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Chemotaxonomic significance of distribution and stable carbon isotopic composition of long-chain alkanes and alkan-1-ols in C_4 grass waxes

Florian Rommerskirchen [a,*], Anna Plader [a], Geoffrey Eglinton [b,1], Yoshito Chikaraishi [c], Jürgen Rullkötter [a]

[a] *Institute of Chemistry and Biology of the Marine Environment (ICBM), Carl von Ossietzky University of Oldenburg, P.O. Box 2503, D-26111 Oldenburg, Germany*
[b] *Hanse Institute for Advanced Study (HWK), Lehmkuhlenbusch 4, D-27733 Delmenhorst, Germany*
[c] *Institute for Research on Earth Evolution, Japan Agency for Marine-Earth Science and Technology, 2-15 Natsushima-cho, Yokosuka 237-0061, Japan*

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Abstract

Grasses (Poaceae) are distributed across the world in broad latitudinal belts and are an important source of C_4 biomass in the geological record of soils as well as lake and marine sediments. We examined long-chain leaf wax components of thirty-five C_4 grasses of the subfamilies Aristidoideae, Chloridoideae and Panicoideae from the southern African grasslands and savannas and three C_3 grasses of the subfamily Pooideae from Peru and Australia and review the relevant botanical, phytogeographic and leaf wax compositional background information. Contents, distribution patterns and molecular stable carbon isotopic compositions of long-chain *n*-alkanes (n-C_{27} to n-C_{35}) and *n*-alkan-1-ols (n-C_{22} to n-C_{32}) were used to estimate the chemotaxonomic relevance of wax signatures of whole plants, separately for different subfamilies and for members of the three C_4 subtypes (NADP–ME, NAD–ME and PCK). Two grass species were separated into flower heads, leaves and stems and the parts analysed separately. Grass flowers contain remarkable amounts of short-chain *n*-alkanes, which may have a significant influence on the chemical signature of the whole plant, whereas *n*-alkanol distribution patterns exhibit no systematics. The stable carbon isotopic composition of both biomarker types in different plant parts is remarkably uniform. Chemotaxonomic differentiation was not possible on a species level based on whole plant samples, but was more successful for averages of subfamily and photosynthetic subtype data. Wax signatures of C_4 grasses are generally distinguishable from those of C_3 species by heavier isotopic values, higher contents of n-C_{31} and n-C_{33} alkanes and the abundance of the n-C_{32} *n*-alkanol, which is largely absent in C_3 grass waxes. Especially the waxes of the NAD–ME and PCK C_4-subtype grasses, which thrive in extremely arid tropical and subtropical areas, contain high relative amounts of longer-chain *n*-alkane homologues. The chemical classification on a subfamily level, which is in agreement with previously reported subfamilial phylogeny of grasses, implies an evolutionary wax adaptation of C_4 grasses to warm and arid habitats. Our results confirm the validity of the contents, distribution patterns and molecular stable carbon isotopic compositions of

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[*] Corresponding author.
E-mail address: f.rommerskirchen@icbm.de (F. Rommerskirchen).
[1] Present address: Biogeochemistry Centre, Department of Earth Sciences, University of Bristol, Bristol BS1 7RU, UK.

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long-chain *n*-alkanes and alkan-1-ols as indirect proxies of continental climate conditions in environmental studies of the tropics.

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1. Introduction

Plants use two principal types of carbon fixation pathways during photosynthesis. The first is the so-called C_3 pathway (Benson–Calvin cycle), which is thought to have been used by higher land plants since their advent some 450 million years ago at relatively high concentrations of atmospheric CO_2 (Sage, 1999, 2004). Of the estimated 250,000 species of land plants, about 85–89% use the C_3 photosynthetic pathway, e.g., almost all trees, shrubs, as well as grasses preferring wet, cool growing seasons (Leegood, 1999a; Sage et al., 1999a). The other principal way, the C_4 pathway (Hatch–Slack cycle), requires a cell anatomy which differs substantially from that of the C_3 plants (Hatch and Slack, 1966). It is believed that the physiological adaptation of the C_4 mechanism evolved independently many times between 7 and 30 million years ago in response to a decline in concentration of CO_2, conditions that would have favoured photorespiration (Kellogg, 1999; Leegood, 1999b; Sage et al., 1999b; Giussani et al., 2001; Keeley and Rundel, 2003; Sage, 2004). This has resulted in three C_4 sub-groups: Those using the NADP–malic enzyme (NADP–ME; malate as CO_2 supplier), those using the NAD–malic enzyme (NAD–ME; aspartate), and those using the PCK enzyme (aspartate) to release CO_2 from their internally stored carbon pools (Hatch et al., 1975). These mechanisms of CO_2 concentration lead to an improved water use efficiency (Downes, 1969) and prevent ineffective photorespiration at locations of high temperature, high light intensity, high salinities, limited water supply and/or low CO_2 concentrations (Björkman, 1976; Ehleringer et al., 1997; Collatz et al., 1998; Winslow et al., 2003; Sage, 2004). Under these conditions, which prevail in certain subtropical and tropical areas, C_4 plants successfully outcompete C_3 plants (e.g., Björkman and Berry, 1973; Osmond et al., 1980; Edwards et al., 1985).

The independent evolutionary events in the history of the C_4 photosynthetic mechanism led to its distribution among a wide range of unrelated plants, and it is represented in 8000–10,000 species in 19 diverse taxonomic families (Sage, 1999, 2004). About 60% of C_4 land plant species belong to the grass family (Poaceae), followed by sedges (about 20%) and dicotyledons (about 16%; according to Sage, 2004). Within the grass family, one half of the around 10,000 species use the C_4 pathway (Hattersley, 1987; Hattersley and Watson, 1992). The grass family is not the largest in term of species and genera, but its environmental importance is great for it provides the principal ground cover of the grasslands and savannas. In savannas, woody species are significant but do not form a closed canopy or a continuous cover (Bourliere, 1983). Thereby, the importance of C_4 plants (predominantly tropical grasses) decreases with increasing abundance of woody (C_3) species, which is governed by edaphic characteristics and the climate, especially the mean annual rainfall (Walter, 1971; Whittaker, 1975). Where grasslands exhibit two distinct growing seasons, C_3 grasses mostly grow in the cooler, moist months (Williams, 1974; Winslow et al., 2003). Grassy vegetation occupies a third of the land surface (Clayton and Renvoize, 1986; Werner, 1991) and is distributed across the world in broad latitudinal belts (Fig. 1a; Hartly, 1950; Cross, 1980). Savannas and grasslands cover 50% of Africa and Australia, about 45% of South America and 10% of India and Southeast Asia (Fig. 1a and b; Werner, 1991).

Grasses may be divided into the major subfamilies Bambusoideae, Chloridoideae, Panicoideae, and Pooideae (Clayton and Renvoize, 1986; Gibbs Russell et al., 1991; Watson and Dallwitz, 1992a,b onwards; Grass Phylogeny Working Group, 2001). Pooideae (C_3) reach their maximum diversity in the temperate zone and Bambusoideae (C_3), though mostly tropical, are mainly confined to humid forest shade. Chloridoids (C_4) and panicoids (mainly C_4), on the other hand, are concentrated in the tropics and subtropics (cf. Fig. 2b; Gibbs Russell et al., 1991). They can be found in savanna zones between deserts and forests (cf. Fig. 1a and b; Clayton and Renvoize, 1986), although the chloridoids in particular grow in extremely arid areas. The subfamilial classification of grass species undergoes frequent reorganisation due to ongoing reevaluation of phylogeny. The current phylogenetic tree of grasses, which was elaborated by the Grass Phylogeny Working Group (2001), consists of 12 subfamilies (Fig. 2a). The C_4 carbon fixation pathway is generally found in three subfamilies: Aristidoideae, Chloridoideae and Panicoideae (Fig. 2a).

The geographical distribution of the C_4-containing subfamilies is linked to the abundance of C_4 subtypes within them, because they prefer different habitats of annual rainfall patterns compared to the rest of the subfamily members (cf. Fig. 2b; Schulze et al., 1996; Taub, 2000; Ghannoum et al., 2001; Wan and Sage, 2001). The NADP–ME grass species (mainly in the Panicoideae and Aristidoideae subfamilies) predominate in all regions where C_4 grasses occur, but they reach their maximum abundance in areas of moderate moisture with more than 500 mm/a precipitation (e.g., Fig. 1c). NAD–ME and PCK species have their maximum diversity in relatively arid regions with less than 500 mm annual precipitation and are concentrated in the subfamily Chloridoideae, where the NADP–ME subtype is unknown (Fig. 1c; according to Ellis et al., 1980; Gibbs Russell et al., 1991; Renvoize and Clayton, 1992; Watson and Dallwitz, 1992a; Schulze et al., 1996; Wan and Sage, 2001).

The distinctions in carbon isotopic composition of land plant biomass due to different carbon fixation pathways can be used to assign plants to the C_3/C_4 photosynthetic pathways. The $\delta^{13}C_{TOC}$ of total organic carbon (TOC) has characteristic ranges from $-23‰$ to $-34‰$ and from $-12‰$ to $-14‰$ in C_3 and C_4 plants, respectively (Schidlowski, 1987). Leaf wax lipids during biosynthesis become more depleted in ^{13}C than the total biomass, e.g., by about 10‰ in *n*-alkanes (Collister et al., 1994), so that their $\delta^{13}C$ values vary between $-32‰$ and $-39‰$ in C_3 and between $-18‰$ and $-25‰$ in C_4 plants (Rieley et al., 1991, 1993; Collister et al., 1994).

Wax lipids cover all aerially exposed organs of higher land plants to control the water balance and protect against mechanical damage to leaf cells, weathering, water stress, and attack by microbes, fungi and insects (Chibnall et al., 1934; Eglinton and Hamilton, 1967; Kolattukudy, 1976, 1980, 1996; Tulloch, 1976; Baker, 1982; Bianchi, 1995; Riederer and Schreiber, 1995). Their development was a prerequisite for the evolutionary step of aquatic to terrestrial plants (Gülz, 1994). The thickness of the wax layer is variable and difficult to evaluate. It depends on many stimulatory effects on wax production like increase in energy flux, decrease in humidity or soil moisture content (Whitecross and Armstrong, 1972; Baker, 1974; Baker and Procopiou, 1980). Exceptionally thick layers are found on leaves of plants which grow under arid conditions (Baker and Procopiou, 1980) with their particular needs for regulating transpiration (Riederer and Schreiber, 1995).

Long-chain *n*-alkanes and *n*-alkan-1-ols are major components of leaf waxes (Tulloch, 1976; Bianchi,

Fig. 1. (a) World map showing a simplified assessment of the global distribution of areas with typical tropical and temperate grasses including grasslands, savannas, wood- and scrublands. The assessment is based on maps of Whittaker (1975), Cross (1980), Bourliere (1983), Coupland (1992), of the Global Land Cover Characteristics Database 2.0 (Loveland et al., 2000; http://edcdaac.usgs.gov) as well as Sala et al. (2001). (b) Map of Africa showing political boundaries as well as simplified phytogeographical units of today, based on maps of White (1983) and of the Global Land Cover Characteristics Database 2.0 (Loveland et al., 2000; http://edcdaac.usgs.gov). Countries where grass samples for this study were collected are labelled. (c) Partial map of Africa showing generalised units of dominating subfamilies of the grass vegetation (grey codes according to grass subfamily superiority; after Gibbs Russell, 1988), of phytogeographical regions (white text and broken boundaries) and of mean annual rainfall based on the 1961–1990 mean monthly climatology of New et al. (1999); alternately dotted and dashed grey lines. Areas of precipitation where more than 40% of rainfall occurs during the winter season are diagonally striped. All other areas are summer rainfall areas.

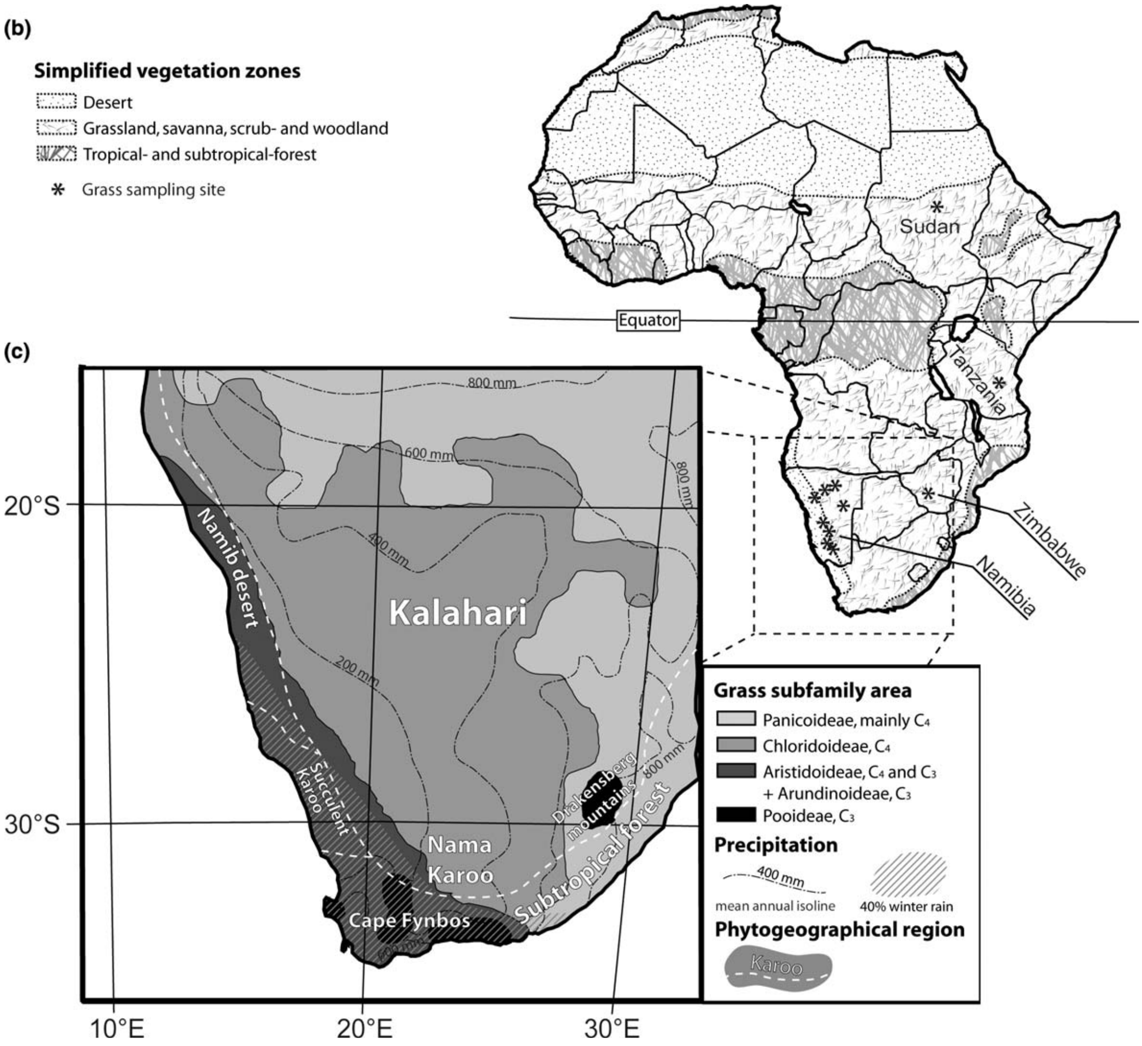


Fig. 1 (*continued*)

1995); *n*-alkanes typically occur in the *n*-C_{27} to *n*-C_{35} range with characteristic odd-over-even (Eglinton and Hamilton, 1967) and *n*-alkanols in the *n*-C_{20} to *n*-C_{34} range with even-over-odd carbon number predominance (Baker, 1982; Bianchi, 1995). These compounds are relatively resistant against degradation after the decay of plants, although the stability decreases from *n*-alkanes to *n*-alkanols (Cranwell, 1981). Epicuticular wax components can easily be dispersed by wind and rivers; they can be associated with plant detritus or adhere to dust particles and can thus end up in soils, lake and ocean sediments, which makes them useful higher-plant biomarkers in palaeoenvironmental studies.

