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Plant and soil lipid modifications under elevated atmospheric CO_2 conditions: I. Lipid distribution patterns

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Abstract

Grassland soils are regarded as one potential sink for atmospheric CO_2 via photosynthetic fixation in plant biomass and subsequent transformation into soil organic matter upon degradation. In the future, an enrichment in atmospheric CO_2 concentration is expected, leading to modified photosynthetic activity in plant biomass. Free air CO_2 enrichment (FACE) experiments provide an opportunity for investigating under field conditions plant behaviour expected under future atmospheric composition. Lipid components are important constituents of plant surfaces, whereby their position at the plant/atmosphere interface leads to a high susceptibility towards environmental change. The main focus of this study was an investigation of the modification in lipid distribution patterns within plant biomass and the translocation of these lipids towards, and fixation within, soil organic matter as a result of enhanced CO_2 concentration. We demonstrate which lipids are mainly influenced under modified CO_2 concentrations and show how this affects the lipid composition of plant biomass and soil. Carboxylic acid, alcohol and aliphatic hydrocarbon distribution patterns of plant biomass and soils are discussed. While short chain acids reveal a uniform depletion in unsaturated C_{18} acids in plants and soils under enhanced CO_2 concentration, the alcohol fraction shows diverse trends for *Lolium perenne* and *Trifolium repens* plants and soil. Long chain alcohols increase in abundance for *L. perenne* and decrease for *T. repens* samples. The *n*-alkanes in soil, as degradation products of plant-derived acids and alcohols, exhibit minor compositional variation. Decreasing amounts of plant-derived acids vs. increasing concentrations of alcohols are noted for *T. repens* samples. The study demonstrates the response on the molecular level of selected plants under enhanced atmospheric CO_2 concentration. Lipid compositional variation is modified by photosynthetic activity and adapted biosynthesis under future atmospheric conditions may be expected.
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1. Introduction

Increasing amounts of anthropogenic emissions of CO_2 to the atmosphere have led to intensive debate about the potential environmental consequences and have initiated activities as defined in the Kyoto Protocol of the United Nations Organization (IGBP, 1998; Prentice et al., 2001; IPCC,

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2007). Meanwhile, it is beyond dispute that, throughout the following decades, a further increase in atmospheric CO_2 concentration cannot be avoided (IGBP, 1998; IPCC, 2007). Consequently, during the 1990s numerous experiments and studies were initiated to determine the reaction of plant biomass and ecosystems to future CO_2 conditions. Parts of these investigations were arranged as free air carbon dioxide enrichment (FACE) experiments, where under otherwise natural conditions plants are grown under ambient and elevated CO_2 conditions on neighbouring plots. However, controversial results were published concerning the bioproductivity and degradability under enhanced CO_2 concentrations. On one hand, productivity increased for numerous C_3 plants (Nowak et al., 2004; Poorter and Navas, 2003; Pendall et al., 2004; Wang et al., 2004), whereas the degradability increased on the other hand (Norby and Luo, 2004; Pendall et al., 2004). For some plants such as *L. perenne* even a decrease in productivity was observed in some experiments (Nijs et al., 1996).

Soils are regarded as one potential sink for atmospheric CO_2 via photosynthetic fixation in plant biomass, which is then transformed into soil organic matter (OM) upon degradation. Soil OM studies are intended to differentiate whether soils act as a source or a sink for CO_2 and to unravel the incorporation and stabilisation processes in plant biomass (Kögel-Knabner, 2002). Recent studies on a European scale described a net carbon uptake in grassland soils (Janssens et al., 2003), indicating the importance of these ecosystems for the global carbon cycle. Soil in FACE experiments did not confirm the previous assumption of an increased translocation of plant-derived carbon towards soil, yielding enhanced carbon content under elevated CO_2 concentration (e.g. van Kessel et al., 2000). Similarly, microbial biomass sequestration was not significantly enhanced under elevated CO_2 concentration (Glaser et al., 2006). Molecular approaches concerning compositional changes in plant biomass and soil organic carbon under elevated CO_2 concentration are scarce, but indicate compositional changes of the soil organic carbon under elevated CO_2 conditions (Giesemann, 2005; Heim and Schmidt, 2006; Bock et al., 2007).

It is well known that lipids constitute a major part of the organic components of fresh plant material and soil (e.g. Gregorich et al., 1996). They play an important role in the incorporation of plant material into soil organic carbon (SOC; Kögel-Knabner, 2002) and contain several diagnostic markers for source apportionment (e.g. Wiesenberg and Schwark, 2006) and turnover rate determination (Lichtfouse et al., 1994, 1997; Cayet and Lichtfouse, 2001; Wiesenberg et al., 2004a). Recent studies in particular subjected agricultural or forest soils to determination of input and turnover of lipids in the soil (e.g. Wiesenberg et al., 2004a; Quénéa et al., 2006). Numerous studies exist on lipid distribution patterns in arable soils (e.g. Lichtfouse et al., 1997; Wiesenberg et al., 2004a; Quénéa et al., 2006), forest soils (e.g. Nierop, 1998; Marseille et al., 1999) or peaty soils (Bol et al., 1996; Jansen et al., 2006), with less attention being paid to grassland soils (van Bergen et al., 1997; Bull et al., 2000; Jansen et al., 2006). In addition, wax lipid composition of pasture plants has been studied with respect to the effects of species, plant age and plant parts (e.g. Dove et al., 1996), whereas only few studies address the influence of modified environmental conditions on lipid composition. These studies include plant lipid analyses under modified atmospheric conditions such as enhanced temperature (e.g. Larkindale and Huang, 2004) or atmospheric CO_2 concentration (e.g. Huang et al., 1999; Hussain et al., 2001; Peñuelas et al., 2002) or input of lipids to grassland soils under ambient vs. elevated CO_2 concentration. In these investigations, modifications in lipid proportions were observed for tree seeds (Huang et al., 1999; Hussain et al., 2001) but were absent for shrub plants (Peñuelas et al., 2002).

