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Decrease in heathland soil labile organic carbon under future atmospheric and climatic conditions

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Abstract Characterization of the impacts of climate change on terrestrial carbon (C) cycling is important due to possible feedback mechanisms to atmospheric CO_2 concentrations. We investigated soil organic matter (SOM) dynamics in the A1 and A2 horizons (~0–5.1 and ~5.1–12.3 cm depth, respectively) of a shrubland grass (*Deschampsia flexuosa*) after 8 years of exposure to: elevated CO_2 (CO_2), summer drought (D), warming (T) and all combinations hereof, with $TDCO_2$ simulating environmental conditions for Denmark in 2075. The mean C residence time was highest in the heavy fraction (*HF*), followed by the occluded light fraction and the free light fraction (*fLF*), and it increased with soil depth, suggesting that C was stabilized on minerals at depth. A2 horizon SOM was susceptible to climate change whereas A1 horizon SOM was largely unaffected. The A2 horizon *fLF* and *HF* organic C stocks decreased by 43 and 23% in response to warming, respectively. Organic nitrogen (N) stocks of the A2 horizon *fLF* and *HF* decreased by 50 and 17%, respectively. Drought decreased the A2 horizon *fLF* N stock by 38%. Elevated CO_2 decreased the A2 horizon *fLF* C stock by 39% and the *fLF* N stock by 50%. Under $TDCO_2$, A2 horizon *fLF* C and N stocks decreased by 22 and 40%, respectively. Overall, our results indicate that shrubland SOM will be susceptible to increased turnover and associated net C and N losses in the future.

Keywords Climate change · SOM · Density fractionation · C cycling · N cycling · FACE · ^{13}C · ^{15}N · C storage · CLIMAITE

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Introduction

Climate change is accelerated by increasing atmospheric concentration of carbon dioxide (CO_2) (IPCC 2013). The extent to which soil carbon (C) sequestration will counterbalance increasing atmospheric CO_2 concentrations depends in part on soil organic matter (SOM) dynamics (Davidson and Janssens 2006; Hofmockel et al. 2011b; Trumbore and Czimczik 2008). However, it is unclear how SOM will respond to climate change (Hofmockel et al. 2011b; Nie et al. 2014) because links and feedback mechanisms between SOM dynamics and climate are not fully understood (Heimann and Reichstein 2008; Trumbore and Czimczik 2008). Changes in C and nitrogen (N) cycling within SOM pools could drastically change long-term C sequestration and soil N availability (Hofmockel et al. 2011b).

SOM contains roughly 50% C and 0.1–6% N (Cotrofo and Gorissen 1997; Schnitzer and Khan 1978) and is mainly derived from plants through exudates, symbiotic fungi and litter (Davidson and Janssens 2006; Trumbore and Czimczik 2008), and to a minor extent from mesofauna, fungi (Mehrabanian 2013) and bacteria/archaea. The incorporation of OM into soil aggregates or sorption onto mineral or organic surfaces slows SOM decomposition by microbes and contributes to its stabilization in soil (Kleber et al. 2007). As changes in bulk SOM stocks can be difficult to observe due to high spatial variability in most natural ecosystems, improved understanding of climate change effects on SOM turnover and changes in soil C and N pools can be gained from SOM fractionation in combination with climate manipulation experiments (Trumbore and Czimczik 2008).

The SOM fractionation approach is particularly valuable in climate change experiments because non-complexed SOM pools often display more sensitive responses to environmental change than the bulk SOM pool (Christensen 2001). SOM fractionation techniques are based on the assumption that the extent and degree to which SOM is adsorbed to mineral soil particles regulates SOM dynamics and function (Gregorich et al. 2006). Soil density fractionation provides a mean to separate SOM inside and outside of aggregates (designated occluded light fraction, *oLF*, and free light fraction, *fLF*, respectively, with densities <1.5 g cm^{-3}) from mineral-associated SOM (heavy fraction, *HF*, with a density of typically 2.5–3.0 g cm^{-3}). Particles that sink in heavy liquid are thought to be absorbed to clay and sesquioxides, and contain variable amounts of humified SOM (Beare and Gregorich 2007; Kogel-Knabner et al. 2008).

In general, the youngest, most labile and least ^{13}C enriched (=^{13}C most negative) SOM prevails as discrete particles of plant origin (*fLF*) whereas older, most processed, recalcitrant and ^{13}C enriched SOM is associated with the *HF* (Gunina and Kuzyakov 2014; Kogel-Knabner et al. 2008; Meyer and Leifeild 2013; Wagai et al. 2009). It is believed that the *HF* can be formed from the *oLF* or directly from *fLF* material (Wagai et al. 2009). The *oLF* is thought to originate from the *fLF* and may partially be more degraded and recalcitrant (Buurman and Roscoe 2011; Wagai et al. 2009). Stabilization of soil organic C (SOC) and soil organic N (SON) is typically connected to mineral association in the *HF* (Bimüller et al. 2014; Marschner et al. 2008; Schrumpf et al. 2013). Organic C pesistence via selective preservation of recalcitrant compounds such as melanoidins, black C, tannins or aliphatic structures in the *oLF* (Mikutta et al. 2006; Poirier et al. 2003) is probably a less important stabilization mechanism (Marschner et al. 2008).

Climate change manipulation experiments have traditionally investigated single-factorial or combined effects of, in particular, elevated atmospheric CO_2 concentrations and warming (reviewed in Dieleman et al. 2012). These experiments, however, lack studying the effect of more severe future drought events (Dieleman et al. 2012) or anticipated changed precipitation patterns in general (IPCC 2013), which may also influence soil C and N turnover. In addition, changes in CO_2, temperature and precipitation may interact, complicating the prediction of the effects of multiple climatic and environmental stress factors from single factor studies (Andresen et al. 2010; Larsen et al. 2011; Scherber et al. 2013). Combined with the fact that changes in bulk SOC are hard to detect on an annual basis (Xu et al. 2011) this calls for research on fractionated soil C and N stocks in long-term multi-factorial climate manipulation experiments.

Shrublands constitute an important component of terrestrial landscapes [~7% of European land area (Carter et al. 2012)] and provide multiple important ecosystem services (Beier et al. 2009). The global area covered by shrublands may further increase as changes in land use cause shrub invasion in many arid and semiarid regions of the world (Schlesinger et al. 1990). Hence, shrublands deserve special attention in climate change impact research (Kröel-Dulay et al. 2015). The objective of this study was to evaluate how eight years of elevated CO_2, increased temperature and extended periods of drought, and all-factorial combinations hereof, affect soil C and N stocks in the A horizon of a temperate shrubland.

In the current work we tested four main hypotheses addressing the interaction between SOM pools and climate change conditions, i.e.:

1. Warming decreases the size of the *fLF* due to increased SOM turnover rates (Amundson and Davidson 1990; Kotroczo et al. 2008). Previous investigations from the shrubland ecosystem revealed a tendency for higher leaf litter decomposition under warming (Andresen et al. 2010), higher N turnover (Larsen et al. 2011), a higher microbial biomass (Haugwitz et al. 2014) and a stimulation of soil respiration (R_s) in most seasons (Selsted et al. 2012).
2. Drought increases SOC and SON stocks at the site. This hypothesis is based on literature evidence demonstrating drought-driven increases in litter input from increased plant senescence (Munné Bosch 2004), and drought-induced reductions in R_s (Linn and Doran 1984; Selsted et al. 2012; Skopp et al. 1990), N mineralization (Larsen et al. 2011) and leaf litter decomposition (Andresen et al. 2010).
3. Elevated CO_2 increases the SOM pool size due to a stimulation of net photosynthesis (Albert et al. 2011) and root biomass (Arndal et al. 2013) under elevated CO_2 at our experimental site.
4. The three-factorial treatment combination of warming, drought and elevated CO_2 is not expected to cause significant changes of the SOM pools after eight treatment years. Previous shorter term experiments at the specific site showed that the stimulating effects of elevated CO_2 and warming on plant biomass, SOM turnover (measured via soil and leaf litter incubation bags after 1 year) and soil fauna cancelled out or were reduced when combined with drought (Andresen et al. 2010; Kongstad et al. 2012; Larsen et al. 2011; Maraldo et al. 2010; Reinsch and Ambus 2013).