It has been suggested that plants of warmer tropical climates biosynthesise longer-chain wax components than do plants in habitats of the temperate regions (e.g., Cranwell, 1973; Gagosian and Peltzer, 1986; Poynter et al., 1989). This concept has been used as a proxy for continental climate development in studies of marine sediments, where contents of longer-chain length alkane homologues covaried with higher sea-surface temperature estimates based on long-chain alkenones (Hinrichs et al., 1997; Rinna et al., 2000). Schefuß et al. (2003b), however, did not find the same relationship in dusts collected off the African continent in a north-south transect. They argued that precipitation (aridity) is more important in governing higher plant *n*-alkane distributions. Other palaeoenvironmental studies found a shift to longer-chain homologues with heavier $\delta^{13}C$ values and attributed these findings to an increase in

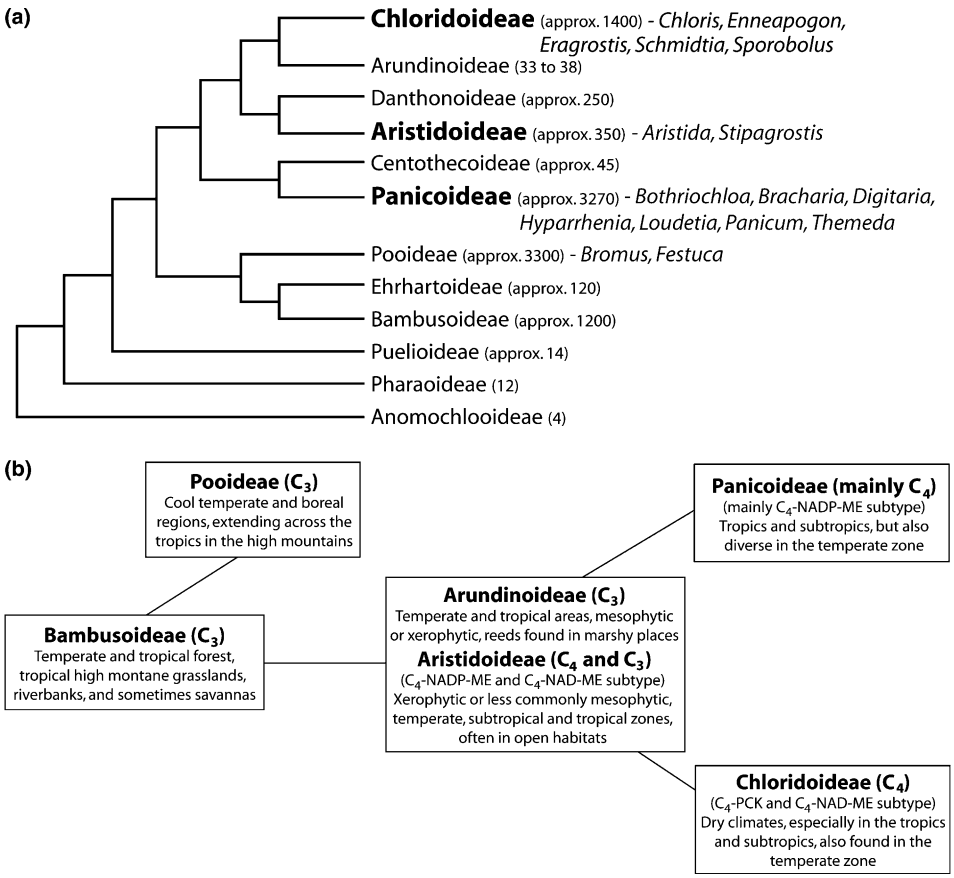


Fig. 2. (a) Phylogeny of the grass family based on the grass subfamily classification of the Grass Phylogeny Working Group (2001). Taxa that contain the C_4 photosynthetic mechanism are boldfaced. Number of species of the whole subfamily (bracketed; gathered from the Grass Phylogeny Working Group, 2001) as well as grass species used for chemical classification, are noted next to the subfamily. (b) Simplified evolutionary sequence of grass embryo types (based on the postulated sequence of Clayton and Renvoize, 1986). Displayed are the four most species-rich subfamilies Bambusoideae, Chloridoideae, Panicoideae and Pooideae as well as the potentially crossing subfamilies Arundinoideae and Aristidoideae. Preferred habitat description of the subfamilies was gathered from the Grass Phylogeny Working Group (2001). C_4-containing subfamilies exhibit further information about the major C_4 physiology within the subfamily (according to Ellis, 1977; Watson and Dallwitz, 1992a,b onwards and Schulze et al., 1996).

abundance of C_4 vegetation (e.g., Ficken et al., 1998; Huang et al., 2000; Zhao et al., 2000; Boom et al., 2001, 2002; Conte and Weber, 2002a,b; Eglinton et al., 2002; Conte et al., 2003; Rommerskirchen et al., 2003; Schefuß et al., 2003a,b; Zhang et al., 2003; Zhao et al., 2003; McDuffee et al., 2004; Zhang et al., in press). The waxes of C_4 grasses are cited in several recent sediment studies as representing important fossil C_4 plant material (e.g., Boom et al., 2002; Rommerskirchen et al., 2003; Zhang et al., 2003; Zhao et al., 2003; Zhang et al., in press). However, analytical data for *n*-alkanes and *n*-alkanols in the leaf waxes of extant C_4 grasses are only sparsely available (e.g., Tulloch, 1981, 1984; Dove and Mayes, 1991; Dove et al., 1996; Chen et al., 1998; Smith et al., 2001). A chemotaxonomic approach to grass waxes on a subfamilial level by Maffei (1996) was mainly based on C_3 grasses.

The present study provides significant background information relevant to the emerging use of long-chain aliphatic biomarkers in environmental studies. We focused our grass wax lipid investigation on the southern African tropical and subtropical savannas and deserts. On a subfamilial and photosynthetic level we evaluate the chemotaxonomic significance of wax *n*-alkane and free wax *n*-alkanol carbon number distribution patterns as well as molecular stable carbon isotopic compositions of thirty-five C_4 and three C_3 grasses. We also make a preliminary assessment of the reliability of wax homologues as proxy parameters by evaluating the contribution of different plant parts of two grass species to the whole plant wax signal. By reviewing the botanical background information and using the analytical data of the present study together with previously published analytical data, we elucidate the significance of C_4 grass waxes for palaeoenvironmental studies of the tropics and subtropics.

2. Description of sampling area, samples and methods

2.1. Description of sampling area: grass diversity and distribution in southern Africa

The vegetation types of Africa grade south- and northwards from the equatorial lowland rain forest to dry forest, wood-, scrub- and grassland, different types of savannas and deserts, and finally to Afroalpine vegetation, southern subtropical and northern Mediterranean forest. For a broad-brush assessment in Fig. 1b we combined grasslands, savannas, wood- and scrublands into a grass vegetation zone, tropical and subtropical forest into a tree-dominated vegetation and defined deserts as sparse vegetation zones. A large part of the African continent is covered by grass-dominated ecosystems (White, 1983).

The predominance of C_4 grass species (Fig. 1c) ranges south and eastward from the central arid desert over the cool temperate highvelds to the humid subtropical east coast (Vogel et al., 1978). Chloridoideae (Ch), Panicoideae (Pa) and Aristidoideae (Ari) are common subfamilies (Fig. 1c) and grass species of *Aristida* (Ari, C_4), *Chloris* (Ch, C_4), *Cynodon* (Ch, C_4), *Eragrostis* (Ch, C_4), *Panicum* (Pa, C_4), *Sporobolus* (Ch, C_4), *Stipagrostis* (Ari, C_4) or the subspecies *Themeda triandra* (Pa, C_4) are dominant (Bayer, 1959; White, 1983; O'Connor and Bredenkamp, 1997). Aristidoideae and Arundinoideae (Aru) are mostly found in areas with more than 40% of rainfall occurring in winter, like at the southwest coast of Africa (cf. Fig. 1c; according to Ellis et al., 1980; Gibbs Russell, 1988; Gibbs Russell et al., 1991). Up to 86% of all plant species in the hot dry environment of the southern Kalahari use the C_4 photosynthetic pathway (Leistner, 1967), and 95% of the grass species are C_4 plants (Ellis et al., 1980). C_3 grasses are sparse, occupying moist places on river banks, in lakes and swamps, where the ubiquitous *Phragmites* species (Aru, C_3) are dominant (White, 1983). The mountain grasslands are characterised by moist and relatively cool conditions so that temperate grasses of Pooideae (Po), like *Festuca* (Po, C_3) and *Bromus* (Po, C_3) become dominant (Bayer, 1959; White, 1983). Thus, the only regions where C_3 grass is more abundant than the C_4 species are the highest parts of the Drakensberg mountains and the winter rainfall areas on the west and south coast of Cape Fynbos, with outliers on the mountains (Fig. 1c; Vogel et al., 1978; Ellis et al., 1980; Werger and Ellis, 1981).

2.2. Samples

For this study we selected thirty-five C_4 and three C_3 grass samples of the most abundant types in southern Africa. Nine grasses belong to the Aristidoideae, 15 to the Chloridoideae, 11 to the Panicoideae and 3 to the Pooideae subfamilies. Grass samples were made available by Dr. R.W. Mayes of the Macaulay Institute in Aberdeen (United Kingdom), Prof. E.-D. Schulze of the Max-Planck Institute of Biogeochemistry in Jena (Germany) and A. Gerecht of the University of Oldenburg (Germany). They collected grasses on different field trips for similar lipid and stable carbon isotopic investigations. Where typical African grass species were not available, we used species of other tropical or subtropical sampling sites in the world. Grasses from R.W. Mayes were collected by D. Smith, R.W. Mayes, H. Ali, M. Daniels and their colleagues during wet seasons on different field trips in Zimbabwe, Tanzania, Sudan, Peru and Australia. Species assignment was performed by using the field guides of Clayton and Renvoize (1986) and Gibbs Russell et al. (1991). The samples were dried at 65 °C, ground and stored in annealed glass. E.-D. Schulze collected plants on a field trip in Namibia in the dry season of 1964. Prof. O. Volk, University of Würzburg (Germany), determined the species. After drying the samples were stored between pieces of paper in a herbarium. A. Gerecht collected grasses in Namibia during the dry season of 2004. The grass species were determined by using the field guide of van Oudtshoorn (1999).

Information about the analysed species, sampling locality and season is compiled in Table 1. Of *Aristida meridionalis*, *Chloris virgata*, *Enneapogon cenchroides*, *Panicum maximum* and *Festuca orthophylla* we used duplicate samples collected during different field trips. Grasses collected during the dry seasons were mainly strawy. Three of the grasses had lost their reproductive parts (cf. Table 1). In general, however, complete grass plants were analysed. Sufficient material of *Sporobolus* sp. and *Brachiaria* sp. (Table 1) was available to separate them

Table 1
Grass species studied: subfamily, tribus, subtribus, species and photosynthetic group membership of analysed subspecies as well as C_4 physiology, sample locality and sampling season

Subfamily	Tribus	Subtribus	Species	Photo-synthetic pathway	Preferred habitat and climate	SW African dispersal				Analysed *Subspecies*	C_4 Physiology	Locality[a] (sampling season)
						West African Rainforest	Namib-Karoo	South Tropical Africa	Kalahari			
Aristidoideae	Aristideae		*Aristida* L.	C_4	Temperate and subtropical	×	×	×	×	*A. adscensionis*[b]	NADP–ME	Namibia[2] (dry)
										A. barbicollis	NADP–ME	Zimbabwe[1] (wet)
										A. congesta	NADP–ME	Namibia[2] (dry)
										A. graciliflora	NADP–ME	Zimbabwe[1] (wet)
										A. meridionalis	NADP–ME	Zimbabwe[1] (wet), Namibia[2] (dry)
			Stipagrostis Nees	C_4	Desert and semidesert, sometimes dunes (*S. ciliate*, a sandbinder)		×	×	×	*S. ciliate*	NAD–ME	Namibia[2] (dry)
										S. hirtigluma	NAD–ME	Namibia[2] (dry)
										S. uniplumis	NAD–ME	Namibia[2] (dry)
Chloridoideae	Cynodonteae	Chloridinae	*Chloris* O. Swartz	C_4	Tropical and warm temperate, diverse habitats, mostly in short grassland on poor soils or disturbed ground	×	×	×	×	*C. gayana*	PCK	Zimbabwe[1] (wet)
										C. virgata	PCK	Zimbabwe[1] (wet), Namibia[2] (dry)
	Eragrostideae	Eleusiminae	*Eragrostis* N.M. Wolf	C_4	Cosmopolitan, subtropical, mostly open habitats, often on poor or sandy soils or disturbed ground	×	×	×	×	*E. nindensis*	NAD–ME	Namibia[2] (dry)
										E. superba	NAD–ME	Zimbabwe[1] (wet)
										E. tremula	NAD–ME	Sudan[1] (wet)
										E. violacea de winter	NAD–ME	Zimbabwe[1] (wet)
										E. viscosa	NAD–ME	Zimbabwe[1] (wet)
		Sporobolinae	*Sporobolus* R.Br.	C_4	Tropical and warm temperate, diverse habitats including coastal sand dunes	×	×	×	×	*S. ioclados*	NAD–ME	Zimbabwe[1] (wet)
										S. pyramidalis	PCK	Zimbabwe[1] (wet)
										Sporobolus sp.	(?)	Tanzania[1] (wet)
	Pappophoreae		*Enneapogon* Desv. Ex P. Beauv.	C_4	In warm regions, open habitats, bushland and semidesert		×	×	×	*E. cenchroides*	NAD–ME	Zimbabwe[1] (wet), Namibia[2] (dry)
										Enneapogon sp.	NAD–ME	Namibia[3] (dry)
			Schmidtia Steud.	C_4	Tropical and dry, open habitats, woods and bushland, on dry sandy soils		×	×	×	*S. kalahariensis*[b]	PCK	Namibia[2] (dry)
Panicoideae	Andropogoneae	Andropogoninae	*Bothriochloa* Kuntze	C_4	Warm regions, open habitats, grassy places	×	×	×		*B. insculpta*	NADP–ME	Zimbabwe[1] (wet)
			Hyparrhenia Anderss.	C_4	Commonly adventive, open habitats, savannah	×	×	×	×	*H. filipendula*	NADP–ME	Zimbabwe[1] (wet)

(*continued on next page*)

Table 1 (*continued*)

Subfamily	Tribus	Subtribus	Species	Photo-synthetic pathway	Preferred habitat and climate	SW African dispersal				Analysed *Subspecies*	C_4 Physiology	Locality[a] (sampling season)
						West African Rainforest	Namib -Karoo	South Tropical Africa	Kalahari			
			Themeda Forssk.	C_4	Commonly adventive, open habitats, warm	×	×			*T. triandra*	NADP–ME	Zimbabwe[1] (wet)
	Arundinelleae		*Loudetia* Hochst.	C_4	Tropical, open habitats, in savannah, woodland, often on poor shallow soils	×	×	×	×	*L. simplex*	NADP–ME	Zimbabwe[1] (wet)
	Paniceae	Digitariinae	*Digitaria* Haller	C_4	Mainly warm regions, mostly open habitats including weedy ground and sandy beaches	×	×	×	×	*D. milanjiana*	NADP–ME	Zimbabwe[1] (wet)
		Setariinae	*Brachiaria* (trin.) Gribseb.	C_4	Shade species or of open habitats	×	×	×	×	*B. erucitormis* *Brachiaria* sp.	PCK PCK	Zimbabwe[1] (wet) Tanzania[1] (wet)
			Panicum L.	C_4	Tropical, subtropical and warm temperate, commonly adventive, shade and open habitats	×	×	×	×	*P. arbusculum*[b] *P. maximum* *Panicum* sp.	NAD–ME PCK (?)	Namibia[2] (dry) Zimbabwe[1] (wet), Namibia[2] (dry) Namibia[3] (dry)
Pooideae	Bromeae		*Bromus* L.	C_3	Temperate and tropical mountains, commonly adventive, shade and open habitats	×		×		*Bromus* sp.		Australia[1] (wet)
	Poeae		*Festuca* L.	C_3	Worldwide temperate and mountains, commonly adventive, hillsides, mountains, plains and meadows	×		×		*F. orthophylla*		Peru[1] (wet)

Taxonomical classification after Clayton and Renvoize (1986), Watson and Dallwitz (1992a,b onwards) and Grass Phylogeny Working Group (2001); photosynthetic pathway, habitat and dispersal areas in southwest Africa after Watson and Dallwitz (1992a,b onwards) as well as C_4 physiology according to Ellis (1977), Watson and Dallwitz (1992a,b onwards) and Schulze et al. (1996).

