Elevated atmospheric CO₂ fuels leaching of old dissolved organic matter at the alpine treeline

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[1] Dissolved organic matter (DOM), the mobile form of soil organic matter (SOM), plays an important role in soil C cycling and in nutrient transport. We investigated the effects of 5 years of CO₂ enrichment (370 versus 570 µmol CO₂ mol⁻¹) on DOM dynamics at the alpine treeline, including the analysis of fast-cycling components such as low molecular weight organic acids (LMWOAs), dissolved organic carbon (DOC) biodegradability, and the decomposition of ¹⁴C-labeled oxalate. Concentrations of DOC in canopy throughfall were 20% higher at elevated CO₂, probably driven by higher carbohydrate concentrations in leaves. In the organic soil layer, 5 years of CO₂ enrichment increased water-extractable organic C by 17% and soil solution DOC at 5 cm depth by 20%. The ¹³C tracing of recently assimilated CO₂ revealed that the input of recent plant-derived C (<15% of total DOC) was smaller than the CO₂-induced increase in DOC. This strongly suggests that CO₂ enrichment enhanced the mobilization of native DOC, which is supported by significant increases in dissolved organic nitrogen (DON). We mainly attribute these increases to a stimulated microbial activity as indicated by higher basal and soil respiration rates (+27%). The ¹⁴C-labeled oxalate was more rapidly mineralized from high CO₂ soils. The concentrations of LMWOAs, but also those of “hydrophilic” DOC and biodegradable DOC (6% of total DOC), were, however, not affected by elevated CO₂, suggesting that production and consumption of “labile” DOC were in balance. In summary, our data suggest that 5 years of CO₂ enrichment speeded up the cycling of “labile” DOM and SOM in a late successional treeline ecosystem and increased the mobilization of older DOM through a stimulated microbial activity. Such a “priming effect” implies that elevated CO₂ can accelerate the turnover of native SOM, and thus, it may induce increasing losses of old C from thick organic layers.


1. Introduction

[2] As the mobile fraction of soil organic matter (SOM), dissolved organic matter (DOM) represents a key vehicle for the transport of nutrients in ecosystems and determines the solubility and mobility of metals and organic compounds [e.g., Kalbitz et al., 2000; Hagedorn et al., 2000]. Fluxes of DOM through the soil are a small but potentially important pathway of C loss [Neff and Asner, 2001] and sorption of DOM in the mineral soil might contribute significantly to the accumulation and preservation of SOM [Kaiser and Guggenberger, 2000]. The current increase in atmospheric CO₂ by 1.5 ppm per year could potentially change DOM dynamics. Previous studies have demonstrated that experimental CO₂ enrichment alters the functioning of ecosystems and consequently DOM mobility in the following ways: (1) plant species specific changes in growth [Saxe et al., 1998; Körner, 2000]; (2) a higher C allocation to belowground sinks often followed by accelerated microbial activities [e.g., Norby et al., 2004; King et al., 2004]; (3) altered plant chemistry with decreasing N and increasing carbohydrate concentrations [Körner, 2000], and thus, changes in litter quality that could affect decomposition processes [e.g., Hättenschwiler and Bretsch, 2001].

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Although the effects of elevated CO\textsubscript{2} on plants and ecosystems have been intensively studied, little attention has been paid to the response in DOM. A better understanding of DOM dynamics under elevated CO\textsubscript{2} could help to elucidate the role of soils as C sinks/sources with ongoing atmospheric change. To date it is controversial whether or not soils may sequester more C under elevated CO\textsubscript{2} [Schlesinger and Lichter, 2001; Hagedorn et al., 2003; Loya et al., 2003; Carney et al., 2007]. Changes in SOM are difficult to detect, because C stocks are large and spatially highly variable [Hungate et al., 1996]. However, since DOM is the leaching product of SOM and its sources (litter and roots) [Kalbitz et al., 2000; Hagedorn et al., 2004], DOM in soil waters might actually represent a highly sensitive indicator for how elevated CO\textsubscript{2} might affect SOM.

In planted microcosms, CO\textsubscript{2} enrichment increased water- and salt-extractable organic C of soils [Cheng and Johnson, 1998; van Ginkel and Gorissen, 1998], suggesting higher soil C solubilization, which agrees with the hypothesis of stimulated belowground activity under elevated CO\textsubscript{2} [Zak et al., 1993; Hungate et al., 1997]. Recently, Freeman et al. [2004] observed that 3 years of CO\textsubscript{2} enrichment increased dissolved organic carbon (DOC) concentrations in peatland microcosms by as much as 60%. They hypothesized that rising CO\textsubscript{2} might even be responsible for the increase in DOC in surface waters of Northern Europe. Other studies, however, observed only negligible CO\textsubscript{2} effects on DOC concentrations in solutions of disturbed mineral soils in aggrading forests and forested mesocosms [King et al., 2001; Hagedorn et al., 2002]. So far, none of the CO\textsubscript{2} studies has looked at different fractions of DOM. For instance, rapidly cycling compounds such as low molecular weight organic acids (LMWOAs) and biodegradable DOM are closely linked to root turnover and litter decay [Yano et al., 2000; van Hees et al., 2005]. Consequently, these DOM fractions are likely responding more sensitively to elevated CO\textsubscript{2} than bulk DOM.