Methods

Experimental field site

Soil samples were collected at the site of the *CLIMAITE* experimental site, a temperate shrubland/grassland ca. 50 km north of Copenhagen, Denmark (55°53′N 11°58′E), matured on moraine deposits (Mikkelsen et al. 2008). The soil is a coarse textured sandy Arenosol (FAO)/Entisol (US Soil Taxonomy) from the Weichsel glaciation with only weak signs of podsolization, a relatively low Cation Exchange Capacity (CEC) and acidic pH (Table 1). The dominating plant types are grasses (ca. 77% coverage by *Deschampsia flexuosa*) and evergreen shrubs (ca. 23% coverage by *Calluna vulgaris*) (Kongstad et al. 2012). The experiment comprises twelve octagon-shaped plots (6.8 m diameter) that have been exposed to multiple environmental treatments since October 2005. The octagons are organized pair-wise in six blocks, where one of the paired octagons is exposed to ambient (A) atmospheric CO_2 concentration (390 ppm) and one is exposed to elevated CO_2 at 510 ppm (CO2) realized by Free-Air CO_2 Enrichment (FACE). All octagons are split into four equal-sized plots exposed to, in addition to ambient or elevated CO_2, either no treatment (A), extended spring/summer droughts (D) via horizontally moving curtains (removing 8–11% of annual precipitation and decreasing soil water content in D compared to A plots by 3.2 ± 0.5 and 5.7 ± 0.6 percentage points on average during the whole drought treatment periods and during the last 7 days of the drought treatments, respectively; Fig. 1a), to passively elevated night-time temperature (T) via a second set of horizontally moving reflective curtains (annual mean temperature at 20 cm above soil surface and at 5 cm soil depth elevated by 0.3 and 0.4 °C, respectively, in T compared to A plots, ranging from 0.1 °C in both air and soil during winter to 0.5 and 0.7 °C, respectively, during spring/summer; Fig. 1b) or a combination of drought and warming (TD). Hence the experimental design allows for the test of eight treatments (A, T, D, CO_2, TD, TCO_2, DCO_2, $TDCO_2$), each replicated six times. The full factorial treatment, $TDCO_2$, simulates as closely as possible a likely Danish climate scenario in 2075, as predicted by the Danish Meteorological Institute (www.DMI.dk). For more details, see Mikkelsen et al. (2008) and Scherber et al. (2013).

Table 1 Soil physical and chemical characteristics

Soil depth (cm)	Sand (% wt)	Silt	Clay	CEC (meq 100 g^{-1})	pH (0.01 M $CaCl_2$)
0–5	88.0 ± 0.7	9.7 ± 0.3	2.3 ± 0.8	3.32 ± 0.17	3.4 ± 0.03
5–10	91.9 ± 0.3	6.5 ± 0.2	1.6 ± 0.2	1.78 ± 0.14	3.7 ± 0.03
10–15	91.8 ± 0.5	5.9 ± 1.0	2.3 ± 0.6	1.37 ± 0.11[a]	4.2 ± 0.06[a]

Soil was collected in 2004 (pre-treatment) adjacent to each experimental octagon (n = 12) for cation exchange capacity (CEC) and pH; four locations (n = 4) were randomly selected in 2004 for textural analysis

[a] data for 10–30 cm soil depth

Soil sampling and sample pre-treatment

Four to five soil cores (Ø 2 cm, depth 12.3 ± 0.3 cm corresponding to the approximate depth of the A horizon) were collected randomly beneath *D. flexuosa* from the outer periphery of each experimental plot in December 2013. Soil cores were divided into an A1 horizon (0–5.1 ± 0.2 cm) and an A2 horizon (5.1 ± 0.2–12.3 ± 0.3 cm) using color- and density differences. Any litter fraction was removed from the samples. The soil was air-dried and large aggregates were gently crushed to pass a 2 mm sieve. The fraction >2 mm was removed by dry sieving. Subsequently, roots and visible plant remains were removed from the samples and the soil was homogenized using the cone and quarter technique (Raab et al. 1990). Three subsamples of 5 g were weighed into 50 mL Falcon tubes (BD Biosciences, DK) for density fractionation, bulk (non-fractionated) soil analysis and pH measurement, respectively. Roots were dried at 70 °C and analyzed as described below.

Soil fractionation

Soil density fractionation was carried out following protocols of Schrumpf et al. (2013) using sodium polytungstate (SPT, Sigma Aldrich No. 71913, Denmark) at a density of 1.6 g mL^{-1}. After addition of 25 mL SPT to the soil samples, the Falcon tubes were shaken gently by hand to release the free light fraction (*fLF*). Suspensions were left to settle for ~1 h prior to 30 min of centrifugation at 4000×*g*. The floating *fLF* and SPT supernatant were pipetted onto glass fibre filters (porosity 4, DUAN, Schott, Germany) and filtered under vacuum. The filtered SPT was checked for density changes and poured back into the Falcon tubes. Density changes were not observed in the current experiment. The *fLF* on the glass fibre filters was washed with milli-Q water to a conductivity of the rinsing water <50 µS. The occluded light fraction (*oLF*) was obtained by treating the re-suspended SPT-soil solution with ultrasound at 26 J mL^{-1}. Calorimetrical calibration of the sonicator (Digital Sonifier No. 450, Branson, USA) was performed according to Schmidt et al. (1999) to provide an estimate for the applied energy. The applied energy level was based on 1) a strong discoloration of the SPT at energy levels higher than 26 J m L^{-1} that indicated reallocation of C (Fig. SI1) and 2) tests on the effect of different levels of sonication energy on the amount and the C concentration of the *oLF* and *HF* (Schmidt et al. 1999) (results not shown). Complete disruption of aggregates was assumed when no further *oLF* was released (i.e. the mass of *oLF* increased) at the next sonication step. After sonication, samples were centrifuged (4000×*g*, 30 min) and the floating *oLF* and SPT were pipetted onto quartz fibre filters and filtered under vacuum. The *oLF* was washed with milli-Q water to a conductivity of the rinsing water <50 µS. The settled *HF* was transferred onto glass microfiber filters (GF/C, Whatman, DK) and washed with milli-Q water to a conductivity of <50 µS of the rinsing water. The density separated soil fractions were transferred quantitatively onto tin trays, dried at 60 °C and weighed.

The recovery of soil mass was calculated from the sum of the mass in the density fractions and the initial bulk soil sample weight. Recovery of soil C was calculated from the sum of the C in the density fractions, the SPT solution and the rinse water versus the amount of C contained in the bulk soil sample. Recovery of soil N was calculated from the sum of the N in the density fractions versus the amount of N contained in the bulk soil sample. Average soil mass, C and N recoveries were 99.1, 111.7 and 87.9%, respectively (Table SI3).

