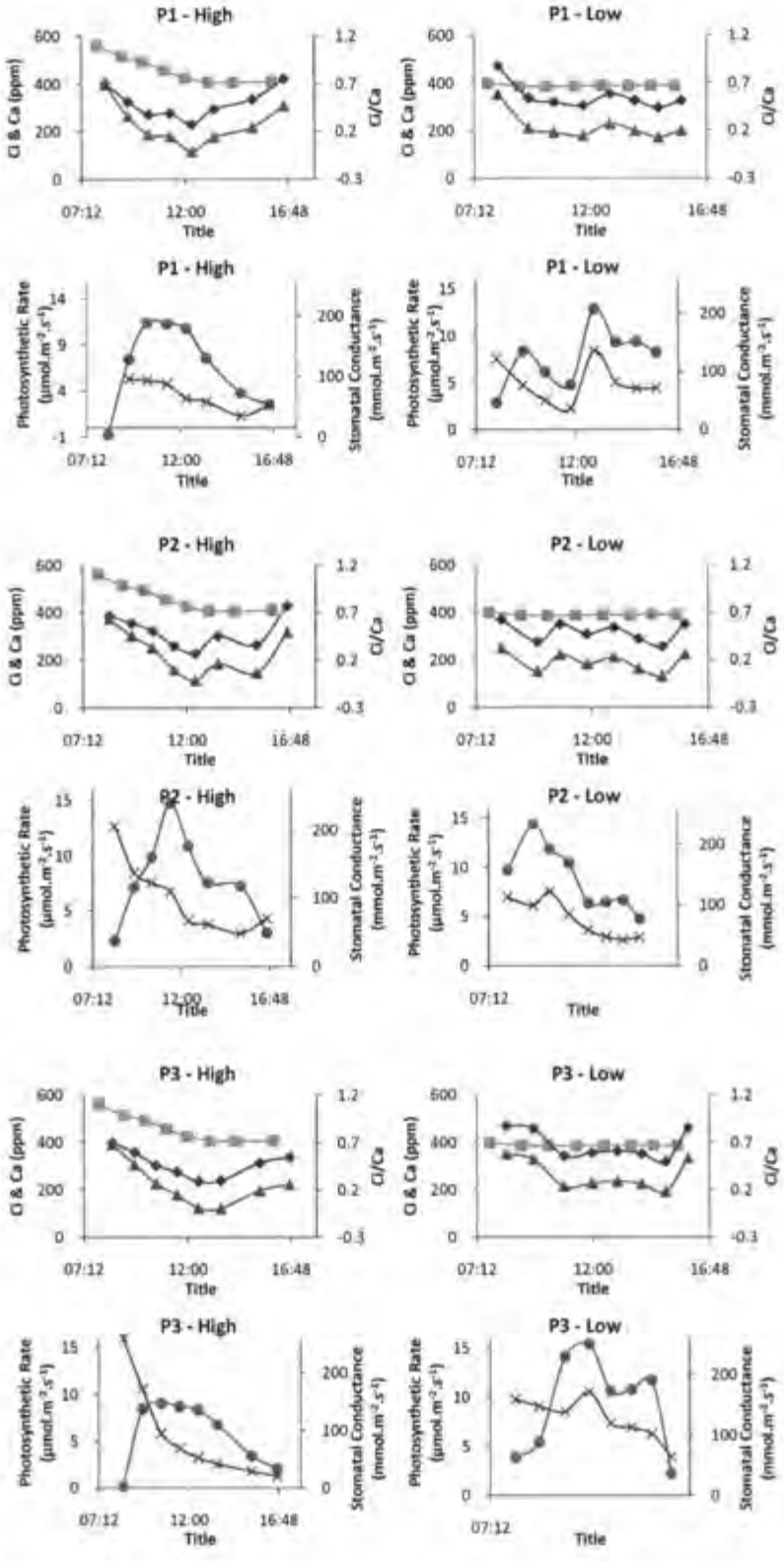

Figure 10: Diurnal trends in C_i, C_a, C_i/C_a, Photosynthetic Assimilation (A) and Stomatal Conductance (g_s) for three *P. pinea* individuals (P1, P2 & P3) on a high traffic and low traffic day. Squares – C_a; Triangles – C_i; Diamonds - C_i/C_a; Circles – A & Crosses – g_s.

