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,
2007). Meanwhile, it is beyond dispute that, throughout the following decades, a further increase in atmospheric CO2 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 CO2 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 CO2 conditions on neighbouring plots. However, controversial results were published concerning the bioproductivity and degradability under enhanced CO2 concentrations. On one hand, productivity increased for numerous C3 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 CO2 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 CO2 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 CO2 concentration (e.g. van Kessel et al., 2000). Similarly, microbial biomass sequestration was not significantly enhanced under elevated CO2 concentration (Glaser et al., 2006). Molecular approaches concerning compositional changes in plant biomass and soil organic carbon under elevated CO2 concentration are scarce, but indicate compositional changes of the soil organic carbon under elevated CO2 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 CO2 concentration (e.g. Huang et al., 1999; Hussain et al., 2001; Penuelas et al., 2002) or input of lipids to grassland soils under ambient vs. elevated CO2 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 (Penuelas et al., 2002).

Here, we demonstrate lipid compositional changes under enhanced CO2 concentration for several pasture plants and show how this lipid signal is transferred from plant biomass to soil. We show that enhanced atmospheric CO2 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 CO2 concentration on a grassland ecosystem. While ambient air
contained ca. 350 ppm CO₂, fumigated plots had received an enhanced CO₂ 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 CO₂ 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 (δ¹³C values) are expressed in permil relative to the Vienna Pee Dee Belemnite (V-PDB) standard:

\[ \delta^{13}C = \left( \frac{^{13}C}{^{12}C_{\text{sample}}} / \frac{^{13}C}{^{12}C_{\text{std}}} \right) - 1 \times 10^3 \]  
where \( ^{13}C/^{12}C_{\text{std}} = 0.0112372. \)

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 CH₂Cl₂/CH₃OH (93/7; v/v) at 5 × 10⁶ 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 vapouriser (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-C₂₀ carboxylic acid, \( d_{37} \) n-C₁₈ alcohol, \( d_{50} \) n-C₂₄ 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 CH₂N₂ 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,
Carbon and lipid contents of plants grown under ambient and elevated atmospheric CO₂ concentrations were almost identical, consistent with literature data (Pen˜uelas et al., 2002). Seed from Pinus taeda may, however, contain significantly more lipids when exposed to elevated CO₂ 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 CO₂ as a result of both higher atmospheric CO₂ content and the 13C-depleted atmospheric CO₂ values due to the added CO₂ being lighter (isotopic composition /C0 &). The isotopic depletion was similar for both plants, around 9 & vs. the plants grown under ambient CO₂ conditions. In comparison to plant material, soil samples showed larger differences between both plants and both CO₂ 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 CO₂ 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 CO₂ conditions by 97 µg/g for the L. perenne soil and only 17 µg/g for the T. repens soil. The δ¹³C value was depleted by 4.4‰ for the soil under L. perenne and 3‰ for that under T. repens, under elevated CO₂ conditions. The smaller changes for the T. repens soil correspond to the lower biomass productivity of T. repens than L. perenne under elevated CO₂ 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 CO₂ 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 CO₂ 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 C₁₈ and saturated C₁₆ components (Fig. 2), typical of a broad variety of plant waxes including L. perenne (Walton, 1990; Bianchi,

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Total carbon [mg/g]</th>
<th>Total organic carbon [mg/g]</th>
<th>Extract yield [µg/g]</th>
<th>Isotopic composition (δ¹³C [%o, V-PDB])</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. perenne plants ambient</td>
<td>443.2 ± 1.1</td>
<td>n.d. a</td>
<td>78460</td>
<td>−28.3 ± 0.0</td>
</tr>
<tr>
<td>L. perenne plants elevated</td>
<td>447.4 ± 0.8</td>
<td>n.d. a</td>
<td>74020</td>
<td>−37.7 ± 0.5</td>
</tr>
<tr>
<td>L. perenne soil ambient</td>
<td>30.4 ± 0.5</td>
<td>27.0 ± 1.2</td>
<td>943</td>
<td>−26.5 ± 0.7</td>
</tr>
<tr>
<td>L. perenne soil elevated</td>
<td>33.6 ± 1.5</td>
<td>28.2 ± 2.2</td>
<td>1040</td>
<td>−30.9 ± 0.3</td>
</tr>
<tr>
<td>T. repens plants ambient</td>
<td>452.0 ± 1.3</td>
<td>n.d. a</td>
<td>101340</td>
<td>−27.0 ± 0.0</td>
</tr>
<tr>
<td>T. repens plants elevated</td>
<td>441.8 ± 2.6</td>
<td>n.d. a</td>
<td>101760</td>
<td>−36.2 ± 0.1</td>
</tr>
<tr>
<td>T. repens soil ambient</td>
<td>26.9 ± 0.9</td>
<td>25.6 ± 1.4</td>
<td>853</td>
<td>−26.9 ± 0.0</td>
</tr>
<tr>
<td>T. repens soil elevated</td>
<td>29.5 ± 2.2</td>
<td>27.1 ± 0.9</td>
<td>870</td>
<td>−29.9 ± 0.0</td>
</tr>
</tbody>
</table>