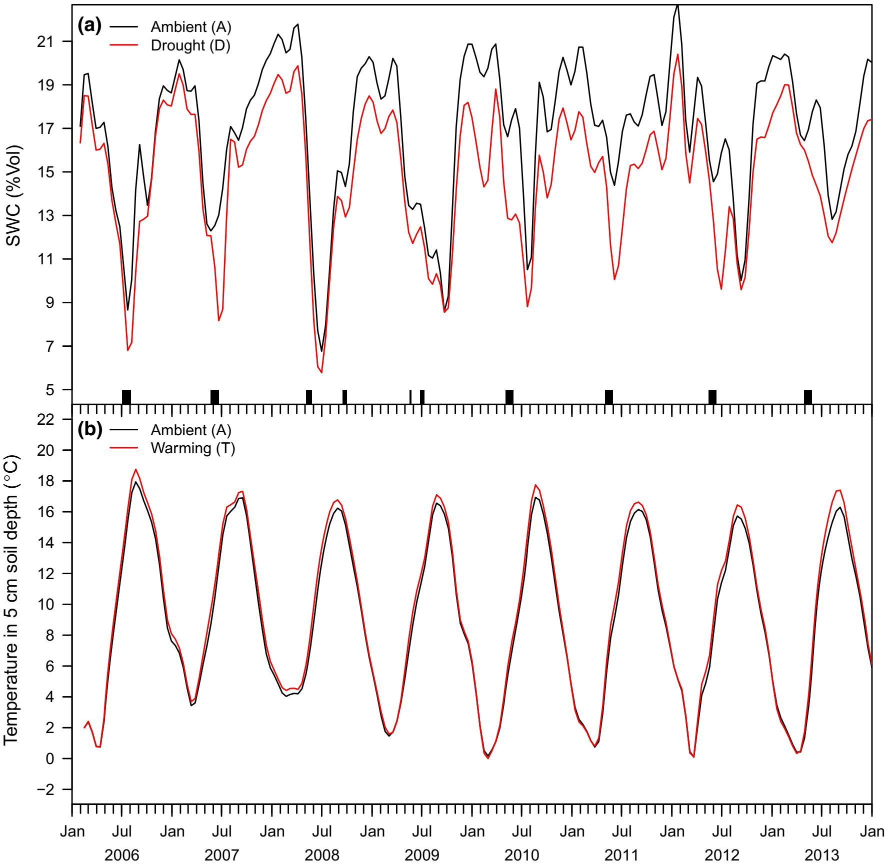

Fig. 1 **a** Volumetric soil water content (SWC) in ambient (*A*) and drought (*D*) plots (*spline curves* of monthly means). *Black bars* indicate drought-treated periods. SWCs were decreased compared to ambient plots by on average 3.2 ± 0.5 percentage points during drought periods (mean decrease increasing to 5.7 ± 0.6 percentage points during the last 7 days of treatment) and showed a longer-lasting effect (mean decrease compared to non-treated plots of 1.9 ± 0.3 percentage points. **b** Mean soil temperatures in ambient (*A*) and warming (*T*) plots (*spline curves* of monthly means). The warming treatment increased the annual mean temperature at 20 cm above soil surface (not shown) and at 5 cm soil depth by 0.3 and 0.4 °C, respectively, ranging from differences of 0.1 °C during winter to 0.5 and 0.7 °C, in air and soil respectively, during spring/summer

Soil solution pH

A soil subsample was gently suspended in milli-Q water (5:25 w:vol) and allowed to stand for 10 min. Soil solution pH was measured using a Radiometer Copenhagen PHM92 Laboratory pH meter.

C loss to fractionation medium and rinsing water

Water soluble components of the SOC pool may easily be lost during SPT suspension and rinsing. In order to quantify this C loss, SPT solutions and collected rinsing water samples were filtered through 0.45 µm nylon filters (Minisart, DK) and analyzed for dissolved organic C (DOC) on a TOC_V CPH Analyzer (Shimadzu Suzhou Instruments, JP). Loss of C to the SPT solution and to the rinse water during density fractionation accounted for 4.8±0.1 and 12.5±0.5% of the bulk C in the A1 and A2 horizon, respectively. Five-mL subsamples of the SPT were freeze-dried and the precipitate was analyzed for total C and the $^{13}C/^{12}C$ isotope ratio.

Total C, N and stable isotope analyses

For analysis of the dry matter C and N concentrations (% C and % N) and isotopic ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$, duplicates of finely ball-milled samples were weighed into tin capsules, using 10, 0.1–1, 20, 20 and 10 mg of the *fLF*, *oLF*, *HF*, bulk soil and root mass, respectively. Samples were measured by Dumas combustion (1020 °C) on an elemental analyzer (CE 1110, Thermo Electron, Milan, Italy) coupled in continuous flow mode to a Finnigan MAT Delta PLUS isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany). The isotope ratios are reported by the delta notation (δ^{13}C and δ^{15}N), i.e. the change in isotopic ratio relative to international reference materials, i.e. Pee Dee Belemnite (PDB) and atmospheric air for C and N, respectively.

Newly-assimilated C and C residence time

The concentrated CO_2 used for the FACE treatment had a distinctly lower ^{13}C isotopic value (signature of the added CO_2, $^{13}CO_{2FACE} = -29‰$; Reinsch and Ambus 2013) than ambient air ($^{13}CO_{2AIR} = -8‰$). Newly assimilated C (C_{new}) in plots subjected to elevated CO_2 was subsequently tracked into the SOM fractions according to the equation proposed by Bock et al. (2007):

$$\%C_{new} = 100 \times \frac{\delta^{13}C_{SOM.CO2} - \delta^{13}C_{SOM.A}}{\delta^{13}C_{root} - \delta^{13}C_{SOM.A}} \tag{1}$$

where $\delta^{13}C_{SOM.CO_2} = \delta^{13}C$ of the SOM fraction in the CO_2 treatment, $^{13}C_{SOM.A} = {}^{13}$C of the SOM fraction in the A treatment, and $^{13}C_{root} = {}^{13}$C of the root material in the CO_2 treatment. The calculation assumes steady state C inputs, an instantaneous change in $^{13}C_{root}$, a temporal persistent value of $^{13}C_{root}$, and a negligible impact of aboveground litter on SOM formation; assumptions that are a simplification of the reality. The ^{13}C values of collected root materials are presented in supplementary Table SI1.

The mean residence time of C (MRT_C) in each SOM fraction was calculated according to:

$$MRT_c = 1/k \tag{2}$$

where $k = -\ln$(proportion of old C)/(years elapsed since the start of the experiment). A negative C_{new} was observed for 7.5% of the samples. Because k requires a positive value for C_{new} to be meaningful, the calculation of k was based on a plot average C_{new} ($n = 6$). Mean turnover rates for C were calculated across treatments by multiplying C_{new} with the grams of C in a given fraction, followed by division with the fraction dry weight and eight years of elevated CO_2 treatment.

Statistical analyses

Results are presented as means ± standard error ($n = 6$) unless indicated otherwise. 182 Outliers (i.e. values lower or higher than the quartile ± interquartile range*1.5) corresponding to 4.5% of the values were removed from the dataset. Statistical analyses of treatment effects were conducted with a linear mixed effect model (lmer, $p < 0.05$) (R Core Team 2014). Data were divided into A1 and A2 horizon samples since almost all variables within the *fLF*, *oLF* and *HF* showed a significant difference between the horizons in Welch's *t* test (Welch 1947) (Table SI2). The same statistical model was used for all variables, with all main climate factors (T, D, CO_2) and their interactions included. The model included a random statement that accounted for the experimental design (block, octagon octagon × D, octagon × T; the CO_2 treatment is accounted for in the octagon as CO_2 is manipulated at octagon level). P-values <0.05 were considered significant, and trends in treatment effects ($p < 0.1$) are indicated.

Results

Distribution and characteristics of density fractions

The *HF* constituted at least 96 and 98% of the total soil mass in the A1 and A2 horizons, respectively. The *HF* was associated with high mineral contents as reflected by lower total soil C and N concentrations than in the bulk soil (Table 2). In contrast to the total mass, the light fractions constituted important reservoirs of OC and ON in both soil horizons (10–24% of the total C and 3–21% of the total N each; Table 2).

^{13}C abundance under ambient CO_2 decreased in the order *oLF* ≥ leaf litter and roots ≥ bulk soil ≥ *HF* > *fLF* and under elevated CO_2 in the order *oLF* and *HF* ≥ bulk soil > *fLF* > roots > leaf litter in both horizons (Tables 2 and SI1). ^{15}N abundance decreased in the order *HF* > bulk soil and *oLF* > *fLF*, leaf litter and roots in the A1 horizon. In the A2 horizon, ^{15}N-enrichment decreased in the order *HF* > bulk soil > *oLF* and *fLF* > roots > leaf litter (Tables 2 and SI1).

