



Leaf wax *n*-alkane distributions in arid zone South African flora: Environmental controls, chemotaxonomy and palaeoecological implications



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ABSTRACT

The environmental controls on leaf wax *n*-alkane distributions and associated interpretation of such distributions in geological archives have long remained rather enigmatic. Studies of contemporary vegetation often conflate changing environmental conditions and species differences between biomes, making it difficult to assess the extent to which variation is driven by plant adaptation to prevailing environmental conditions and/or more fixed chemotaxonomic patterns. We present a case study of arid and semi-arid regions of South Africa that considers these issues. We anticipate that such conditions may impart strong controls upon leaf wax synthesis.

Leaf wax *n*-alkane data from 215 plants and 93 soils from the Succulent Karoo and Fynbos biomes of South Africa revealed yield and distributions to be highly variable. While many plants exhibited concentrations comparable with previous reports, several succulent plants produced *n*-alkane yield up to 100× the modal *n*-alkane concentration for their biome. The data demonstrate that, on average, leaf wax *n*-alkane distributions in the Succulent Karoo are different from those of the Fynbos biome, with the former associated with longer maximum chain length, less dispersed distributions and stronger odd numbered chain length preference. The patterns were closely mirrored in the soils. Average chain length was weakly correlated with climate and this is interpreted to be a function of multiple factors. We hypothesise that a key control is the shifting proportion of plant functional type between biomes, with a greater abundance of succulent growth forms in the more arid Succulent Karoo (which tended to produce longer and less dispersed *n*-alkane chain length distributions), differentiating this biome from the Fynbos, in which woody shrubs are more dominant. Inter-biome variability was also apparent, with some same taxonomic or functional groups common to both biomes tending to produce longer chain length *n*-alkane distributions under more arid conditions. There was, however, considerable individual plant variability and we observed both insensitivity to environmental conditions (i.e. consistent *n*-alkane distributions) and marked variability in chain length distributions.

A key finding is the high yield of *n*-alkanes from multiple succulent plants, many of which use the CAM photosynthetic pathway. Compound specific $\delta^{13}\text{C}/\delta\text{D}$ analyses (e.g. estimates of C_3 and C_4 biomass) for regions with potential succulent plant contributions should therefore be carried out cautiously.

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1. Introduction

Leaf waxes comprise complex mixtures of fatty acids, *n*-alkanes (C_{21} – C_{35}), *n*-alkanols (C_{20} – C_{32}), aldehydes and wax esters, which

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form a protective barrier around plant leaves (Jenks and Ashworth, 1999). They are hypothesised to serve various specific functions, including internal water retention, external water repellence and protection from UV radiation and pathogens (Jenks and Ashworth, 1999; Shepherd and Griffiths, 2006). Wax abraded or ablated to the atmosphere is replaced throughout a plant's life (Conte et al., 2003), potentially very quickly (Gao et al., 2012). As a result, waxes

are widely distributed in the environment and are commonly a component of atmospheric aerosols (Peltzer and Gagosian, 1989; Conte and Weber, 2002).

Leaf waxes are preserved in a range of sedimentary and geological contexts, and *n*-alkanes have attracted particular interest for palaeoenvironmental research as they are both relatively recalcitrant in the sedimentary context (i.e. high preservation potential) and amenable to compound specific stable carbon and hydrogen isotope analysis (e.g. Feakins et al., 2005; Schefuß et al., 2005; Sachse et al., 2012). Such data have been used to infer past changes in the abundance of plants using the C₃ and C₄ pathways (Collister et al., 1994; Boom et al., 2002) or to assess palaeo-hydrological change (e.g. Niedermeyer et al., 2010). Various studies have considered spatial, temporal and botanical patterns in the distributions of *n*-alkane homologues (i.e. the relative proportions of the different *n*-alkane homologues, usually C₂₁–C₃₅). Such patterns have also been considered in sedimentary contexts and used to infer palaeoenvironmental or palaeoecological change (inter alia, Poynter et al., 1989; Ficken et al., 2000; Schwark et al., 2002; Zhang et al., 2006; Bai et al., 2008).

Given the functions of leaf waxes, it is reasonable to assume that their synthesis is, at least to some extent, regulated and optimised by plants (Conte et al., 2003; Shepherd and Griffiths, 2006). However, the underlying significance of changes in *n*-alkane distributions is often not simple to interpret (Dodd and Afzal-Rafii, 2000; Bush and McInerney, 2013). In particular, the question of whether or not leaf wax distributions provide chemotaxonomic information has long been debated and there have been numerous attempts to link carbon chain length distributions to specific plant species, genera or plant functional type (Maffei, 1996), as well as different photosynthetic pathways (e.g. Rommerskirchen et al., 2006a). However, it remains rather unclear to what extent changes in leaf wax composition within palaeoenvironmental archives can be interpreted in terms of shifts in the relative proportions of different plant functional types or plant species and/or the response (i.e. adjustment in leaf wax composition) of a plant community to environmental conditions. For example, distinct shifts in *n*-alkane and *n*-alkanol chain length are seen in north–south transects in southwest Africa. They are associated with the transition from tropical rainforest to savannah (Vogts et al., 2009) and the pattern is mirrored in wax components transported offshore to the southeast Atlantic (Rommerskirchen et al., 2003; Vogts et al., 2012). However, resolving the processes driving such patterns, and thus the (palaeo)environmental significance of records obtained from the same area (e.g. Rommerskirchen et al., 2006b; Maslin et al., 2012), is difficult because the patterns in modern atmospheric aerosols conflate changing environmental conditions and changing plant species/functional type. A recent meta-analysis of *n*-alkane distributions from > 2000 specimens highlighted the great variability in leaf wax *n*-alkane production and distribution, suggesting that there is a need for caution when interpreting leaf wax distributions (Bush and McInerney, 2013). Comparative data concerning leaf wax concentration/production are less often reported (Bush and McInerney, 2013), but the importance of the issue for the interpretation of sedimentary archives has recently been demonstrated (Diefendorf et al., 2011).

Here we have built on such analyses to present a detailed case study that tracks patterns in leaf wax distribution within and between two arid zone biomes in South Africa. It was anticipated that, in arid environments, where stomatal conductance is low for a greater proportion of the time, water loss through the cuticle would be of increased significance (Shepherd and Griffiths, 2006). Hence, there would be environmental pressure for plants to optimise their wax composition (e.g. Macková et al., 2013). Drought adaptation is of further interest as the Succulent Karoo is renowned for an abundance of stem and leaf succulents, many of

which utilise the crassulacean acid metabolism (CAM) pathway (Mooney et al., 1977; Rundel et al., 1999). Leaf wax distributions from CAM plants (which include > 10,000 angiosperm species and ca. 7% of vascular plants) have received relatively little study (cf. Maffei et al., 1997; Feakins and Sessions, 2010; Boom et al., 2014). Many terrestrial CAM plants are drought-adapted and have thick, waxy cuticles that potentially yield high leaf wax concentration (Eglinton et al., 1962; Maffei et al., 1997). In large parts of the Succulent Karoo, they are the dominant growth form/functional type and their contribution to, and influence on, various marine sedimentary archives in the region (e.g. Rommerskirchen et al., 2006a; Maslin et al., 2012), as well as emerging terrestrial palaeoenvironmental archives (Carr et al., 2010; Chase et al., 2012), is poorly defined.

We also sought to address a “scale gap” that exists between the analysis of individual plants (e.g. Maffei, 1996; Vogts et al., 2009; Bush and McInerney, 2013) and the integrated signals that characterise atmospheric aerosols (e.g. Huang et al., 2000; Vogts et al., 2012), lake basin sediments (Castañeda et al., 2009) and marine archives (Schefuß et al., 2005). This was achieved via the combined analysis of plant specimens and local soils sampled across the study area. There have been relatively few studies that directly link data from plants and associated soils (cf. Otto and Simpson, 2005), but soils are a potentially significant store and source of (re-worked) wax, both to atmospheric aerosols (e.g. Poynter et al., 1989) – particularly in desert regions – and via runoff within catchments (Schefuß et al., 2005). This step is thus pertinent for the use of leaf wax component distributions in palaeoenvironmental reconstruction, not only in terms of understanding and assessing the influence of post-depositional diagenesis (e.g. Buggle et al., 2010), but also in terms of understanding the effect of differences in leaf wax production between plant species, genera or plant functional type.

We present a data set comprising 215 samples of modern plants and 93 soils from the Succulent Karoo and Fynbos biomes of southwest South Africa (Fig. 1). The environmental (climatic and edaphic) gradients in the region, as well as the occurrence of several cosmopolitan plant species throughout the study area allowed us to consider both biome-scale differences in leaf wax *n*-alkane distributions and some chemotaxonomic responses. Overall, the aims were to:

- (i) Characterise the *n*-alkane leaf wax yield/concentration for contemporary plants and soils across the dryland biomes of South Africa.
- (ii) Assess whether the major biomes/associated eco-regions/ plant functional type in the sampling area produce characteristic *n*-alkane leaf wax concentrations and distributions.
- (iii) Assess the extent to which comparable plant leaf wax signatures are preserved within the extractable lipids of the region's soils.
- (iv) Assess the significance of the results for application of leaf wax distributions as palaeoenvironmental proxies in southern Africa and for the interpretation of compound-specific stable isotope analysis.