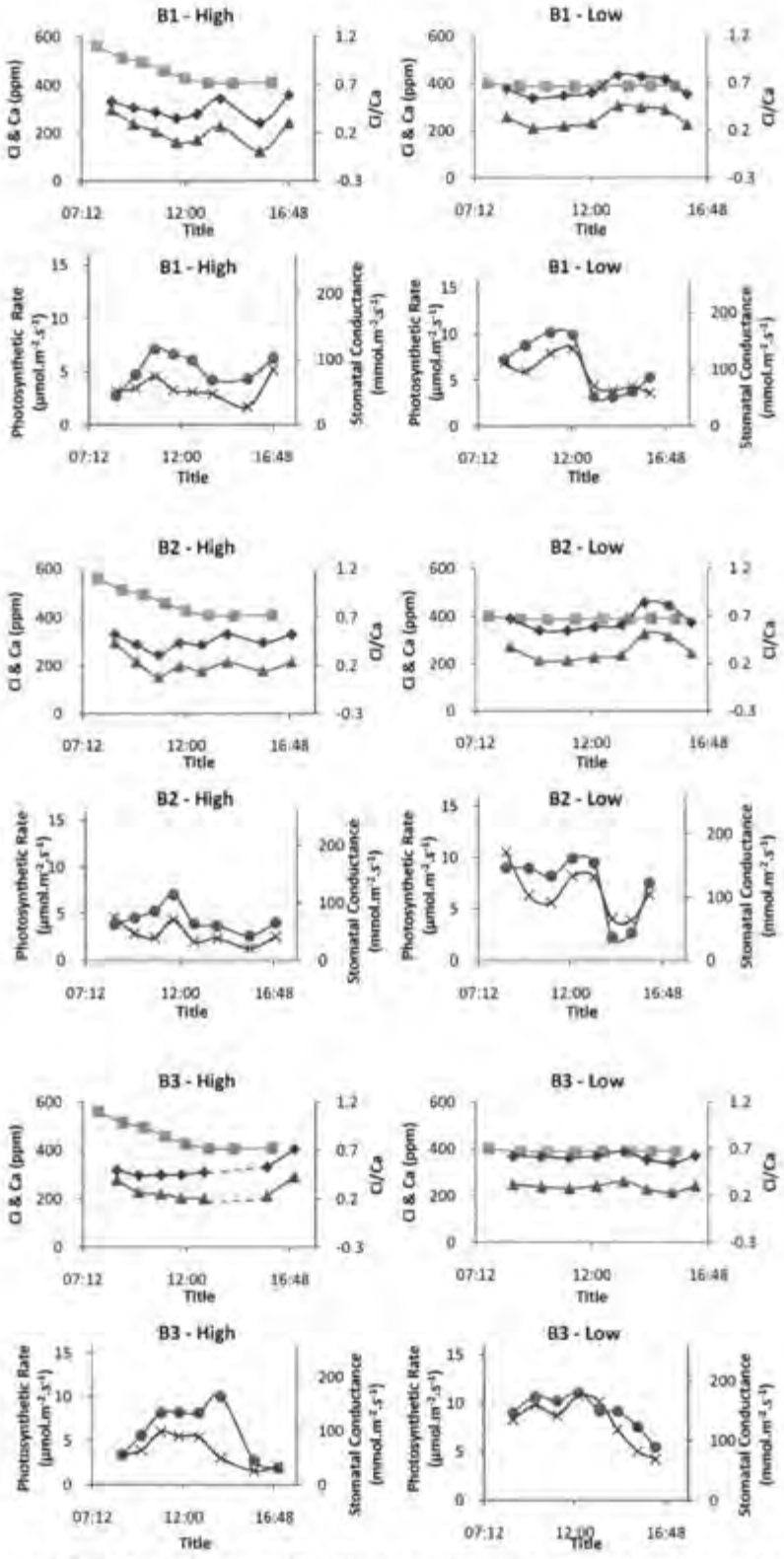

Figure 11: Diurnal trends in C_i, C_a, C_i/C_a, Photosynthetic Assimilation (A) and Conductance (g_s) for three *E. lysistemon* individuals (B1, B2 & B3) on a high traffic day and a low traffic day. Squares – C_a; Triangles – C_i; Diamonds - C_i/C_a; Circle – A & Crosses – g_s.

Table 2: Isotopic signature of atmospheric CO_2 and gas exchange measurements of each plant. Weighted $[CO_2]_{Atmosphere}$ – Photosynthetically weighted CO_2 concentration; $\delta^{13}C_{Atmosphere}$ – Output of Keeling plot from photosynthetically weighted CO_2 concentration; Weighted C_i – Photosynthetically weighted C_i; Photosynthetically weighted C_i/C_a; A – Photosynthetic Rate ($\mu mol_{CO2}.m^{-2}_{leaf\ area}.s^{-1}$) and g_s – Photosynthetically weighted stomatal Conductance ($mmol_{H2O}.m^{-2}_{leaf\ area}.s^{-1}$). Percentage Change from low to high traffic day.

Sample	Weighted $[CO_2]_{Atmosphere}$		$\delta^{13}C_{Atmosphere}$			Weighted C_i		C_i/C_a			A ($\mu mol.m^{-2}.s^{-1}$)			g_s ($mm.m^{-2}.s^{-1}$)		
	High	Low	High	Low	Difference	High	Low	High	Low	Difference	High	Low	% Change	High	Low	% Change
PF1	427	392	-10.07	-8.62	-1.45	190	210	0.48	0.54	-0.06	5.53	7.45	25.7	74.28	123.61	39.9
PF2	431	391	-10.22	-8.62	-1.61	213	211	0.53	0.54	-0.01	4.36	6.67	34.6	58.51	82.55	29.1