Changes in chemistry of bulk soil and density fractions in the climate treatments

Effects of climate treatments on plant and soil C and N concentrations, and total C and N pools (OC and ON) were investigated (Figs. 2 and 3). In general, treatment effects appeared more frequently in the A2 horizon than in the A1 horizon (Table 3). An exception to this was ^{13}C, which was decreased by elevated CO_2 in both horizons in all measured C pools (Tables 2, 3; Table SI1). Likewise, root material ^{13}C was markedly reduced in all plots exposed to elevated CO_2, ranging from −27.2 ± 0.1 to −35.0 ± 0.5‰, independent of soil depth (Table SI1). Samples generally showed large variability, and hence some of the statistical results have to be interpreted with reservation. It is worth mentioning that all climate treatments reduced the A2 horizon *fLF* N stock relative to the ambient treatment, while treatments hardly differed from each other (Fig. 3m; Table 3). This could indicate that the treatments are non-additive but it could also reflect that the higher *fLF* N stock of the ambient treatment was caused by high variability between replicates.

Responses to warming

Across all treatment combinations, warming (T) significantly decreased soil C and N stocks in the A2 horizon *fLF*, *HF* and the bulk soil (Figs. 2m and 3m, 2o and 3o, and 2p and 3p, respectively; Table 3). When combined with CO_2 and drought, warming reduced the bulk soil C stock from 1765 ± 61 g C m^{-2} in the A2 horizon to 1355 ± 138 g C m^{-2} (Fig. 2p; Table 3), which was linked to a decreased C concentration (Fig. 2h; Table 3). The dominant source of C loss was associated with the *HF* (-272 g C m^{-2}), and to lesser extent with the *fLF* (–74 g C m^{-2}).

Much in parallel to the reduction in soil C (C/N ratio remained unchanged, Fig. SI4), the N pool decreased in the A2 horizon *HF*, from 81.5 ± 6.2 g N m^{-2} to 67.1 ± 8.1 g N m^{-2} (Fig. 3o; Table 3) due to a decrease in the N content of the fraction (Fig. 3g; Table 3); for the *fLF*, the N pool decreased by 2.1 g N m^{-2} (Fig. 3m; Table 3). Overall, the bulk soil showed a substantial 17 g N m^{-2} (19%) decrease of the A2 horizon N pool in response to warming (Fig. 3p; Table 3).

Responses to drought

Drought decreased the A2 horizon *fLF* N stock from 4.2 ± 0.7 to 2.6 ± 0.5 g N m^{-2}, probably due to a combination of non-significant decreases in the N concentration, the *fLF* weight fraction, and the soil bulk density. Drought also increased the ^{15}N abundance in the *oLF* from 0.2 ± 0.3‰ to 1.9 ± 0.5‰ but only in plots under ambient CO_2 (significant DCO2 interaction; Table 3, Table SI1). Drought responses often acted in combination with CO_2 and/or warming (Table 3). A noticeable example is the temperature-driven loss of N from the *HF* in the A2 horizon. The warming-induced N loss was 14.4 g N m^{-2} but when combined with drought, the N loss was reduced to 2.2 g N m^{-2} (Fig. 3o; Table 3).

Responses to elevated CO_2

With respect to elevated CO_2 as a driver for soil C and N stocks in this ecosystem, we observed responses in the A2 horizon *fLF* in particular. The C stock of this soil fraction was reduced by ~ 67 g C m^{-2} under elevated CO_2 to a total size of 104 ± 22 g C m^{-2} (Fig. 2m; Table 3), despite a concurrent increase in C from 43.6 ± 0.8% to 51.6 ± 1.1% (Fig. 2e; Table 3). A concurrent reduction of the relative weight proportion of the A2 horizon *fLF* from 0.20 ± 0.002% to 0.12 ± 0.002% was measured under elevated CO_2, but only when the CO_2 was not combined with warming (significant antagonistic TCO2 interaction, Table 3; data not shown).

The loss of C under elevated CO_2 was lower in combinations with both warming and drought (Fig. 2m). The A2 horizon *fLF* N stock also decreased under elevated CO_2, from 4.2 ± 0.7 to 2.1 ± 0.6 g N m^{-2} (Fig. 3m; Table 3), but as for C in the *fLF*, the elevated CO_2-induced loss of N was reduced by significant interactions with both, warming and drought.

A change in N concentration was not observed for any of the density fractions. However elevated CO_2 decreased the bulk A2 horizon soil N concentration from 0.06 ± 0.003% to 0.05 ± 0.003%, but only when not combined with warming (significant TCO2 interaction; Table 3).

Table 2 Characteristics of bulk soil and soil organic matter density fractions with respect to mass proportion, concentrations of carbon (C) and nitrogen (N), isotopic composition (^{13}C and ^{15}N), pH and bulk density

Soil profile	Fraction	Mass proportion (% of total)	Total C (% of dry matter)	Fraction of C (% of total)	Total N (% of dry matter)	Fraction of N (% of total)	C:N	^{13}C (‰ vs. PDB)		^{15}N (‰ vs. air)	pH (H_2O)	Bulk density (g cm^{-3})
								Ambient CO_2	FACE			
A1 horizon (depth)	*fLF*	2.3 ± 0.2	37.6 ± 0.6	24±1	1.7 ± 0.03	21 ± 2	21.4 ± 0.4	−28.9 ± 0.1	−31.1 ± 0.2	−1.4 ± 0.2	NA	NA
(0–5.1 ± 0.2 cm)	*oLF*	1.0 ± 0.2	46.9 ± 1.3	12±1	1.6 ± 0.04	8 ± 1	29.4 ± 1.3	−26.9 ± 0.2	−28.1 ± 0.2	−0.5 ± 0.1	NA	NA
	HF	95.9 ± 0.2	2.2 ± 0.05	65±2	0.1 ± 0.01	71 ± 2	17.0 ± 0.3	−27.9 ± 0.07	−28.5 ± 0.03	0.2 ± 0.1	NA	NA
	Bulk	100	3.1 ± 0.09	NA	0.2 ± 0.01	NA	15.6 ± 0.4	−27.9 ± 0.06	−29.0 ± 0.1	−0.4 ± 0.1	4.7 ± 0.02	0.99 ± 0.02
	Roots	NA	46.0 ± 0.6	NA	0.9 ± 0.03	NA	55.3 ± 2.8	−27.2 ± 0.1	−34.8 ± 0.8	−1.4 ± 0.1	NA	NA
	Leaf litter	NA	45.8 ± 0.05	NA	1.8 ± 0.03	NA	27.0 ± 0.4	−27.1 ± 0.01	−36.1 ± 0.1	−1.8 ± 0.4	NA	NA
A2 horizon (depth)	*fLF*	0.2 ± 0.01	47.7 ± 1.0	10±1	0.9 ± 0.03	3 ± 1	52.0 ± 2.2	−28.6 ± 0.07	−29.9 ± 0.2	0.1 ± 0.4	NA	NA
(5.1 ± 0.2–12.3 ± 0.3 cm)	*oLF*	0.3 ± 0.1	47.7 ± 2.1	15±2	0.9 ± 0.04	4 ± 1	51.1 ± 1.8	−27.0 ± 0.1	−28.7 ± 0.4	0.7 ± 0.4	NA	NA
	HF	98.4 ± 0.4	0.6 ± 0.02	76±1	0.04 ± 0.01	93 ± 1	14.5 ± 0.2	−27.7 ± 0.06	−28.3 ± .0.5	3.7 ± 0.1	NA	NA
	Bulk	100	0.8 ± 0.04	NA	0.06 ± 0.01	NA	14.5 ± 0.5	−27.6 ± 0.05	−28.2 ± 0.07	3.4 ± 0.1	5.0 ± 0.03	1.45 ± 0.01
	Roots	N	39.6 ± 0.8	NA	0.7 ± 0.03	NA	59.9 ± 3.0	−27.0 ± 0.1	−34.9 ± 0.8	−1.0 ± 0.1	NA	NA