Essentially all published data on CO\textsubscript{2} effects on DOM and SOM are based on artificial ecosystems with highly disturbed soils or plantations of rapidly growing plant communities. It is highly uncertain how well-established plant communities and undisturbed soils will respond to a CO\textsubscript{2}-enriched atmosphere. Here, we have studied the effects of 5 years of CO\textsubscript{2} enrichment on DOM dynamics in a late successional treeline ecotone in the Swiss Alps. Ecosystems at the alpine treeline are characterized by a relatively low net primary productivity, little developed soils with typically a thick organic layer [Bednorz et al., 2000]. Soil organic matter dynamics and DOM fluxes are generally very little understood in alpine soils. A recent study at the Norwegian treeline suggests that the relative importance of DOC leaching as compared to other C fluxes is greater at the treeline than at lower altitudes [Clarke et al., 2005].

In our study we specifically aimed to determine (1) how elevated CO\textsubscript{2} affects concentrations and characteristics of DOM at the alpine treeline, (2) how sensitive rapidly cycling DOM components respond to high CO\textsubscript{2} concentration, (3) if CO\textsubscript{2} enrichment rather affects recent plant-derived DOM or older DOM leached from SOM, and (4) the consequences of altered DOM dynamics for the overall SOM cycling under elevated CO\textsubscript{2}.

### Study Site

The study was carried out at 2180 m.a.s.l. at Stillberg in the Central Alps near Davos, Switzerland, where a long-term research site was established in the late 50 s to study climate–plant growth relationships [Schönenberger and Frey, 1988]. The terrain is rather steep with northeast exposed slopes of 25 to 30°. Long-term average annual precipitation is 1050 mm, mean maximum snow depth is 1.50 m, and average January and July temperatures are −5.8°C and 9.4°C, respectively. Parent rock material is Paragneiss. Soil types are Rankefs and weakly developed Podzols [Blaser, 1980] with characteristic properties shown in Table 1. The organic layers are Humimors dominated by an Oa horizon [Bednorz et al., 2000] and have thicknesses between 5 and 15 cm. The plant community is dominated by ericaceous dwarf shrubs such as Vaccinium myrtillus, Vaccinium uliginosum, and Empetrum hermaphroditum. Common herbaceous species are Gentiana punctata, Homogyne alpina, Melampyrum pratense, Avenella flexuosa, and Leontodon helveticus. Individual trees not taller than 1.5–2.5 m of the two species Larix decidua and Pinus uncinata form a sparse open canopy. The trees originate from a large-scale afforestation experiment established in 1975 [Schönenberger and Frey, 1988], when small seedlings (1 to 3 years old) were planted into the intact and undisturbed dwarf shrub community.

### Methods

#### CO\textsubscript{2} Enrichment

Our free air CO\textsubscript{2} enrichment (FACE) study site is located at or slightly above the actual natural treeline within a relatively homogeneous 2500 m\textsuperscript{2} area [Hätteneschwiler et al., 2002]. Forty plots were assigned to 10 groups of four neighboring plots (two each with an individual Larix and Pinus tree in the plot center per group) in order to facilitate logistics of CO\textsubscript{2} distribution and regulation [Hätteneschwiler et al., 2002]. Half of these groups were randomly assigned to an elevated CO\textsubscript{2} treatment, while remaining groups served as controls, resulting in a split plot design. The CO\textsubscript{2} release system was installed by fixing a hexagonal stainless steel frame with an area of 1.1 m\textsuperscript{2} on three wooden posts. From each frame, 24 laser-punched drip irrigation tubes were hung vertically around the ring (15 cm apart from each other), weighted with a 3 mm stainless steel rod (to maintain rigidity) and the bottom ends were buried 2–4 cm into the soil, which resulted in a CO\textsubscript{2} enrichment of the trees and all understory vegetation in the plot.

#### Table 1. Properties of Organic Layer Material (Mainly Oa Horizon) Collected From 0–5 cm Depth

<table>
<thead>
<tr>
<th>Soil Organic C, Percent</th>
<th>C/N</th>
<th>pH (CaCl\textsubscript{2})</th>
<th>CEC eff\textsuperscript{a} mmol/kg</th>
<th>BS\textsuperscript{b}, Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient CO\textsubscript{2}</td>
<td>4.80 ± 2.5</td>
<td>27.2 ± 0.7</td>
<td>4.2 ± 0.1</td>
<td>169 ± 8</td>
</tr>
<tr>
<td>Elevated CO\textsubscript{2}</td>
<td>4.03 ± 2.4</td>
<td>27.2 ± 0.5</td>
<td>4.2 ± 0.1</td>
<td>167 ± 13</td>
</tr>
</tbody>
</table>

\textsuperscript{a}CEC eff: effective cation exchange capacity.

\textsuperscript{b}BS: base saturation.
concentrations were measured and regulated at the group level (four trees) with a control system. Growing season average was 566 ± 75 ppm, CO₂ under elevated CO₂ and 370 ± 3 ppm under ambient CO₂. More details about the experimental setup and the performance of the CO₂ enrichment are given by Hättenschwiler et al. [2002].

3.2. Sampling

[9] Soil solution was collected in all plots by installing two ceramic suction cups (SoilMoisture Equipment Corporation, Santa Barbara, California, United States) diagonally (60° to surface) at 3 to 7 cm depth in each plot. All of the suction cups were located within the Oa horizon that dominated the organic layer. In addition, we installed 10 suction cups (5 per treatment) at depths of 15 cm (E horizon) and 30 cm depth (BC horizon). The suction cups were installed at fixed depths and not at defined horizons, because space within the 1.1 m² plots was too limited to open pits for identifying diagnostic horizons and inserting horizontal lysimeters. In order to minimize sorption of DOC to the ceramic cups, we only used “aged” suction cups that had been used in other experiments before. Prior to their installation, they were flushed first with 1 M HCl, and then with distilled water. All lysimeters at a given depth were connected to one 1-L glass bottle per plot buried in the ground. Residence time of soil waters in the sampling devices was kept as short as possible. At each sampling, we collected soil waters by evacuating suction cups with a constant 400 hPa for about 16 h (overnight). However, during the dry summer 2003, we had to apply the suction for a week to get sufficient amounts of water.