2. Material and methods

2.1. Study area

The study area encompasses the Fynbos (part of the Cape Floristic Region; CFR) and Succulent Karoo biomes of South Africa (Fig. 1). Fynbos is a Mediterranean-type shrubland occupying ca. 90,000 km² at the southwest tip of South Africa. It exhibits some of the highest levels of plant diversity and endemism in the world (Goldblatt and Manning, 2002) and is characterised by

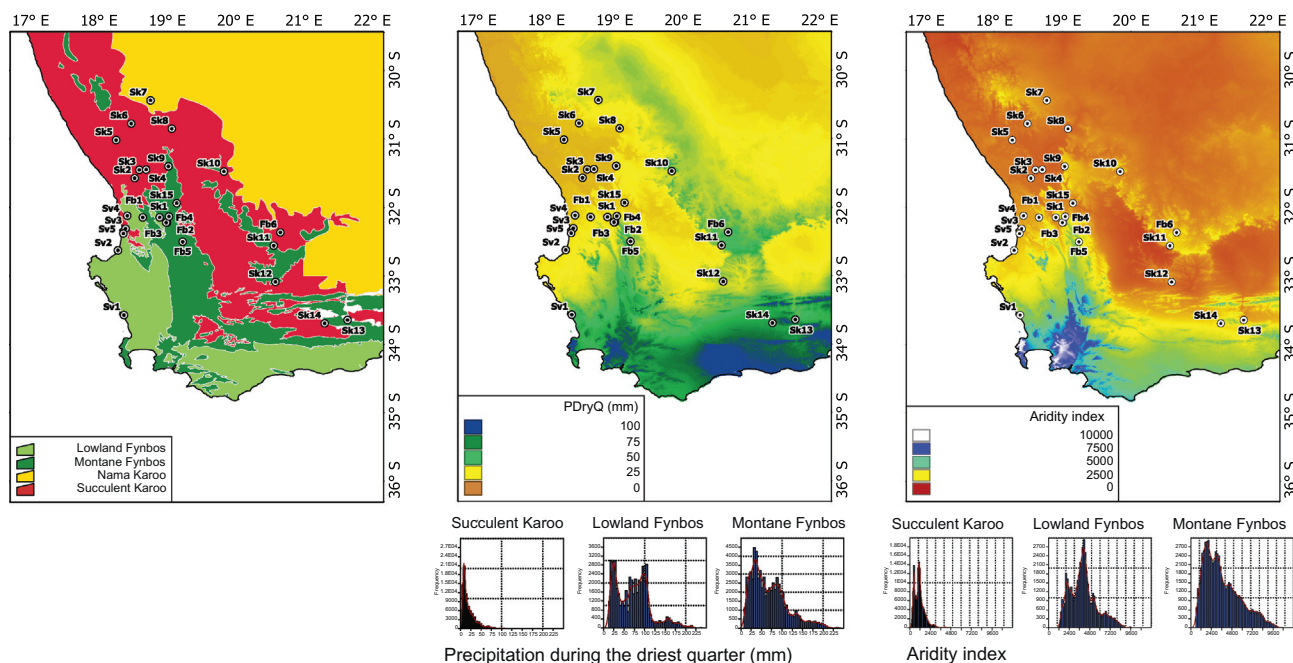


Fig. 1. Distribution of biomes and major eco-regions in south west South Africa, along with sample site locations (upper diagram). The field codes for the sites relate to the different biomes/eco-regions and are as follows: SK, Succulent Karoo; FB, Montane Fynbos and SV, Lowland Fynbos (often referred to locally as “Sandveld”). The lower two diagrams provide contextual information on climatic conditions across the study area, specifically, precipitation of the driest quarter and aridity index (after [Trabucco and Zomer, 2009](#)). Note the broader (and overlapping) distributions of climatic conditions in the two Fynbos eco-regions compared with the Succulent Karoo, which occupies a much narrower range of climatic conditions. The Montane Fynbos sites represent the relatively drier end of spectrum of conditions under which this eco-region is found.

sclerophyllous proteoid shrubs, small-leaved ericoid shrubs (dominantly from the Ericaceae family), Cape reeds (Restionaceae) and a diverse array of geophytes from the Liliaceae and Iridaceae families ([Cowling et al., 1997](#)). The vegetation is for the most part structurally uniform and characterised by an absence of trees. It is adapted to summer drought, frequent burning and low nutrient soil ([Cowling and Holmes, 1992](#)). Fynbos is broadly divided into two major eco-regions: Lowland Fynbos, found on the coastal plains (often locally referred to as “Sandveld”), and Montane Fynbos, associated with the uplands of the Cape Fold Belt Mountains ([Campbell and Werger, 1988](#)). Mean annual rainfall in the Fynbos Biome is variable and is strongly influenced by the topography of the Cape Fold Belt Mountains ([Fig. 1](#)). At the southern limit of the study area (Lowland Fynbos) mean annual rainfall is ca. 480 mm/yr, >95% of which falls during the austral winter. As such, apart from some CAM species, all Fynbos plants, including grass, use the C_3 pathway ([Vogel et al., 1978](#)).

The Succulent Karoo occupies > 100,000 km² ([Cowling and Hilton-Taylor, 1999](#)). Its vegetation is also distinguished by the limited occurrence of trees and large shrubs, and it experiences a winter rainfall climate regime. As a result, it shares some affinity with the Fynbos Biome ([Born et al., 2006](#)). Summer aridity – and therefore growing season length – is, however, a key environmental factor (along with geological substrate) differentiating these biomes, with the Succulent Karoo generally being more arid and experiencing higher summer temperature ([Cowling et al., 1997](#)). At the northern limit, mean annual rainfall is ca. 160 mm/yr and rainfall seasonality is markedly reduced (ca. 50% during austral winter; [Fig. 1](#)). Rainfall is, however, unusually reliable for a desert ecosystem ([Cowling et al., 1999](#)). Mean annual temperature ranges between 14 and 19 °C, with summer temperature ranging between 27 and 34 °C ([Hijmans et al., 2005](#)). As a result of these relatively arid conditions, there is a greater occurrence of drought-adapted species and the Succulent Karoo is renowned for a high concentration of leaf succulents and dwarf shrubs from the Aizoaceae, Crasulaceae and Euphorbiaceae families ([Milton et al., 1997](#); [Cowling](#)

and [Hilton-Taylor, 1999](#)). Many use CAM photosynthesis ([Rundel et al., 1999](#)) and are characterised by other (some relatively unusual) drought adaptations, such as a predominance of dwarf succulence and shallow rooting systems ([Cowling et al., 1999](#)), along with more common drought adaptations such as thick, waxy cuticles.

2.2. Field sampling

Sampling was carried out in April 2010 and September 2011 ([Supplementary material, Tables S1 and S2](#)). Sample plots were placed across the north–south climate gradient in the Fynbos/southern Succulent Karoo biomes as well as across an east–west gradient from the coast, across the Cape Fold Belt Mountains into their rain shadow and the Fynbos/Succulent Karoo ecotone ([Fig. 1](#)). All material was obtained from detailed 10 × 10 m vegetation survey plots. Small samples of fresh leaf or photosynthetic material (e.g. stem succulents) were obtained from each plant within the survey plot and were identified with reference to herbarium material at the University of Cape Town. Assignment to genus and species was not always possible, particularly for some families (notably Aizoaceae) and for specimens for which flower material could not be found in the field. All plants were, however, further classified into five plant functional types. Soil samples (200–400 g) were obtained from the upper 10–15 cm of the soil (i.e. the A horizon, with the litter layer first removed) from 4 fixed locations within each plot (see [Carr et al., 2013](#) for further details). It was anticipated that the leaf wax preserved within the soils [specifically the soil organic matter (SOM), not fresh leaf litter] would provide an opportunity to consider the extent that survey plot/eco-region/biome-averaged plant leaf wax *n*-alkane signatures are represented in local SOM. It was hypothesised that the soil samples would integrate larger spatial and temporal scales and therefore be less susceptible to potential sampling bias caused by annual/seasonal variation in wax production.

2.3. Sample preparation and extraction

All samples were stored in paper bags and immediately air dried at the University of Cape Town prior to shipping to the UK, whereupon they were freeze-dried. In total, from 26 survey plots across the study area, 133 plants from the Succulent Karoo, 54 plants from the Montane Fynbos eco-region and 28 plants from the Lowland Fynbos eco-region, along with 102 soil samples from across the whole study region were analysed (Fig. 1). Sample preparation and analysis were conducted at the University of Leicester Environmental Stable Isotope Laboratory. In most cases plant leaves were removed for analysis, with stem material and/or flowers excluded. Exceptions included stem succulents such as *Euphorbia mauritanica* and some grass samples where whole specimens had to be processed in order to obtain sufficient leaf wax yield. Sub-samples of the air-dried soils were sieved at 2 mm to remove clasts and leaf litter/roots, freeze dried and homogenised. Ca. 0.5 g powdered leaf material and ca. 25 g soil, along with 5 α -cholestane as a standard were subject to Soxhlet extraction [24 h; in hexane/CH₂Cl₂/MeOH, 1:2:2). Each extract was rotary evaporated and purified over a Na₂SO₄ column. The apolar fraction was obtained via Al₂O₃ column chromatography, eluted using hexane/CH₂Cl₂ (9:1).