Selected variables are displayed for roots and leaf litter. Data are means of observations across all treatments in 2013 (n = 48 ± SE), except for ^{13}C isotope values shown separately for plots exposed to ambient and elevated CO_2, respectively (n = 24 ± SE). NA = not available. Data on leaf litter are from Boesgaard (2013)

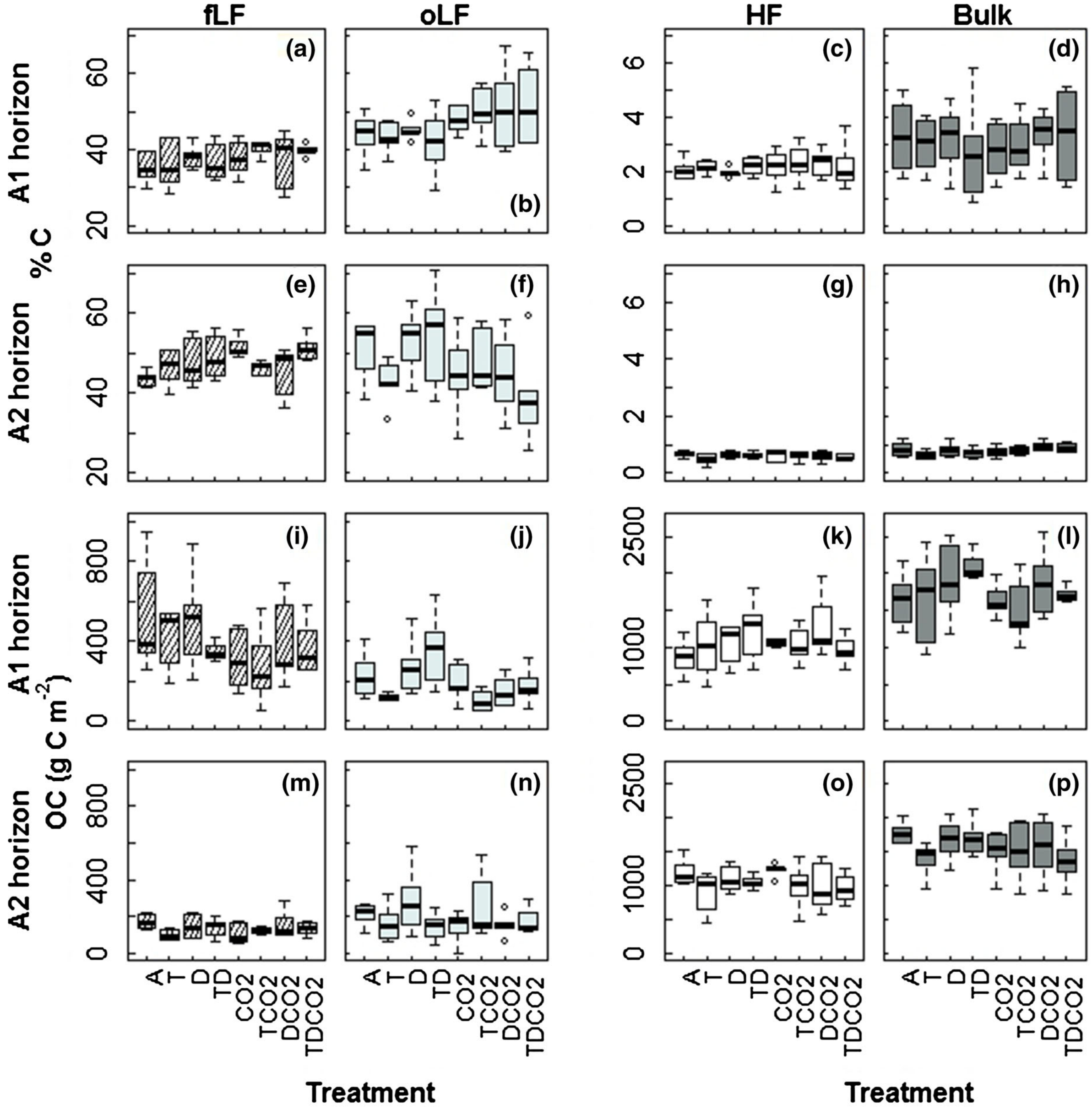

Fig. 2 *Boxplots* of effects of the climate treatments in soil A1 and A2 horizons (5.1 ± 0.2 and 5.1 ± 0.2 to 12.3 ± 0.3 cm, respectively) on dry matter C concentration (**a**–**h**) and the stocks of organic C (OC) (**i**–**p**) in soil organic matter (SOM) fractions and the bulk soil. *fLF* free light fraction, *oLF* occluded light fraction, *HF* heavy fraction, bulk = bulk soil. *Boxes* represent interquartile ranges, whiskers represent 1.5 interquartile ranges from the *boxes*. Thirty outliers (3.9% of the values) were removed from the figure

Responses to future environmental conditions

The combination of all three imposed climate drivers ($TDCO_2$), i.e. the simulation of future climate scenario, decreased the A2 horizon *fLF* C stock from 171 ± 17 g C m^{-2} in control plots to 133 ± 15 g C m^{-2} (Fig. 2m; Table 3); this decrease was observed in spite of the increase in relative C concentration (Fig. 2e; Table 3). In contrast, the relative C concentration in the A2 horizon *oLF* decreased in the combined treatment (Fig. 2f; Table 3), but this was not accompanied by a concurrent decrease of the C

Table 3 Effects of climate treatments (D = drought, T = warming, CO_2 = elevated CO_2, and combinations) on ^{13}C, ^{15}N, organic C- and N stocks of bulk soil, soil organic matter fractions (*fLF* = free light fraction, *oLF* = occluded light fraction, *HF* = heavy fraction) and roots

Variable	Horizon	Sample	Significant and near-significant treatments and direction of movement
Weight fraction	A1	fLF	CO$_2^{**}$↓
		oLF	ns
		HF	ns
	A2	fLF	T§↓, CO$_2^{*}$↓, TCO$_2^{*}$, DCO$_2^{\S}$
		oLF	ns
		HF	ns
%C	A1	Bulk	ns
		fLF	D§↑
		oLF	ns
		HF	ns
		Roots	ns
	A2	Bulk	T*↓, TCO$_2^{*}$, DCO$_2^{\S}$
		fLF	CO$_2^{*}$↑, DCO$_2^{**}$, TCO$_2^{\S}$, TDCO$_2^{*}$
		oLF	T§↓, TD§, TCO$_2^{\S}$, TDCO$_2^{*}$
		HF	T*↓
		Roots	ns
%N	A1	Bulk	ns
		fLF	ns
		oLF	ns
		HF	T§↓
		Roots	ns
	A2	Bulk	T*↓, CO$_2^{*}$↓, TCO$_2^{*}$, TDCO$_2^{**}$
		fLF	ns
		oLF	ns
		HF	T*↓, TD§, TCO$_2^{\S}$
		Roots	ns
C stock	A1	Bulk	ns
		fLF	CO$_2^{\S}$↓
		oLF	TD§
		HF	ns
	A2	Bulk	T*↓, TD§, TCO$_2^{\S}$, TDCO$_2^{\S}$
		fLF	T**↓, CO$_2^{*}$↓, TD*, TCO$_2^{*}$, DCO$_2^{*}$, TDCO$_2^{*}$
		oLF	ns
		HF	T*↓
N stock	A1	Bulk	ns
		fLF	CO$_2^{\S}$↓
		oLF	TD§
		HF	T*↑, TCO$_2^{\S}$
	A2	Bulk	T*↓, TD§
		fLF	T**↓, D**↓, CO$_2^{**}$↓, TD**, TCO$_2^{*}$, DCO$_2^{*}$, TDCO$_2^{*}$
		oLF	ns
		HF	T*↓, TD*