Here, we demonstrate lipid compositional changes under enhanced CO_2 concentration for several pasture plants and show how this lipid signal is transferred from plant biomass to soil. We show that enhanced atmospheric CO_2 concentration, expected to occur within the next century, modifies plant biomass and soil OM on a molecular level, leading to a different plant biomass and soil OM quality.

2. Materials and methods

2.1. Sampling site

Samples were obtained from the free air carbon dioxide enrichment (FACE) experiment of the Swiss Federal Institute of Technology (ETH) experimental trial near Eschikon, 20 km NE of Zurich. The main objective of the trial was to determine the impact of an elevated atmospheric CO_2 concentration on a grassland ecosystem. While ambient air

contained ca. 350 ppm CO_2, fumigated plots had received an enhanced CO_2 concentration of ca. 600 ppm for 10 years when soil was sampled in 2002. The soil was classified as Eutric Cambisol (FAO, 1994) and experimental details have been described (Zanetti et al., 1997; Hebeisen et al., 1997).

2.2. Samples

Soil and plant samples were derived from separate plots with clover [*Trifolium repens* (L.)] and ryegrass [*Lolium perenne* (L.)] grown under ambient and elevated CO_2 without N fertilization. While plants were harvested for above ground biomass during the growing period in 1998, soil samples (0–10 cm depth) were taken as cores at the end of the experimental period (2002). Plant materials from ambient and FACE plots were pooled separately and air dried after sampling. For each ambient and FACE plots three soil cores were taken and stored in a freezer (−32 °C) until aliquots of samples from each plot were taken and combined with corresponding samples from analogous replicate experiments and homogenized. Air-dried samples were ground finely using a ball mill.

2.3. Bulk elemental analysis

Samples were analysed for stable carbon isotope composition using a continuous flow Heraeus CHN-O-Rapid elemental analyser coupled to a Finnigan MAT Delta-S mass spectrometer. Carbon isotope compositions ($\delta^{13}C$ values) are expressed in permil relative to the Vienna Pee Dee Belemnite (V-PDB) standard:

$$\delta^{13}C = [(^{13}C/^{12}C_{sample}/^{13}C/^{12}C_{std}) - 1] \times 10^3 \quad (1)$$

where $^{13}C/^{12}C_{std} = 0.0112372$.

2.4. Lipid extraction and separation

The complete extraction and separation procedure has been described in detail (Wiesenberg et al., 2004b). For lipid extraction, an accelerated solvent extractor (Dionex ASE 200) was used. Stainless steel extraction vessels were filled with 5 g plant material or 30 g soil, respectively. Free lipids were extracted with CH_2Cl_2/CH_3OH (93/7; v/v) at 5×10^6 Pa and 75 °C. The heating phase was 5 min and static extraction time 20 min. Extraction was repeated under identical conditions except for a higher temperature (140 °C) with a heating phase of 7 min and both extracts were combined. Total lipids were sequentially separated into eight fractions of different polarity (Wiesenberg et al., 2004b). A heterocompound, medium pressure liquid chromatography separation (H-MPLC) described by Willsch et al. (1997) yielded 6 fractions: (i) a low polarity fraction containing aliphatic and aromatic hydrocarbons as well as acyclic ketones; (ii) an intermediate polarity fraction comprising straight chain and branched alcohols and sterols; (iii) a carboxylic acid fraction; (iv) a fraction containing organic bases; (v) a high polarity and/or high molecular weight fraction containing very long chain wax esters and (vi) a polar fraction of undefined composition. The low polarity fraction was rechromatographed using the MPLC separation scheme described by Radke et al. (1980) to give three additional fractions: (i) aliphatic hydrocarbons, (ii) aromatic hydrocarbons and (iii) low polarity hetero compounds. Volume reduction was performed via a turbo vaporiser (Zymark) or rotary evaporation.

2.5. GC/MS (gas chromatography–mass spectrometry)

For identification and quantification, a defined amount of deuteriated standards (d_{39} n-C_{20} carboxylic acid, d_{37} n-C_{18} alcohol, d_{50} n-C_{24} alkane) was added to the carboxylic acid, alcohol and aliphatic hydrocarbon fractions, respectively. Compound identification was performed with a HP 5890 Series II gas chromatograph coupled to a HP 5989A mass spectrometer. For quantification, a HP 5890 Series II GC equipped with a flame ionisation detector (FID) was used. Acids were derivatised with CH_2N_2 and detected as methyl esters, whereas alcohols were silylated using BSTFA [N,O-bis(trimethylsilyl)trifluoroacetamide].