2.4. Gas chromatography-mass spectrometry (GC-MS)

GC was carried out with a Perkin Elmer Clarus 500 GC-MS instrument equipped with a CP-Sil 5CB-MS (30 m \times 0.25 mm \times 0.25 μ m) column. The GC oven programme was: 60 $^{\circ}$ C to 120 $^{\circ}$ C at 20 $^{\circ}$ C/min, then to 310 $^{\circ}$ C (held 15 min) at 4 $^{\circ}$ C/min. Components were assigned on the basis of mass spectra and retention times. Retention times were confirmed with an authenticated standard. Absolute concentrations were determined in Turbomass 5.2.0 using the peak areas of the *n*-alkanes and that of the internal standard. Response factors for the standard and each *n*-alkane homologue were determined using an authenticated standard (C₇–C₄₀). Linearity between peak area and concentration was checked for all *n*-alkanes in the concentration range 0–500 μ g/mg.

2.5. Data analysis

Using the concentration data, various indices were used to characterise the *n*-alkane distributions. The carbon preference index (CPI) was calculated using the following equation:

$$\text{CPI}_{23-33} = \frac{1}{2} \left(\frac{C_{23} + C_{25} + C_{27} + C_{29} + C_{31} + C_{33}}{C_{22} + C_{24} + C_{26} + C_{28} + C_{30} + C_{32}} \right) + \left(\frac{C_{23} + C_{25} + C_{27} + C_{29} + C_{31} + C_{33}}{C_{24} + C_{26} + C_{28} + C_{30} + C_{32} + C_{34}} \right) \quad (1)$$

where C_x is the concentration of the *n*-alkane with *x* carbons. Distributions exhibiting odd/even preference have CPI > 1, which is considered indicative of a terrestrial plant leaf wax origin and/or limited diagenesis and/or no petroleum contamination.

The average chain length (ACL_{21–35}) was calculated as per Eq. (2) following Poynter et al. (1989):

$$\text{ACL}_{21-35} = \frac{21 * C_{21} + 23 * C_{23} + 25 * C_{25} + 27 * C_{27} + 29 * C_{29} + 31 * C_{31} + 33 * C_{33} + 35 * C_{35}}{C_{21} + C_{23} + C_{25} + C_{27} + C_{29} + C_{31} + C_{33} + C_{35}} \quad (2)$$

where C_x is the concentration of the *n*-alkane (C) with *x* carbons.

Dispersion (*d*) around the weighted average *N* (i.e. the spread in the *n*-alkane distribution) was calculated following Dodd and Afzal-Rafii (2000). Here the weighted mean chain length (*N*) for all detected *n*-alkanes is:

$$N = \sum (\%C_n n) / 100 \quad (3)$$

from which dispersion is calculated as:

$$d = \sum \%C_n (n - N)^2 \quad (4)$$

where *n* is the number of carbons (C₂₁–C₃₅). The ratio *d*/*N* is of relevance to consideration of lipid phase transition. Lipid distributions that are longer and narrower (i.e. low *d*/*N*) are anticipated to have a higher melting point to maintain their crystalline structure (Riederer and Schneider, 1990; Shepherd and Griffiths, 2006).

Various studies have also found the ratio between the C₂₉ and C₃₁ *n*-alkanes (Norm31) to be an environmentally sensitive parameter. This is calculated as follows:

$$\text{Norm31} = C_{31} / (C_{31} + C_{29}) \quad (5)$$

where C_x is the concentration of the *n*-alkane (C) with *x* carbons

We also used multivariate analysis to consider inter-/intra-biome variability in leaf wax distribution. The analysis was complicated by the strongly non-normal distributions of the proportions of the different *n*-alkane homologues (tested using a Wilks-Shapiro test). Consequently, non-metric multidimensional scaling (NMDS) was used as a non-parametric ordination approach to explore the underlying structure in the data. The technique uses measures of dissimilarity to graphically represent the relationships between samples in multidimensional space. The use of rank order similarity makes fewer assumptions concerning data structure (Clarke, 1993). Analysis was performed on the C₂₇–C₃₃ data (present in every sample). The data were square root transformed using Wisconsin double standardization, and dissimilarity between samples was measured using the Bray–Curtis index (Bray and Curtis, 1957). Three dimensional NMDS (stress 0.038) was subsequently performed on the dissimilarity matrix using 9999 random starts to avoid local minima and reach a stable solution. The output NMDS data space was rotated via principal components analysis so that the first axis expressed the maximum amount of variation in *n*-alkane distribution (Clarke, 1993). The significance of biome in determining the distribution of individual samples in ordination space was also tested. Ordination was undertaken using the VEGAN package in R (Oksanen et al., 2008; R Development Core Team, 2009). In addition, climatic data for the study sites were obtained from WorldClim 1.4 (Hijmans et al., 2005), with site aridity indices derived following Trabucco and Zomer (2009).

3. Results

The apolar lipid fraction from fresh plant material revealed a suite of compounds typical of plant leaf waxes (e.g. Jenks and Ashworth, 1999), including *n*-alkanes as dominant components, along with *iso*-alkanes, alkenes and wax esters (>C₃₅) in subordinate amounts. Data pertaining to the *n*-alkane yield are presented in Table S2.

3.1. Leaf wax *n*-alkane concentration and distributions from plants

Long chain *n*-alkanes occurred in all plant samples and spanned a range from C₂₁ to C₃₇. C₃₆ and C₃₇ homologues were occasional found in very low concentration (<1% of total *n*-alkanes), while short chain (<C₂₄) homologues were detected in very low concentration. The total yield was extremely variable, ranging between 0.009 mg/g dry weight (dw) and 32.5 mg/g dw (Table 1). The distribution was highly skewed, particularly for the Succulent Karoo specimens (Fig. S1). The majority (90%) of plants in the study area afforded 5 mg/g dw (mode 1 mg/g dw), consistent with previous studies (e.g. Vogts et al., 2009). However, some specimens with high *n*-alkane yield were found. All were succulents from the Euphorbiaceae and Aizoaceae families. The lowest concentration was found in plants from the Restionaceae family (ca. 0.01 mg/g dw). In

Table 1

n-Alkane concentration averaged (with standard deviation) per biome and per plant functional type for plant samples and averaged per biome for soil samples. The number of samples is in parentheses. The PFT averages exclude 4 unassigned specimens (i.e. $n = 211$).

<i>Total n-alkane content (mg/g dry plant wt.)</i>	
Succulent Karoo (133)	2.82 ± 5.15
Montane Fynbos (54)	0.65 ± 1.11
Lowland Fynbos (28)	0.51 ± 0.82
Grass (6)	0.81 ± 1.07
Herb (19)	0.57 ± 1.15
Woody shrub (93)	1.10 ± 2.55
Succulent (23)	2.73 ± 4.71
Woody Succulent (70)	3.34 ± 5.94
<i>Total n-alkane content (µg/g dry soil wt.)</i>	
Succulent Karoo (53)	2.95 ± 3.84
Montane Fynbos (21)	2.67 ± 2.83
Lowland Fynbos (19)	1.37 ± 1.38

terms of plant functional type, succulents and woody succulents gave on average the largest (but also the most variable) amount (Table 1).

ACL_{21–35} ranged from 25.5 to 33.4, while CPI ranged between 3.58 and 139, i.e. an odd preference, consistent with recent studies (Bush and McInerney, 2013). As with the *n*-alkane concentration, the distribution of CPI values was highly skewed. High values were rare, with the majority of plants (>90%) having CPI between 3 and 40 (mode 15). Those with the highest values (*Psilocaulon junceum* and *Aizoon* sp.) were associated with distributions dominated by a single homologue (C₃₁). The lowest values were clearly associated with the broadest *n*-alkane distributions, with dispersion and CPI negatively correlated (Fig. 2). Succulent Karoo plants tended to exhibit lower dispersion (*d*) than plants from the Fynbos (Table 2 and Fig. 2).

Biome/eco-region averaged leaf wax distributions are shown in Fig. 3. In the Succulent Karoo C_{max} for the majority of plants was at C₃₁ (81% of specimens), followed by C₃₃ (10%) and C₂₉ (3%). One specimen maximised at C₃₅. For the Montane Fynbos eco-region most plants had C_{max} at C₂₉ (28%) or C₃₁ (43%), with the shorter homologues (C₂₅–C₂₇) markedly more common than the Succulent Karoo (Fig. 3).

The Lowland Fynbos specimens produced distributions intermediate between Succulent Karoo and Montane Fynbos, with the majority exhibiting C_{max} at C₃₁ (61%), but with a significant proportion maximising at C₂₉ (29%). ACL_{21–35} thus increased from

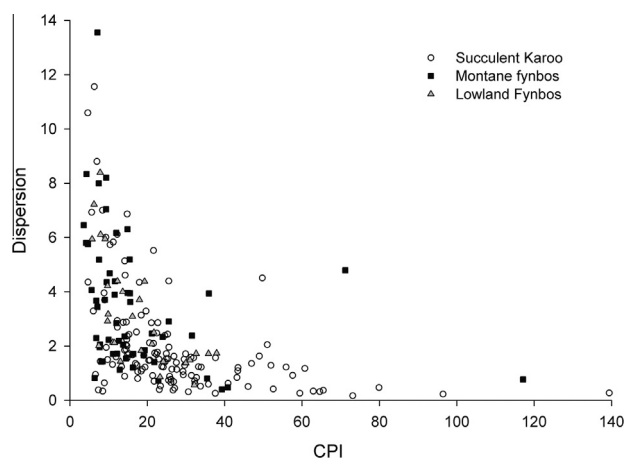


Fig. 2. Relationship between CPI and *n*-alkane dispersion (“*d*”, following Dodd and Afzal-Rafii, 2000) presented by biome/eco-region.