P. falcatus	**429**	**391**	**-10.14**	**-8.62**	**-1.53**	**201**	**211**	**0.50**	**0.54**	**-0.03**	**4.95**	**7.06**	**29.9**	**66.40**	**103.08**	**35.6**
P1	455	389	-11.05	-8.52	-2.54	185	207	0.46	0.52	-0.05	6.74	7.71	12.6	45.37	84.55	46.3
P2	464	390	-11.34	-8.53	-2.81	199	189	0.50	0.47	0.02	7.82	8.80	11.1	95.67	85.09	12.4
P3	457	389	-11.13	-8.52	-2.61	197	239	0.49	0.60	-0.10	5.87	9.25	36.5	81.00	132.16	38.7
P. pinea	**459**	**389**	**-11.18**	**-8.52**	**-2.65**	**194**	**212**	**0.48**	**0.53**	**-0.05**	**6.81**	**8.59**	**20.7**	**74.01**	**100.60**	**26.4**
B1	453	389	-10.98	-8.49	-2.49	202	241	0.50	0.60	-0.10	5.27	6.42	17.8	57.99	102.83	43.6
B2	454	389	-11.02	-8.52	-2.50	204	241	0.51	0.60	-0.09	4.35	7.22	39.8	48.09	118.26	59.3
B3	457	389	-11.11	-8.52	-2.58	176	238	0.44	0.59	-0.15	6.06	9.05	33.0	99.22	136.85	27.5
E. lysistemon	**454**	**389**	**-11.04**	**-8.51**	**-2.52**	**194**	**240**	**0.48**	**0.60**	**-0.12**	**5.23**	**7.56**	**30.9**	**68.43**	**119.32**	**42.6**

Table 3: Discrimination – calculated from photosynthetically weighted C_i/C_a; $\delta^{13}C_{plant}$ – calculated from discrimination model; $\delta^{13}C_{soluble\ carbohydrates}$ - measured; Difference = $\delta^{13}C_{plant} - \delta^{13}C_{soluble\ carbohydrates}$. Agreement between modelled and measured values: X = No, ✓ = Yes; O = unclear.