[10] Throughfall was sampled with small polyethylene (PE) funnels (Ø 10 cm) just above the soil surface connected to 250 mL aluminium-foliated PE bottles. In order to capture the spatial variability, we changed the placement of the PE funnels after each sampling (n = 4 per season) within each plot. Samples from throughfall and soil waters were collected every month during the snow-free season. The site is not accessible in winter. All samples of soil and throughfall water were kept as short as possible. To minimize sorption of DOC, the samples were flushed first with 1 M NaOH inside the tube, on top of which a 14C-labeled oxalate in soils. Biodegradable DOC was quantified by incubating “fresh” soil solution from the Oa horizon (collected for 24 h and filtered to 0.45 µm) in the laboratory for 4 weeks. Soil solution (230 mL) was filled into 300 mL incubation flasks. To avoid nutrient limitation, we added 4 mL of a standard solution (4 mM CaCl₂, 2 mM K₂HPO₄, 1 mM K₂SO₄, 1 mM MgSO₄, 25 µM H₃BO₃, 2 µM MnSO₄, 2 µM ZnSO₄, 0.5 µM CuSO₄) and NH₄NO₃ yielding 15 mg N L⁻¹ in the solution. Then all samples were inoculated with 5 mL extracts of fresh Oa horizon material collected outside the experimental plots (extraction of soils with 5 mM CaCl₂ in a ratio 1:5 and filtration through a Schleicher and Schuell 790 1/2). To provide surfaces for microbial growth, we added 5 g of fiberglass and one fiberglass filter to each flask. The flasks were sealed, incubated in the dark at 20°C for 4 weeks and gently shaken by hand every day. In order to check the activity of microorganisms, we used a glucose solution of 30 mg C L⁻¹ as a control. Another control with ultrapure water was used to quantify the CO₂ production from the added inoculum. Biodegraded DOC was quantified by two methods: first, by determining the difference in DOC in 0.45 µm filtrates before and after incubation; and second by measuring the increase in CO₂ in the headspace of the flasks during two biweekly intervals. The samples and headspace were flushed with CO₂-free compressed air before the incubation. The CO₂ concentration in the gas phase was calculated using the general gas equation, from which we calculated the CO₂ in the liquid phase by using solubility constants and the measured pH.

[14] Oxalate biodegradation kinetics in soil: In late August 2003, after 3 years of CO₂ enrichment, composite Oa horizon samples were taken from all plots and bulked for all blocks, yielding n = 5 for both soils from ambient and elevated CO₂ plots, respectively. 14C-oxalate (1,2-14C; 185 MBq mmol⁻¹; ARC, Saint Lewis, Missouri, United States) was used in the assay. A 14C-radio labeled solution (100 µl, specific activity 1.7 kBq mL⁻¹) with concentrations ranging from 10 to 1000 µM (1–100 nmol; pH 4.5) was added to 1.00 ± 0.02 g of moist soil contained in 50 mL polypropylene tubes. Following addition, the soil was gently shaken to ensure mixing and incubated at 4°C in sealed tubes. The 14CO₂ produced by biodegradation of the substrate was collected by placing a plastic scintillation vial containing 1.0 mL of 1 M NaOH inside the tube, on top of the soil but separated from direct contact with the soil by a spacer. 14CO₂ trapped as NaH14CO₃ in the NaOH was determined by liquid scintillation (Wallac 1414 scintillation
samples were immediately frozen after collection. In the relatively dry year 2003 and 12 h in 2004. Unfiltered time for the soil solution was kept as short as possible, 3 days run to eliminate interference from Al and Fe ions. Sampling (final concentration 250

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swinity at 285 nm in DOC, a measure for aromaticity [Swaine and Hills 1959]. DOC was calculated by difference. The molar UV absorp-

counter, Wallac, Tampere, Finland) using alkali compatible scintillation fluid (Wallac Optiphase 3; Wallac, Tampere, Finland). $^{14}$C-CO$_2$ production was measured during the linear initial phase of mineralization, which was assessed in a separate experiment (not shown). The sampling time for $^{14}$CO$_2$ trap removal was 1 h.

The concentration-dependent experimental data were fitted to a single Michaelis–Menten equation:

$$V = \left(\frac{V_{\text{max}}C_s}{C_s + K_M}\right)$$

where $V$ is microbial substrate mineralization rate, $V_{\text{max}}$ the maximum mineralization rate, $C_s$ soil solution concentration and $K_M$ is the concentration at which half maximal mineralization occurs. $C_s$ was calculated assuming perfect mixing of the added oxalate with the solution present in the soil (estimated from the moisture content) and sorption onto the solid phase. Sorption characteristic (Langmuir) were taken from an Oe horizon of a South Swedish forest soil [van Hees et al., 2003].

3.4. Chemical Analysis

Hydrophobic and hydrophilic DOM was estimated by passing samples acidified to a pH of 2 through XAD-8 columns [Aiken and Leenheer, 1993]. In the effluent of the XAD-8 columns, representing the hydrophilic fraction, C concentration was measured. The hydrophobic fraction of DOC was calculated by difference. The molar UV absorptivity at 285 nm in DOC, a measure for aromaticity [Chin et al., 1994] was determined with a Cary 50 UV spectrophotometer (Varian, Incorporated, Palo Alto, California, United States). Phenol concentrations were measured with the Folin-Denis assay according to Swaine and Hills [1959]. Low molecular weight organic acids (LMWOAs) were determined in soil solutions sampled in mid September 2003 and early September 2004 by capillary electrophoresis using the method of Dahle ´n et al. [2000] employing electrokinetic injection. To determine oxalate and citrate, EDTA (final concentration 250 $\mu$M at pH 9) was added in a separate run to eliminate interference from Al and Fe ions. Sampling time for the soil solution was kept as short as possible, 3 days in the relatively dry year 2003 and 12 h in 2004. Unfiltered samples were immediately frozen after collection.