Montane Fynbos (28.7 ± 2.0) to Lowland Fynbos (29.3 ± 1.7) to Succulent Karoo (30.7 ± 1.7). The Norm31 ratio followed a similar trend, increasing from 0.49 ± 0.28 for Montane Fynbos to 0.57 ± 0.31 for Lowland Fynbos, rising to 0.84 ± 0.17 for Succulent Karoo (Table 2). Succulent Karoo plants were significantly different from the two Fynbos eco-regions (Kruskal Wallis test, $p < 0.001$) for all of the parameters derived using Eqs. (1)–(5). The Lowland and Montane Fynbos eco-regions were not significantly different for any parameter. It is also important to note the high standard deviation associated with the biome-averaged data, reflecting significant inter-specimen variation in the dataset as a whole.

The ordination analysis revealed that the largest differences in *n*-alkane composition (reflected in the first two axes of the rotated NDMS) were driven by the significance of C₂₇–C₂₉ relative to C₃₁ and C₃₃ in the individual distributions (Fig. 4). There was also a clear role for C₃₃ in distinguishing a significant proportion (but not all) of the Succulent Karoo plants. The third axis separated C₂₅ from the other homologues (data not shown). The average ordination scores for each biome are represented by the centre of each ellipse in the ordination space in Fig. 4, illustrating the separation of the Succulent Karoo from the two closely associated Fynbos eco-regions. The goodness of fit ($r^2 = 1 - SS_w/SS_t$, where SS_w and SS_t are the within-group and total sums of squares) was used to consider the separation of individual biomes. The results suggest that biome type explains a statistically significant, but low proportion of the total variation in the dataset (r^2 0.23, $p < 0.001$ based on 999 permutations). This, in conjunction with the overlap of the 95% confidence intervals (Fig. 4), further illustrates the considerable variation associated with individual specimens. On the basis of these data it would therefore be difficult to confidently assign an unknown specimen to a single biome, other than perhaps one characterised by a very short or a very long *n*-alkane distribution. Bush and McInerney (2013) recently reported similar results.

3.2. Individual variability: cosmopolitan species

As illustrated in Fig. 4, there was significant variability in individual plant *n*-alkane distribution. Disentangling the causes of such variability is challenging, but one obvious approach is to consider individuals of the same species across the study area and associated climatic gradients (e.g. Dodd et al., 1998; Dodd and Poveda, 2003). Unfortunately, in this study there were relatively few species common to multiple sampling plots. This reflects the high species turnover in and between the biomes (Cowling et al., 1997, 1999). Two cosmopolitan species, *Galenia africana* (Aizoaceae) and *Salsola tuberculata* (Chenopodiaceae) were, however, identified. *G. africana* is the most widespread and occurred in sites with mean annual rainfall ranging from 180 mm/a to > 300 mm/a (aridity index from 1200 to 1900 – equivalent to half of the total aridity index variability in the study area). Despite this, *G. africana* produced quite consistent chain length distributions, characterised by co-dominant C₂₉ and C₃₁ homologues, low dispersion and high CPI values (Fig. 5). Six specimens of *S. tuberculata* were sampled from sites spanning a narrower climatic range (mean annual rainfall 157–217 mm/yr and aridity index 980–1390), including two specimens from the same sample plot (SK4). In contrast to *G. africana*, *Salsola* produced highly variable *n*-alkane distributions (Fig. 5). These were highly dispersed, spanned the range C₂₁–C₃₃ and maximised at either C₂₅ or C₃₁. CPI values were lower and more variable. Due to the low replicate numbers it would be highly speculative to correlate the distributions for individual *S. tuberculata* specimens with climatic parameters, but the rather limited environmental range over which this species was sampled implies that some of the variability may represent (a) response(s) to micro-scale soil conditions and thus flexibility in leaf wax synthesis.

Table 2

n-Alkane distribution descriptive parameters [see Eqs. (1)–(5)] averaged (with standard deviation) per biome/eco-region for both plants and soils (ND, not determined).

Biome/eco-region	Number of samples	CPI _{23–33}	ACL _{21–35}	ACL _{27–33}	Dispersion (<i>d</i>)	<i>d</i> / <i>N</i> ^a	Norm31 C ₃₁ /(C ₂₉ + C ₃₁)
Succulent Karoo Plants	133	27 ± 19	30.7 ± 1.7	31.0 ± 0.77	2.04 ± 1.98	0.07 ± 0.07	0.84 ± 0.17
Succulent Karoo Soils	53	12 ± 4	30.4 ± 0.75	31.0 ± 0.36	6.94 ± 3.46	ND	0.83 ± 0.08
Montane Fynbos Plants	54	18 ± 19	28.7 ± 2.03	29.7 ± 0.87	3.47 ± 2.52	0.12 ± 0.09	0.49 ± 0.28
Montane Fynbos Soils	21	9 ± 5	27.9 ± 1.58	29.7 ± 0.93	7.99 ± 1.51	ND	0.52 ± 0.14
Lowland Fynbos Plants	28	18 ± 10	29.3 ± 1.70	30.0 ± 0.91	3.17 ± 2.02	0.11 ± 0.17	0.57 ± 0.31
Lowland Fynbos Soils	19	10 ± 2	29.3 ± 0.71	30.2 ± 0.47	8.46 ± 2.55	ND	0.60 ± 0.18

^a Calculated only for plant material (i.e. fresh/unaltered wax).

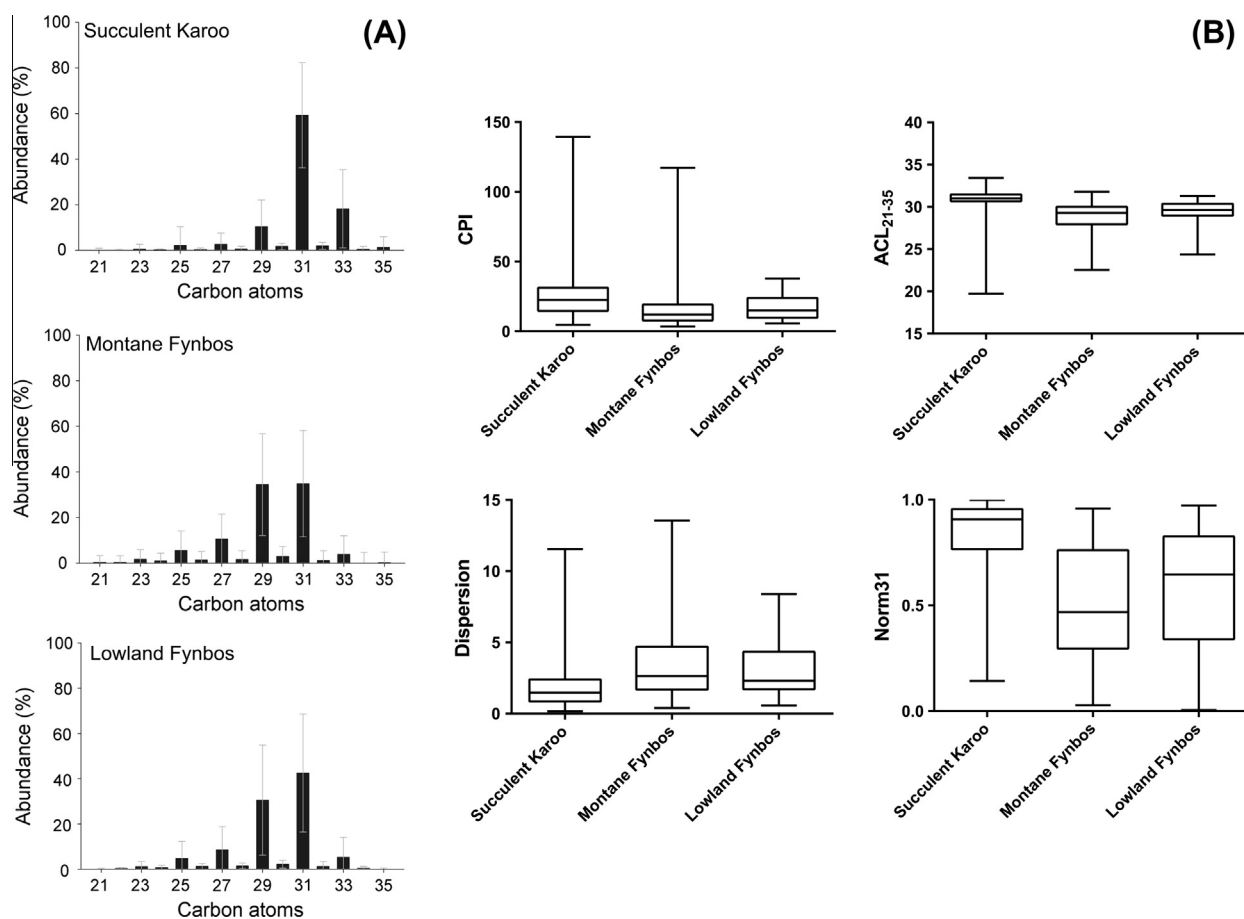


Fig. 3. Panel A: Biome/eco-region average *n*-alkane distributions, with standard deviation plotted. Panel B: Box and whisker plots for the four main *n*-alkane distribution descriptive parameters.