3. Results and discussion

3.1. Elemental analysis and lipid content

L. perenne and *T. repens* plants showed almost identical carbon content (Table 1) of ca. 445 mg/g, typical for many plants. In contrast, extractable lipid content was greater for *T. repens*. Most likely, this can be attributed to the higher content of epicuticular wax in *T. repens* leaves, whose abundance varies throughout the growing season (Moseley,

Table 1
Carbon content and extract yield under ambient and elevated CO_2 concentration

	Total carbon [mg/g]	Total organic carbon [mg/g]	Extract yield [μg/g]	Isotopic composition ($\delta^{13}C$ [‰ V-PDB])
L. perenne plants ambient	443.2 ± 1.1	n.d.[a]	78460	−28.3 ± 0.0
L. perenne plants elevated	447.4 ± 0.8	n.d.[a]	74020	−37.7 ± 0.5
L. perenne soil ambient	30.4 ± 0.5	27.0 ± 1.2	943	−26.5 ± 0.7
L. perenne soil elevated	33.6 ± 1.5	28.2 ± 2.2	1040	−30.9 ± 0.3
T. repens plants ambient	452.0 ± 1.3	n.d.[a]	101340	−27.0 ± 0.0
T. repens plants elevated	441.8 ± 2.6	n.d.[a]	101760	−36.2 ± 0.1
T. repens soil ambient	26.9 ± 0.9	25.6 ± 1.4	853	−26.9 ± 0.0
T. repens soil elevated	29.5 ± 2.2	27.1 ± 0.9	870	−29.9 ± 0.0

[a] Not determined, but similar to total carbon content.

1983). Carbon and lipid contents of plants grown under ambient and elevated atmospheric CO_2 concentrations were almost identical, consistent with literature data (Peñuelas et al., 2002). Seed from *Pinus taeda* may, however, contain significantly more lipids when exposed to elevated CO_2 concentration (Hussain et al., 2001), which is most likely related to a different plant habit.

As expected, the stable carbon isotopic composition changed significantly for the plants grown under elevated CO_2 as a result of both higher atmospheric CO_2 content and the ^{13}C-depleted atmospheric CO_2 values due to the added CO_2 being lighter (isotopic composition −48‰). The isotopic depletion was similar for both plants, around 9‰ vs. the plants grown under ambient CO_2 conditions.

In comparison to plant material, soil samples showed larger differences between both plants and both CO_2 fertilisation experiments. In general, soil under *L. perenne* contained approximately 10% more total carbon, organic carbon and extractable lipids than soil under *T. repens*, which can be attributed to a larger biomass production and input in the *L. perenne*-cropped soil. Under elevated CO_2 concentration, the total carbon content increased in soil by approximately 3 mg/g, whereas the organic carbon content increased by 0.8–1.5 mg/g in both plots. Like the carbon content, the lipid extract yield rose under elevated CO_2 conditions by 97 μg/g for the *L. perenne* soil and only 17 μg/g for the *T. repens* soil. The $\delta^{13}C$ value was depleted by 4.4‰ for the soil under *L. perenne* and 3‰ for that under *T. repens*, under elevated CO_2 conditions. The smaller changes for the *T. repens* soil correspond to the lower biomass productivity of *T. repens* than *L. perenne* under elevated CO_2 conditions (Teyssonneyre et al., 2002).

The highest proportion of plant lipids could be attributed to the high molecular weight fraction containing mainly wax esters (Fig. 1). While *T. repens* biomass contained twice as much carboxylic acid as low polarity lipids including, e.g. alkanes and ketones, *L. perenne* was characterized by equal abundances of both fractions. All other fractions occurred only in trace amounts in the plant biomass, consistent with previous determinations for a variety of agricultural crops (Wiesenberg, 2004). Proportions of lipid fractions revealed only minor differences between plants grown under ambient and elevated CO_2 conditions (Fig. 1). In contrast to plants, the highest lipid proportions in soils were related to low polarity lipids, followed by smaller amounts of high molecular weight compounds and carboxylic acids, whereas the other fractions contributed only traces to total soil lipids. This distribution pattern of soil lipids is similar to that for several arable soils (Wiesenberg et al., 2004a). Changes in lipid proportion could not be observed for soil under *T. repens*, whereas for *L. perenne* a slight increase in most lipid fractions at the expense of the polar fraction was noted.

3.2. Carboxylic acids

Carboxylic acids, as main contributors to plant leaf waxes, are primary products generated during biosynthetic processes and so are prone to react to a modified CO_2 environment. Their amounts should therefore give information on the extent to which the different environment might change primary plant biosynthetic products.

3.2.1. Distribution patterns

The acids of *L. perenne* showed a predominance of unsaturated C_{18} and saturated C_{16} components (Fig. 2), typical of a broad variety of plant waxes including *L. perenne* (Walton, 1990; Bianchi,