Sample	Discrimination			$\delta^{13}C_{plant}$			$\delta^{13}C_{soluble\ carbohydrate}$			Difference		Agreement?
	High	Low	Difference	High	Low	Difference	High	Low	Difference	High	Low	
PF1	15.14	16.55	-1.41	-24.8	-24.8	-0.1	-25.7	-28.0	2.4	0.86	3.28	X
PF2	16.42	16.57	-0.14	-26.2	-24.8	-1.4	-28.0	-26.2	-1.9	1.82	1.40	✓
P. falcatus	**15.78**	**16.56**	**-0.78**	**-25.5**	**-24.8**	**-0.8**	**-26.9**	**-27.1**	**0.2**	**1.34**	**2.34**	**O**
P1	14.88	16.12	-1.24	-25.6	-24.2	-1.3	-25.3	-27.3	2.0	-0.28	3.03	X
P2	15.63	15.11	0.52	-26.6	-23.3	-3.3	-26.2	-26.4	0.2	-0.35	3.15	X
P3	15.54	17.88	-2.34	-26.3	-25.9	-0.3	-25.6	-26.3	0.7	-0.65	0.35	O
P. pinea	**15.35**	**16.37**	**-1.02**	**-26.1**	**-24.5**	**-1.6**	**-25.7**	**-26.7**	**1.0**	**-0.43**	**2.18**	**X**
B1	15.80	18.03	-2.24	-26.4	-26.1	-0.3	-26.7	-25.2	-1.5	0.33	-0.81	O
B2	15.93	18.02	-2.09	-26.5	-26.1	-0.5	-25.6	-26.8	1.2	-0.95	0.72	X
B3	14.35	17.83	-3.48	-25.1	-25.9	0.8	-25.8	-25.1	-0.7	0.71	-0.80	O
E. lysistemon	**15.36**	**17.96**	**-2.60**	**-26.0**	**-26.0**	**0.0**	**-26.0**	**-25.7**	**-0.3**	**0.03**	**-0.30**	**✓**

When comparing the two forms of the model, the simple model (Eq. 2) and the expanded model (Eq. 9 & 10) the importance of directly including internal conductance (g_i) and photorespiration becomes clear. The simple model, which assumes a constant g_i does not accurately model C_i/C_a when the g_i is low but as it increases the model predicted C_i/C_a more accurately (Table 4). The results for C_i/C_a using the expanded model (Table 5) indicate the same trend of increasing accuracy with increasing g_i. The relative accuracy of the predictions made by the expanded model was greater.

Table 4: Results from simple model indicating how calculated C_i/C_a values can vary when the internal conductance is not considered. Actual C_i/C_a values calculated from measurements and back-calculated C_i/C_a values derived from isotope measurements using the simple discrimination model (Eq. 9 & 10). Calculations were based on the assumptions of constant photosynthetic assimilation (6 $\mu mol.m^{-2}.s^{-1}$), boundary layer conductance (1.5 $mol.m^{-2}.s^{-1}$), leaf temperature (30°C) and light compensation point (30 $\mu mol.mol^{-1}$) (Modified from Seibt et al. 2008).

g_i ($mol.m^{-2}.s^{-1}$)	C_a ($\mu mol.mol^{-1}$)	C_s ($\mu mol.mol^{-1}$)	C_i ($\mu mol.mol^{-1}$)	C_c ($\mu mol.mol^{-1}$)	Actual C_i/C_a	Δ	Back-calculated C_i/C_a	C_i
0.1	370	366	260	200	0.7	17.2	0.56	209
0.2	370	366	260	230	0.7	19.4	0.67	246
0.4	370	366	260	245	0.7	20.6	0.72	265