3.3. Plant families

Grouping the plants by family allowed some limited consideration of chemotaxonomic patterns. Ten families had six or more representatives across the study area. Considering the associated standard deviations, the results suggest that *n*-alkane distributions cannot be considered diagnostic for these families (Fig. 6). The plots do, however, broadly indicate that the families associated with succulent growth forms (Euphorbiaceae, Crassulaceae, Aizoaceae) tend to be dominated by C₃₁ and C₃₃. The Poaceae samples, consistent with previous findings (Smith et al., 2001; Rommerskirchen et al., 2006a; Bush and McInerney, 2013) were also associated with a higher proportion of the longer chain length homologues (avg. ACL_{21–35} 31.2 ± 0.5). The Aizoaceae (60 specimens) was the best represented family and, with the exception of *G. africana*, all were succulents. Although on average Aizoaceae were associated

with relatively high ACL and low dispersion (avg. ACL_{21–35} 31.4 ± 0.8, average dispersion 1.42 ± 0.9), in detail they showed some variability. For example, the three specimens of *Cephalophyllum* sp. were all co-dominated by C₃₁ and C₃₃, as were specimens of *Ruschia burtoiae*, *Ruschia tumidula* and *Ruschia stricta*. However, one *Ruschia* sp. sample produced a single large peak at C₃₁, while *Antimima* sp. (also Aizoaceae) maximised at C₃₃.

3.4. Plant functional type

As it was not possible to identify all specimens to generic and species level, the samples were also classified into plant functional types, which allowed the data to be considered in the context of a plant's broader ecophysiology (Table S1). The plants were grouped into one of five broad PFTs (modified from Todd and Hoffman, 2009). This is a rather coarse division (cf. the more detailed

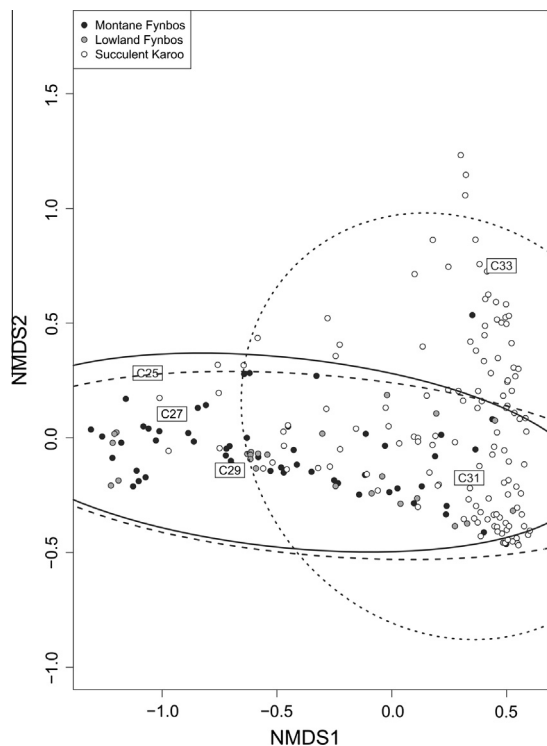


Fig. 4. Two dimensional NMDS ordination plots of *n*-alkane composition for the sampled biomes/eco-regions. The closer points plot together, the more similar their *n*-alkane distributions. The variable score for each *n*-alkane is also plotted in the ordination space (text in boxes). The ellipses represent the confidence limits for the biome/eco-region averaged ordination scores. The average ordination score for each biome therefore lies at the centre of each ellipse. Solid line, Montane Fynbos; long dash, Lowland Fynbos; short dash, Succulent Karoo. Note the close overlap of the Lowland and Montane Fynbos ellipses.

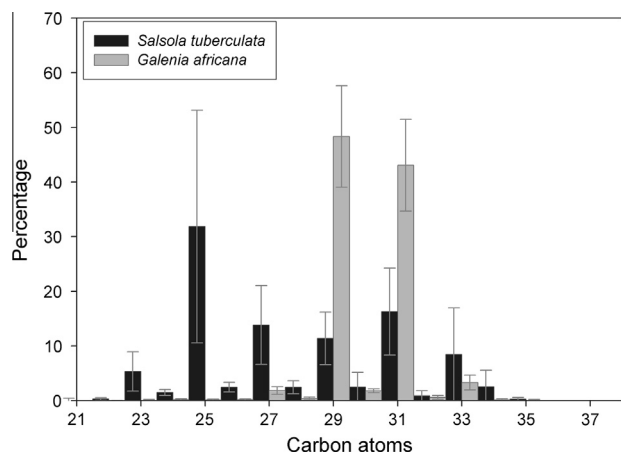


Fig. 5. Comparison of averaged *n*-alkane distributions for *Salsola tuberculata* ($n = 6$) and *Galenia africana* ($n = 5$). Standard deviation is also shown.

division of Todd and Hoffman, 2009), but it was considered sufficient to both distinguish the main plant physiologies/form and to retain (given the variability for individual plants) a significant number of specimens within each category. When aggregated by PFT (Fig. 7) the various homologue descriptors revealed clear distinction of the grasses, succulents and woody succulents from woody shrubs and to a lesser extent, herbs (latter PFT highly variable). This implies that the greater abundance of woody shrubs in the Fynbos Biome, particularly the Montane Fynbos sites, along with an absence of grasses and the rarer occurrence of succulents,

can explain some proportion of the differences between the biome-averaged distributions. Succulents and grasses tended to be associated with higher ACL and lower dispersion, while the herbs (in the Fynbos) and woody shrubs were associated with lower ACL and more dispersed chain length distributions. It was also apparent that for those PFTs found in multiple biomes/eco-regions (herbs, woody shrubs and woody succulents), the average values for CPI and ACL tended to be higher (dispersion lower) in the Succulent Karoo. Thus, for the same PFT, plants in the Succulent Karoo, on average, produced longer *n*-alkane distributions. This was most apparent for the herbs and woody shrubs (Fig. 7).

3.5. *n*-Alkane concentration and distribution in soils

The concentration of SOM in the region is generally low (Carr et al., 2013) and 9 of 102 original soil samples had insufficient *n*-alkanes for analysis. The *n*-alkane concentration was markedly lower than for the plant material, ranging between 0.1 and c. 20 $\mu\text{g/g}$ soil dw (Fig. S1), i.e. highly variable and, as seen in the plants, the distribution of *n*-alkane yields was positively skewed. This is significant in the Succulent Karoo samples, which produced the highest soil concentration, both on average and in terms of maximum values (Fig. S1). Average concentration was lowest in the Lowland Fynbos (Table 1). CPI values in the soils were consistently lower than those in the plants (Table 2), although they all retained a strong odd preference and so were not therefore suggestive of hydrocarbon contamination (Bray and Evans, 1961). Shorter chain length (C_{21} – C_{24}) homologues were more prominent in the soils, averaging 16%, 10% and 7% of the total *n*-alkane yield, vs. 3%, 2% and 1% in the plants (Montane Fynbos, Lowland Fynbos and Succulent Karoo, respectively), which probably reflects some microbial contribution (e.g. Ladygina et al., 2004).

The biome-average ACL_{21-35} values for soils increased from Montane Fynbos (27.9 ± 1.58), to Lowland Fynbos (29.3 ± 0.71) and maximised in Succulent Karoo (30.4 ± 0.75). These values were very similar to the plant averages (Table 2). The slightly lower values for Succulent Karoo and Montane Fynbos probably reflected the increased prominence of the C_{21} – C_{24} in the soils. For most parameters, the biome average standard deviation was lower than for the plants, which is interpreted as indicative of spatial/temporal averaging of the *n*-alkanes in the soils. When the *n*-alkane distributions in the plants and soils were compared as plot averages (Fig. 8) there was a strong correlation (r^2 0.66; $p < 0.01$; $n = 24$), particularly for ACL_{27-33} . The correlation, although still positive and significant (r^2 0.45; $p < 0.01$; $n = 24$), was weaker for ACL_{21-35} , reflecting the admixture of shorter homologues in the soils. Interestingly, CPI was not significantly correlated (r^2 0.11; p 0.1), nor was there a correlation between average dispersion in soils and average dispersion in the associated plants. The Norm31 index and ACL_{27-33} for the soils were, given the uncertainties, indistinguishable from biome/ecoregion plant averages (Table 2). The near identical values for C_{27} – C_{33} (Table 2) suggest that either the lifetime of *n*-alkanes in the soils is either very short (i.e. removing potential for the soils to reflect seasonal or inter-annual variation in leaf wax composition), or that there is little to no seasonal variability in leaf wax distributions, or that leaf wax production dominantly occurs in a single season.