Table 3 continued

Variable	Horizon	Sample	Significant and near-significant treatments and direction of movement
^{13}C	A1	Bulk	CO_2***↓
		fLF	CO_2***↓, DCO_2*
		oLF	CO_2*↓, $T^§$↓
		HF	CO_2*↓, TCO_2*
		Roots	CO_2***↓, TCO_2*
		SPT	$CO_2^§$↓
	A2	Bulk	CO_2*↓, TCO_2**
		fLF	CO_2**↓, $TCO_2^§$
		oLF	CO_2*↓, TCO_2*
		HF	CO_2*↓, $TCO_2^§$
		Roots	CO_2***↓, $TD^§$, $TCO_2^§$, $DCO_2^§$
		SPT	CO_2**↓
^{15}N	A1	Bulk	ns
		fLF	ns
		oLF	ns
		HF	T*↓, TD*, $TCO_2^§$
		Roots	ns
	A2	Bulk	$DCO_2^§$
		fLF	$TD^§$
		oLF	D**↑, DCO_2***, $TDCO_2$*
		HF	ns
		Roots	DCO_2*, $TCO_2^§$
C_{new}	A1	fLF	ns
		oLF	ns
		HF	ns
	A2	fLF	ns
		oLF	$D^§$↓
		HF	$TD^§$
Bulk density	A1	Bulk	TD*
	A2	Bulk	ns
pH	A1	Bulk	$CO_2^§$↑, $TCO_2^§$
	A2	Bulk	T**↑, TCO_2**

Effects of climate treatments are assessed using a linear mixed effect model (lmer)

* 5% level, ** 1% level, *** 0.1% level, § $p < 0.1$, ns non-significant. Arrows indicate an increase or decrease due to the climate treatment

stock (Fig. 2n). The full treatment combination also tended to decrease the C stock of the A2 horizon bulk soil and the *HF* ($p < 0.1$; Table 3; Fig. 2p and o, respectively). Furthermore, the full treatment combination caused a 40% reduction in N from the A2 horizon *fLF*, from 4.2 ± 0.7 g N m^{-2} under ambient conditions to 2.5 ± 0.5 g N m^{-2} (Fig. 3m). This N loss was neither driven by reduced N %, a smaller *fLF* weight fraction or by a lower soil bulk density alone (Table 3) but was probably caused by a combination of non-significant decreases in these variables.

New C and mean C residence time in SOM

The specific $^{13}C/^{12}C$ isotopic composition of the atmospheric CO_2 in experimental plots exposed to elevated CO_2 enabled the calculation of C_{new} into the two soil horizon SOM fractions. The C_{new} generally

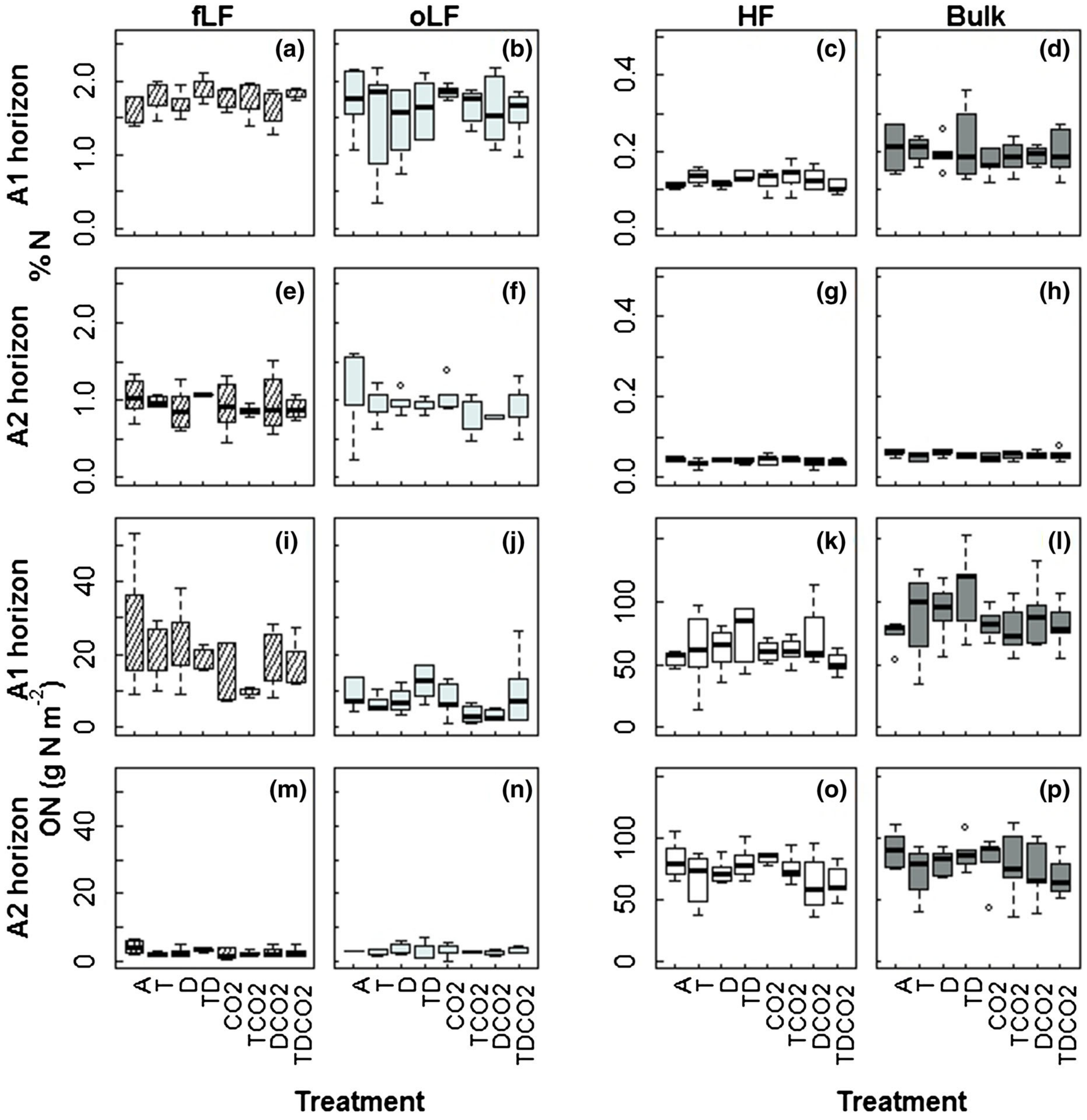

Fig. 3 *Boxplots* of effects of the climate treatments in soil A1 and A2 horizons (5.1 ± 0.2 and 5.1 ± 0.2 to 12.3 ± 0.3 cm, respectively) on dry matter N concentration (**a**–**h**) and stocks of organic N (ON) (**i**–**p**) in soil organic matter (SOM) fractions and the bulk soil. *fLF* free light fraction, *oLF* occluded light fraction, *HF* heavy fraction, bulk = bulk soil. *Boxes* represent interquartile ranges, whiskers represent 1.5 interquartile ranges from the boxes. Thirtyseven outliers (4.8% of the values) were removed from the figure

decreased in the order *fLF* ≥ *oLF* ≥ *HF* with an overall maximum of 46% C_{new} in the A1 horizon *fLF*, and a minimum of 6% C_{new} in the A2 horizon *HF* (Fig. 4a–c). None of the treatments affected the formation of new C, although the drought treatment tended to decrease C_{new} formation in the *oLF* of the A2 horizon (Fig. 4b; Table 3). The incorporation of new C during the eight years of the experiment in relation to the current C stock further enabled an assessment of the MRT_C. The MRT_C in the *HF* (overall 99 ± 10 years) exceeded the MRT_C in the *fLF* (26 ± 4 years) and *oLF* (39 ± 4 years),