Table 5: Results from expanded model indicating how the back-calculated C_i/C_a values are closer to the actual values than those of the simplified model.

g_i ($mol.m^{-2}.s^{-1}$)	C_a ($\mu mol.mol^{-1}$)	C_s ($\mu mol.mol^{-1}$)	C_i ($\mu mol.mol^{-1}$)	C_c ($\mu mol.mol^{-1}$)	Actual C_i/C_a	Δ	Back-calculated C_i/C_a	C_i
0.1	370	366	260	200	0.7	16.63	0.66	243
0.2	370	366	260	230	0.7	18.83	0.68	250
0.4	370	366	260	245	0.7	19.94	0.69	255

Discussion

Is there a significant roadside build-up of CO_2 in Cape Town and can it be attributed to increased fossil fuel emissions?

A significant difference was found in the number of cars passing the sites (Fig. 4) as well as a significant difference in atmospheric CO_2 concentrations (Fig. 5) on high and low traffic days. This was an indication that higher traffic pressures increased the atmospheric CO_2 concentration.

The collected air samples produced a Keeling Plot with a strong predictive power. This allowed us to determine the $\delta^{13}C$ of the source ($\delta^{13}C_{source}$ = -26.078‰). The $\delta^{13}C_{source}$ indicated a strong fossil fuel influence on the atmosphere particularly from petrol which had an isotopic signature almost identical to the source air ($\delta^{13}C_{petrol}$ = -26.08‰). This supported the suggestion that fossil fuels strongly influence the atmosphere's isotopic signal. Further support was gained from the $\delta^{13}C_{source}$ resembling the typical fossil fuel isotopic signatures of other studies (Table 1).

When using the Keeling Plot method the scatter and range of data must be considered since the method requires extrapolation beyond the range of measured data in order to obtain the $\delta^{13}C_{source}$ from the intercept. If there is too much scatter and/or insufficient range within the data, small uncertainties in the regression slope to lead to large uncertainties in the accuracy of the intercept (Tans 1998 in Pataki et al. 2003b). However this uncertainty can be reduced by having a range of CO_2 concentrations greater than 75ppm (Pataki et al. 2003b). The Keeling Plot produced during this study had a strong predictive power (R^2 = 0.98), little scatter in the data and a large range of CO_2 concentrations (195ppm). Thus the intercept value was a good representation of the $\delta^{13}C_{source}$.

The significant difference in isotopic signature between high and low traffic days suggested that it should be possible to trace the signal into the plant's biomass. Brugnoli et al. (1988) estimated that between 80 and 90% of recently fixed assimilate is accounted for in the sucrose and starch fraction of the plant. The soluble carbohydrates were therefore chosen as the plant material to analyse isotopically as they would have been produced with the CO_2 taken up on the day that the gas exchange measurements were performed. Since the

isotopic signatures of the high traffic day atmospheric samples were more negative it was expected that the isotopic signature of the soluble carbohydrates would be more negative when sampled at the end of a high traffic day.

Can the effects of elevated anthropogenic CO_2 emissions be detected in the daily isotopic signals of soluble carbohydrates of roadside plants in Cape Town?

Farquhar et al. (1989) noted that to distinguish variations in the $\delta^{13}C_{source}$ from the effects of plant metabolic processes it is necessary to translate the $\delta^{13}C$ of plant organic matter into photosynthetic ^{13}C discrimination (Δ). There is a substantial amount of variation in leaf discrimination that occurs not only between different species but also among the individuals of a population. A large proportion of this variation is genetically based (Ehleringer et al., 1993) but environmental influences have also been attributed with some of the variation. This potential source of complication led to the requirement that the plants studied were not water stressed, which was confirmed by the xylem pressure potential measurements.