[a] Grasses were made available by: ^{1} = R.W. Mayes, ^{2} = E.-D. Schulze, ^{3} = A. Gerecht.

[b] Sample without inflorescence part.

into flower heads, stems and leaves and, thus, to estimate the contribution of different plant parts to the whole wax signal of these species.

To extend the significance of our results by comparison, we collected analytical data of odd-carbon-numbered n-C_{27} to n-C_{33} alkanes and even-carbon-numbered free n-alkanols of grass waxes from the literature. Data of 291 grasses from different sampling sites in the world were used to evaluate the general characteristics in wax lipid distribution patterns of a given subfamily as well as averages of the three C_4 subtypes (cf. Figs. 4–6, and their legends).

2.3. Analytical and evaluation methods

The analytical procedures were similar to those previously described by Mangelsdorf et al. (2000) apart from the details noted below. For lipid analyses grass aliquots of 0.5–2 g were extracted. Two different extraction methods, optimised to ascertain comparability with respect to efficiency and selectivity, were used. E.-D. Schulze's and A. Gerecht's non-ground grass samples were extracted ultrasonically using a mixture of dichloromethane and methanol (99/1, v/v; 5 times 60 ml, each 60 s). R.W. Mayes' ground grass samples were extracted in an accelerated solvent extractor (ASE) using dichloromethane and methanol (99/1, v/v; three times 70 bar and 100 °C, each 5 min). Squalane, erucic acid (n-$C_{22:1}$), 5α-androstan-17-one and 5α-androstan-3β-ol were added to the extracts as internal standards. The n-hexane-soluble plant lipids were separated by medium-pressure liquid chromatography (Radke et al., 1980) into fractions of aliphatic/alicyclic hydrocarbons, aromatic hydrocarbons and polar heterocomponents (NSO). Carboxylic acids were separated from the NSO fraction by using a column with potassium hydroxide-impregnated silica gel.

n-Alkanols were isolated by urea adduction. The lipid fraction was dissolved in 1 ml n-hexane. To facilitate crystallisation, 3 μg of n-dotetracontane (n-$C_{42}H_{86}$) were added. While shaking, 1.0 ml of acetone and 1.5 ml of a saturated methanolic urea solution was added. Crystallisation of the urea adduct started immediately. During a short heating period the crystals redissolved and then recrystallised slowly by cooling over night. The crystals were dried under a stream of nitrogen without heating. The nonadduct fraction was extracted four times with 8 ml of n-hexane. After each addition of n-hexane, the sample was dispersed ultrasonically (30 s) and centrifuged (10 min at 3000 min^{-1}). The solution above the crystals was removed each time using a pipette. The entire adduction procedure was repeated with the resulting crude nonadduct. Both adduct fractions were then dissolved in 2 ml water, combined and extracted five times with 4 ml n-hexane. The solution was dried with sodium sulphate (1 h) and filtered. The solvent was removed under reduced pressure. Urea adduction was repeated once with this adduct fraction. n-Alkanols were converted to their trimethylsilyl ether derivatives before analysis by gas chromatography (GC).

The wax lipids were analysed by gas chromatography with a flame ionisation detector (GC-FID), gas chromatography–mass spectrometry (GC–MS), and a GC coupled to a Finnigan MAT 252 isotope mass spectrometer for compound-specific stable carbon isotope ratios (GC–irm-MS). The purity of lipid fractions for isotopic measurements was checked by GC–MS. GC–irm-MS analyses were run in duplicate or triplicate with standard deviations better than 0.5‰. Isotopic ratios are expressed as $\delta^{13}C$ values in per mil relative to the V-PDB standard. Contents as well as $\delta^{13}C$ values of n-alkanols are corrected for the contribution of the trimethylsilyl group from derivatisation. The $\delta^{13}C$ values are expressed as single weighted mean averages for the odd-carbon-numbered n-C_{27} to n-C_{35} alkanes ($\delta^{13}C_{WMA27\text{–}35}$) as well as for the even-carbon-numbered n-C_{22} to n-C_{32} alkanols ($\delta^{13}C_{WMA22\text{–}32}$) in order to encompass the variability of data for individual homologues.

Plant wax biomarker contents were calculated as $\mu g\,g^{-1}$ dry plant material (DM) based on signal intensities of biomarkers and internal standards in the GC-FID traces. For an assessment of systematics in the distribution patterns of biomarkers, contents of individual homologues were converted into percentage of that biomarker within the homologous series to allow comparison of samples after averaging. In order to find systematic patterns of chemical composition, we performed an agglomerate hierarchical cluster analysis by using the SYSTAT 11 software for Windows and the minimum variance Ward linkage (squared Euclidean distances; for more details see Kaufman and Rousseeuw, 1990, as well as Legendre and Legendre, 1998).

Seven aliphatic/alicyclic fractions of grass lipid samples were contaminated by fossil fuel refinery products (Tables 2a and 3), albeit in a small proportion relative to the main n-alkane homologues. For a correction of the distribution patterns and

Table 2a

n-Alkane data of grass subspecies studied: subfamily, subspecies, sampling location, individual *n*-alkane content in μg g^{-1} dry matter (DM), $TCOC_{27–35}$, $CPI_{27–35}$ and $ACL_{27–35}$ values

	Subfamily/*Subspecies*	Sampling location	*n*-Alkane content[a](μg g^{-1} DM)											$TCOC_{27–35}$[b]	$CPI_{27–35}$[c]	$ACL_{27–35}$[d]
			26	27	28	29	30	31	32	33	34	35	36			
Whole plant samples	Aristidoideae															
	Aristida adscensionis[e,f]	Namibia	7.4	17.8	8.6	63.3	16.9	**411.1**	12.4	80.4	2.6	5.3	n.d.	577.9	14.1	30.97
	Aristida barbicollis	Zimbabwe	0.6	13.0	2.1	30.3	4.4	**112.2**	4.5	49.4	1.5	6.9	n.d.	211.8	16.2	31.07
	Aristida congesta	Namibia	3.1	19.4	3.9	50.5	7.8	**225.5**	6.2	62.8	2.3	6.8	n.d.	365.0	17.1	30.93
	Aristida graciliflora	Zimbabwe	0.6	4.6	1.5	45.1	7.8	**230.6**	5.6	44.2	0.6	3.4	n.d.	327.8	20.9	30.98
	Aristida meridionalis	Zimbabwe	1.2	17.2	3.1	27.4	3.4	**44.9**	2.0	12.4	0.4	1.0	n.d.	103.0	10.6	30.08
	Aristida meridionalis	Namibia	3.7	26.9	4.4	31.7	4.0	**62.3**	2.8	21.4	0.5	1.3	n.d.	143.6	11.3	30.14
	Stipagrostis ciliata[e]	Namibia	2.0	4.3	0.9	8.1	1.3	**69.3**	1.2	25.2	0.1	1.0	n.d.	107.9	30.2	31.19
	Stipagrostis hirtigluma[e]	Namibia	14.1	87.2	29.9	156.1	32.8	**593.9**	23.5	162.3	5.9	11.3	n.d.	1010.8	10.5	30.71
	Stipagrostis uniplumis	Namibia	4.7	18.7	3.5	41.4	4.7	**100.8**	2.6	16.5	0.5	1.5	n.d.	178.8	14.9	30.63
	Chloridoideae															
	Chloris gayana	Zimbabwe	1.5	20.6	4.2	35.3	6.3	188.3	10.4	**287.6**	8.9	63.4	n.d.	595.2	18.5	32.14
	Chloris virgata	Zimbabwe	2.0	17.8	3.5	27.5	3.6	42.2	2.8	**51.9**	3.3	22.1	n.d.	161.5	10.7	31.41
	Chloris virgata[e]	Namibia	3.2	13.8	3.2	15.1	3.1	**26.4**	2.2	16.2	2.3	14.0	0.9	85.5	6.7	31.03
	Enneapogon sp.	Namibia	3.1	30.5	5.3	69.8	11.0	352.4	13.7	**407.9**	7.9	79.8	1.4	940.4	23.7	31.93
	Enneapogon cenchroides	Zimbabwe	0.7	9.4	2.2	30.4	5.0	**215.4**	6.9	200.1	3.2	21.2	0.6	476.6	26.7	31.81
	Enneapogon cenchroides[e]	Namibia	4.3	14.9	2.9	21.9	3.7	**103.6**	2.3	32.7	1.0	3.1	n.d.	176.2	16.8	30.85
	Eragrostis nindensis	Namibia	3.7	12.1	2.7	14.9	2.1	**33.7**	2.3	29.3	1.2	5.2	n.d.	95.2	10.2	31.01
	Eragrostis superba	Zimbabwe	2.8	**60.2**	6.1	44.7	3.4	42.1	3.1	54.7	2.4	14.5	0.2	216.2	11.8	30.25
	Eragrostis tremula	Sudan	2.2	17.1	3.5	41.8	7.4	**178.4**	5.6	78.2	1.6	8.0	n.d.	323.3	17.1	31.11
	Eragrostis violacea de winter	Zimbabwe	1.5	25.5	4.7	31.7	7.3	**104.0**	4.8	76.8	1.9	8.4	0.4	246.5	12.3	31.09
	Eragrostis viscosa	Zimbabwe	7.1	79.5	11.5	**149.1**	12.8	139.4	7.5	77.7	2.3	7.1	0.3	452.7	12.0	30.05
	Schmidtia kalahariensis[e,f]	Namibia	1.4	61.6	18.6	35.6	13.6	225.6	19.8	**805.6**	16.2	166.9	1.3	1295.3	17.0	32.51
	Sporobolus ioclados	Zimbabwe	2.1	28.0	3.4	41.3	5.9	116.5	6.5	**119.6**	3.4	16.8	1.0	322.2	15.5	30.90
	Sporobolus pyramidalis	Zimbabwe	2.8	66.6	9.0	95.8	11.1	185.2	10.2	**222.4**	6.0	41.1	1.3	611.1	15.4	31.25
	Panicoideae															
	Bothriochloa insculpta	Zimbabwe	1.9	20.7	3.8	24.5	3.5	**39.5**	1.7	18.5	0.5	2.4	n.d.	105.6	9.8	30.19
	Brachiaria erucitormis	Zimbabwe	1.1	3.9	1.4	8.1	1.8	34.1	3.1	**64.2**	1.0	2.9	n.d.	113.1	15.1	31.96
	Digitaria milanjiana	Zimbabwe	0.8	18.4	3.9	44.2	6.3	102.6	6.3	**113.9**	4.4	36.1	0.9	315.1	13.8	31.67
	Hyparrhenia filipendula	Zimbabwe	2.7	37.7	5.5	43.6	6.6	183.1	13.3	**265.6**	8.1	59.4	n.d.	589.4	16.2	31.90
	Loudetia simplex	Zimbabwe	0.8	8.6	1.7	23.2	2.8	**76.1**	2.4	41.3	1.4	9.9	n.d.	159.0	18.1	31.26
	Panicum sp.	Namibia	5.3	39.7	6.5	68.2	9.7	145.1	11.2	**236.1**	10.4	117.2	0.8	606.2	13.9	32.06
	Panicum arbusculum[e,f]	Namibia	5.3	12.1	5.2	20.7	6.8	146.3	15.4	**357.0**	13.9	95.7	1.0	631.8	14.0	32.59
	Panicum maximum	Zimbabwe	2.1	36.2	4.3	51.1	5.2	**81.9**	2.8	24.5	0.6	2.4	n.d.	196.0	13.7	30.04
	Panicum maximum	Namibia	3.2	15.1	3.5	40.5	5.3	**84.4**	4.1	34.4	0.9	2.4	0.3	176.8	12.0	30.64
	Themeda triandra	Zimbabwe	1.8	35.2	2.9	25.2	3.9	**69.2**	3.2	33.6	1.2	4.8	n.d.	168.1	13.2	30.38

Plant part samples	Pooideae														
	Bromus sp.	Australia	1.1	14.6	2.1	49.9	3.6	**95.3**	2.5	63.3	0.9	14.5	n.d.	237.6	24.6	31.11
	Festuca orthophylla	Peru	1.6	29.1	4.6	**89.4**	1.9	14.7	n.d.	3.8	n.d.	1.4	n.d.	138.5	18.9	28.96
	Festuca orthophylla	Peru	1.6	21.6	4.8	**88.6**	3.0	28.3	n.d.	4.3	n.d.	n.d.	n.d.	142.9	16.9	29.21
	Chloridoideae															
	Sporobolus sp. (flowers)	Tanzania	5.7	92.7	9.7	98.1	8.8	176.0	7.8	**216.3**	4.3	27.4	0.6	610.4	17.9	30.96
	Sporobolus sp. (leaves)	Tanzania	1.1	4.4	1.6	8.2	2.1	23.7	1.8	**32.9**	1.1	10.6	0.5	79.6	11.0	31.93
	Sporobolus sp. (stems)	Tanzania	0.7	1.4	0.6	2.5	0.5	8.5	0.6	**15.8**	0.5	6.7	0.3	34.8	13.4	32.37
	Panicoideae															
	Brachiaria sp. (flowers)	Tanzania	14.7	**238.7**	16.3	226.5	10.1	202.9	8.2	147.7	7.2	43.9	0.0	859.8	17.2	29.91
	Brachiaria sp. (leaves)	Tanzania	1.5	12.5	1.8	17.3	2.6	59.1	4.2	**101.8**	3.1	35.2	0.9	226.0	17.4	32.15
	Brachiaria sp. (stems)	Tanzania	0.5	2.1	0.5	6.4	0.9	23.2	1.4	**31.9**	1.1	12.5	0.4	76.1	17.5	32.21

Boldface content values of individual *n*-alkanes refer to distribution pattern maxima.

n.d., not determined.

[a] Numbers according to individual *n*-alkane carbon numbers.