Water-extractable organic carbon (WEOC) was measured by gently shaking 5 g of field fresh soils with 100 mL 0.05 mM NaCl using an overhead shaker within 12 h after sampling. Roots were removed and soils were homogenized prior to the extraction. We chose a 0.05 mM NaCl solution because it had approximately the same ionic strength as the soil solution for the extraction. We chose a 0.05 mM NaCl solution within 12 h after sampling. We chose a 0.05 mM NaCl solution within 12 h after sampling. We chose a 0.05 mM NaCl solution within 12 h after sampling. We chose a 0.05 mM NaCl solution within 12 h after sampling.

Concentrations of dissolved and water-extractable organic C and total dissolved N (TDN) was determined with a Shimadzu TOC/TN analyser (TOC-V, Shimadzu Corporation, Tokyo, Japan). Dissolved organic N was estimated by subtracting concentrations of DIN from those of TDN. In all soil waters, the error of this indirect estimate was small because the fraction of DIN in TN was below 5% (Table 1). When inorganic N concentrations were below the detection limits (0.008 and 0.010 mg N L$^{-1}$ for NO$_3$ and NH$_4$, respectively), we subtracted half of these values from TDN. Nitrate concentrations were determined by ion chromatography (DX-120, Dionex, Sunnyvale, California, United States); those of NH$_4$ were measured colorimetrically by automated flow injection analysis (PE FIAS-300, PerkinElmer, Incorporated, Waltham, Massachusetts, United States). The $\delta^{13}$C values of plant and freeze-dried samples were determined with an automated elemental analyser–continuous flow isotope ratio mass spectrometer (EA-1110, Carlo Erba, Milan, interfaced with a Delta-S Finnigan MAT, Bremen). Results of the C isotope analysis are expressed in $\delta$ units (‰). The $\delta^{13}$C values were referenced to the Pee Dee Belemnite (PDB) standard.

3.5. Calculations and Statistical Analysis

Fluxes of DOM from the organic layer were roughly estimated by multiplying modeled water fluxes with DOM concentrations. Water fluxes were modeled with DyDOC [Michałzik et al., 2003], its hydrological subroutine is a simple “bucket” model assuming that precipitation falls as snow at temperature below 0°C and snowmelts above 5°C. Since we did not have DOM concentrations for the winter when the study site was not accessible, we roughly estimated them by assuming a Q$_{10}$ of 2.

Effects of CO$_2$ enrichment on all measured parameters were tested by ANOVA using a full factorial split plot model. Concentrations of DOC, dissolved organic nitrogen (DON), water-extractable OC, and in situ soil respiration were log transformed before the analysis. All statistical analyses were performed with R (version 1.9.1, R Development Core Team, 2004).

4. Results

4.1. DOM Concentrations and Fluxes

Concentrations of DOC and DON show a typical depth distribution with concentrations peaking in throughfall and the organic layer (Figure 1). In the sandy and stony mineral horizons (E and Bhs), concentrations of DOC decreased, but they still had values of approximately 10 mg C L$^{-1}$ at 30 cm depth. Dissolved organic N was the dominant form of N in all soil waters at 5, 15, and 30 cm depth comprising more than 95% of total dissolved N. The DOC to DON ratios increased with passage through the soil from 30 ± 5 in throughfall to 57 ± 3 in the mineral soil at 30 cm depth. Within the Oa horizon, DOC/DON was almost twice as high as the C to N ratio of solid organic matter (Tables 1 and 2).

Fluxes: Modeled drainage from the organic layer was 1200, 650, and 1000 mm a$^{-1}$ in 2002, 2003, and 2004, respectively. Evapotranspiration was estimated to be between 250 to 300 mm a$^{-1}$, which closely matched evapo-transpiration losses measured in lysimeter experiments with dwarf shrub communities at our study site ranging from 200 to 300 mm a$^{-1}$ [De Jong et al., 2002]. Under the assumption that DOC concentrations during winter months followed a Q$_{10}$ of 2 [Michałzik et al., 2003], we estimated that the...
DOC exports from the organic layer ranged between 20 and 35 g C m$^{-2}$ a$^{-1}$. The DON export was about 6 g N m$^{-2}$ a$^{-1}$ making up more than 95% of the total N export (inorganic + organic N).

[23] CO$_2$ enrichment significantly increased concentrations of DOC in throughfall (Figure 1; +20%; $P < 0.05$). The CO$_2$ effect on throughfall DON was smaller (+12%) and not significant. The slightly different response of DOC and DON shows that the DOC to DON ratio in throughfall increased under elevated CO$_2$ (35 ± 2 versus 31 ± 1) but this difference was only marginally significant ($P < 0.06$).

[24] In the soil solution of the organic layer at 3 to 7 cm depth, CO$_2$ enrichment clearly increased DOC concentrations over time (Figure 2). While the DOC concentrations were approximately the same in 2002 in both CO$_2$ treatments (+3% at high CO$_2$), the CO$_2$ effect on DOC increased from year to year (2003: +10%, 2004: +13%, 2005: +23%). Because of the large spatial variability, the CO$_2$ effect on DOC was not significant, not even in 2005 (main CO$_2$ effect: $P = 0.12$). However, the increase in DOC concentrations of DOC between 2002 and 2005 (Figure 2) was significantly greater at elevated CO$_2$ (+36%) as compared to ambient CO$_2$ (+14%; $P < 0.05$). The response in DON concentration was essentially the same as found for DOC. Again, the main CO$_2$ effect was not statistically significant in either year ($P = 0.11$ in 2005), but the increase in DON concentrations between 2002 and 2005 was significantly greater under elevated (+27%) than under ambient CO$_2$ (+5%; $P < 0.05$). The increase in DOC and DON concentrations under elevated CO$_2$ did not result from a concentration effect through decreasing amounts of water.