4. Discussion

4.1. Alkane production and variability in the Succulent Karoo and Fynbos biomes

The leaf wax distributions from plants in the Fynbos and Succulent Karoo are consistent with numerous studies in revealing a

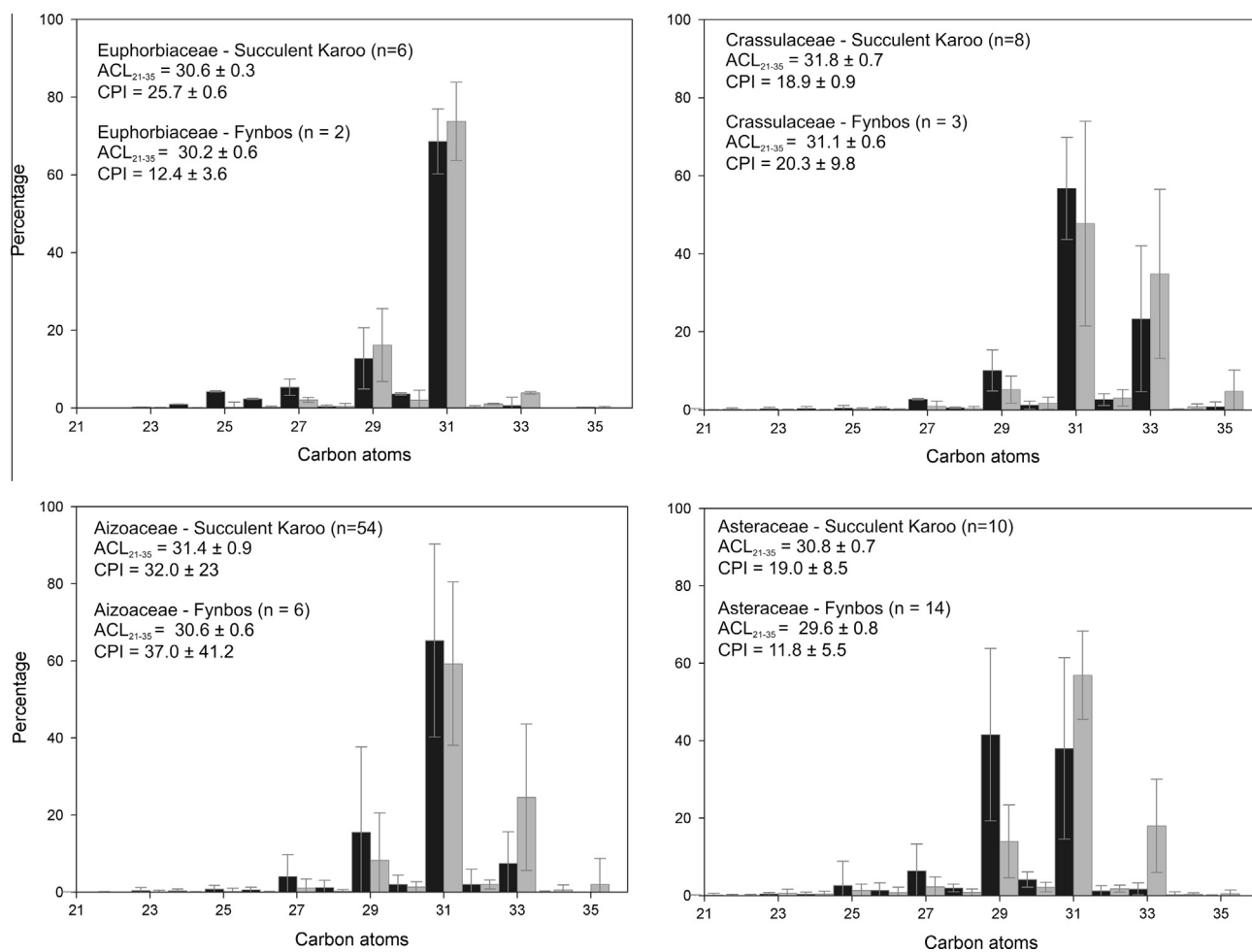


Fig. 6. Examples of average distributions for four families across the Fynbos (black) and Succulent Karoo (grey) biomes. Note that in each case the *n*-alkane distribution is characterised by longer chain length for plants sampled in the Succulent Karoo biome.

dominance of C_{27} – C_{33} *n*-alkanes and strong odd preference ($CPI > 1$). Modal *n*-alkane yield, particularly from non-succulent growth forms, is comparable with other studies and angiosperms in general (Bush and McInerney, 2013 and references therein). Several succulent plants show some unusual features, most notably the high CPI in some specimens which is in excess of ranges previously reported for angiosperms (Bush and McInerney, 2013; Fig. 9). The highest values are seen in plants from the Aizoaceae and Montiniaceae families. These plants also tend to produce high *n*-alkane yield, which in some cases exceeds 10,000 $\mu\text{g/g}$ (dw), but this is not always the case (Fig. 9). Although the yields are high compared with other African vegetation communities (particularly grasses; e.g. Smith et al., 2001; Rommerskirchen et al., 2006a), Maffei et al. (1997) reported an *n*-alkane concentration of 1–10 mg/g fresh weight for various Cactaceae, and the dw concentrations here are also comparable with several succulent Crassulaceae species from arid areas of the Canary Islands (Eglinton et al., 1962). Thus, the findings provide further evidence that lipid concentration for some plant types (in this case succulents) may be several orders of magnitude greater than other plant types (see also Diefendorf et al., 2011). Our data therefore suggest that such differences should be carefully considered in the application of plant biomarker proxies to arid regions, or sites that might source *n*-alkanes from arid zones.

The data further suggest that on average there are clear biome-scale differences between *n*-alkane distributions in Succulent Karoo plants and plants in Fynbos Biome. The former tend to maximise

at longer chain length, have narrower distributions (lower *d*) and are more likely to be dominated by C_{31} and C_{33} (Fig. 3). The latter tend to maximise at shorter chain length, show greater dispersion and have a lower tendency for odd dominance. However, considerable variation is apparent at the scale of individual plants, which is reflected in the large standard deviations and skewed distributions of the biome-averaged descriptive parameters (Fig. 3). Interestingly, although the maximum values (and thus total range) for the various descriptive parameters for the Succulent Karoo plants tend to be highest (i.e. there are some quite extreme values), the inter-quartile range for Succulent Karoo is actually smaller than both Fynbos eco-regions for three of the four parameters (Fig. 3). Such complexity and variability is underlined by the *n*-alkane distributions obtained from the multiple *G. africana* and *S. tuberculata* specimens, which demonstrate both consistency across the study area (*G. africana*), and enormous variability in closely related (geographically) specimens/locations (*S. tuberculata*).

4.2. Relationship with climatic parameters

Across the study sites, mean annual temperature varies by 5.5 °C, maximum temperature of the warmest month by 9 °C and mean annual precipitation by 326 mm/yr. We found no statistically significant relationship between any *n*-alkane distribution descriptor and any temperature-related parameter [including mean annual temperature, maximum temperature of the warmest month or growing season (wettest quarter) temperature].