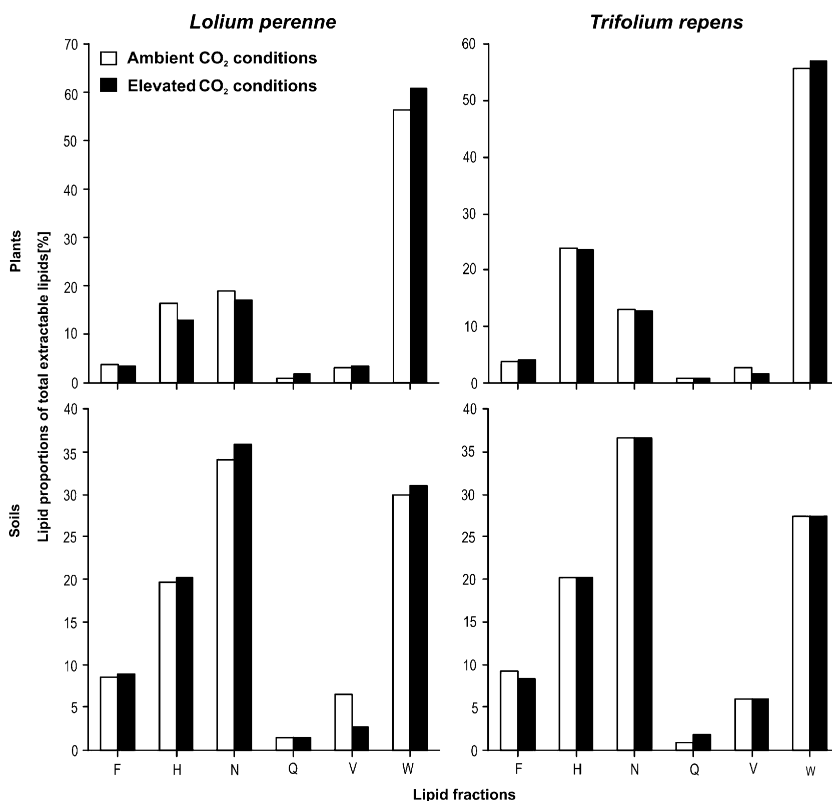

Fig. 1. Lipid proportions after heterocompound medium pressure liquid chromatography with: (F) intermediate polarity fraction (straight chain and branched alcohols and sterols); (H) carboxylic acid fraction; (N) low polarity fraction (aliphatic and aromatic hydrocarbons as well as acyclic ketones); (Q) organic bases fraction; (V) polar fraction (undefined content); (W) high polarity and/or HMW-fraction (mainly long chain wax esters).

1994). Even numbered long chain homologues were present in minor amount and predominated over the odd numbered homologues. Higher abundances of n-C_{26} than n-C_{24} acid are typical for C_3 grasses (Wiesenberg and Schwark, 2006). The plants grown under elevated CO_2 conditions were depleted for most acids in comparison to the control plants. The depletion for unsaturated C_{18} acids was particularly obvious. This is similar to the situation for grasses grown under heat stress, which produce more saturated C_{16} acid at the expense of some unsaturated C_{18} homologues (Larkindale and Huang, 2004). Thus, the carboxylic acid composition of the plant material changed as a result of increased CO_2 concentration.

Soil under *L. perenne* showed a different distribution pattern of acids in comparison to the plants. In addition to unsaturated C_{18} and saturated C_{16} acids, unsaturated C_{16} acids were present in large amount and could be attributed to soil microbiota. Furthermore, a minor predominance of short chain ($\leqslant$ 19 carbons) vs. long chain ($\geqslant$ 20 carbon) acids was observed for soil vs. plant biomass. The predominance of even numbered homologues was still present in the soil. Like plant biomass, soil from plots under elevated CO_2 conditions was depleted in short chain acids, except for unsaturated C_{16} components, which remained almost unchanged, suggesting similar microbial activity in both soils, as previously documented for this site (Glaser et al., 2006). Surprisingly, long chain acids showed a slight enrichment in the soil of the elevated vs. the ambient plots. This effect cannot be related to a change in the microbial community structure as previously determined for several other soils under enhanced CO_2 conditions (Sadowsky and Schortemeyer, 1997). No significant variation in microbial community was observed for the soil from the Eschikon FACE experiment (Montealegre et al., 2002; Glaser et al., 2006). The higher amounts of long chain acids under enhanced CO_2 conditions can be attributed to selective preservation of long chain components

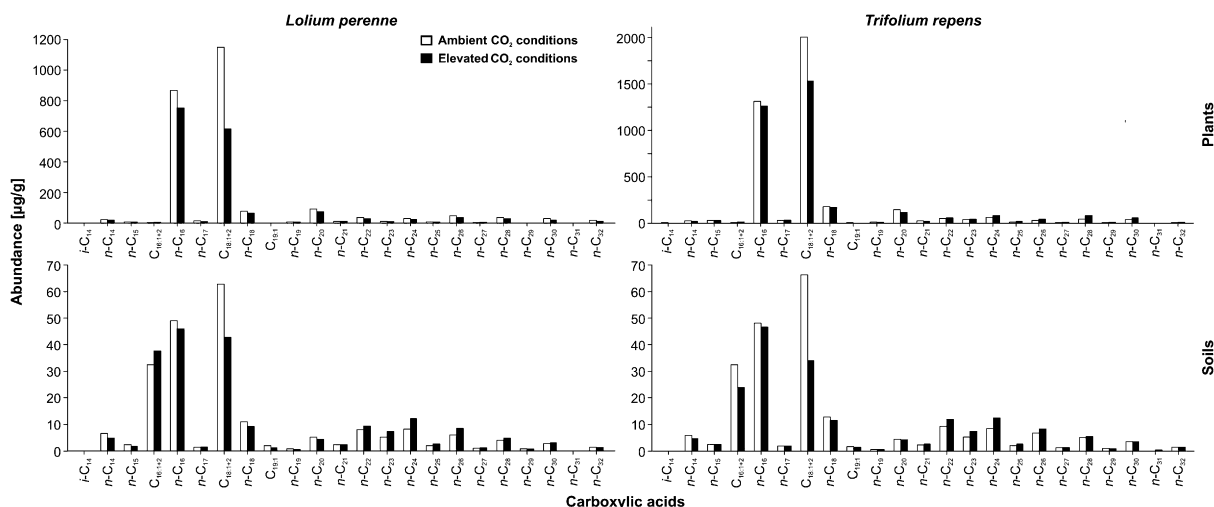

Fig. 2. Carboxylic acid distribution patterns of *Lolium perenne* and *Trifolium repens* plants and soils.

at the expense of short chain acids. The latter were degraded faster due to their greater accessibility for microbial degradation.