[b] $TCOC_{27–35}$: Total content of odd- carbon-numbered *n*-C_{27} to *n*-C_{35} alkanes in μg/g DM.

[c] $CPI_{27–35}$: Carbon preference index of *n*-alkanes (carbon number 27–35).

[d] $ACL_{27–35}$: Averaged chain length of odd-carbon-numbered *n*-alkanes (carbon number 27–35).

[e] After Huang et al. (2000) corrected *n*-alkane contents (see text for details).

[f] Sample without inflorescence part.

$\delta^{13}C$ values we applied the mass balance approach of Huang et al. (2000). Generally, the odd-carbon-number predominance of wax *n*-alkanes of terrestrial higher plants is expressed as a high carbon preference index ($CPI > 5$; e.g., Collister et al., 1994), whereas oil-derived *n*-alkanes have no significant odd-over-even carbon number predominance and, thus, a CPI close to 1 (Bray and Evans, 1961). In our slightly contaminated samples we detected a decrease in CPI values and a shift to lighter $\delta^{13}C$ values in the range of the *n*-C_{19} to *n*-C_{29} alkanes. The contamination maximised at the *n*-C_{23} alkane. For correction, we assumed that (a) due to significant variations in CPI of higher land plants the averaged CPI of our unpolluted grass samples ($CPI_{av} = 15.6$) represents the typical grass wax CPI, (b) the *n*-alkanes of the fossil fuel have a CPI of 1, and (c) the averaged $\delta^{13}C$ values of the *n*-C_{20} to *n*-C_{24} alkanes in each polluted sample represent the stable carbon isotopic composition of the fossil fuel *n*-alkanes. Assuming that the relative contribution of contamination to the *n*-C_n and *n*-C_{n+1} alkanes ($n = 25, 27, 29$) is equal, we can write:

$$CPI_{av} = \frac{A_{W,n}}{A_{W,n+1}} = \frac{A_n - A_{x,n}}{A_{n+1} - A_{x,n}}$$

or

$$A_{x,n} = \frac{CPI_{av} \times A_{n+1} - A_n}{CPI_{av} - 1},$$

where n and $n + 1$ refer to *n*-C_n and *n*-C_{n+1} alkanes, A = abundance, W = grass wax *n*-alkane, x = contamination. A_n and A_{n+1} are the measured abundances of *n*-C_n and *n*-C_{n+1} alkanes in grass waxes and $A_{x,n}$ is the abundance of the *n*-C_n alkane in the fossil fuel (after Huang et al., 2000). Simple subtraction of $A_{x,n}$ from A_n leads to $A_{W,n}$. By using $A_{x,n}$ and a bimodal mixture model we corrected the molecular $\delta^{13}C$ values in the following way:

$$\delta^{13}C_n \times A_n = \delta^{13}C_{W,n} \times (A_n - A_{x,n}) + \delta^{13}C_x \times A_{x,n},$$

where $\delta^{13}C_n$ refers to the measured $\delta^{13}C$ value of the *n*-C_n alkane and $\delta^{13}C_x$ refers to the averaged $\delta^{13}C$ values of the *n*-C_{20} to *n*-C_{24} alkanes in the same sample.

3. Results

3.1. *Contents and carbon number distributions of long-chain n-alkanes of grass waxes*

Lipids in the fractions of the aliphatic/alicyclic hydrocarbons of the organic solvent extracts of

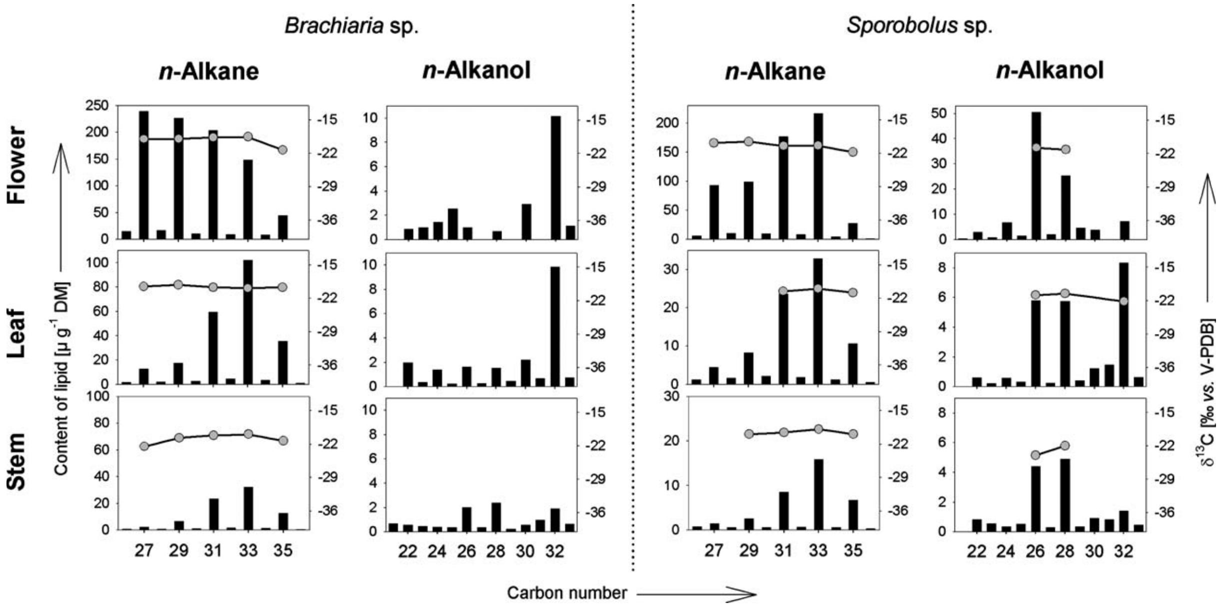
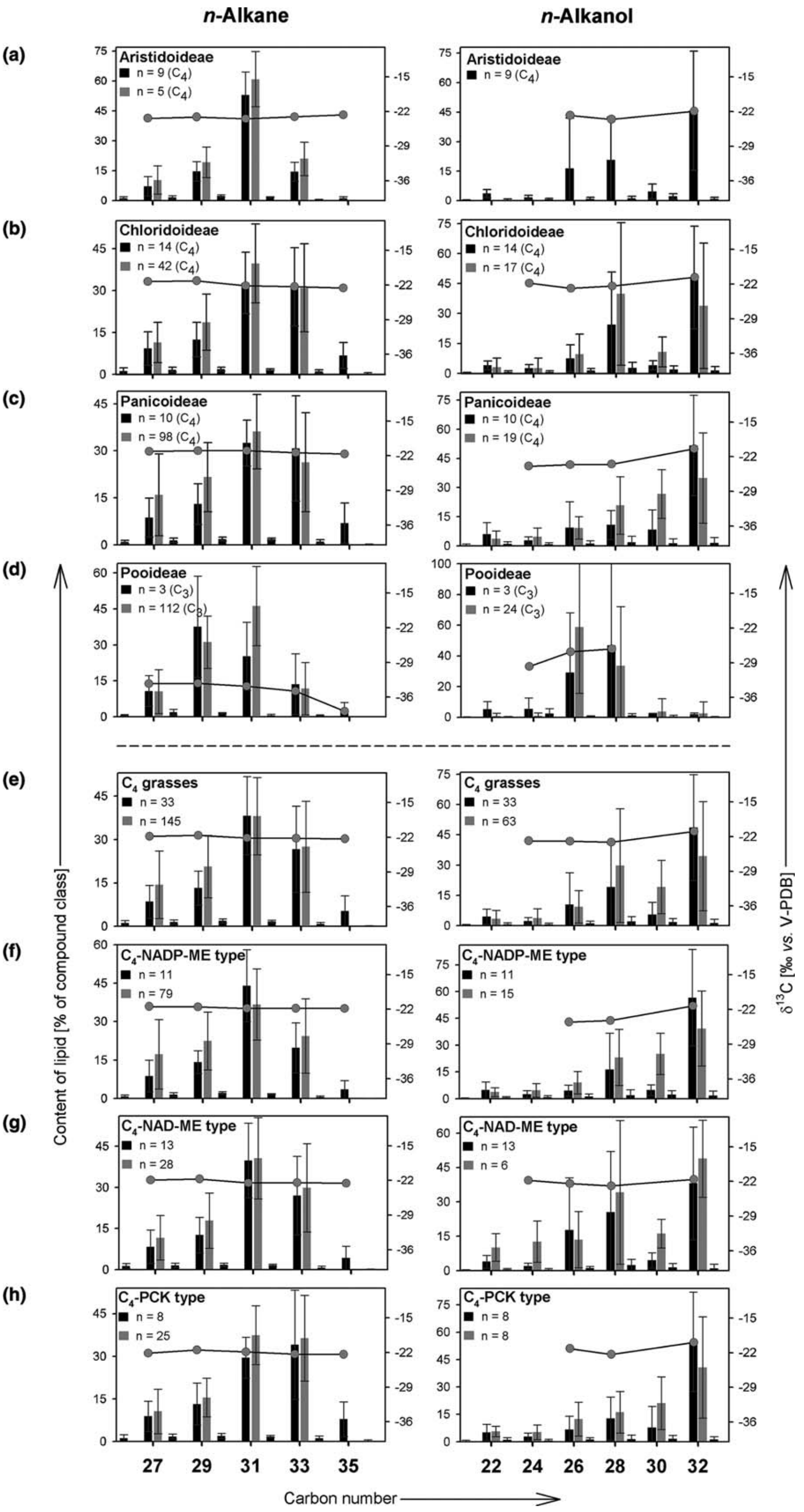


Fig. 3. Histogram representations of long-chain *n*-alkane and *n*-alkanol contents (in μg g^{-1} DM, left *Y*-axis), overlain by molecular stable carbon isotope data (○; $\delta^{13}C$ in ‰ versus V-PDB, right *Y*-axis) of individual homologues in waxes of grass flowers, leaves and stems of one species each of *Brachiaria* (Panicoideae, C_4) and *Sporobolus* (Chloridoideae, C_4). The histograms of the compounds in the flowers are individually normalised to the most abundant homologue, whereas each leaf and stem pair are normalised together to the most abundant homologue.

grass samples are dominated by homologous series of *n*-alkanes. They have 27–35 carbon atoms in the long-chain range. Shorter- and longer-chain *n*-alkanes were detected, but they are present in small quantities and, thus, have less chemotaxonomic significance. The CPI_{27-35} values are high and range from 6.7 to 30.2 (Table 2a).

The total content of odd-carbon-numbered *n*-C_{27} to *n*-C_{35} alkanes ($TCOC_{27-35}$) of whole grass samples varies significantly between 85.5 μg g^{-1} DM and 1295 μg g^{-1} DM. Of the plant parts studied separately, the flower heads of *Brachiaria* sp. and *Sporobolus* sp. have the highest $TCOC_{27-35}$ values (860 and 610 μg g^{-1} DM, respectively), followed by leaves (226 and 80 μg g^{-1} DM), whereas stems have less of these alkanes (76.1 and 34.8 μg g^{-1} DM; Table 2a). The total *n*-alkane contents of grasses may be affected by different contributions of flowers, leaves and stems. In the present study, limited to two species, analysis reveals that flower heads contain markedly higher amounts of the *n*-C_{27} and *n*-C_{29} alkanes compared to leaves and stems of the same species (Fig. 3). Especially in the *Brachiaria* sp. sample the distribution pattern maxima differ significantly, i.e., they shift from *n*-C_{33} in the leaves and stems to the *n*-C_{27} alkane in the flowers. In addition, the flowers contain significant amounts of shorter-chain *n*-alkanes (an additional 16% of the

Fig. 4. Averaged histogram representations of long-chain *n*-alkane and *n*-alkanol content (content in % of compound class, left *Y*-axis) based on data for odd *n*-C_{27} to *n*-C_{35} alkanes and even *n*-C_{22} to *n*-C_{32} alkanols (black bars), overlain by molecular stable carbon isotope data (○; $\delta^{13}C$ in ‰ versus V-PDB, right *Y*-axis) of individual homologues (this study) as well as averaged histogram representation of published data of odd *n*-C_{27} to *n*-C_{33} alkanes and even *n*-C_{22} to *n*-C_{32} alkanols (grey bars; from Smith and Martin-Smith, 1978; Tulloch, 1981, 1982, 1984; Spencer and Chapman, 1985; Mayes et al., 1986; Dove et al., 1990; Malossini et al., 1990; Dove and Mayes, 1991; Laredo et al., 1991; Dove, 1992; Mayes et al., 1994; Dove et al., 1996; Maffei, 1996; Chen et al., 1998; Dawson et al., 2000; Delgado et al., 2000; Smith et al., 2001; Boadi et al., 2002; Ali, 2003; Chikaraishi and Naraoka, 2003, unpublished data). Displayed are averages of four grass subfamilies (a–d) and different types of C_4 photosynthesis (e–h). Data of duplicate samples are averaged. The diagrams are individually normalised to the most abundant homologue. *n*, number of species used for the averaging of data from this study (■) and from published data (▨).



n-Alkane
n-Alkanol
(a)
Aristidoideae
n = 9 (C4)
n = 5 (C4)
Aristidoideae
n = 9 (C4)
(b)
Chloridoideae
n = 14 (C4)
n = 42 (C4)
Chloridoideae
n = 14 (C4)
n = 17 (C4)
(c)
Panicoideae
n = 10 (C4)
n = 98 (C4)
Panicoideae
n = 10 (C4)
n = 19 (C4)
(d)
Pooideae
n = 3 (C3)
n = 112 (C3)
Pooideae
n = 3 (C3)
n = 24 (C3)
(e)
C4 grasses
n = 33
n = 145
C4 grasses
n = 33
n = 63
(f)
C4-NADP-ME type
n = 11
n = 79
C4-NADP-ME type
n = 11
n = 15
(g)
C4-NAD-ME type
n = 13
n = 28
C4-NAD-ME type
n = 13
n = 6
(h)
C4-PCK type
n = 8
n = 25
C4-PCK type
n = 8
n = 8
0
15
30
45
60
75
80
100
-15
-22
-29
-36
27
29
31
33
35
22
24
26
28
30
32
Content of lipid [% of compound class]
δ13C [‰ vs. V-PDB]
Carbon number

n-C_{25} and 1% of the n-C_{23} alkane; cf. Fig. 3). The overall chain length distribution, best expressed by the average chain length parameter in the odd-carbon-number range 27–35 ($ACL_{27–35}$; Poynter et al., 1989), is more or less the same in leaves and stems. The $ACL_{27–35}$ values decrease from around 32.17 for stem and leaf n-alkanes in *Bracharia* sp. and *Sporobolus* sp. to 29.91 and 30.96 for flower head n-alkanes (Table 2a). Thus, the amount of shorter-chain n-alkane homologues outside the main long-chain range of the whole plant wax n-alkane distribution can be described by the flower/leaf alkane ratio.

The n-alkane distribution patterns of whole grass plant waxes maximise either at the n-C_{29}, n-C_{31} or n-C_{33} alkane (Table 2a). The subspecies of *Aristida*, *Stipagrostis* and *Sporobolus*, as well as the duplicate samples of *Aristida meridionalis*, *Festuca orthophylla* and *Panicum maximum* exhibit consistent n-alkane patterns. The members of other subspecies have n-alkane distribution patterns which resemble each other, but are not constant. Variations on the species level are difficult to evaluate due to inconsistent flower/leaf ratios and different habitats and sampling seasons of the grasses. However, on a subfamily level the distribution patterns are more systematic, e.g., the C_4 species of Aristidoideae always show a distribution maximum at the n-C_{31} alkane. The n-alkanes of grasses from the other C_4 subfamilies (Chloridoideae and Panicoideae) have a maximum either at the n-C_{31} or the n-C_{33} homologue. On the other hand, the two different C_3 species of the Pooideae subfamily contain more abundant shorter-chain n-alkanes, and the long-chain homologues maximise at n-C_{29} or n-C_{31} (Table 2a).