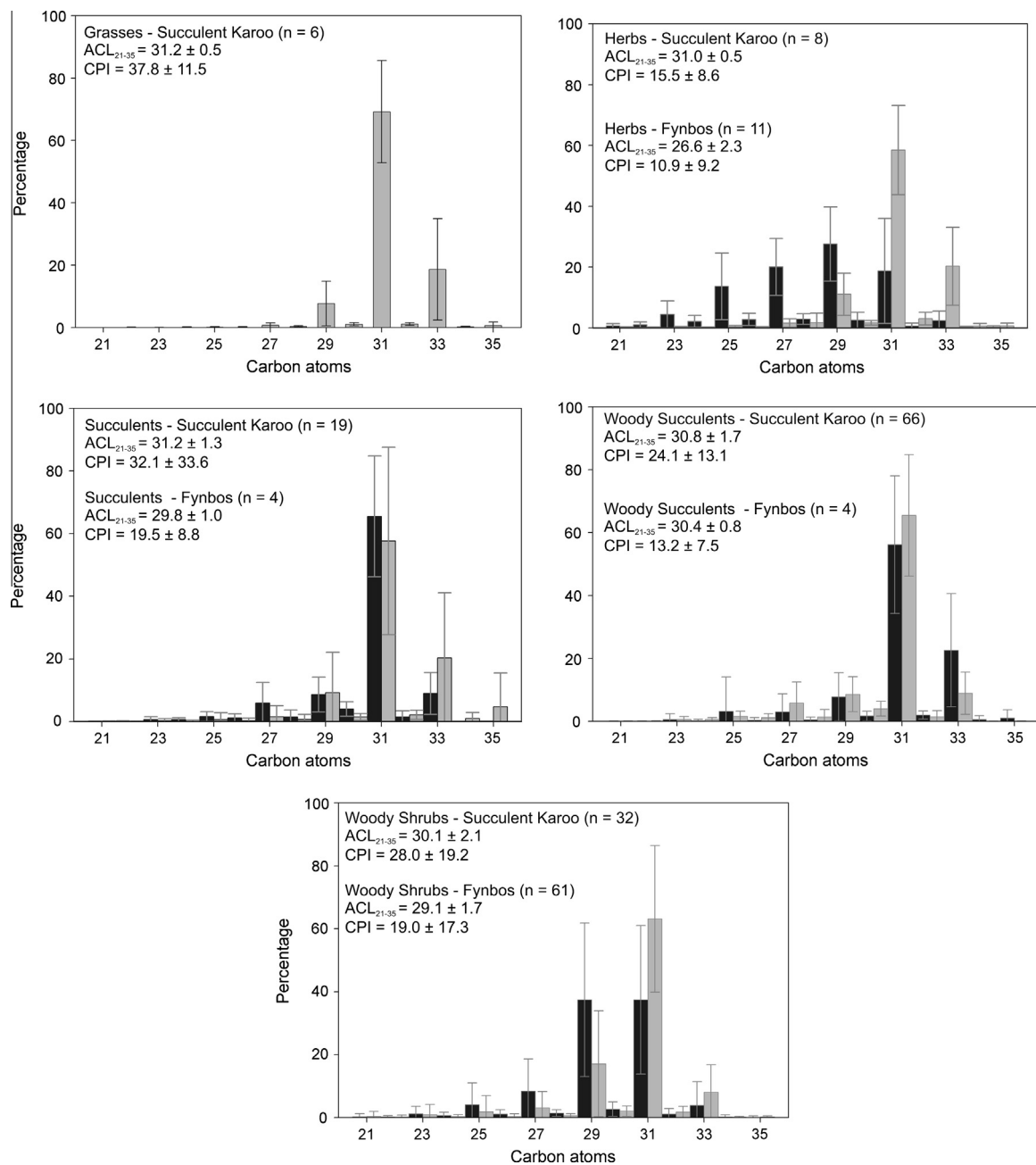


Fig. 7. *n*-Alkane distributions averaged by functional type. The associated PFT averaged ACL₂₁₋₃₅ and CPI are shown, along with the standard deviation. Grey bars represent specimens from the Succulent Karoo biome and black bars specimens from the Fynbos biome.

Considered in its entirety, the dataset shows a weak (r^2 0.14) but significant ($p < 0.01$) relationship between ACL₂₁₋₃₅ and aridity index. The relationship is stronger (r^2 0.21) when log aridity is used and substantially stronger (r^2 0.35) when a single outlier site (SV1) from the Lowland Fynbos is removed. The ACL₂₇₋₃₃ data produce very similar results (r^2 0.16 for all data, 0.22 for all data with log aridity and 0.28 with the removal of SV1). The reasons for site SV1 being an outlier are unclear. CPI does not correlate with any climatic variable. These patterns are the same for both soil and plant data, with the former associated with higher r^2 values and statistical significance (Fig. S3), which presumably reflects greater averaging of individual plant variation for the soil samples (Table 2).

When biomes are considered individually, the relationships between rainfall/aridity and *n*-alkane distribution are no longer

significant. There is, however, a clear division between the two biomes (Fig. 10). When the data are plotted in terms of plant functional type it is apparent that the distinction at least partly reflects differences in the predominant plant functional types between the two biomes (Fig. 10). When individual plant functional types are considered, the relationships between *n*-alkane distribution and climate are also insignificant (though the small number of replicates for some PFTs should be noted). Thus, although at face value the total data set could be interpreted as indicating that terrestrial leaf wax component distributions in western South Africa are weakly linked with aridity (Fig. 10), such a correlation is more indirect, and is seemingly driven to some extent by the differences in several key plant functional types along an environmental gradient, particularly the lower significance of succulents relative to woody shrubs in the Fynbos Biome (Fig. S4). This does not mean

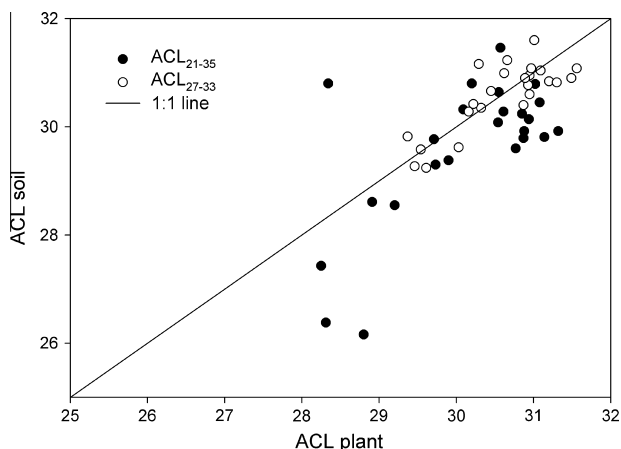


Fig. 8. Plot averaged *n*-alkane distribution parameters for plants and soils. The data comprise the averaged ACL_{21–35} for each measured plant or soil sample from a 10 × 10 m survey plot. The former ranged between 3 and 14 individuals (see Table S2) and the latter 3–4 soil samples. The 1:1 line is shown for comparison.

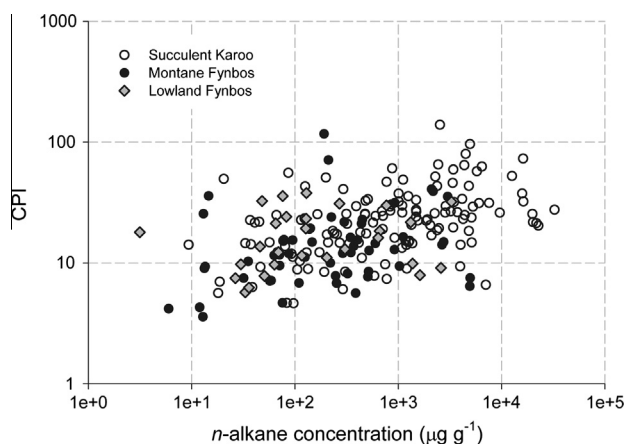


Fig. 9. A log–log plot of *n*-alkane concentration vs. CPI. For comparison, the reader is also directed to a comparable compilation presented by Bush and McInerney (2013).

that plants do not/cannot respond individually to their environmental conditions. Such additional variability is seen here to be superimposed on the overall patterns in the dataset (Figs. 6 and 7). For instance, in the PFTs common to both biomes there is a tendency to longer and less dispersed distributions for the specimens from the Succulent Karoo (Fig. 7). Furthermore, the data demonstrate that some plant species, such as *S. tuberculata*, are able to adjust their leaf wax *n*-alkane composition substantially, while some species (e.g. *G. africana*) and some families (Aizoaceae) show much less variability. It is known that fluctuation in leaf wax concentration and composition can be induced experimentally in response to drought (e.g. Koch et al., 2006; Macková et al., 2013). In natural habitats, Dodd et al. (1998) demonstrated an increased abundance of the longest chain length homologues with increasingly arid conditions in the leaf wax of *Austocedrus chilensis*. We cannot resolve such trends in terms of an individual species, but we do see some tendency for succulent plants/plants in the Succulent Karoo to be more frequently associated with the longer chain length distributions. C₃₃, in particular, is much more significant in succulents (Figs. 3 and 4).

Various hypotheses concerning specific controls on leaf wax lipid distributions have centred on their role as a protective coating, particularly in terms of water retention (i.e. a hydrophobic coating

to limit non-stomatal water loss). Given such a role, a response to aridity and/or temperature would be anticipated (Riederer and Schneider, 1990; Dodd et al., 1998; Shepherd and Griffiths, 2006). Riederer and Schneider (1990) proposed a model in which hydrocarbon distribution in leaf waxes is optimised such that water loss is minimised by the co-alignment and organisation of hydrocarbon molecules. Concerning lipid phase transition, it is anticipated that hydrocarbon melting point would increase with chain length (i.e. the melting point of C₃₁ *n*-alkane is nearly 9 °C higher than for C₂₇) and that hydrocarbon mixtures would influence the temperature range of phase transition (Shepherd and Griffiths, 2006). Thus, a low *d*/*N* ratio is associated with a high crystallinity and is more resistant to phase transition with rising temperature (Shepherd and Griffiths, 2006). Our data suggest an association between succulence (i.e. drought adaptation) and high ACL/low dispersion *n*-alkane distribution and a (weaker) association between aridity and longer chain length, which is consistent with these mechanisms. Similarly, Eglinton et al. (1962) presented *n*-alkane distributions for > 60 plants from the Crassulaceae family, including several succulent plant forms. These plants also tended to exhibit high ACL, low dispersion and high CPI distributions which, in conjunction with high *n*-alkane yield, are consistent with our findings in implying that *n*-alkane distributions are optimised for water retention in some succulent plants.

4.3. Implications for palaeoenvironmental reconstruction

This study has several implications for the use of leaf wax distributions as palaeoenvironmental proxies. The comparison of the soil and plant data indicates that, although *n*-alkane concentration in soils is often low, the distributions, particularly when the longer homologues are considered, show a close correspondence with local plants. This implies that a signal representative of local plant communities is retained within local soils, including the strongly positively skewed distribution of *n*-alkane concentration in the Succulent Karoo Biome (Fig. S1). We would therefore anticipate that (excluding the potential influence riparian vegetation in wetland and lake contexts; e.g. Gao et al., 2011) representative leaf wax distributions ought to be preserved in local sedimentary and palaeoecological archives. Some preliminary data from the study region are consistent with this statement (see Carr et al., 2010, Table 3).

However, the results also indicate that there is significant variability in leaf wax lipid yield from plants (cf. Diefendorf et al., 2011). In this respect, the tendency of the succulent plant functional type to be associated with the highest yield is an important finding, suggesting that caution is required when interpreting fossil leaf wax data in the region (or other regions where such plants are present). This may be particularly significant for studies in which there is a large or poorly understood/characterised source area for leaf waxes. In these circumstances it is important to consider information on likely wax lipid yield rather than broad estimates of plant biomass in a geographic area (Diefendorf et al., 2011). The issue is perhaps most relevant for studies seeking to estimate proportions of C₃ and C₄ plants using *n*-alkane δ¹³C (Boom et al., 2014) and sites associated with long transport distance (e.g. marine archives), as opposed to more confined terrestrial locales in which local source vegetation can more easily be characterised.