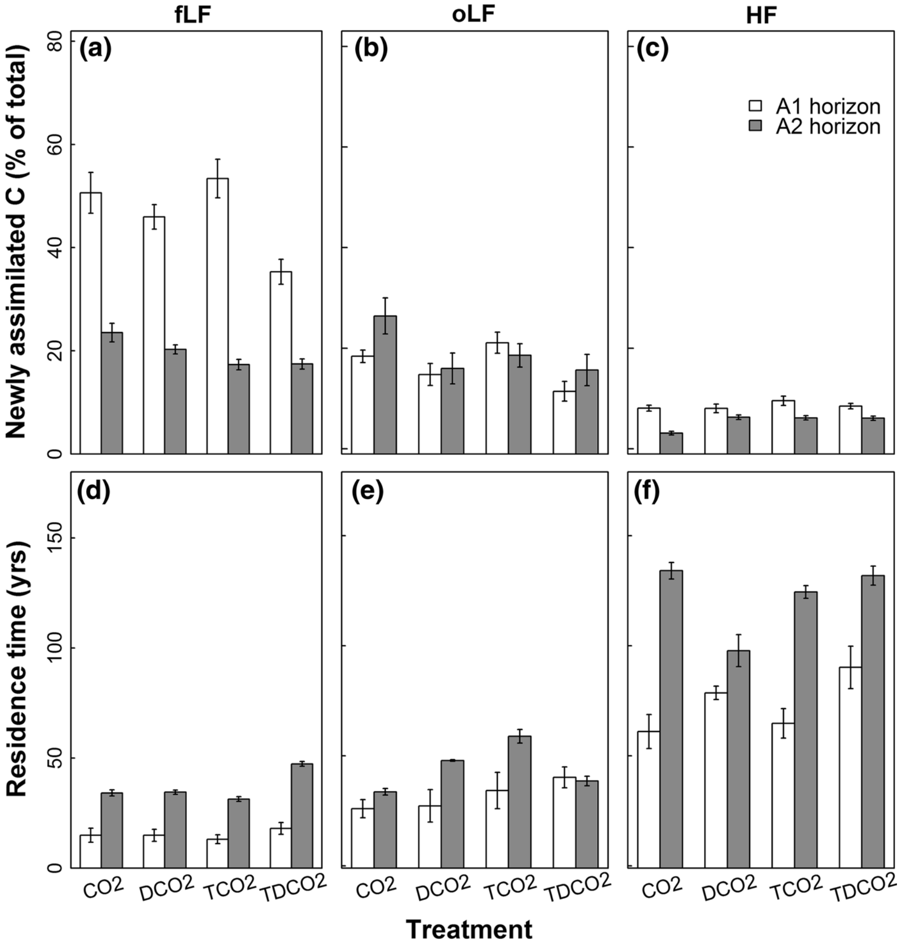

Fig. 4 Newly assimilated C in the free light fraction (fLF) (**a**), occluded light fraction (oLF) (**b**) and heavy fraction (HF) (**c**) and mean C residence time in the fLF (**d**), oLF (**e**) and HF (**f**) of the A1 and A2 horizon in treatments with elevated CO_2. *Error bars* represent ±1 SE

independently of the applied treatments and horizons (Fig. 4d–f).

Effect of soil depth on soil C and N

With increasing soil depth, i.e. the transition from the A1 to the A2 horizon, the pool of bulk soil C decreased from 1745 ± 52 g C m^{-2} to 1550 ± 72 g C m^{-2} (Fig. 2l and p; Table SI2). The pool of C bound in the *fLF* also decreased from 395 ± 32 g C m^{-2} in the A1 horizon to 133 ± 9 g C m^{-2} in the A2 horizon, despite a slight increase in C concentration (Fig. 2e, i, and m; Table 3). DOC followed the same pattern and decreased with depth, as indicated by the DOC concentration in the SPT solution ($p < 0.001$; Fig. SI2a). The ^{13}C of the *fLF, HF* and bulk soil increased with depth for ambient CO_2 (0.3‰) and elevated CO_2 (0.8‰) treatments (Tables 2, SI2; Fig. SI3). In parallel to the depth-related distribution of C, the N concentrations and N pools generally also decreased with depth in the SOM fractions and bulk soil (Table 2; Fig. 3). The C:N ratio was generally higher in the deeper soil layer, most pronounced in the *fLF* where A2 horizon $C:N > 50$ (Table 2). Similarly, the ^{15}N generally increased with soil depth, up to 3.8‰ for the bulk soil (Table 2; Fig. SI3). Newly assimilated C in the *fLF* and *HF* decreased with soil depth ($p < 0.001$ and 0.01, respectively; Fig. 4a, c) and correspondingly, the MRT_C of the *fLF* and *HF* increased with depth ($p < 0.001$ and 0.01, respectively; Fig. 4d, f).

Discussion

An ecosystem in transition

The different patterns of ^{13}C signatures between the SOM fractions and plant roots under elevated CO_2 and ambient CO_2, respectively, show that our ecosystem had not yet established a new equilibrium in terms of C allocation after eight years of continuous exposure to ^{13}C depleted CO_2. The reported MRT_Cs and changes in C and N allocation to SOM pools under elevated CO_2 discussed in the following sections have hence to be interpreted with reservation. Given the violation of the steady-state assumptions (Derrien and Amelung 2011), higher C-input under eCO_2 into our ecosystem potentially leads to an under-estimate of the actual MRT_Cs. The relative magnitude of potential errors in MRT_C estimates diminishes with the duration of the experiment and is inversely related to the decay-rate (k). This suggests that in particular the MRT_Cs for the *HF* may be underestimated in the current study.

Origins of the *oLF* and *HF* and their relative roles in SOC stabilization

This section discusses possible origins of the *oLF* and *HF* under ambient CO_2 concentration using the indicators C:N ratio, ^{13}C, and ^{15}N, and localizes the stabilization of SOC using the indicators MRT_C and SOM weight fraction. Considering the general pattern of enrichment in ^{13}C of SOM with age, the observed higher ^{13}C enrichment of the *oLF* relative to the *HF* ($p < 0.001$) suggests that the *HF* was mainly formed from the more ^{13}C depleted *fLF* (Table 2). Meanwhile, selective degradation of ^{13}C depleted compounds within the *oLF* such as plant or microbial lipids, lignin or aliphatic compounds (Badeck et al. 2005; Park and Epstein 1961) provides a pathway for *HF* formation from the *oLF*. A MRT_C of the *HF* in the A2 horizon of more than 100 years and a rather slow mean C turnover in the *HF* and *oLF* of 0.03 and 1.7 mg C mg dry weight^{-1} yr^{-1}, respectively, further suggest little transfer of C from the *HF* to the *oLF*. Chemical analysis of the SOM fractions is needed to ultimately determine the predominant sources of C for the *oLF* and *HF*. The differences in ^{15}N and C:N ratios between *fLF* and *oLF* in the A1 horizon (but not the A2 horizon) suggest that the *oLF* had undergone additional chemical transformation, possibly due to a longer inclusion period (Buurman and Roscoe 2011).

To our best knowledge this is the first study that consistently shows a higher ^{13}C enrichment of *oLF* C relative to *HF* C. John et al. (2005) also observed higher or equal ^{13}C-enrichment of *oLF* C relative to *HF* C for some of their samples, but mainly reported ^{13}C signatures of *oLF* C intermediate between C in the *HF* and *fLF*. The latter was also observed for a loamy soil with three different plant covers (Gunina and Kuzyakov 2014) and for most of the sandy loam or loamy sand grassland soils in Baisden et al. (2002). Other researchers have reported similar ^{13}C signatures of *oLF* C and *fLF* C, e.g. across 12 European study sites of different land use (Schrumpf et al. 2013) or more ^{13}C depleted C in the *oLF* compared to the *fLF* (Buurman and Roscoe 2011; Roscoe et al. 2004). The apparent variance in the origin of the *oLF* suggests that SOM dynamics are indeed dependent on initial precursors and soil type, which is in line with findings by Thockmorton et al. (2012) and Baisden et al. (2002), but contrary to findings by Gunina and Kuzyakov (2014) and Schrumpf et al. (2013).