Like *L. perenne* plant biomass, the biomass of *T. repens* showed a predominance of unsaturated C_{18} and saturated C_{16} acids (Fig. 2), in agreement with previous analyses (Body, 1974). The CO_2-fertilized plants showed only a significant depletion in unsaturated C_{18} acids, whereas the abundances of the other acids were virtually identical in plants grown under elevated and ambient CO_2 conditions. Thus, *T. repens* plant wax seems not to be influenced by an enhanced CO_2 level to the same extent as *L. perenne* wax. This is in agreement with literature data, where legumes like *T. repens* have been documented as being less affected by atmospheric CO_2 concentration than grasses like *L. perenne* (Teyssonneyre et al., 2002).

Like soil under *L. perenne*, soil under *T. repens* revealed a microbial contribution of unsaturated C_{16} acids in addition to predominantly plant-derived saturated C_{16} and unsaturated C_{18} acids. Long chain acids were slightly enriched in soil under elevated CO_2, manifesting the assumption that under enhanced CO_2 concentration selective preservation of long chain acids and preferred degradation of other plant-derived components by soil biota seems to be possible.

3.2.2. Selected molecular ratios

To illustrate the changes in lipid distribution patterns, selected ratios were determined (Table 2). Under elevated CO_2, an increase in saturated vs. unsaturated C_{16} and C_{18} acids was observed for plant material. A larger change could be observed for *L. perenne* than for *T. repens*, which agrees with known stronger reaction to environmental change for grasses than legumes (Teyssonneyre et al., 2002). Controversially, soil under *T. repens* showed a greater relative enrichment in saturated short chain acids than *L. perenne* soil, probably due to slightly different microbial activity in each soil.

To illustrate the different behaviour of various chain lengths, the ratio of long chain (saturated C_{20} to C_{30}) to short chain (C_{16} and C_{18} saturated and unsaturated) acids was determined (Table 2). As expected, the ratio was rather low for the plants, because plants mainly consist of short chain homologues (Walton, 1990). However, under elevated CO_2 an increase of 20–100% could be determined for both plants, with a greater change noted for *T. repens*. This is supposed to be related to modification of the plant wax composition under enhanced CO_2 concentration. For *T. repens* the acid elongation mechanism seems to be favoured under enhanced CO_2, leading to both a relative decrease in short chain acids and an absolute enrichment in long chain acids, as described above. For soil, the ratio was significantly higher, mainly because the plant-derived short chain acids were degraded, while the long chain ones may have been selectively preserved and stabilized. A significant increase in the ratio, of between 79 and 108%, in soil under enhanced CO_2 conditions argues for a simultaneous selective preservation of long chain acids and decreased input of short chain homologues.

Table 2
Molecular ratios for plant and soil lipids under ambient and elevated CO_2 concentration

			Acids sat./unsat. $C_{16} + C_{18}$ [a]	Long (C_{20-30})/short chain ($C_{16+18sat.+unsat.}$) [b]	CPI_{acids} [c]	Alcohols C_{26}/($C_{26} + C_{30}$)	$CPI_{alcohols}$ [d]	Alkanes C_{29}/($C_{29} + C_{33}$)	$CPI_{alkanes}$ [e]
Lolium perenne	Plant	Ambient	0.8	0.4	5.9	0.98	32.5	0.64	16.3
		Elevated	1.3	0.5	5.5	0.99	32.5	0.66	17.4
	Soil	Ambient	0.6	1.3	2.7	0.87	13.6	0.60	8.7
		Elevated	0.7	2.7	2.8	0.85	14.8	0.63	9.2
Trifolium repens	Plant	Ambient	0.7	0.3	3.9	0.03	22.7	0.98	12.8
		Elevated	0.9	0.6	4.0	0.04	24.2	0.99	12.5
	Soil	Ambient	0.6	1.4	2.8	0.74	11.1	0.68	8.5
		Elevated	1.0	2.5	2.9	0.73	14.1	0.70	9.4

[a] $(C_{16:0} + C_{18:0})/(\Sigma C_{16:1+2} + \Sigma C_{18:1-3})$.
[b] $(\Sigma C_{20-30})/(\Sigma C_{16:0-2} + C_{18:0-3})$.
[c] $[(\Sigma C_{20+22+24+26+28+30}/\Sigma C_{19+21+23+25+27+29}) + (\Sigma C_{20+22+24+26+28+30}/\Sigma C_{21+23+25+27+29+31})]/2$.
[d] $[(\Sigma C_{22+24+26+28+30+32}/\Sigma C_{21+23+25+27+29+31}) + (\Sigma C_{22+24+26+28+30+32}/\Sigma C_{23+25+27+29+31+33})]/2$.
[e] $[(\Sigma C_{23+25+27+29+31+33}/\Sigma C_{22+24+26+28+30+32}) + (\Sigma C_{23+25+27+29+31+33}/\Sigma C_{24+26+28+30+32+34})]/2$.