On the contrary, measured water contents were slightly greater at elevated CO$_2$ (+9 and +7% in 2003 and 2004; n.s.). At the lower soil depths, 15 and 30 cm, CO$_2$ enrichment did not affect DOC and DON concentrations (Figure 1). The DOC/DON ratios were not affected by the CO$_2$ enrichment in none of the soil depths.

[25] Water-extractable organic C from “root-free fresh” organic layers comprised roughly 0.25% of total soil C. In line with in situ DOM, water extractable organic C in 2003 and 2004 was on average 17% higher under elevated CO$_2$ (Figure 3, $P < 0.05$). Since concentrations of solid SOM (measured from the same samples as water-extractable organic C) were the same in both CO$_2$ treatments, the larger soluble C pool indicates that elevated CO$_2$ increased DOM production.

[26] Soil respiration: CO$_2$ enrichment stimulated basal respiration from “root-free, fresh” Oa horizons by 30% (Figure 4; $P < 0.05$). Consistently, mean soil CO$_2$ efflux during the fifth season in 2005 was 27% greater under elevated CO$_2$ than under ambient CO$_2$, but this effect was only marginally significant ($P < 0.07$).

### 4.2. DOM Properties

[27] Enrichment with CO$_2$ had no effects on the molar UV absorptivity, the concentrations of phenolics, and on the fractions of hydrophilic DOC in the organic layer (Table 2). A number of LMWOAs were identified in the soil solutions (Table 3). Average concentrations varied between <0.1–3 μM with the highest values seen for the monoprotic acids (1–3 C atoms per molecule). In addition to the LMWOAs presented, propionate was occasionally detected in

<table>
<thead>
<tr>
<th>DOC/DON</th>
<th>Molar UV Absorptivity, L cm$^{-1}$ mol$^{-1}$</th>
<th>Hydrophilic DOC (Percent of DOC)</th>
<th>Phenolics (Percent of DOC)</th>
<th>$\Sigma$ LMWOAs (Percent of DOC)</th>
<th>Biodegradable DOC (Percent of DOC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient CO$_2$</td>
<td>47.0 ± 2.4</td>
<td>402 ± 20</td>
<td>19.4 ± 1.0</td>
<td>12.6 ± 1.7</td>
<td>0.46 ± 0.05</td>
</tr>
<tr>
<td>Elevated CO$_2$</td>
<td>45.4 ± 1.8</td>
<td>411 ± 8</td>
<td>19.5 ± 1.4</td>
<td>12.1 ± 0.7</td>
<td>0.39 ± 0.06</td>
</tr>
</tbody>
</table>

*Means and standard errors of n = 20.*
The contribution of LMWOAs to total DOC was small (0.42% ± 0.04) and overall little affected by CO2 enrichment (Table 3). The LMWOA composition is comparable to most studies involving forest soils, but the concentrations are at the lower end of the scale [Strobel, 2001]. Concentrations of acetate were significantly lower at elevated CO2, while those of lactate were significantly higher in 2003.

4.3. DOM Turnover

[28] Biodegradation: The 4-week incubation of “field-fresh” soil solution sampled during 1 day from the Oa horizon showed that only a relative small fraction (6% of total DOC) was rapidly biodegradable (Table 2). The low mineralization rate cannot be attributed to an insufficient activity of microbes since 90% of the glucose added as a control was lost during the incubation (and the rest was very likely tied up in microbial biomass; data for glucose not shown). Mineralization of DOC did not change in response to the previous 4-year CO2 exposure of soils.

[29] Mineralization of 14C-labeled oxalate was fast and conformed to a hyperbolic (Michaelis-Menten) type of kinetics over the concentration interval studied (R2 > 0.8; Figure 5). Both maximum mineralization rates (Vmax) and the concentration at which half maximal mineralization occurs (Km) are within the range reported for forest soils [van Hees et al., 2005]. On average, soils from elevated CO2 plots showed marginally significant larger Vmax values (+43%) and higher Km values (+29%), indicating that high CO2 stimulated mineralization of added 14C-labeled oxalate in soils. In order to get an estimate of the CO2 effects on the oxalate mineralization under field conditions, we used the measured mineralization kinetics to calculate the mineralization rates for in situ soil oxalate concentrations measured in 2003 (Table 3). Figure 5 (inset) shows that they were 40% higher at elevated CO2 compared to ambient CO2 (P < 0.05).

5. Discussion

5.1. DOM Fluxes in a CO2 Enriched Treeline Ecotone

[31] Our first estimates of DOM fluxes at an alpine treeline indicate that DOM in throughfall and leachates from the organic layer contributes significantly to C and N fluxes in these ecosystems. DOC inputs via throughfall amounted to 5 g C m⁻² during the 4-month growing season corresponding to 10% of the annual C input through leaf litterfall (T. Handa, unpublished data, 2006). The DOC export of about 20 to 35 g C m⁻² a⁻¹ from the organic layer found here is in the range of values reported for temperate forest ecosystems [Michalzik et al., 2001]. However, in relative terms, DOC appears to be far more important for the overall C cycle in the treeline ecotone than in temperate forests which are typically characterized by other large C fluxes through litterfall and SOM mineralization. At our study site, Reichstein et al. [2000] estimated a mineralization rate of 70 to 120 g C m⁻² a⁻¹ from the organic layer, which agrees well with our measured heterotrophic soil CO2 effluxes (for the vegetation period, see Figure 4). Thus, DOC export accounted for approximately 20 to 30% of the total annual C losses from the organic layer.