Averaged n-alkane distribution patterns of subfamilies and plants having the same photosynthetic pathway or physiology are shown in Fig. 4; numerical data of averaged proxies are compiled in Table 4. The subfamilies have averaged $ACL_{27–35}$ values, which increase from 30.10 for Pooideae to 30.74 for Aristidoideae, 31.24 for Chloridoideae and 31.27 for Panicoideae (Table 4). Generally, the averaged distribution patterns separate the Pooideae from the subfamilies comprising the C_4 grasses. Thus, distributions maximising at slightly shorter-chain n-alkanes are typical of C_3 grasses, whereas longer-chain maxima characterise C_4 grasses (Fig. 4d and e). A further separation was observed between the aristidoid C_4 species and the remaining C_4 containing subfamilies. The chloridoids and panicoids are similar in averaged n-alkane distribution patterns (Fig. 4a–c). Within the three C_4 subtypes the n-C_{33} alkane contents increase from the NADP–ME to the NAD–ME and PCK subtypes (Fig. 4f–h). Accordingly, the average $ACL_{27–35}$ values increase from 30.87 (NADP–ME) to 31.09 (NAD–ME) and 31.37 (PCK; Table 4).

3.2. Molecular carbon isotope signatures of long-chain n-alkanes of grass waxes

The compound-specific $\delta^{13}C$ values of the individual odd-carbon-numbered n-alkanes (Table 3, Figs. 3 and 4) are consistent with their origins in waxes of C_3 and C_4 higher land plants. The weighted mean average of $\delta^{13}C$ values ranges from −18.7‰ to −25.8‰ and centres at − 21.7‰ for C_4 grasses, compared to a range of −30.4‰ to −36.4‰ and a centre at −33.8‰ for the few C_3 grasses examined (Tables 3 and 4). The wax n-alkanes of individual plant parts exhibit no significant distinctions in the $\delta^{13}C_{WMA27–35}$ values for the two species (*Bracharia* sp. and *Sporobolus* sp.) sampled (Table 3, Fig. 3).

Three characteristic $\delta^{13}C_{WMA27–35}$ ranges occur within the subfamilies. As expected, the lightest values belong to the C_3 pooid grasses. The $\delta^{13}C_{WMA27–35}$ values of the C_4 species separate the aristidoid grasses from the other C_4 subfamilies by the former having about 2‰ lighter $\delta^{13}C$ values (Table 4). The variations of $\delta^{13}C_{WMA27–35}$ within the C_4 subtypes spread from −21.3‰ for NADP–ME subtype species to −21.7‰ for NAD–ME subtypes and to −22.0‰ for PCK-subtypes (Table 4). This trend differs slightly from that of bulk $\delta^{13}C$ values of grasses of the three C_4 subtypes determined by Hattersley (1982) and Schulze et al. (1996). The differences among the $\delta^{13}C_{WMA27–35}$ values of the subtypes are within the standard deviation of molecular $\delta^{13}C$ values of 0.5‰, i.e., significantly smaller than the standard deviation of 1.3‰ and 2.0‰ of the averaged values (Table 4), which suggests that carbon isotope values cannot be used reliably for chemotaxonomic classification at this level.

3.3. Contents and carbon number distributions of long-chain n-alkanols of grass waxes

Free wax n-alkanols exhibit a pronounced even-over-odd carbon number predominance of long-chain components, which mainly comprise the n-C_{22} to n-C_{32} homologues. The n-alkanol $CPI_{22–32}$

Table 2b

n-Alkanol data of grass subspecies studied: subfamily, subspecies, sampling location, individual n-alkanol content in µg g^{-1} dry matter (DM); TCEC$_{22–32}$, CPI$_{22–32}$, and ACL$_{22–32}$ values

	Subfamily/*Subspecies*	Sampling location	n-Alkanol content[a](µg g^{-1}DM)													TCEC$_{22-32}$[b]	CPI$_{22-32}$[c]	ACL$_{22-32}$[d]
			21	22	23	24	25	26	27	28	29	30	31	32	33			
Whole plant samples	Aristidoideae																	
	Aristida adscensionis[e]	Namibia	0.2	1.1	0.3	0.8	0.4	1.1	0.4	3.1	0.3	1.9	0.9	**12.8**	0.2	20.8	5.9	30.07
	Aristida barbicollis	Zimbabwe	n.d.	16.3	2.7	9.3	3.1	23.7	n.d.	12.4	2.8	7.7	6.4	**257.9**	7.6	327.4	12.7	30.64
	Aristida congesta	Namibia	0.2	2.1	0.3	0.6	0.6	3.3	0.8	25.4	0.4	4.4	0.9	**45.3**	0.5	81.1	19.9	30.08
	Aristida graciliflora	Zimbabwe	0.3	4.9	0.3	1.8	n.d.	10.1	3.7	**119.8**	5.6	4.9	2.0	8.8	n.d.	150.4	12.5	27.92
	Aristida meridionalis	Zimbabwe	0.8	11.6	0.8	2.2	2.1	6.2	1.3	38.7	4.7	41.6	15.9	**676.3**	6.6	776.6	17.5	31.47
	Aristida meridionalis	Namibia	n.d.	1.3	0.1	0.3	0.2	1.0	0.2	2.7	0.4	0.2	1.6	**29.3**	0.3	34.8	7.9	31.06
	Stipagrostis ciliata	Namibia	n.d.	0.6	n.d.	2.1	1.3	**139.3**	1.3	18.6	1.3	0.4	n.d.	4.3	0.5	165.2	41.4	26.35
	Stipagrostis hirtigluma	Namibia	n.d.	6.6	n.d.	2.1	0.4	18.6	0.5	12.5	1.4	5.1	2.9	**39.9**	0.1	84.8	11.9	29.00
	Stipagrostis uniplumis	Namibia	0.1	0.8	0.1	0.4	0.1	3.5	0.1	**5.2**	0.3	2.0	0.3	3.3	0.4	15.2	15.6	28.24
	Chloridoideae																	
	Chloris gayana	Zimbabwe	n.d.	3.3	0.9	2.7	1.2	4.4	1.6	15.9	4.2	3.6	2.7	**21.9**	2.7	51.8	3.8	29.07
	Chloris virgata	Zimbabwe	n.d.	8.6	2.5	9.0	1.2	15.8	6.7	**86.7**	9.3	12.8	4.1	82.0	6.8	215.0	7.1	29.09
	Chloris virgata	Namibia	n.d.	2.1	0.1	0.4	0.1	1.2	0.9	3.3	0.7	3.8	1.4	**95.9**	0.3	106.6	18.1	31.52
	Enneapogon sp.	Namibia	0.7	3.9	0.4	3.4	0.2	15.3	0.7	6.0	0.9	1.8	0.6	**38.5**	0.9	68.8	17.1	29.31
	Enneapogon cenchroides	Zimbabwe	0.8	20.2	1.1	6.5	0.9	16.8	3.0	14.1	8.8	11.1	n.d.	**174.5**	n.d.	243.2	10.6	30.22
	Enneapogon cenchroides	Namibia	0.1	1.0	0.2	0.4	0.3	3.1	1.1	**62.7**	0.5	0.5	0.5	25.4	n.d.	93.0	31.4	28.95
	Eragrostis nindensis	Namibia	0.1	1.3	n.d.	0.4	n.d.	1.5	0.1	5.7	0.1	4.0	0.8	**31.3**	0.1	44.3	26.4	30.72
	Eragrostis superba	Zimbabwe	0.2	3.7	0.4	3.0	n.d.	5.0	n.d.	**146.0**	15.8	4.6	n.d.	20.0	n.d.	182.4	10.5	28.24
	Eragrostis tremula	Sudan	n.d.	2.7	1.0	1.6	n.d.	2.0	0.4	1.4	n.d.	3.0	n.d.	**46.3**	n.d.	56.9	23.2	30.89
	Eragrostis violacea de winter	Zimbabwe	n.d.	1.9	0.3	0.9	0.3	2.4	0.3	2.6	1.3	1.3	1.3	**9.9**	1.6	19.0	3.7	29.18
	Eragrostis viscosa	Zimbabwe	0.3	3.8	0.4	3.2	n.d.	17.3	8.1	**209.0**	11.4	9.6	3.4	15.4	n.d.	258.4	10.6	28.04
	Schmidtia kalahariensis[e]	Namibia	n.d.	3.2	0.1	1.9	0.2	6.2	0.3	4.9	n.d.	0.5	n.d.	**87.4**	0.6	104.1	93.8	30.99
	Sporobolus ioclados	Zimbabwe	n.d.	4.7	0.9	1.9	1.2	4.3	1.9	7.7	3.8	5.6	3.2	**44.8**	1.6	69.0	4.0	30.12
	Sporobolus pyramidalis	Zimbabwe	n.d.	1.5	0.3	2.4	0.7	9.1	0.9	5.5	n.d.	1.1	1.9	**13.8**	0.7	33.4	6.8	28.62
	Panicoideae																	
	Bothriochloa insculpta	Zimbabwe	n.d.	3.7	n.d.	1.9	n.d.	2.4	n.d.	5.7	n.d.	n.d.	n.d.	**412.7**	n.d.	426.3	n.d.	31.79
	Brachiaria erucitormis	Zimbabwe	n.d.	10.6	1.1	3.3	0.7	8.3	1.2	4.5	1.1	2.6	2.4	**92.4**	2.5	121.7	30.5	30.31
	Digitaria milanjiana	Zimbabwe	n.d.	2.6	2.8	2.4	2.9	2.6	2.0	3.2	5.5	2.8	2.9	**12.5**	2.6	26.1	10.3	28.15
	Hyparrhenia filipendula	Zimbabwe	n.d.	3.0	0.9	2.4	1.5	2.8	2.1	15.8	4.4	6.8	n.d.	**38.1**	2.1	69.0	5.4	29.92
	Loudetia simplex	Zimbabwe	n.d.	0.9	0.3	0.8	0.3	1.6	0.7	2.3	1.4	1.3	0.9	**3.7**	1.1	10.5	2.3	28.57
	Panicum sp.	Namibia	0.5	2.3	0.6	2.1	1.3	12.5	1.8	21.0	3.3	7.8	1.5	**44.6**	0.3	90.4	7.9	29.63
	Panicum arbusculum[e]	Namibia	0.1	0.9	0.2	2.5	1.3	**101.0**	2.8	38.7	n.d.	5.4	0.7	66.8	n.d.	215.4	36.3	28.28
	Panicum maximum	Zimbabwe	3.2	3.4	4.5	5.4	3.3	14.4	4.4	9.3	11.1	**18.7**	4.7	n.d.	n.d.	51.2	15.3	28.52
	Panicum maximum	Namibia	0.1	1.9	n.d.	0.6	n.d.	5.9	n.d.	34.1	n.d.	19.1	1.4	**201.5**	n.d.	263.1	112.2	31.11
	Themeda triandra	Zimbabwe	0.3	1.7	0.4	1.4	0.2	0.9	n.d.	1.7	n.d.	1.7	n.d.	**28.4**	n.d.	35.8	31.6	30.79

(*continued on next page*)

Table 2b (*continued*)

	Subfamily/*Subspecies*	Sampling location	*n*-Alkanol content[a](μg g^{-1}DM)													TCEC$_{22\text{-}32}$[b]	CPI$_{22\text{-}32}$[c]	ACL$_{22\text{-}32}$[d]
			21	22	23	24	25	26	27	28	29	30	31	32	33			
	Pooideae																	
	Bromus sp.	Australia	0.9	7.2	n.d.	1.9	n.d.	7.9	2.8	**430.0**	1.9	11.2	n.d.	7.1	n.d.	465.3	99.2	27.97
	Festuca orthophylla	Peru	n.d.	20.1	3.6	49.6	20.4	**232.0**	n.d.	13.3	5.4	18.3	11.5	24.9	3.1	358.2	8.5	25.83
	Festuca orthophylla	Peru	n.d.	10.3	2.5	29.8	19.3	**224.0**	2.7	10.8	n.d.	8.8	n.d.	5.9	n.d.	280.6	8.9	25.60
Plant part samples	Chloridoideae																	
	Sporobolus sp. (flowers)	Tanzania	0.4	3.0	0.9	6.6	1.4	**50.4**	1.9	25.2	4.6	3.7	0.0	7.2	0.0	96.2	10.4	26.87
	Sporobolus sp. (leaves)	Tanzania	0.0	0.6	0.2	0.6	0.3	5.8	0.2	5.7	0.4	1.2	1.5	**8.3**	0.6	22.2	6.9	28.83
	Sporobolus sp. (stems)	Tanzania	0.0	0.8	0.5	0.3	0.5	4.4	0.3	**4.9**	0.3	0.9	0.8	1.4	0.4	12.7	4.7	27.4
	Panicoideae																	
	Brachiaria sp. (flowers)	Tanzania	0.0	0.8	1.0	1.4	2.5	1.0	0.0	0.7	0.0	2.9	0.0	**10.1**	1.1	17.0	3.3	29.98
	Brachiaria sp. (leaves)	Tanzania	0.0	1.9	0.3	1.4	0.2	1.6	0.3	1.5	0.5	2.2	0.7	**9.8**	0.7	18.4	6.4	29.27
	Brachiaria sp. (stems)	Tanzania	0.6	0.6	0.5	0.4	0.3	2.0	0.3	**2.4**	0.2	0.6	0.9	1.9	0.6	7.7	2.8	27.97

Boldface content values of individual *n*-alkanols refer to distribution pattern maxima.

n.d., not determined.

[a] Numbers according to individual *n*-alkanol carbon numbers.

[b] TCEC$_{22–32}$: Total content of even-carbon-numbered *n*-C_{22} to *n*-C_{32} alkanols in μg/g DM.

[c] CPI$_{27–33}$: Carbon preference index of *n*-alkanes (carbon number 22–32).

[d] ACL$_{22–32}$: Averaged chain length of even-carbon-numbered *n*-alkanols (carbon number 22–32).

[e] Sample without inflorescence part.