* Not determined, but similar to total carbon content.
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 (\( \leq 19 \) carbons) vs. long chain (\( \geq 20 \) 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.

![Diagram of lipid proportions](image-url)
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\textsubscript{18} and saturated C\textsubscript{16} acids (Fig. 2), in agreement with previous analyses (Body, 1974). The CO\textsubscript{2}-fertilized plants showed only a significant depletion in unsaturated C\textsubscript{18} acids, whereas the abundances of the other acids were virtually identical in plants grown under elevated and ambient CO\textsubscript{2} conditions. Thus, *T. repens* plant wax seems not to be influenced by an enhanced CO\textsubscript{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\textsubscript{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\textsubscript{16} acids in addition to predominantly plant-derived saturated C\textsubscript{16} and unsaturated C\textsubscript{18} acids. Long chain acids were slightly enriched in soil under elevated CO\textsubscript{2}, manifesting the assumption that under enhanced CO\textsubscript{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\textsubscript{2}, an increase in saturated vs. unsaturated C\textsubscript{16} and C\textsubscript{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\textsubscript{20} to C\textsubscript{30}) to short chain (C\textsubscript{16} and C\textsubscript{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\textsubscript{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\textsubscript{2} concentration. For *T. repens* the acid elongation mechanism seems to be favoured under enhanced CO\textsubscript{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\textsubscript{2} conditions argues for a simultaneous selective preservation of long chain acids and decreased input of short chain homologues.
The carbon preference index of long chain acids (CPI\textsubscript{acids}; Table 2) was determined as follows:

\[
\text{CPI}_{\text{acids}} = \left( \frac{R_{20}+22+24+26+28+30 + R_{19}+21+23+25+27+29}{R_{16}+18+R_{21}+23+25+27+29+31} \right) + \left( \frac{R_{20}+22+24+26+28+30 + R_{21}+23+25+27+31+33}{R_{22}+24+26+28+30+32+33} \right) \right) / 2.
\]

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 \textit{L. perenne} by 7% but increased slightly for \textit{T. repens} under enhanced CO\textsubscript{2} conditions. This is probably due to biosynthetic inhibition in \textit{L. perenne} under enhanced CO\textsubscript{2} concentration. For both soils only minor changes over a similar range as for \textit{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

\textit{L. perenne} was characterized by large amounts of even numbered long chain C\textsubscript{26} alcohols (Fig. 3), consistent with results obtained by Alleborne et al. (1970) and Bull et al. (2000). The n-C\textsubscript{28} homologue was present in lower abundance, whereas all the others occurred in trace amounts. Under enhanced CO\textsubscript{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 \textit{L. perenne} revealed higher abundances of all even numbered long chain alcohols (especially C\textsubscript{22–C30}) when compared to the most abundant n-C\textsubscript{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\textsubscript{2} conditions revealed higher abundances of alcohols, due to a selective preservation of long chain alcohols from the modified plant biomass.

In comparison to \textit{L. perenne}, alcohols were less abundant in \textit{T. repens} (Fig. 3). A predominance of the n-C\textsubscript{30} homologue is consistent with results of Body (1974), although C\textsubscript{28} or C\textsubscript{30} alcohols are usually most abundant in C\textsubscript{4} plants (Rommerskirchen et al., 2006). Under elevated CO\textsubscript{2}, \textit{T. repens} contained notably lower amounts of individual alcohols (ca. 50%). Thus, we conclude that selected n-alcohols become more depleted under enhanced CO\textsubscript{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\textsubscript{2} conditions (Table 1, Fig. 1). The soil under \textit{T. repens} showed no obvious change under ambient vs. elevated CO\textsubscript{2} conditions. In comparison to the plant