Our data demonstrate that, on average, differences between plant functional type and major biomes are apparent in southwestern South Africa. Various studies have sought to use shifts in dominant chain length, ACL or CPI in sedimentary records to infer palaeoenvironmental or palaeoecological change. They have been interpreted in terms of the changing occurrence/presence of plant types in both terrestrial (e.g. Schwark et al., 2002; Bai et al., 2008) and aquatic/riparian (e.g. Ficken et al., 2000; Gao et al., 2011)

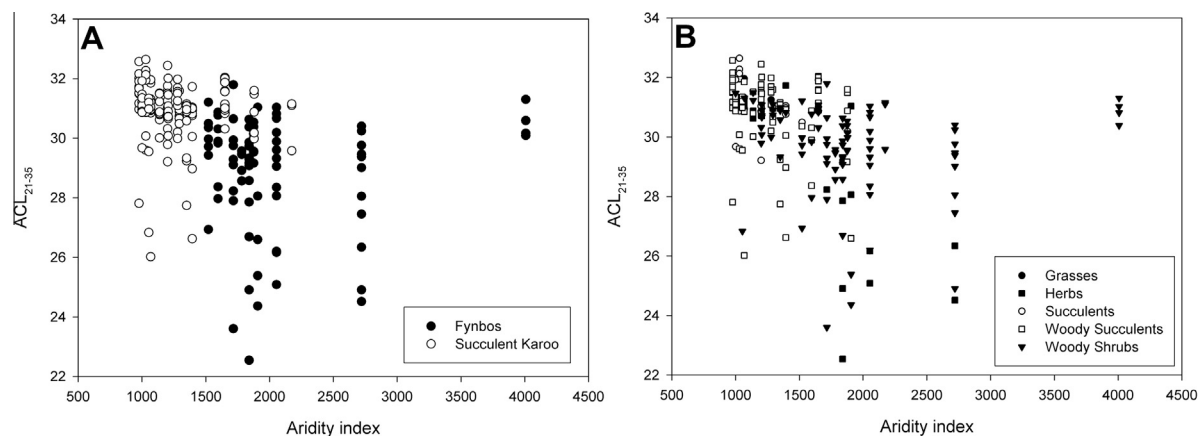


Fig. 10. (A) Relationship between ACL_{21-35} and aridity index. Note the outlier site (SV1) on the right, removal of which markedly increases the r^2 value for the relationship (from 0.14 to 0.35). This site is the most southerly and is on the Atlantis coastal dune plume. (B) Same data but with plants marked according to functional type.

contexts. In other circumstances such changes have been considered more in terms of fluctuation in climatic conditions, for instance changing temperature (Castañeda et al., 2009). Fig. 11 compares data from this study with various other southern African vegetation types (taken from Rommerskirchen et al., 2006a and Vogts et al., 2009). As previously noted, C_4 grasses tend to produce the longest distributions (Rommerskirchen et al., 2006a), but here these are exceeded by some of the succulents of the Succulent Karoo biome, particularly when the Norm31 parameter is considered. Considerable overlap is seen between the woody and herbaceous vegetation of the Fynbos and Savanna biomes and it is therefore unlikely that such woody/shrubby vegetation types could ever be reliably distinguished in a fossil sample. The variability in the Succulent Karoo and Fynbos vegetation (Fig. 11; note for instance the inter-quartile ranges and total ranges for Norm31), compared with some savanna vegetation types and the grasses is also noteworthy and perhaps reflects some combination of the high edaphic variability in the Succulent Karoo and Fynbos (Cowling et al., 1999), as well as the larger number of plants sampled (across a topographically and climatically diverse region) in this study.

From our data it is argued that the link between n -alkane distribution and climate is rather indirect in that it reflects a combination of shifting proportions of plant functional types across the study area and/or the adjustment of n -alkane distributions within plant type or taxa (Figs. 6 and 7). The latter aspect is currently poorly characterised, but there is potentially considerable variation in the propensity or ability of different taxa to modify their n -alkane distributions (Fig. 5). Considering these data in terms of palaeoenvironmental research for the region, it is not likely that long term, large scale changes in the occurrence and distribution of

various plant functional types occurred independently of climatic change and, as such, an understanding of site context should assist with the interpretation of fossil n -alkane data. For instance, at sites close to the ecotone between the Fynbos and Succulent Karoo (e.g. Carr et al., 2010; Chase et al., 2011), leaf wax distributions may be useful as an environmental proxy as palaeoclimatic changes may induce fluctuations in the extent, distribution and/or relative proportions of the various functional types. While it would be prudent to interpret such data in conjunction with other proxy data, in this situation leaf wax distribution data can be envisaged to fluctuate in a manner that reflects larger scale climate changes. Some evidence for such changes can be seen in palynological records from the region (e.g. Scott and Woodborne, 2007; Valsecchi et al., 2013). Furthermore, the data presented here suggest that, in such a context, significant fluctuations in leaf wax distributions associated with the shifting balance of plant functional type, need to be considered very carefully in the interpretation of n -alkane $\delta^{13}C$ and δD records (e.g. Feakins and Sessions, 2010; Sachse et al., 2012; Douglas et al., 2012), particularly given the generally higher production of n -alkanes in succulent plants in the region. Furthermore, Boom et al. (2014) show that CAM photosynthesising plants produce leaf wax with highly variable n -alkane $\delta^{13}C$ values, potentially confounding simple mixing model-based reconstructions of C_3/C_4 plant occurrence.

5. Conclusions

This study presents n -alkane leaf wax distributions for 215 plants and 93 soils from arid and semi-arid Fynbos and Succulent Karoo biomes of South Africa. In terms of biome average, the

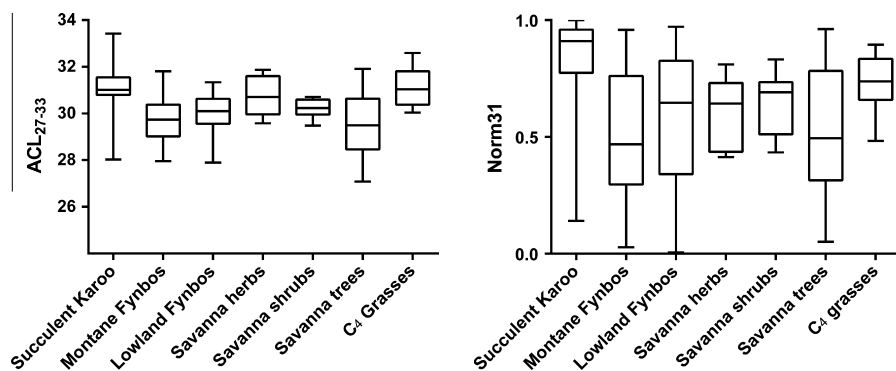


Fig. 11. Box plots summarising n -alkane distributions from this study with data from the savannas of southern Africa (Namibia and Zimbabwe) taken from Vogts et al. (2009) and Rommerskirchen et al. (2006a) $n = 8$ for savanna herbs, $n = 9$ for savanna shrubs, $n = 28$ for savanna trees, $n = 31$ for C_4 grasses. Due to different reported n -alkane chain length ranges, the data used here comprise only the C_{27} – C_{33} range, which is the reported n -alkane range common to all studies.

n-alkane distributions of the Fynbos and Succulent Karoo biomes are distinct and the patterns are closely mirrored in the contemporary soils. There is a tendency for chain length distribution to be longer (increased C₃₃ in particular) and less dispersed for plants and soils in the Succulent Karoo. The individual Fynbos eco-regions show considerable overlap and are not distinguishable. Importantly, the data also demonstrate considerable variability in *n*-alkane yield from individual plant specimens. Succulent growth forms are associated with the highest yield. The data also reveal the complexity and variability in leaf wax production when individual plant specimens are considered, and it is apparent that some species, such as *S. tuberculata* can synthesise highly variable leaf wax distributions.

Although the more arid biome (the Succulent Karoo) is, on average, associated with higher ACL, lower dispersion and higher CPIs, correlation with multiple climatic parameters is weak. Taking the dataset as a whole, there is a weak but significant relationship between average chain length and aridity/rainfall, but in detail it can be seen that this is at least partly a function of the changing proportions of distinct (in terms of leaf wax) plant functional types as climatic conditions change (i.e. increased abundance of succulent growth forms). There are several implications for palaeoenvironmental research. In the study locality itself, the data suggest that plant wax *n*-alkane distributions are preserved in local soils with some fidelity. Furthermore, although any correlation between leaf wax distribution and climatic parameters is rather indirect, there may be potential for using such data to infer shifts in the abundance of plant functional types in locations near to major ecotones. More generally, the data illustrate the great variability in *n*-alkane yield from different plant functional types and particularly the high *n*-alkane concentration associated with some succulent growth forms. We suggest that the influence of succulent plant-derived waxes on sedimentary leaf wax records should not be discounted in arid regions, even if the apparent biomass of such plants is low. As many plants with this growth form are CAM plants, their potential to influence the interpretation of compound-specific stable carbon and hydrogen isotope analyses should be considered cautiously.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.orggeochem.2013.12.004>.

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