The carbon preference index of long chain acids (CPI_{acids}; Table 2) was determined as follows:

$$CPI_{acids} = [(\Sigma C_{20+22+24+26+28+30}/\Sigma C_{19+21+23+25+27+29}) + (\Sigma C_{20+22+24+26+28+30}/\Sigma C_{21+23+25+27+29+31})]/2.$$

As expected, it was higher for plants than for soil, because fresh plant material was characterized by a predominance of even carbon numbered homologues. Surprisingly, it decreased for *L. perenne* by 7% but increased slightly for *T. repens* under enhanced CO_2 conditions. This is probably due to biosynthetic inhibition in *L. perenne* under enhanced CO_2 concentration. For both soils only minor changes over a similar range as for *T. repens* plants could be observed.

3.3. Alcohols

Although less abundant than acids in plants and soils, alcohols are potential precursors of alkanes, the most simple and supposedly most stable lipids in soil, as determined previously for arable (Wiesenberg et al., 2004a) and peaty soils (Bol et al., 1996).

3.3.1. Distribution patterns

L. perenne was characterized by large amounts of even numbered long chain C_{26} alcohols (Fig. 3), consistent with results obtained by Alleborne et al. (1970) and Bull et al. (2000). The n-C_{28} homologue was present in lower abundance, whereas all the others occurred in trace amounts. Under enhanced CO_2 conditions, an increase in the most abundant alcohol by ca. 20% is remarkable. The other alcohols showed only minor changes. In contrast to the plant biomass, the soil under *L. perenne* revealed higher abundances of all even numbered long chain alcohols (especially C_{22}–C_{30}) when compared to the most abundant n-C_{26} alcohol. This is due to the soil matrix, where alcohols may originate from sources other than the above ground plant biomass. Additionally, the distribution pattern of fresh plant biomass is modified during degradation and incorporation processes in soils. Like the plant biomass, soil under enhanced CO_2 conditions revealed higher abundances of alcohols, due to a selective preservation of long chain alcohols from the modified plant biomass.

In comparison to *L. perenne*, alcohols were less abundant in *T. repens* (Fig. 3). A predominance of the n-C_{30} homologue is consistent with results of Body (1974), although C_{28} or C_{30} alcohols are usually most abundant in C_4 plants (Rommerskirchen et al., 2006). Under elevated CO_2, *T. repens* contained notably lower amounts of individual alcohols (ca. 50%). Thus, we conclude that selected n-alcohols become more depleted under enhanced CO_2 concentration.

Concomitantly, some sterols were enriched (unpublished results), leading to constant proportions of this intermediate polarity fraction in the total lipid extract under ambient and elevated CO_2 conditions (Table 1, Fig. 1). The soil under *T. repens* showed no obvious change under ambient vs. elevated CO_2 conditions. In comparison to the plant

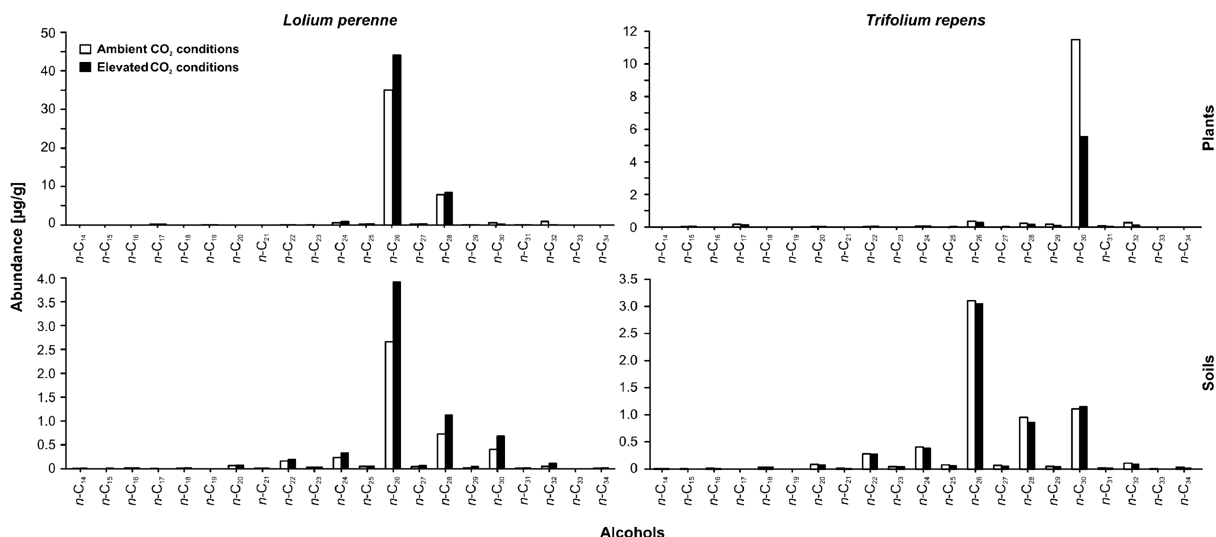

Fig. 3. Alcohol distribution patterns of *Lolium perenne* and *Trifolium repens* plants and soils.

material, a predominance of the n-C_{26} alcohol could be determined. This similar pattern to soil under *L. perenne* indicates that the observed distribution still reflects the arable and grassland land use prior to the FACE experiment. The enhanced proportion of n-C_{30} in soil under *T. repens* is related to the input of the corresponding plant material in comparison to the soil under *L. perenne*, where this pattern is missing. Like the soil under *L. perenne*, a relative enrichment in all even numbered long chain alcohols was observable.