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Plant Ambient</th>
<th>Elevated</th>
<th>Plant Elevated</th>
<th>Soil Ambient</th>
<th>Elevated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acids sat./unsat.</td>
<td>C\textsubscript{16} + C\textsubscript{18}</td>
<td>C\textsubscript{16} + C\textsubscript{18}</td>
<td>C\textsubscript{16} + C\textsubscript{18}</td>
<td>C\textsubscript{16} + C\textsubscript{18}</td>
<td>C\textsubscript{16} + C\textsubscript{18}</td>
</tr>
<tr>
<td>Long (C\textsubscript{20–30})/short chain</td>
<td>C\textsubscript{16} + 18 sat. + unsat.</td>
<td>C\textsubscript{16} + 18 sat. + unsat.</td>
<td>C\textsubscript{16} + 18 sat. + unsat.</td>
<td>C\textsubscript{16} + 18 sat. + unsat.</td>
<td>C\textsubscript{16} + 18 sat. + unsat.</td>
</tr>
<tr>
<td>CPI\textsubscript{acids} (^a)</td>
<td>0.8</td>
<td>1.3</td>
<td>0.6</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>CPI\textsubscript{alcohols} (^b)</td>
<td>0.4</td>
<td>0.5</td>
<td>1.3</td>
<td>2.7</td>
<td>2.7</td>
</tr>
<tr>
<td>CPI\textsubscript{alkanes} (^c)</td>
<td>5.9</td>
<td>5.5</td>
<td>2.7</td>
<td>2.8</td>
<td>2.8</td>
</tr>
<tr>
<td>Alcohol C\textsubscript{26}/(C\textsubscript{26} + C\textsubscript{30}) (^d)</td>
<td>0.98</td>
<td>0.99</td>
<td>0.87</td>
<td>0.85</td>
<td>0.85</td>
</tr>
<tr>
<td>Alcohol C\textsubscript{26}/(C\textsubscript{29} + C\textsubscript{33}) (^e)</td>
<td>32.5</td>
<td>32.5</td>
<td>13.6</td>
<td>14.8</td>
<td>14.8</td>
</tr>
<tr>
<td>Alcohol C\textsubscript{26}/(C\textsubscript{29} + C\textsubscript{33}) (^e)</td>
<td>0.64</td>
<td>0.66</td>
<td>0.60</td>
<td>0.63</td>
<td>0.63</td>
</tr>
<tr>
<td>Alcohol C\textsubscript{26}/(C\textsubscript{29} + C\textsubscript{33}) (^e)</td>
<td>16.3</td>
<td>17.4</td>
<td>8.7</td>
<td>9.2</td>
<td>9.2</td>
</tr>
</tbody>
</table>

\(^a\) \((C_{16:0} + C_{18:0})/(\Sigma C_{16:1} + \Sigma C_{18:1–3})\).  
\(^b\) \((\Sigma C_{20–30})/(\Sigma C_{16:0} + \Sigma C_{18:0–3})\).  
\(^c\) \([(\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 + 31 + 33)]/2.  
\(^e\) \([(\Sigma C_{22–24} + 26 + 28 + 30 + 32 + 33 + \Sigma C_{21–23} + 25 + 27 + 29 + 31 + 33)]/2.  
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, \( \text{CPI}_{\text{alcohols}} \) (Table 2) was determined as follows:

\[
\text{CPI}_{\text{alcohols}} = \frac{\left(\sum C_{22+24+26+28+30+32}/\sum C_{21+23+25+27+29+31}\right) + \left(\sum C_{22+24+26+28+30+32}/\sum C_{23+25+27+29+31+33}\right)}{2}.
\]

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 \( \text{CPI}_{\text{alcohols}} \) under elevated vs. ambient \( \text{CO}_2 \) 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 \( \text{CO}_2 \) 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 \( \text{CO}_2 \) 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 \( \text{CO}_2 \) 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 \( \text{CO}_2 \) 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. Contrarily, 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.
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:

$$\text{CPI}_{\text{alkanes}} = \left[ \frac{\sum C_{23}+25+27+29+31+33}{\sum C_{22}+24+26+28+30+32+33} + \frac{\sum C_{23}+25+27+29+31+33}{\sum C_{24}+26+28+30+32+34} \right] / 2.$$

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. Consequently, 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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