It was necessary to consider how C_i changed as well as the physiological responses of the plant between the two days to understand the differences in discrimination values, since discrimination is a reflection of the influence of environmental conditions on stomatal behaviour (Comstock & Ehleringer 1992). Numerous FACE experiments have found that A increased with elevated CO_2 concentrations because of increased carboxylation rates and competitive inhibition of oxygenation. The studies found that g_s decreased because the stomata responded to increased CO_2 concentrations by decreasing the aperture size (Ainsworth & Rogers 2007). C_i/C_a values in turn, responded to elevated CO_2 concentrations however the direction of response varied because some species have the potential of maintaining a constant C_i/C_a while it varies in other species (Ehleringer & Cerling 1995).

The results of the isotopic analysis indicated that the plants were not simply influenced by the isotopic CO_2 source since only *E. lysistemon* had a more negative $\delta^{13}C$ on the high traffic day. Although the difference between the two days was within the limit of error associated with the measurements. *P. falcatus* and *P. pinea* both indicated trends of more negative $\delta^{13}C$ values on low traffic days although the *P. falcatus* trend was also within the limit of error associated with the measurements. These results indicated that the isotopic

signatures of the plants were not simply records of the source's isotopic signature but were being influenced by the physiological responses of the plant.

Farquhar et al. (1989) noted that their model suggested that if C_i increased at the same rate as C_a (i.e. $C_a - C_i$ = constant) then the $\delta^{13}C$ would become more negative as a result of increased discrimination. Saurer et al. (2004) studied century long tree-ring records of *Larix*, *Pinus* and *Pinea* growing at 26 high-latitude sites. They found that when the C_i increased less but in a proportional manner (i.e. C_i/C_a = Constant), discrimination would remain the same and in turn so would the isotopic signature. Martin et al. (1988) exposed woody and herbaceous plants to a range of air pollutants. The woody plants experienced smaller effects on their $\delta^{13}C$ but when there was an effect, the isotopic signals usually became less negative. The results were attributed to decreased stomatal conductance under elevated CO_2 which caused a decrease in C_i (i.e. C_i/C_a = decrease). Therefore the physiological responses had more of an effect on the isotopic signature of the plant than did the isotopic signature of the source air.

The C_i values were slightly, but significantly, lower on high traffic days which may have been an indication that C_i values were being adjusted to the changes in C_a. The physiological responses provided a clearer explanation for the observed C_i/C_a changes. The percentage decrease in g_s from low to high traffic days was greater than that of A in each of the plants. This essentially led to a decreased supply and only a slight decrease in demand. This led to a lower CO_2 concentration in the sub-stomatal cavity on high traffic days. Therefore the discrimination and thus the $\delta^{13}C$ of the plant were being influenced not only by the source of CO_2 but also by the physiological responses to elevated CO_2 concentrations. The decrease in g_s was expected and supported by findings of FACE experiments while the decrease in A was unexpected. von Caemmerer & Quick (2000) found that as CO_2 concentration increased, the rate of carbon fixation increased, this led to an increased demand for ATP required for RubP regeneration. Photosynthesis therefore would no longer be Rubisco limited but rather be limited by the capacity to regenerate RubP. Ainsworth & Rogers (2007) suggested that this response was more important in the short term as long term responses have been shown as an acclimation of decreased Rubisco content and activity. The high CO_2 concentrations experienced by the plants, in Cape Town, were only short term pulses therefore it could be speculated that they had not acclimated to increased

CO_2 concentrations but rather were experiencing short term responses during each pulse of elevated CO_2. The response of becoming RubP regeneration limited implied that although A would be stimulated by increased carboxylation rates and competitive inhibition, it would be slightly slowed by the RubP regeneration limitation if ATP could not be produced fast enough (Davis 1987, Moore et al. 1999). Thus if the plants were experiencing short-term responses then it may be an explanation for the decrease in A seen in the results of this study.