Table 3
Isotopic data for grass subspecies studied: subfamily, subspecies, photosynthetic pathway, physiology (according to Ellis, 1977; Watson and Dallwitz, 1992a,b onwards; Schulze et al., 1996), sampling location, and molecular stable isotopic composition of *n*-alkanes and *n*-alkanols

	Subfamily/*Subspecies*	Photosynthetic pathway	Physiology	Sampling location	δ^{13}C of *n*-alkanes[a] (‰ vs. V-PDB)						δ^{13}C of *n*-alkanols[a] (‰ vs. V-PDB)						
					27	29	31	33	35	$\delta^{13}C_{WMA\text{-}27\text{–}35}$[b]	22	24	26	28	30	32	$\delta^{13}C_{WMA\text{-}22\text{–}32}$[b]
Whole plant samples	Aristidoideae																
	Aristida adscensionis[c]	C_4	NADP–ME	Namibia	−29.0	−24.9	−25.3	−23.8	n.d.	−25.2	n.d.	n.d.	n.d.	−24.4	n.d.	−24.1	−24.1
	Aristida barbicollis	C_4	NADP–ME	Zimbabwe	−20.0	−21.4	−23.9	−22.1	−21. 2	−22.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Aristida congesta	C_4	NADP–ME	Namibia	−20.4	−22.9	−24.3	−24.0	−24. 2	−23.9	n.d.	n.d.	−24.7	−22.3	n.d.	−18.0	−19.7
	Aristida graciliflora	C_4	NADP–ME	Zimbabwe	−25.6	−23.9	−24.1	−23.9	n.d.	−24.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Aristida meridionalis	C_4	NADP–ME	Zimbabwe	−21.4	−22.4	−22.8	−22.4	n.d.	−22.4	n.d.	n.d.	n.d.	n.d.	n.d.	−19.5	−19.5
	Aristida meridionalis	C_4	NADP–ME	Namibia	−20.8	−21.4	−21.9	−22.7	n.d.	−21.7	n.d.	n.d.	−24.3	−24.7	n.d.	−22.2	−22.4
	Stipagrostis ciliata[c]	C_4	NAD–ME	Namibia	−24.7	−21.7	−19.8	−20.7	n.d.	−20.4	n.d.	n.d.	−25.1	−25.3	n.d.	n.d.	−25.1
	Stipagrostis hirtigluma[c]	C_4	NAD–ME	Namibia	−25.3	−25.0	−23.2	−24.0	n.d.	−23.9	n.d.	n.d.	−16.7	−19.1	n.d.	−20.2	−19.1
	Stipagrostis uniplumis	C_4	NAD–ME	Namibia	−23.2	−24.5	−25.9	−24.0	n.d.	−25.1	n.d.	n.d.	−23.1	−25.7	n.d.	−27.8	−25.5
	Chloridoideae																
	Chloris gayana	C_4	PCK	Zimbabwe	−23.0	−22.5	−23.4	−24.9	−24. 0	−24.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Chloris virgata	C_4	PCK	Zimbabwe	−20.3	−19.6	−20.2	−21.1	−21. 9	−20.6	n.d.	n.d.	n.d.	−23.4	n.d.	−19.8	−21.6
	Chloris virgata[c]	C_4	PCK	Namibia	−24.2	−20.6	−21.7	−21.1	−20. 9	−21.7	n.d.	n.d.	n.d.	n.d.	n.d.	−20.2	−20.2
	Enneapogon sp.	C_4	NAD–ME	Namibia	−22.3	−23.0	−22.7	−22.5	−23. 2	−22.7	n.d.	−21.8	−24.5	−23.7	n.d.	−21.5	−22.5
	Enneapogon cenchroides	C_4	NAD–ME	Zimbabwe	−19.5	−19.4	−20.9	−22.7	−23. 7	−21.6	n.d.	n.d.	n.d.	n.d.	n.d.	−19.7	−19.7
	Enneapogon cenchroides[c]	C_4	NAD–ME	Namibia	−19.5	−18.3	−20.1	−20.1	n.d.	−19.8	n.d.	n.d.	n.d.	−20.3	n.d.	−20.6	−20.4
	Eragrostis nindensis	C_4	NAD–ME	Namibia	−24.4	−22.7	−24.6	−24.0	n.d.	−24.0	n.d.	n.d.	n.d.	−25.2	n.d.	−20.6	−21.3
	Eragrostis superba	C_4	NAD–ME	Zimbabwe	−20.4	−20.8	−21.8	−22.7	−23. 0	−21.5	n.d.	n.d.	n.d.	−21.8	n.d.	n.d.	−21.8
	Eragrostis tremula	C_4	NAD–ME	Sudan	−24.0	−25.1	−25.9	−26.3	−26. 4	−25.8	n.d.	n.d.	n.d.	n.d.	n.d.	−21.5	−21.5
	Eragrostis violacea de winter	C_4	NAD–ME	Zimbabwe	−18.0	−18.1	−19.6	−19.2	−19. 3	−19.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Eragrostis viscosa	C_4	NAD–ME	Zimbabwe	−20.0	−19.6	−21.8	−22.4	−21. 8	−20.8	n.d.	n.d.	n.d.	−20.1	n.d.	n.d.	−20.1
	Schmidtia kalahariensis[c]	C_4	PCK	Namibia	−21.8	−22.0	−22.9	−20.5	−21. 3	−21.2	n.d.	n.d.	n.d.	n.d.	n.d.	−21.6	−21.6
	Sporobolus ioclados	C_4	NAD–ME	Zimbabwe	−19.9	−21.8	−22.4	−22.0	−21. 8	−19.6	n.d.	n.d.	n.d.	n.d.	n.d.	−20.6	−20.6
	Sporobolus pyramidalis	C_4	PCK	Zimbabwe	−21.2	−22.0	−22.1	−22.7	−23. 8	−22.3	n.d.	n.d.	−21.2	n.d.	n.d.	−20.4	−20.7
	Panicoideae																
	Bothriochloa insculpta	C_4	NADP–ME	Zimbabwe	−18.0	−19.2	−18.7	−18.6	n.d.	−18.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Brachiaria erucitormis	C_4	PCK	Zimbabwe	−21.9	−20.4	−20.9	−20.3	n.d.	−20.6	n.d.	n.d.	n.d.	n.d.	n.d.	−18.6	−18.6
	Digitaria milanjiana	C_4	NADP–ME	Zimbabwe	−20.5	−18.9	−19.5	−19.7	−20. 4	−19.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Hyparrhenia filipendula	C_4	NADP–ME	Zimbabwe	−21.5	−21.9	−21.9	−22.8	−23. 0	−22.4	n.d.	n.d.	n.d.	−25.4	n.d.	−22.7	−23.5
	Loudetia simplex	C_4	NADP–ME	Zimbabwe	−19.8	−18.6	−18.2	−20.0	−22. 0	−19.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Panicum sp.	C_4	?	Namibia	−22.2	−22.6	−23.0	−22.7	−22. 3	−22.7	n.d.	−23.9	−24.4	−22.6	n.d.	−20.0	−21.5
	Panicum arbusculum[c]	C_4	NAD–ME	Namibia	−24.1	−22.0	−24.0	−21.4	−21. 6	−22.2	n.d.	n.d.	−22.8	−24.8	n.d.	−21.8	−22.8
	Panicum maximum	C_4	PCK	Zimbabwe	−20.0	−20.8	−19.4	−23.3	n.d.	−20.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Panicum maximum	C_4	PCK	Namibia	−24.7	−24.2	−24.5	−24.8	n.d.	−24.5	n.d.	n.d.	n.d.	−21.3	n.d.	−19.0	−19.3
	Themeda triandra	C_4	NADP–ME	Zimbabwe	−18.7	−21.3	−19.6	−20.0	−20. 5	−19.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Pooideae																
	Bromus sp.	C_3		Australia	−36.0	−35.7	−36.0	−37.5	−38. 9	−36.4	n.d.	n.d.	n.d.	−26.2	n.d.	n.d.	−26.2
	Festuca orthophylla	C_3		Peru	−30.2	−30.3	−31.3	−31.2	n.d.	−30.4	n.d.	−29.8	−25.2	n.d.	n.d.	n.d.	−26.1
	Festuca orthophylla	C_3		Peru	−31.0	−31.2	−32.2	−33.1	n.d.	−31.4	n.d.	n.d.	−28.4	n.d.	n.d.	n.d.	−28.4

(*continued on next page*)

Table 3 (*continued*)

	Subfamily/*Subspecies*	Photosynthetic pathway	Physiology	Sampling location	$\delta^{13}C$ of *n*-alkanes[a] (‰ vs. V-PDB) 27	29	31	33	35	$\delta^{13}C_{WMA\text{-}27\text{–}35}$[b]	$\delta^{13}C$ of *n*-alkanols[a] (‰ vs. V-PDB) 22	24	26	28	30	32	$\delta^{13}C_{WMA\text{-}22\text{–}32}$[b]
Plant part samples	Chloridoideae																
	Sporobolus sp. (flowers)	C_4	?	Tanzania	−19.9	−19.6	−20.5	−20.4	−21. 8	−20.3	n.d.	n.d.	−20.8	−21.2	n.d.	n.d.	−20.9
	Sporobolus sp. (leaves)	C_4	?	Tanzania	n.d.	n.d.	−20.6	−20.0	−20.9	−20.3	n.d.	n.d.	−20.9	−20.5	n.d.	−22.2	−21.3
	Sporobolus sp. (stems)	C_4	?	Tanzania	n.d.	−20.0	−19.6	−18.9	−20.0	−19.4	n.d.	n.d.	−24.0	−22.0	n.d.	n.d.	−23.0
	Panicoideae																
	Brachiaria sp. (flowers)	C_4	PCK	Tanzania	−19.1	−19.0	−18.8	−18.6	−21. 4	−19.0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Brachiaria sp. (leaves)	C_4	PCK	Tanzania	−19.6	−19.2	−19.7	−19.9	−19. 8	−19.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Brachiaria sp. (stems)	C_4	PCK	Tanzania	−22.5	−20.7	−20.2	−20.0	−21. 3	−20.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d., not determined.

[a] $\delta^{13}C$: Molecular stable carbon isotopic composition of *n*-alkanes and *n*-alkanols (numbers according to individual carbon numbers).

[b] $\delta^{13}C_{WMA27\text{–}35}$: Weighted mean average of molecular stable carbon isotopic composition of odd-carbon-numbered *n*-alkanes (carbon number 27–35) [‰ versus V-PDB]; $\delta^{13}C_{WMA22\text{–}32}$: Weighted mean average of measured molecular stable carbon isotopic composition of even-carbon-numbered *n*-alkanols (carbon number 22 to 32) [‰ versus V-PDB] (note that mean average values for a given sample were only calculated of the alkane and alkanol homologues for which carbon isotopic ratios were available).

[c] *n*-Alkane stable carbon isotopic values corrected after Huang et al. (2000) (see text for details).

values range from 2.3 to 112.2 and centre at about 22.3 (Table 2b). The total content of even-carbon-numbered *n*-alkanols ($TCEC_{22\text{–}32}$) varies significantly from 10.5 to 777 μg g^{-1} DM, whereas in general the *n*-C_{22} to *n*-C_{31} alkanols are less abundant than the *n*-C_{27} to *n*-C_{35} alkanes in the same species (cf. Tables 2b and 2a). $TCEC_{22\text{–}32}$ values of individual parts of the two grass species examined exhibit a trend similar to that found for the *n*-alkanes and are highest in flower heads followed by leaves and are lowest in stems (Table 2b). The corresponding carbon number distributions do not reveal relationships with plant classification similar to those found for the *n*-alkanes (Fig. 3). The *n*-alkanol pattern of *Sporobolus* sp. flower heads is dominated by *n*-C_{26} and *n*-C_{28} alkanols, whereas in the leaves the *n*-C_{32} compound appears as an additional major homologue. The *n*-alkanol pattern in the stem is similar to that in flower heads. In flower heads and leaves of *Brachiaria* sp. the *n*-C_{32} homologue is the principal *n*-alkanol, whereas stems are dominated by the *n*-C_{28} alkanol.

In most samples studied, the *n*-alkanol distribution patterns are not unimodal or in a bell-shaped curve as found for the *n*-alkanes. Generally, one or two *n*-alkanols, mainly *n*-C_{26}, *n*-C_{28} or *n*-C_{32}, dominate (Table 2b). The *n*-C_{30} alkanol is not a prominent homologue, as it is typically for higher plant waxes (Bianchi, 1995). The C_{30} alkanol has been reported to have hormonal activity as a plant growth regulator (Ries et al., 1977) and, thus, may be partly retained in the interior of the cell.

The waxes of the few C_3 grasses included in this study mainly contain *n*-C_{26} and *n*-C_{28} alkanols (Fig. 4d), with either one dominating (Table 2b). In contrast to this, in C_4 grass waxes (Fig. 4e) the *n*-C_{32} alkanol generally appears as the major homologue. The *n*-C_{32} alkanol is almost totally absent in the C_3 grasses (cf. Fig. 4d and e). This separates the Pooideae from those subfamilies using C_4 photosynthesis (cf. Fig. 4a–d). The averaged $ACL_{22\text{–}32}$ values increase from Pooideae (26.84) to Aristidoideae (29.43), Chloridoideae (29.64) and finally Panicoideae (29.71; Table 4). This trend is similar to that found for *n*-alkane patterns. The alkanol distribution patterns of the C_4 subtypes (Fig. 4f–h) generally exhibit the *n*-C_{32} compound as the major homologue, whereas the NAD–ME subtype additionally has a higher content of the shorter-chain *n*-C_{26} and *n*-C_{28} alkanols; this property separates it from the PCK and NADP–ME subtypes (Fig. 4f–h).

Table 4
Averaged biomarker and isotope data

	n-Alkane				*n*-Alkanol			
	$CPI_{27–35}$[a] (SD)	$ACL_{27–35}$[b] (SD)	$ACL_{LIT\text{-}27\text{-}33}$[c] (SD)	$\delta^{13}C_{WMA27–35}$[d] (SD) ‰	$CPI_{22–32}$[a] (SD)	$ACL_{22–32}$[b] (SD)	$ACL_{LIT\text{-}22–32}$[c] (SD)	$\delta^{13}C_{WMA22–32}$[d] (SD) ‰
Aristidoideae (C_4)	16.2 (±6.3)	30.74 (±0.40)	30.67 (±0.68)	−23.4 (±0.8)	16.1 (±10.4)	29.43 (±1.68)	–	−22.2 (±2.8)
Chloridoideae (C_4)	15.3 (±5.4)	31.24 (±0.68)	30.79 (±0.66)	−21.8 (±1.8)	19.1 (±23.2)	29.64 (±1.10)	29.08 (±1.62)	−21.0 (±0.8)
Panicoideae (C_4)	14.0 (±2.2)	31.27 (±0.90)	30.46 (±0.88)	−21.0 (±1.6)	28.0 (±34.0)	29.71 (±1.29)	29.34 (±1.09)	−21.1 (±2.1)
Pooideae (C_3)	21.2 (±4.0)	30.10 (±1.18)	30.19 (±0.51)	−33.8 (±3.8)	54.0 (±52.3)	26.84 (±1.31)	26.92 (±1.05)	−26.7 (±0.7)
C_4 grasses	15.2 (±4.9)	31.11 (±0.71)	30.52 (±0.81)	−21.7 (±1.8)	22.3 (±26.7)	29.37 (±1.55)	28.98 (±1.52)	−21.4 (±1.9)
C_4-NADP–ME type	14.7 (±3.4)	30.87 (±0.61)	30.35 (±0.86)	−21.3 (±2.0)	12.6 (±8.6)	30.04 (±1.32)	29.49 (±1.01)	−21.8 (±2.2)
C_4-NAD–ME type	16.6 (±6.4)	31.09 (±0.69)	30.77 (±0.71)	−21.7 (±2.1)	18.7 (±12.1)	29.04 (±1.26)	28.78 (±2.06)	−21.7 (±2.0)
C_4-PCK type	13.6 (±3.8)	31.37 (±0.82)	30.99 (±0.65)	−22.0 (±1.3)	35.9 (±42.5)	29.90 (±1.21)	29.32 (±1.26)	−20.3 (±1.2)

SD: Standard deviation.

[a] $CPI_{27–35}$: Carbon preference index of *n*-alkanes (carbon number 27–35); $CPI_{22–32}$: Carbon preference index of *n*-alkanols (carbon number 22–32).

[b] $ACL_{27–35}$: Averaged chain length of odd-carbon-numbered *n*-alkanes (carbon number 27–35); $ACL_{22–32}$: Averaged chain length of even-carbon-numbered *n*-alkanols (carbon number 22–32).