[32] Fluxes of DOM appear to have increased in response to 5 years of CO2 enrichment in this late successional treeline ecotone. Throughfall DOC, water-extractable OC in the organic layer, and concentrations of “in situ” DOM...
and DON in soil solutions at 3 to 7 cm depth in the Oa horizon, all increased under elevated CO2. The composition of “in situ” DOM did, however, not change. The 20% increase in throughfall DOC in response to elevated CO2 can probably be attributed to an increased availability of soluble C in and on leaves. Concentrations of nonstructural carbohydrates, in particular starch, increased significantly in tree needles and dwarf shrub leaves under elevated CO2 [Handa et al., 2005; Asshoff and Hättenschwiler, 2005]. The wider DOC/DON ratios at elevated CO2 supports the idea that an enhanced accumulation of “labile” C compounds was mainly responsible for the increase in throughfall DOC. In addition, enhanced aboveground growth, and thus, increased canopy area might have contributed to the increase in throughfall DOC at the higher CO2 level (Table 4) [Handa et al., 2006; T. Zumbrunn, unpublished data, 2006].

Our results are in line with the 50% increase in throughfall DOC under elevated CO2 reported from a loblolly pine plantation [Lichter et al., 2000]. They suggested that although the absolute increase in throughfall DOC is small as compared to other C fluxes, it may fuel soil microbial activity because it is composed of mainly metabolically readily accessible forms of organic matter. We indeed found strong evidence for a stimulating CO2 effect on belowground activity. Basal respiration increased by 30% (Figure 4), indicating that more substrate was available under higher CO2 concentrations. Likewise, we measured a marginally significant 27% greater CO2 efflux from soils of high CO2 plots. Such a stimulation of soil microbial activity by elevated CO2 can be explained by the greater direct input of labile throughfall DOC, but also by an increased C allocation to roots, which could lead to increased root exudation. Although total root biomass remained unchanged [Handa et al., 2008], we found a 32% higher accumulation of starch in fine roots (Table 4).

### 5.2. Elevated CO2 Induced Mobilization of Native DOM

[34] Since we exposed late successional plant communities on naturally developed thick organic soil layers to elevated CO2 and given that bulk DOM is mainly made up of relatively “old” components [Fröberg et al., 2003; Hagedorn et al., 2004], we did not expect a CO2 effect on DOM after a relatively short experimental duration of 5 years. The increased DOM concentrations in response to CO2 enrichment found here is in contrast to other experiments with forest ecosystems on mineral soils with juvenile and expanding tree communities. After 2 to 4 years of CO2

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**Table 3. Effects of 3 and 4 Years of CO2 Enrichment on Low Molecular Weight Organic Acids in Soil Solutions of Oa Horizons (3 to 7 cm From the Surface)**

<table>
<thead>
<tr>
<th></th>
<th>Acetic, pM</th>
<th>Butyric, pM</th>
<th>Citric, μM</th>
<th>Formic, μM</th>
<th>Maloni, μM</th>
<th>Lactic, μM</th>
<th>Oxalic, μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient CO2</td>
<td>2.2 ± 0.7a</td>
<td>0.6 ± 0.7a</td>
<td>&lt;0.1a</td>
<td>1.9 ± 0.5a</td>
<td>0.1 ± 0.0a</td>
<td>1.0 ± 0.2a</td>
<td>1.0 ± 0.2a</td>
</tr>
<tr>
<td>Elevated CO2</td>
<td>0.8 ± 0.2a</td>
<td>1.3 ± 0.4a</td>
<td>0.2 ± 0.1a</td>
<td>1.1 ± 0.3a</td>
<td>0.1 ± 0.0a</td>
<td>1.0 ± 0.3a</td>
<td>1.3 ± 0.4a</td>
</tr>
</tbody>
</table>

*2003

<table>
<thead>
<tr>
<th></th>
<th>Acetic, pM</th>
<th>Butyric, pM</th>
<th>Citric, μM</th>
<th>Formic, μM</th>
<th>Maloni, μM</th>
<th>Lactic, μM</th>
<th>Oxalic, μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient CO2</td>
<td>1.6 ± 0.4a</td>
<td>1.1 ± 0.1a</td>
<td>0.1 ± 0.1a</td>
<td>1.1 ± 0.2a</td>
<td>0.1 ± 0.0a</td>
<td>1.8 ± 0.6a</td>
<td>0.9 ± 0.1a</td>
</tr>
<tr>
<td>Elevated CO2</td>
<td>0.8 ± 0.2a</td>
<td>1.3 ± 0.4a</td>
<td>0.2 ± 0.1a</td>
<td>1.1 ± 0.3a</td>
<td>0.1 ± 0.0a</td>
<td>1.0 ± 0.3a</td>
<td>0.9 ± 0.1a</td>
</tr>
</tbody>
</table>

*2004

*P<0.05 n.s. n.s. n.s. n.s. n.s. n.s. n.s.