3.3.2. Selected molecular ratios

Because of the low abundances of most alcohols, fewer molecular ratios could be calculated for this fraction than for the acids. To illustrate the sources or state of preservation of long chain alcohols, $CPI_{alcohols}$ (Table 2) was determined as follows:

$$CPI_{alcohols} = [(\Sigma C_{22+24+26+28+30+32}/\Sigma C_{21+23+25+27+29+31}) + (\Sigma C_{22+24+26+28+30+32}/\Sigma C_{23+25+27+29+31+33})]/2.$$

The ratio showed the highest values for *L. perenne* plants, which also contained the largest absolute amounts of these lipids. Lower ratio values were observed for *T. repens*, resulting from the lower predominance of the most abundant alcohols. For soils, relatively uniform and low values can be attributed to the degradation of the primary plant products and the dilution effect in soils caused by "old alcohols" incorporated before the FACE experiment was introduced. Obvious changes in $CPI_{alcohols}$ under elevated vs. ambient CO_2 conditions could not be observed. The minor variations, especially for soil, presumably relate to soil heterogeneity in the different plots.

To illustrate the different response of the individual plants to elevated atmospheric CO_2 concentration within the n-alcohols, the $C_{26}/(C_{26}+C_{30})$ ratio was used (Table 2). As described above, the predominance of n-C_{26} increased under elevated CO_2 conditions in *L. perenne* plants, leading to slightly higher values for the ratio. For *T. repens* it differed markedly, with a value of 0.03, but remained nearly constant under ambient and elevated CO_2 conditions, due to the predominance of n-C_{30}. Within the soils, higher values were observed for *L. perenne* soil as a result of the plant biomass input. Surprisingly, the value decreased in soil under enhanced CO_2 conditions, whereas that for the plant biomass increased. A notable fraction of the soil alcohols in the *L. perenne* plots comprised relicts of old material incorporated prior to the establishment of the FACE experiment and enhanced degradation of the primary plant products led to a relative decrease in n-C_{26}, while the long chain components were selectively preserved. For *T. repens* soil, uniformly lower $C_{26}/(C_{26}+C_{30})$ values than for *L. perenne* soil document the plant-derived input of *T. repens* biomass, which was enriched in the n-C_{30} alcohol.

3.4. Alkanes

Long chain *n*-alkanes are predominantly produced during degradation of carboxylic acids and alcohols and represent a relatively stable lipid fraction when compared to the other lipids (e.g. Bol et al., 1996; Wiesenberg et al., 2004a).

3.4.1. Distribution patterns

L. perenne was characterized by large amounts of n-C_{31} alkane and decreasing abundances of neighbouring odd numbered homologues (Fig. 4). This agrees with distribution patterns determined by Dove (1992), Dove et al. (1996) and Bull et al. (2000). Under enhanced CO_2 conditions, a slight decrease in the abundances was determined and must be related to the decrease in the supposedly main precursors of alkanes, the long chain acids within the *L. perenne* plants. For soil under *L. perenne* a similar alkane distribution pattern was obtained. As within plants under enhanced CO_2 conditions, the amounts of long chain *n*-alkanes (C_{31} and C_{33}) decreased slightly in the soil. Contrastingly, a slight increase in n-C_{27} and n-C_{29} alkanes was observed, which can be ascribed to incorporation of larger amounts of long chain *n*-alcohols derived from plants grown under enhanced CO_2 conditions. Thus, different sources of soil alkanes led to depletion in selected homologues and enrichment in other components.

The alkane distribution pattern differed between *L. perenne* and *T. repens*. The latter was dominated by the n-C_{29} alkane and had slightly lower amounts of n-C_{31}, whereas the other odd numbered homologues were present only in minor amounts. This confirms hydrocarbon distribution patterns for *T. repens* described by Body (1974), Dove (1992), Dove et al. (1996) and Chen et al. (1998). The decrease under enhanced CO_2 conditions was greater than for *L. perenne* and can be attributed to the obvious decrease in the alcohols in *T. repens*. Thus, a large proportion of *T. repens* alkanes seems to be derived from alcohols. For soil under *T. repens*, yields of most long chain *n*-alkanes were stagnant or even decreased slightly, whereby the n-C_{29} homologue increased. This might result from the increased amounts of the C_{30} *n*-alcohol in the soil, which, during degradation, afforded the n-C_{29} alkane.

The similar alkane distribution patterns of all the soil samples might be influenced by a contribution from aerosols containing alkanes derived from, e.g. abraded plant waxes, biomass burning, and/or anthropogenic emissions. Such aerosols containing alkanes were previously observed to influence the alkane compositions of soils and sediments elsewhere (e.g. Conte and Weber, 2002). Due to the vegetation cover and the in situ incorporation of plant-derived waxes on the individual plots, aerosols are thought to influence only marginally the alkane distribution patterns determined