[c] $ACL_{LIT\text{-}27–33}$: Averaged chain length of odd-carbon-numbered *n*-alkanes (carbon number 27–33) from bibliographic data; $ACL_{LIT-22–32}$: Averaged chain length of even-carbon-numbered *n*-alkanols (carbon number 22–32) from bibliographic data (cf. Fig. 4).

[d] $\delta^{13}C_{WMA27–35}$: Weighted mean average of molecular stable carbon isotopic composition of odd-carbon-numbered *n*-alkanes (carbon number 27–35) [‰ versus V-PDB]; $\delta^{13}C_{WMA22–32}$: Weighted mean average of measured molecular stable carbon isotopic composition of even-carbon-numbered *n*-alkanol (carbon number 22–32) [‰ versus V-PDB] (note that mean average values for a given sample were only calculated of the alkane and alkanol homologues for which carbon isotopic ratios were available).

3.4. Molecular carbon isotope signatures of long-chain n-alkanols of grass waxes

The $\delta^{13}C$ values of the *n*-alkanols of the C_4 grasses are in the range of those found for the *n*-alkanes. The $\delta^{13}C_{WMA22–32}$ values vary from – 18.6‰ to −25.5‰ and centre at about −21.4‰. However, the C_3 grass wax *n*-alkanols are markedly less ^{13}C-depleted than the associated *n*-alkanes, and their $\delta^{13}C_{WMA}$ values range from −26.1‰ to 28.4‰, centring at about −26.7‰ (Tables 3 and 4), although the few data points limit the significance of this remarkable observation. More extensive study of C_3 grasses is desirable if this unexpected inconsistency between the *n*-alkane and *n*-alkanol $\delta^{13}C$ data is to be resolved. The *n*-alkanols of individual plant parts of *Sporobolus* sp. show decreasing $\delta^{13}C_{WMA22–32}$ values from the flower head and leaves (about −21.1‰) to the stem (−23.0‰; Table 3). Due to low *n*-alkanol contents in the plant parts of *Brachiaria* sp. no reliable $\delta^{13}C$ values are available. On the subfamilial level the averaged $\delta^{13}C_{WMA22–32}$ values separate the C_4 aristidoids from the other C_4 subfamilies by having about 1‰ lighter $\delta^{13}C_{WMA22–32}$ values. This offset is consistent with that found for the *n*-alkane $\delta^{13}C_{WMA27–35}$ values (Table 4). However, the alkanols of the three C_4 subtypes exhibit a trend opposite to that of their *n*-alkanes. The $\delta^{13}C_{WMA22–32}$ values range from −20.3‰ (PCK) to about −21.8‰ (NADP–ME and NAD–ME) and have no correlation with the $ACL_{22–32}$ values (Table 4).

4. Discussion

The fully developed grass plants we analysed (Table 1) grew in the wild in a variety of habitats and were collected in different seasons. The total contents of the most significant wax *n*-alkanes and *n*-alkan-1-ols in these grasses, expressed by their $TCOC_{27–35}$ and $TCEC_{22–32}$ values (Table 2a), do not reveal any systematics on a species or subfamily level. Variations of *n*-alkane contents of grass waxes as a function of different climatic conditions at the respective sampling sites were also described by Malossini et al. (1990) and Zhang et al. (2004). In three-fourths of our grass samples we observed lower $TCEC_{22–32}$ values compared to the $TCOC_{27–35}$ values of the same plants (cf. Tables 2a,2b). It has been reported that the amount of free *n*-alkanols decreases during maturation of the plant (Tulloch, 1973; Bianchi et al., 1989; Avato et al., 1990). Normally, they are classified as major components of plant waxes with yields exceeding 60% (Baker, 1982; Avato et al., 1987), whereas *n*-alkanes are usually considered subordinate components (3–40%; Tulloch, 1976; Tulloch et al., 1980). Whereas the content of free *n*-alkanols decreases after full development of the plant, wax esters rapidly gain in relative importance. Shrivelling and loss of old leaf blades reduces the *n*-alkanol content of the total plant wax and increasing ester contents indicate new wax production (Tulloch, 1973). Lower *n*-alkanol than *n*-alkane contents may possibly be explained by the degree of senescence of the plants at the time of collection.

The total wax content of an entire plant depends on the different contributions by the different parts of the plants. Generally, the *n*-alkane contents decrease from flowers to leaves and finally to stems (Dove et al., 1996; Smith et al., 2001; Zhang et al., 2004). These findings are confirmed in this study by the $TCOC_{27–35}$ and $TCEC_{22–32}$ values of individual parts, although this type of analysis was performed for two grasses only, *Brachiaria* sp. and *Sporobolus* sp. The total *n*-alkane and *n*-alkanol contents of flower heads were about three times larger than in leaves and six times larger than in stems (Tables 2a and 2b). Different wax quantity contributions by different parts of a plant may lead to significant seasonal variations of the whole-plant wax content. The flower/leaf wax ratio of a plant is thus an important factor for its characterisation. Loss of flower heads and leaves during senescence reduces the total wax biomarker contents of a plant (Smith et al., 2001).

The processes controlling the carbon number distributions of aliphatic wax components have not been clearly described in the literature. The variations observed in this study concerning different parts of the grass plant exhibit a tendency to higher contents of shorter-chain homologues in flowers (Fig. 3; cf. Dove et al., 1996; Smith et al., 2001). Nishimoto (1974), Smith et al. (2001) and Zhang et al. (2004) found that the dominant alkane of plant waxes moves to shorter-chain-length homologues as soon as the plant enters the reproductive phase. Smith et al. (2001) also concluded that the difference in distribution patterns of wax *n*-alkanes of the same grass species, sampled during wet and dry seasons, respectively, may be attributed to the presence or absence of flower heads. Furthermore, they did not find any significant seasonal changes of individual *n*-alkane contents of stem and leaf samples, and there was also no change in the ranking of the more abundant alkanes. However, Tulloch's (1973) findings point to an additional influence on the *n*-alkane distribution pattern of different leaf parts. He found high contents of the n-C_{29} alkane in grass sheath and flag leaf waxes, whereas the third-last leaf generation contained high amounts of the n-C_{31} alkane. Changes in composition are related to the stage of development, particularly to completion of the development of flag leaf and sheath. A similar effect on *n*-alkanes was noted by Laredo et al. (1991). However, the wax biomarker distribution patterns of the same grass species from different habitats and collecting seasons or within the same subspecies are not generally consistent (Tables 2a and 2b), at least not in this limited survey.

Tulloch and Hoffmann (1976) and Tulloch et al. (1980) did not observe changes in *n*-alkanol distribution patterns due to the effects described before and also saw no variations in repeated analyses of a number of perennial species. In contrast to this, Sauvaire et al. (1987) found evidence for lipid profile changes during the development of a leaf. We conclude that chemical classification of grass wax lipids based on homologue distribution patterns of individual subspecies or related species is not suitable to establish a phylogenetic influence on wax homologue distribution patterns.

Averaging the *n*-alkane and *n*-alkanol distributions and carbon isotope patterns on a subfamilial level (Table 4 and Fig. 4, black bars) exhibits clearer systematics and allows the grasses to be divided chemotaxonomically into two main groups: In the first group, the C_3 grasses of the Pooideae subfamily (Fig. 4d) have shorter-chain-length aliphatic compounds, mainly the n-C_{29} and n-C_{31} alkanes and the n-C_{26} and n-C_{28} alkanols. They also have lighter $\delta^{13}C_{WMA}$ values. In the second group, the C_4 grasses of the Chloridoideae and Panicoideae subfamilies are generally characterised by their dominant n-C_{31} and n-C_{33} alkanes and the prominent n-C_{32} alkanol, as well as heavier $\delta^{13}C_{WMA}$ values (Fig. 4 and Table 4). In this comparison, the distribution patterns of Aristidoideae (Fig. 4a) are intermediate. In addition, the isotopic $\delta^{13}C$ values of the aristidoid C_4 species exhibit, on average, 1–2‰ lighter values compared to the remaining C_4 grass subfamilies (Table 4). Possibly, this may be attributed to a specific property of *Aristida* having an uncommon triple layer C_4 anatomy: a mesophyll cell, inner bundle sheath and outer bundle sheath (Voznesenskaya et al., 2005). Anyway, it appears from the present analytical study of more than 30 subspecies that the n-C_{33} alkane and n-C_{32} alkanol are rather characteristic of C_4 grasses overall (Fig. 4e), whereas the n-C_{29} alkane and the n-C_{26} and n-C_{28} alkanols are prominent in the pooid C_3 grasses (Fig. 4d).

Contents and distribution patterns of *n*-alkanes and *n*-alkanols in the leaf waxes of grasses have been reported in a number of studies scattered widely throughout the literature (Fig. 4; legend). Sampling, extraction and analysis procedures varied considerably but a useful collection of data exists for 257 grass subspecies in the case of the *n*-alkanes; data sets are less numerous for *n*-alkanols (69 subspecies). These literature data were used to compile the average histograms shown in Fig. 4 (grey bars; numerical data compiled in Table 4) where they are displayed in parallel with the limited but more uniformly generated data from the present study (black bars). Literature data for compound-specific $\delta^{13}C$ values are extremely sparse and are not presented in Fig. 4. Agreement between the average histograms for the subfamilies examined in the present study and those of the literature survey is generally good for the Aristidoideae, Chloridoideae and Panicoideae (Fig. 4a–c) which all comprise C_4 species. Hence, the use of the average histograms (Fig. 4e) for the *n*-alkanes and *n*-alkanols as being characteristic of the C_4 grasses appears justified. The situation for the Pooideae, however, is in a much more preliminary state, since only two grass species were analysed in the present study. However, the bibliographic data (of 112 subspecies for *n*-alkanes and of 24 subspecies for *n*-alkanols; cf. Fig. 4d) are sufficient to give confidence in the average histograms (grey bars) for this C_3 grass subfamily.

Cluster analysis of our data illustrates the chemical affinity of the waxes of the grass subfamilies. Fig. 5a and b displays cluster trees based on the averaged *n*-alkane and *n*-alkanol distribution patterns and on averaged compound-specific stable carbon isotopic compositions of the grass species studied. Fig. 5c and d is based on the averaged *n*-alkane and *n*-alkanol distribution patterns of bibliographic data. The distance length exhibits the affinity of the nearest cluster. The *n*-alkane cluster tree (Fig. 5a) is similar to the evolutionary sequence (Fig. 2b) and exhibits a separation in the subfamilies comprising C_4 plants that is slightly different from that in the *n*-alkanol tree (Fig. 5b), which in turn matches the phylogenetic tree of

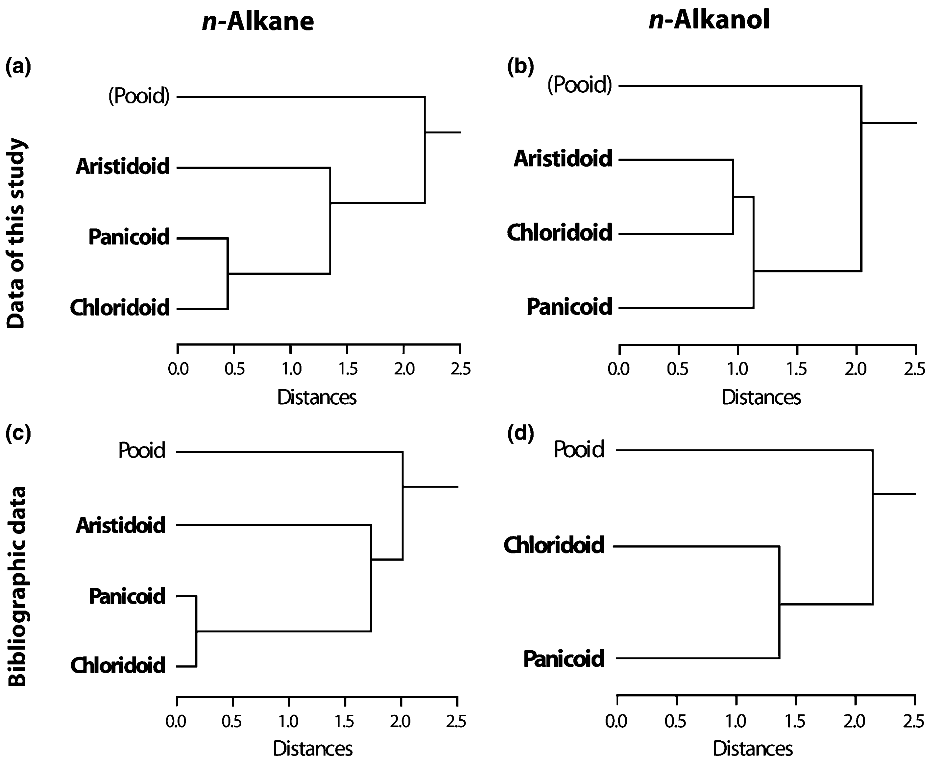


Fig. 5. Results of agglomerate hierarchical cluster analysis using averaged subfamilial (a) *n*-alkane and (b) *n*-alkanol data of contents and stable molecular carbon isotopic compositions of individual homologues based on analytical data of this study as well as (c) *n*-alkane and (d) *n*-alkanol data of contents of individual homologues based on bibliographic data (cf. Fig. 4) in pooid, aristidoid, chloridoid and panicoid waxes. Taxa that contain the C_4 photosynthetic mechanism are boldfaced. In (d), the aristidoid data are missing due to lacking bibliographic data.

the subfamilies (Fig. 2a). The same is evident for the bibliographic data (Fig. 5c and d). Thus, the aristidoids in the *n*-alkane tree are completely separated from the panicoids and chloridoids (cf. Fig. 5a and c), the other C_4 subfamilies, whereas in the *n*-alkanol tree the chloridoids as well as the aristidoids are connected to each other in one cluster arm (cf. Fig. 5b). This confirms the ambivalent position of aristidoid waxes. Maffei (1996) observed a similar separation of *n*-alkane distribution patterns between Pooideae and the C_4-plant-containing subfamilies based on the *n*-C_{33} alkane content. However, his cluster analysis used *n*-alkane and *iso*-alkane data and was limited mainly to pooid (C_3) species.

Averaged C_4 grass subtype distribution patterns of wax components, both in the literature and in this study, exhibit an increase in *n*-C_{33} alkane content from the NADP–ME to the NAD–ME and finally the PCK subtype, but there is no such trend in the averaged *n*-alkanol data. All subtype members generally contain the *n*-C_{32} alkanol as the major homologue (Fig. 4f–h). Nine out of thirteen NAD–ME and five out of eight PCK subtype grasses belong to the Chloridoideae subfamily, whereas twelve NADP–ME subtype grasses are almost equally from the Aristidoideae and Panicoideae (Table 3).

5. C_4 grass wax adaptation and implications for palaeoenvironmental studies

5.1. C_4 grass wax adaptation to the climatic conditions of the habitat

Mean annual temperatures (about 25 °C) and precipitation (2000 mm/a) in the northern part of our sampling area, the equatorial tropical rainforest, are relatively high, whereas southwards, the annual temperatures and precipitation decrease to about 19 °C and 300 mm/a, respectively, in the grass-dominated Kalahari savanna and finally to 15 °C and 15 mm/a, respectively, in the Walvis Bay area (cf. Fig. 1b and c; data from climatograms published by Walter et al., 1975). The rainforest has a closed canopy and humid overcast climate, which reduces direct sunlight irradiation of the vegetation and inhibits grass growth. The absolute maximum temperatures are at about 35 °C. In contrast, the sparse vegetation of the Namib Desert is exposed to much higher absolute maximum temperatures of 40–50 °C (according to van der Merwe, 1983) with the plants growing in the direct sunlight. Generally, tropical C_4 grasses occur in open environments, where temperature at the leaf surface may rise to extreme maximum temperatures eventually exceeding even those cited for the Namib Desert.