*Means and standard errors of n = 20; n.s.: not significant.*
treatment, no effects on DOC concentrations in topsoils have been found, despite considerable increases in fine-root biomass and greater soil respiration rates [King et al., 2001; Hagedorn et al., 2002]. However, our results agree well with a microcosm experiment in peatlands where DOC concentrations increased by 14 to 61% after 3 years of CO2 enrichment [Freeman et al., 2004]. Integrating across these few existing CO2 studies suggests that the type of soil plays a decisive role on the CO2 effects on DOM: in pure organic horizons (peatland soils in the Freeman et al. [2004] study and organic layers in our experiment), elevated CO2 increased DOC leaching, while DOC concentrations in mineral soils did not respond. Apparently, mineral surfaces sorbing DOM particularly the lignin-derived DOM fraction [Kaiser and Guggenberger, 2000] are dampening the response of DOM to CO2 enrichment.

A higher production of “recent” DOM through increased plant growth, increased belowground C allocation, and higher DOM inputs via throughfall is the most evident mechanism for the increased DOC and DON concentrations under elevated CO2. Interestingly, our data show that the fraction of “recent” labile DOC is too small in bulk DOC to account for the observed increase. The “labile” DOM fractions such as low molecular weight organic acids and biodegradable DOC, which both presumably derive from recent C inputs from roots and decomposing litter, were below 10% of total DOM and did not respond to CO2 enrichment. Moreover, tracing the 13C signal added by the CO2 treatment indicates that recent less than 5-year-old photosynthates contributed only to 15% to total DOM, which is actually smaller than the CO2-induced increase in DOC (+23%; Figure 6). The water-extractable OC in 2003 after 3 years of CO2 enrichment shows the same pattern with an increase of 17% under elevated CO2, while the net input of recent C amounted only to 10% of total water-extractable OC (Figure 3). These small fractions of recent C and “labile” C in DOM provide evidence for an accelerated mobilization of “old” DOM through a stimulated microbial breakdown, as an additional more indirect mechanism for increased DOM under elevated CO2. Unfortunately, we cannot compare the fractions of plant-derived recent C in DOM at the two CO2 concentrations, because the 13C label lacks at ambient CO2. However, even under the extreme assumption that elevated CO2 would increase the input of recent C as much as photosynthesis (+50%, Handa et al. [2005]), it would increase total DOC leaching by a mere 5%, which strongly suggests that the major fraction of the 23% increase in DOC at elevated CO2 was derived from an accelerated mobilization of older native SOM. Our conclusion is supported by the similar increase in DON leaching (Figure 2), which cannot be attributed to higher plant inputs since (1) N concentrations of dwarf shrubs and trees declined by 14% under elevated CO2 [Handa et al., 2005; Asshoff and Härtenschwiler, 2005], (2) the DOC-to-DON ratio in throughfall was greater under CO2-enriched plants, and (3) DOM leached from

Table 4. Effects of Elevated CO2 on Cumulative Shoot Growth (2001–2004) and Starch Contents in Needles and Fine Roots

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Species</th>
<th>Shoot Growth, mm</th>
<th>Starch Needles, %</th>
<th>Starch Fine Roots, %</th>
<th>Fine Roots Protein, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient CO2</td>
<td>Larix</td>
<td>274 ± 8</td>
<td>9.9 ± 0.9</td>
<td>5.6 ± 0.5</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Elevated CO2</td>
<td>Larix</td>
<td>319 ± 10</td>
<td>10.6 ± 1.2</td>
<td>7.9 ± 1.0</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Ambient CO2</td>
<td>Pinus</td>
<td>157 ± 7</td>
<td>7.1 ± 0.8</td>
<td>6.5 ± 0.4</td>
<td>1.0 ± 0.0</td>
</tr>
<tr>
<td>Elevated CO2</td>
<td>Pinus</td>
<td>183 ± 21</td>
<td>9.6 ± 1.4</td>
<td>8.0 ± 0.6</td>
<td>0.7 ± 0.0</td>
</tr>
<tr>
<td>FC02 &lt;0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*From Handa et al. [2005].
decomposing litter was significantly depleted in N as compared to C [Hagedorn and Machwitz, 2007].

[36] An accelerated mobilization of old DOC through a stimulated microbial activity (the so-called “priming” effect) is discussed controversial (see reviews by Kuzyakov et al. [2000] and Fontaine et al. [2003]). A common explanation for priming is that an increased availability of microbial substrate induces enzyme production and/or increases enzyme activity leading to a cometabolic decomposition of soil organic matter. Although the production of DOM is assumed to be closely linked to enzymatic activity [Pregitzer et al., 2004] and to be a by-product of lignin degradation [Kalbitz et al., 2006], very little is known about priming effects on the release of DOM. Park and Matzner [2003] showed that experimental additions of glucose to forest soils stimulated microbial activity and doubled DOC and DON fluxes of DOM and DON far beyond an increase from the rapidly decomposing glucose itself. Altered soil microbial communities at elevated CO2 might have contributed to the greater mobilization of older DOM. For instance, Carney et al. [2007] observed that CO2 enrichment increased the abundance of fungi and accelerated losses of old SOM. Since fungi are the main lignin degrader in soils, it could be that a change in microbial communities under elevated CO2 led to an increased production of lignin-derived DOM. Our 13C-based study is the first to suggest that elevated CO2 can induce “priming” by mobilising old DOM in undisturbed organic layers. The composition of the DOM being typical for DOM leached from organic layers into mineral soils with the high-molecular, lignin-derived hydrophobic fraction dominating (Table 2) suggests that similar effects might occur in other forest soils. The CO2-induced increase in DOC concentration found here, corresponds to an increase in DOC export from the organic layer by 4 to 6 g C m⁻², which is negligible in comparison to other C fluxes. With respect to N, however, the 25% increase in DON leaching at elevated CO2 is more relevant, because the treeline ecosystem is N-poor with undetectable inorganic N concentrations in soil solutions and the DON export from the organic layer is similar in magnitude as total atmospheric N deposition in alpine regions [Schmitz et al., 2005].