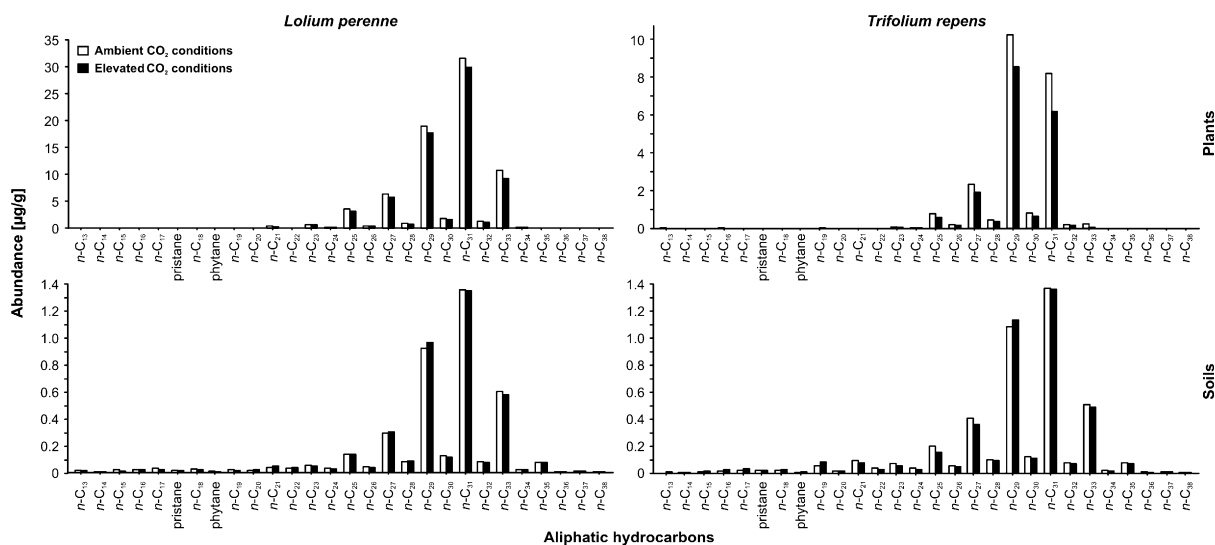

Fig. 4. Aliphatic hydrocarbon distribution patterns of *Lolium perenne* and *Trifolium repens* plants and soils.

in this study, resulting in a low, non-specific background signal.

3.4.2. Selected molecular ratios

Like the other components, the CPI ($CPI_{alkanes}$) can be used to determine the plant-derived input to sediments or soils:

$$CPI_{alkanes} = [(\Sigma C_{23+25+27+29+31+33}/\Sigma C_{22+24+26+28+30+32}) + (\Sigma C_{23+25+27+29+31+33}/\Sigma C_{24+26+28+30+32+34})]/2.$$

As expected, plants exhibited high values (Table 2), with *L. perenne* having the highest. *T. repens* plants revealed results intermediate between *L. perenne* biomass and the soils. The value increased slightly for *L. perenne* and both soils under elevated CO_2 conditions. To determine if there was a predominance of the C_{29} homologue or if long chain homologues were still present in higher amount, the $C_{29}/(C_{29}+C_{33})$ ratio was calculated. *L. perenne* biomass and soil revealed uniformly low values, due to only slightly higher amounts of C_{29} than C_{33}, in accord with literature data (Dove, 1992; Dove et al., 1996). For *T. repens* biomass the value was higher than for *L. repens* due to the fact that *n*-C_{29} was the most abundant compound. Consequentially, soil samples under *T. repens* initially had higher $C_{29}/(C_{29}+C_{33})$ values than soil samples under *L. perenne*. In any case the ratio increased in plant biomass and soils from *T. repens* and *L. perenne* plots under enhanced CO_2 conditions as compared to ambient conditions. This illustrates the predominant biosynthesis of *n*-alkane homologues with a shorter chain length under enhanced CO_2 conditions.

4. Conclusions

The effects of 10 years of elevated atmospheric CO_2 concentration (600 ppmv) on plant and soil lipid composition in the Eschikon grassland experiments were:

- In plants, the amount of total extractable lipid and the *n*-alkane distributions remained almost unchanged. In contrast, modification of carboxylic acid and alcohol distributions occurred under enhanced CO_2 conditions. A notable decline in unsaturated vs. saturated fatty acids indicated deterioration in wax composition due to environmental change, similar to effects noted for heat stress. The proportion of long chain acids increased by a factor of 20–100%, if plants were grown under enhanced CO_2 conditions. The value of the *n*-alcohol $C_{26}/(C_{26}+C_{30})$ ratio increase for both plants. In general, wax lipids of *T. repens* were less affected by enhanced CO_2 concentration than those of *L. perenne.* For alcohols, controversial trends by way of increasing or decreasing lipid concentration were observed for different plants, related to distinct differences in lipid biosynthesis reactions under enhanced CO_2 conditions.
- In soils, minor modifications were observed due to degradation effects and the slow turnover of lipids within soil. Lipid yield for *L. perenne* soil (97 mg/g) increased much more than for *T. repens* soil (17 mg/g). Soil *n*-alkanes showed slight modifications, because they were derived from a variety of plant precursor compounds. These lipid fractions may exhibit contrasting trends of depletion or enrichment under enhanced CO_2 concentration, thus cancelling each other out and minimizing modification of soil alkanes. Functionalized soil lipid fractions reflected modification of plant lipid biomass under elevated CO_2 concentration but were notably affected by degradation processes. For all soil samples, the CPI values of *n*-alkanes, *n*-alcohols and *n*-carboxylic acids increased moderately, pointing to less effective degradation of these lipids under enhanced CO_2 conditions. The observed increase in long chain *n*- acids and *n*-alcohols is attributed to a change in biomass input rather than selective preservation.

Elevated CO_2 concentration did not significantly change bulk lipid and alkane compositions, but modifications could be observed in plant alcohol and carboxylic acid distribution patterns. Under enhanced atmospheric CO_2 conditions, which can be envisaged as occurring within the next century, major changes in plant lipids can be expected, leading to a lower efficiency of surface-protecting plant waxes. Deterioration of plant surface waxes may negatively affect water use efficiency, protection against ultraviolet (UV) radiation or insect attack, leading to a reduction in crop yield.

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