Modelled vs Measured Isotopes

The modelled and measured $\delta^{13}C$ values of *E. lysistemon* were within the error limits suggesting that they could be considered to be the same. However, the $\delta^{13}C$ values for *P. falcatus* and *P. pinea* did not agree in direction of change.

This uncertainty and lack of predictive power of the model could be attributed to the uncertainty of the response of C_i/C_a to elevated CO_2 concentrations. This uncertainty was seen in the study by Saurer et al. (2004) who presented three plausible scenarios of C_i/C_a responses (discussed above). Of the three scenarios their results favoured the scenario of a constant C_i/C_a. A study by Feng et al. (1998) found that C_i/C_a ratios increased in some species, decreased in others and even remained constant in others. They also found that in most species C_i increased monotonically over the last 200 years although it did remain constant in some. Ehleringer & Cerling (1995) found a decrease in the C_i/C of *Prosopis alba* the last 100 years while the C_i remained constant. Polley et al. (1993) found that C_i/C_a remained constant in oats and mustard but increased by 0.03 in wheat. The variability of results in these studies as well as the current study indicate that predicting the change in $\delta^{13}C$ of a plant between high traffic and low traffic days with this model (Eq. 1 & 2) is more complex than simply using the C_i/C_a values to follow the source CO_2 into the plant. This complexity was due to the plants' isotopic signatures not being solely influenced by the $\delta^{13}C_{source}$ but also by the plants' physiological responses to changing atmospheric CO_2 concentrations. And the effect of the plants' physiological responses was not fully captured by the changes in C_i/C_a.

Expanded Discrimination Model

When comparing the calculated values of C_i/C_a from the simple model and the expanded model the importance of including the effects of internal conductance and photorespiration becomes apparent. The differences between the measured and simple model values were much greater than the differences between the measured and expanded model values. Interestingly the same trend of increasing accuracy with increasing internal conductance was seen in the results of both models.

The manner in which the effect of internal conductance in included in the models gives an indication as to why this difference occurs. The simple model implicitly includes the effect of internal conductance in the value of b (Eq. 2). This leaves the model vulnerable to over- or under-estimating the contribution of g_i which will cause a mismatch between the calculated and actual C_i/C_a values (Seibt et al. 2008). The expanded model, however explicitly includes a fractionation term for internal conductance (a_m).

Results in Tables 4 and 5, in combination with the results of this study indicate that not directly including internal conductance could lead to inaccurate results from the models, whether working with a known C_i/C_a to determine the isotopic signature or using the isotopic signature to determine the C_i/C_a value. This leaves the question of whether the results of previous studies that have used the linear model to interpret long-term isotopic tree-ring records are reliable or whether they have excluded a variable that will fundamentally alter the results and interpretation thereof.

Conclusion

The increase in roadside CO_2 concentrations can be attributed to increased anthropogenic fossil fuel emissions by cars. The isotopic signature of these anthropogenic fossil fuels cannot simply be traced into plants because of the complicated nature of physiological responses to elevated CO_2 concentrations. The linear model proposed by Farquhar et al. (1989) was not a good predictor of trends in isotopic signatures between high and low traffic days. Previous studies have shown that it was not a good long-term predictor as C_i/C_a responses are too variable with changing CO_2 concentrations and environmental conditions. If one employs the expanded model suggested by Seibt et al. (2004), which included the

effects of internal conductance and photorespiration, the results appear more reliable (Table 5). Therefore if isotopes are used to determine C_i/C_a values of past or future climates it is important to consider which model is appropriate in terms of accuracy, likely errors within the results as well as practicality of measuring the variables.

This study has highlighted the complexity of the relationship between isotopic signatures of plants and their isotopic signatures. This relationship is not simply defined on what the plant was exposed to but rather a combination of what it was exposed to and how it reacted to environmental changes. The relationship between these two variables is important to understand as it has many practical implications which would allow the scientific world a good opportunity to study past climatic conditions and how plants responded to those climatic conditions as well as how plants are likely to respond to future climate scenarios.

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