5.3. Increased Turnover of “Labile” DOC

[37] Initially, we hypothesized that “labile” DOM compounds would respond most sensitively to the CO2-induced stimulation of belowground activity and to increased C inputs via throughfall. Our analysis of DOM composition, however, revealed that bulk DOC but none of the DOC fractions, not even the most labile ones were affected by elevated CO2. Neither the “hydrophilic” DOC, dominated by carbohydrates from decomposing plant residues and microbial metabolites [Kaiser et al., 2001], nor the rapidly cycling DOC compounds such as LMWOAs and biodegradable DOC showed a response to increased atmospheric CO2.

[38] Measured concentrations of labile DOC, however, must not necessarily correspond with production rates of labile DOC, because elevated CO2 might have simultaneously stimulated the consumption of “labile” compounds. Indeed, our mineralization experiment with 14C-labeled oxalate, a common root and fungal exudate, showed that the added oxalate decomposed more rapidly from soils that were previously exposed to higher atmospheric CO2 concentration. This result provides evidence for a faster cycling of labile DOM with no net effect on labile DOM content in soil solutions. The stimulated oxalate mineralization supports our conclusion that the observed increase in DOM at elevated CO2 was not primarily caused by an increased input of recent labile organic matter into DOM, because an accelerated microbial activity under CO2 enrichment led to a more rapid decomposition of labile DOM but apparently induced an increased DOM mobilization (Figure 7).

[39] Although concentrations of LMWOAs are small, they may contribute significantly to the CO2 effluxes from soils because of their rapid mineralization, usually within hours. For instance, van Hees et al. [2005] estimated that LMWOA mineralization in a boreal forest accounts for approximately 45 g C m⁻² a⁻¹. Therefore, our result showing that CO2 enrichment stimulated oxalate mineralization by 40% suggests that an increased turnover of “labile” DOM could substantially contribute to the CO2-induced increase in soils C availability and respiration rates (+60 g CO2-C m⁻² during the growing season 2005). In contrast to soil respiration, however, labile rapidly cycling DOM plays a less important direct role for DOM leaching from the 5 to 15 cm thick organic layer at this alpine treeline, because Oa horizon DOM is dominated by older nonreadily biodegradable organic matter (Table 2 and Figure 6).

5.4. Implications for Soil Carbon Cycling

[40] In contrast to most previous studies on the response of SOM to elevated CO2 in aggregating ecosystems on disturbed soils [e.g., Schlesinger and Lichter, 2001; Hagedorn et al.,
2003; Loya et al., 2003], we have run our CO2 enrichment experiment in an unproductive treeline ecotone with naturally developed alpine soils. Since spatial heterogeneity is high and C stocks of the 5 to 15 cm thick organic layers are big, the direct detection of any CO2 effect on SOM is impossible after some years. In our study, however, we may make use of our measured DOC dynamics to infer indirectly on SOM cycling because DOC comprises a small fraction of SOM, which is in a microbially driven dynamic equilibrium with the much greater solid SOM fraction [Kalbitz et al., 2000]. Our results showed that elevated CO2 increased DOC concentrations by 20% and that this increase was mainly related to a stimulated microbial activity and not to a greater net input of recent C into DOC. It implies that CO2 enrichment accelerated the mobilization of native SOM and thus, the turnover of SOM. This so-called “priming” effect counteracts an increased storage of C in soils. If we can translate the increased leaching of DOM to SOM cycling, it would indicate that elevated CO2 induces losses of older C from soils. Several studies have indicated that living roots and greater inputs of labile C stimulate native SOM decomposition [Cheng and Coleman, 1990; Kuzyakov et al., 2000]. Microcosm studies and plantations on agricultural C4 soils indicate that CO2 enrichment can induce priming through an increased input of labile C [Cheng and Johnson, 1998; Hoosbeek et al., 2004]. Recently, Carney et al. [2007] found in young scrub oak ecosystems that elevated CO2 reduced soil organic matter storage by altering soil microbial communities with higher abundances of fungi and higher activities of soil carbon-degrading enzymes. There is, however, no experimental evidence that elevated CO2 leads to priming in natural “old” ecosystems, because the SOM pool is too large and/or the fractions of recent C are not known under ambient CO2. Therefore, our result suggesting that CO2 enrichment induces an increased mobilization of older DOM in thick organic layers is a first indication for priming in undisturbed nutrient-poor acidic soils. As the DOM in the Oa horizon was very typical for DOM exported from organic layers with the lignin-derived hydrophobic fraction dominating, CO2-induced priming might occur in many forest soils. How quantitatively relevant it is for total soil C storage and thus for C sequestration remains, however, uncertain.

6. Conclusions

[41] Atmospheric CO2 enrichment in a late successional treeline ecotone with thick organic layers on acidic soils influenced a number of key soil processes. Higher basal and in situ soil respiration rates indicate a sustained increased microbial activity after 5 years of CO2 enrichment. This stimulation was also reflected in a higher turnover of the labile DOC fraction: 14C-oxalate was more rapidly mineralized in high CO2 soils, but as the concentrations of labile DOC components (including oxalate) remained unchanged, elevated CO2 increased both the microbial consumption and the production of “labile” DOC. In the Oa horizon, CO2 enrichment significantly increased concentrations of total DOC which had a typical composition with an 80% contribution of the high-molecular, lignin-derived hydrophobic fraction. A dominance of older C in DOM and an accompanying increase in DON suggests that this increase was mainly caused by an accelerated mobilization of native SOM through a stimulated microbial activity under elevated CO2. This so-called “priming” effect would counteract an increased storage of C in soils, but its quantitative importance for different ecosystems with other soil types needs to be explored.

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