Kawamura et al. (2003) remarked that higher plants growing in tropical regions biosynthesise higher-molecular-weight waxes in response to higher ambient temperatures to maintain the hardness of their leaf surfaces. Leaf waxes occur as carpets of microcrystallites in the micrometer range, varying greatly in size, shape and cuticular distribution from species to species (Martin and Juniper, 1970; Juniper and Southwood, 1986). Loss of microstructure due to melting at elevated temperatures is deleterious to the protective role and transpiration control of leaf waxes. Lipid melting results in increased permeability (Gibbs, 2002). The melting points of the pure *n*-alkane homologues are reported as follows: *n*-C_{27}, 59.2 °C; *n*-C_{29}, 63.7 °C; *n*-C_{31}, 67.9 °C; *n*-C_{33}, 71.2 °C and *n*-C_{35}, 74.6 °C. Those of the *n*-alkanol homologues are: *n*-C_{22}, 72.5 °C; *n*-C_{24}, 77.0 °C; *n*-C_{26}, 80.0 °C; *n*-C_{28}, 83.4 °C and *n*-C_{30}, 88.0 °C (Lide, 2004), i.e., with an ACL increase of 2 the melting point rises by approximately 4 °C. Little is known about the actual effect of increased ACL values on the melting points of leaf waxes. Furthermore, waxes contain a wide range of aliphatic and other compounds. The mixing ratio of straight-chain, branched, saturated or unsaturated, as well as cyclic and heterocompounds will affect the melting point of an epicuticular wax. Chain length has a relatively small effect on the melting point compared to differences in lipid class (alkenes, methylalkanes, etc.; Gibbs, 2002). For example, Patel et al. (2001) found that a mixture of synthetic wax esters with *n*-alkanes melted 3–5 °C lower than predicted from the melting points of the individual lipids. Gibbs (1995) examined two-component mixtures of long-chain cuticular hydrocarbons as a model for lipid interactions. Pure *n*-alkanes melted abruptly over a 2–3 °C range, whereas mixtures melted over a range of 5–20 °C. In addition, for *n*-alkane/*n*-alkene mixtures the melting temperatures were higher than the calculated weighted average temperatures by as much as 17 °C.

Gülz (1994) described fundamental differences between leaf wax compositions of gymnosperms and angiosperms and attributed them to fundamental evolutionary developments. Dodd and Afzal-Rafii (2000) analysed waxes of plant species of the family Cupressaceae and proposed that the hydrocarbon composition displays a strong genetic influence of adaptation to environmental conditions. As yet, little attention has been given to the evolutionary pathways which must underlie the current plant phylogeny and the chemotaxonomic distribution of the lipid biomarkers.

The postulated evolutionary sequence of the major grass subfamilies is shown in Fig. 2b. The original grasses were plants of forest margins or deep shade, characteristics that are retained today in the bamboos and the basal pooid grasses (Kellogg, 2001). Members of the subfamily Bambusoideae traditionally have been speculated to be the most primitive members of the grass family (Soderstrom, 1981). Their adaptation to forest habitats precluded them from the main evolutionary line of the whole family. C_3 grasses of the subfamily Arundinoideae appear to be the descendants of an ancestral line closest to the earliest true grasses and members of this subfamily may have been the first to move to open "savanna ecosystems" from savanna/forest ecotone. Chloridoideae and Panicoideae have come to dominate the open environments of the tropical and subtropical zones. They are the descendants of the early arundinoid grasses which, through the evolution of the C_4 photosynthetic pathway, gained a competitive edge over their C_3 grass ancestors (Fig. 2b; Renvoize and Clayton, 1992). The close relationship of the chloridoid and panicoid grasses, along with other C_4 species, suggests the possibility of underlying physiological similarities (Kellogg, 2001).

The results of our cluster analysis of the distribution patterns of aliphatic wax components (Fig. 5a–d), combined with the known grass subfamilial phylogeny and preferred habitat of subfamilies or C_4 subtypes (e.g., Fig. 2b) suggest that plant wax compositions reflect adaptation to the climate of the habitat. This adaptation may have developed as a secondary effect during the evolutionary succession of the grasses, especially of C_4 grasses during their adaptation to low concentrations of atmospheric CO_2. The carbon number distributions of the *n*-alkanes of the panicoids and chloridoids and the NAD–ME and PCK C_4-subtype species may have evolved to give the increased content of the n-C_{33} alkane and the n-C_{32} alkanol with their higher melting points. Presumably, C_4 grasses acquired an advantage over C_3 plants in hot and arid regions by having higher-melting-point waxes as a result of increased content of longer-chain wax components. An evolutionary adaptive role of plant waxes seems certain but requires further investigation.

5.2. Leaf wax n-alkane and n-alkanol homologues as palaeoclimatic proxies

The potential for chemical adaptation of C_3 and C_4 plants to habitat occupancy has implications for the use of wax compositions in fossil records as biomarker proxies. Thus, palaeoenvironmental studies use chain-length distributions and stable carbon isotopic measurements of aliphatic biomarkers to deduce the C_3 and C_4 plant contribution to sediments from continental vegetation. The averaged C_4 grass wax signatures (Fig. 6), based on the bibliographic data in Fig. 4 as well as on data of this study, may be representative of the world's tropical grasslands and savannas, and as the most important group of plants they may dominate the fossil signatures related to the proportions of C_3 and C_4 vegetation. Chikaraishi and Naraoka (2003) analysed wax n-alkanes of higher land plants which were collected in Japan and Thailand. Histograms and molecular isotopic data of n-alkanes as well as averaged numerical data are shown in Fig. 7a–d. The C_3 and C_4 grasses mainly contain the n-C_{31} alkane, whereas the C_4 grasses have also a higher relative content of the n-C_{33} alkane (cf. Fig. 7c and d). The angiosperm trees (C_3, Fig. 7a), which represent the second-most important group in the C_3 versus C_4 competition in tropical vegetation, contain mainly the n-C_{29} alkane. This supports the findings in palaeoenvironmental studies, which deduce the C_3 and C_4 plant contribution to sediments from continental vegetation by using chain-length distributions of aliphatic biomarkers and isotopic measurements. For example, Eglinton et al. (2002), Schefuß et al. (2003b) and Rommerskirchen et al. (2003) found high relative amounts of the n-C_{29} and n-C_{31} alkanes combined with lighter $\delta^{13}C$ values in those marine sediments on the West African continental margin off the Congo river which received terrestrial organic matter from the predominantly C_3 tropical rainforest in the hinterland. On the other hand, their samples from marine settings near the adjacent African savannas and deserts mainly exhibited the n-C_{31} and n-C_{33} alkane homologues, which were isotopically enriched in ^{13}C relative to the n-C_{29} alkane. The molecular isotope signatures determined in these sediment studies are not as uniform within the homologous series as found for the C_4 and C_3 grasses in this study (Figs. 4 and 6). This underlines, as expected, the occurrence of complex mixtures of C_3 and C_4 plant wax components in geological samples.

As a result of the present study, we propose, as a working paradigm, that the carbon number histograms of the C_4 grasses (Fig. 6) have the n-C_{31}

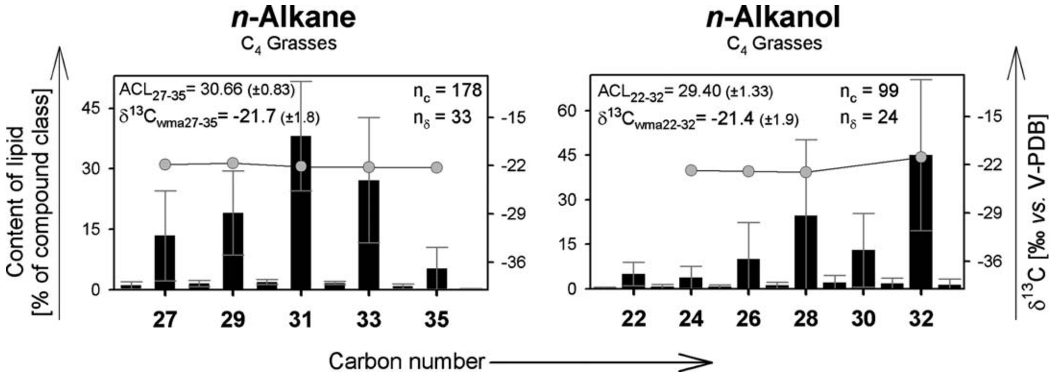


Fig. 6. Averaged histogram representation for C_4 grasses of n-alkane and n-alkanol homologue contents (in % of compound class, left Y-axis) of leaf waxes, based on combined bibliographic data and data of this study, for odd n-C_{27} to n-C_{35} alkanes as well as even n-C_{22} to n-C_{32} alkanols (black bars), overlain by molecular stable carbon isotope data of this study (●; $\delta^{13}C$ in ‰ versus V-PDB, right Y-axis; cf. Fig. 4). The diagrams are individually normalised to the most abundant homologue. ACL: mean average chain length of odd-carbon-numbered n-alkanes (n-C_{27} to n-C_{35}; $ACL_{27–35}$) as well as even-carbon-numbered n-alkanols (n-C_{22} to n-C_{32}; $ACL_{22–32}$) including the standard deviation (in brackets). $\delta^{13}C_{WMA}$: Mean weighted mean average of carbon isotopic values of odd-carbon-numbered n-C_{27} to n-C_{35} alkanes ($\delta^{13}C_{WMA27–35}$) as well as of even-carbon-numbered n-C_{22} to n-C_{32} alkanols ($\delta^{13}C_{WMA22–32}$) including the standard deviation (in brackets). n_c: number of species used for the averaging of content data. n_δ: number of species used for the averaging of isotopic data.

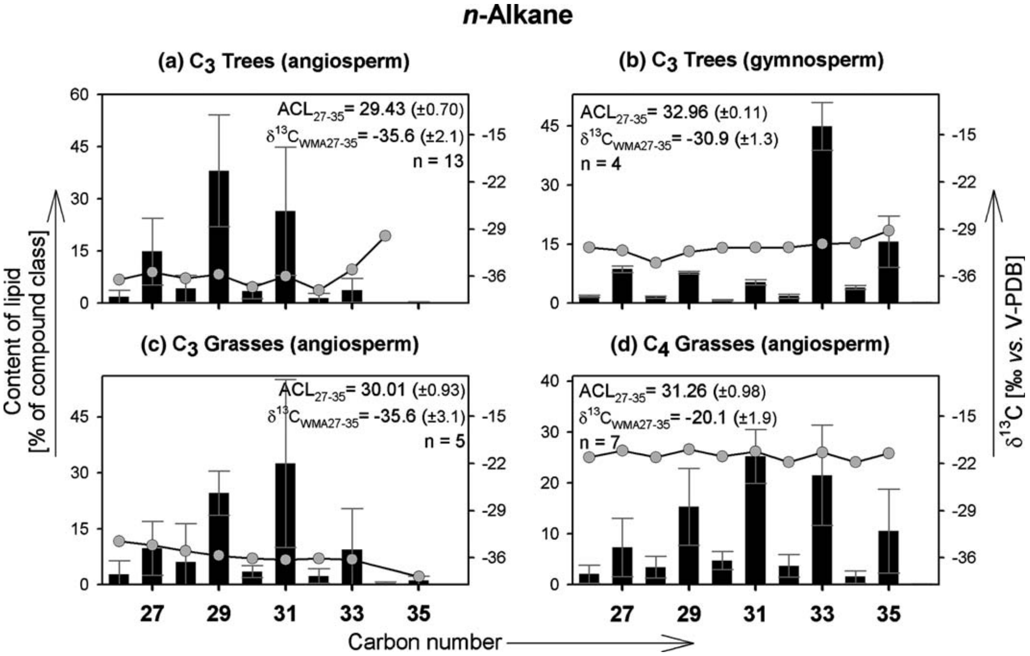


Fig. 7. Averaged histogram representation for (a) C_3 angiosperm and (b) C_3 gymnosperm trees as well as (c) C_3 and (d) C_4 grasses of contents of *n*-C_{26} to *n*-C_{36} alkanes in leaf waxes (in % of compound class, left *Y*-axis; black bars), overlain by averaged molecular stable carbon isotope data (●; $\delta^{13}C$ in ‰ versus V-PDB, right *Y*-axis). *n*-Alkane isotopic values based on data of Chikaraishi and Naraoka (2003). The diagrams are individually normalised to the most abundant homologue. ACL: mean average chain length of odd-carbon-number *n*-alkanes (*n*-C_{27} to *n*-C_{35}; ACL_{27-35}) including the standard deviation (in brackets). $\delta^{13}C_{WMA}$: mean weighted mean average of carbon isotopic values of odd-carbon-numbered *n*-C_{27} to *n*-C_{35} alkanes ($\delta^{13}C_{WMA27-35}$) including the standard deviation (in brackets). *n*: number of species used for the averaging of content data as well as of isotopic data.

and *n*-C_{33} alkanes and the *n*-C_{32} alkanol as dominant homologues with each carrying a $\delta^{13}C$ value of approximately −22‰. We thus have an experimentally derived proxy for the C_4 tropical and subtropical grasslands of the continents as registered in aquatic sediments. While the expansions and contractions of these grassland zones can serve as indirect measures of continental climate change, the actual assessment and modelling of such phenomena can now – in addition to mineral composition, pollen and plant fossil records and estimates of pCO_2, temperature and precipitation – be based upon lipid biomarker distributions and compound-specific stable carbon isotope data.

6. Conclusions

In this paper we have attempted to establish a reliable biomarker proxy for tropical and subtropical grasslands, based on molecular characteristics of C_4 grasses which are predominant in these biomes. The analytical data are for the long chain *n*-alkanes and *n*-alkanols in the epicuticular waxes extracted from more than 30 grass species native to the African grasslands. These lipid distributions have allowed a chemotaxonomic survey which has been extended by a detailed assessment of the respective literature data. The combined findings may be summarised as follows:

- C_4 grasses display considerable variation in the content and distribution of their lipids at the subspecies and species level. At this level the data reveal little in the way of useful chemotaxonomic systematics.
- The C_4 grasses do display characteristic lipid distributions at the subfamily level. The dominant *n*-alkanes are *n*-C_{31} and *n*-C_{33} homologues and the dominant *n*-alkanol is the *n*-C_{32} compound. The ACL values are higher than those of the C_3 grasses and also those reported for many C_3 plants of the temperate zones. The carbon number distribution patterns found in this study are broadly substantiated by the data recorded in the literature for more than 200 species of C_3 and C_4 grasses.

- The dominant n-C_{31} and n-C_{33} alkanes and n-C_{32} alkanol in the C_4 grasses are also each characterised by consistently heavy $\delta^{13}C$ values of circa $-22‰$.
- The higher ACL values shown by the C_4 grasses may reflect evolutionary adaptation to high-temperature arid habitats. If so, the explanation may be a need for retention of crystallinity and hence micromorphology of leaf surface waxes at high temperatures, as in plants exposed to high-intensity insolation in deserts and other exposed and sparsely vegetated areas.
- In terms of palaeoenvironmental studies, the distributions and isotopic characteristics of the *n*-alkanes and *n*-alkanols constitute useful biomarker proxies for the C_4-dominated tropical and subtropical grasslands. These proxies may afford estimates of the areal extent of these biomes and hence the palaeoclimatic conditions.

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