The long MRT_C of the *HF* relative to *fLF* and *oLF* and the high weight fraction of the *HF* (>95% of the bulk soil) suggest that most C in the investigated soil was stabilized by association with minerals. The *oLF* constituted only a small part of the bulk SOM in terms of weight (0.3−1%) due to little aggregate formation in sandy soils (Juo and Franzluebbers 2003), as shown previously (Roscoe et al. 2004). However, due to the high C concentration in the *oLF*, C storage within aggregates at intermediate MRT_Cs was considerable in our ecosystem (4−12% of total C).

Effect of soil depth on SOM turnover

The increases in ^{15}N and ^{13}C in the SOM fractions and the bulk soil with soil depth (except the ^{13}C of the *oLF*) were presumably caused by isotopic discrimination by the microbial community, and suggest that SOM age increases with depth, in accordance with the general conceptual understanding of SOM formation and turnover (Brunn et al. 2014; Schrumpf et al. 2013). The relatively higher C input to the A1 horizon reflected a substantial contribution from aboveground litter to C_{new}, as also indicated by the different ^{13}C signatures of the *fLF* and the roots (Table 2). Decreases in C and N concentration with depth have been reported previously (e.g., Johnsen et al. 2013; Ostrowska and Porębska 2012) and are probably due to a lower SOM input (lower C_{new}) in the A2 horizon combined with a different quality of the SOM entering the soil (Bowden et al. 2014). The increases in C:N ratios of the *oLF* and *fLF* with depth were probably due to concurrent increases in the C:N ratio of the roots but could also originate from higher concentration of recalcitrant compounds (Brunn et al. 2014). The higher MRT_C of the *HF* in the A2 horizon compared to the A1 horizon suggests increased C stabilization with depth.

Effect of climate treatments on SOM cycling

Depth-dependent responses to climate treatments

While ^{13}C labeling of the SOC occurred in both horizons, the majority of all changes in response to climate treatments were observed in the A2 horizon (Figs. 2, 3; Table 3). This was contrary to the expected, as C turnover was generally higher in the A1 horizon. The higher responsiveness to climate change of the A2 horizon compared to the superior A1 horizon may be caused by the observed pattern of relatively large changes of belowground plant processes, in particular increased deep root productivity (Arndal et al. 2013), compared to relatively small changes in the aboveground plant biomass in response to the climate treatments at the experimental site (Kongstad et al. 2012).

Warming

Decreases of the *fLF* C and N pools were in accordance with hypothesis 1. The *HF* lost less C and N compared to the *fLF,* which confirms the previous observations by Leifeld et al. (2013) of a higher temperature sensitivity of labile SOM (high C:N ratio) relative to slowly decomposing/recalcitrant SOM. However, other studies have shown a higher temperature sensitivity of slowly decomposing SOM (e.g., Follett et al. 2012; Suseela et al. 2013). In their review, Conant et al. (2011) concluded that most long-term, cross-site studies indicate that the degradation of slowly decomposing SOM is relatively insensitive to temperature. In contrast, the majority of incubation studies, which typically capture mostly the responses of readily decomposable SOM, presenting only 5–15% of the total SOM pool, show that the decomposition of slowly decomposing SOM is more temperature sensitive than labile SOM (Conant et al. 2011).

The combined annual loss of C from the *fLF* C and *HF* C stocks of 43 g m^{-2} y^{-1} was similar to the increase in R_s induced by warming of 56–58 g m^{-2} y^{-1} at our site (Selsted et al. 2012). These values are in line with an increase in R_s in a tall-grass prairie of 59 g C m^{-2} y^{-1} in response to 2 °C warming (Luo et al. 2009) but slightly higher than the estimated decrease in OC at temperature increase of 3 °C in a range of grassland soils (19 g C m^{-2} y^{-1}; Follett et al. 2012), however in the latter study only C stocks from 0 to 10 cm depth were considered. Our results imply an increased CO_2 release due to soil decomposition in a warming world. Additionally, a stronger decrease of the *fLF* N stock (−51%) compared to the *fLF* C stock (−43%) may indicate progressive N limitation of the ecosystem under warming.

Drought

Contrary to hypothesis 2, the *fLF* C stock and *fLF* C and N concentrations did not increase in response to drought and the *fLF* N stock furthermore decreased. Possibly, the duration and timing of the drought (applied during selected periods each spring or summer, Fig. 1) was not long enough to manifest the predicted changes in the SOM pool. In addition, any changes manifested during the relatively short-term drought events (3−4 weeks) may rapidly diminish due to the fast recovery of photosynthetic rates, R_s and plant growth after rewetting (Albert et al. 2011; Kongstad et al. 2012; Selsted et al. 2012). Our results therefore contrast previous reports of attenuated N turnover (Bimüller et al. 2014), increases in the labile SOC stocks and labile SOM C and N concentrations, and a generally slower SOM turnover (Garten et al. 2009) under drought.

Elevated CO_2

Contrary to hypothesis 3, elevated CO_2 concentration decreased A2 horizon *fLF* C and *fLF* N stocks and tended to decrease the A1 horizon *fLF* C and *fLF* N stocks. Decreases in the *fLF* C and N stocks under elevated CO_2 were the direct consequence of the decrease of the weight fractions of the *fLF* in both horizons as the concentrations of C and N in the *fLF* were either unchanged or increased under elevated CO_2 (Table 3). Given the simultaneous increases in net photosynthesis (Albert et al. 2011) and R_s (Selsted et al. 2012), increased root growth (Arndal et al. 2013) and unchanged aboveground biomass (Kongstad et al. 2012) at the experimental site, the decreased weight fractions of the *fLF*, and decreased *fLF* C and N stocks indicate a faster turnover of labile SOM under elevated CO_2. Our finding is in agreement with previous studies showing that elevated CO_2 may not lead to a higher content of SOC since not only the C input, but also C turnover in the soil is stimulated (Carney et al. 2007; Hofmockel et al. 2011b; Van Groenigen et al. 2014). Increased C turnover is possibly triggered by the stimulation of microbial degradation by enhanced labile C input under elevated CO_2 (Van Groenigen et al. 2014). An altered microbial community structure and composition under elevated CO_2, potentially involving the up-regulation of functional genes and enzymes involved in labile C decomposition (Carney et al. 2007; He et al. 2010; Nie et al. 2014) and decreased soil aggregation (Henry et al. 2005) provide alternative explanations. Progressive N limitation is often anticipated to hinder increases in SOC stocks under increased atmospheric CO_2, (e.g. Hungate et al. 2006). While plant growth was not N limited under elevated CO_2, increased leaf C:N ratios, both measured after two treatment years (Larsen et al. 2011), may have reduced ecosystem N availabilty after eight years of treatment and may have contributed to the decreases in the *fLF* C and N stocks. Changes in more stable SOM (*HF*) and in the bulk soil C and N stocks under elevated CO_2 were not detected, perhaps due to longer turnover times of SOM within the *HF*.

The average loss of C from the *fLF* observed under elevated CO_2 (ca. 8 g C m^{-2} y^{-1} after eight treatment years in this study) was much smaller than the increase in R_s (124–146 g C m^{-2} y^{-1}) during the initial three treatment years (Selsted et al. 2012). This suggests a substantial increase in root respiration and/or flux of labile organic compounds such as root exudates rapidly utilized and respired by the soil microbial community, but also potentially additional losses of C from deeper soil layers than those sampled in this study (the average sampling depth was 17.4 cm).

The loss of N from the *fLF* under elevated CO_2 averaged ca. 0.25 g N m^{-2} y^{-1}. However, neither N-leaching [0.1–0.6 g N m^{-2} y^{-1}; Larsen et al. (2011)] nor nitrous oxide (N_2O) degassing [$<8.8 \times 10^{-4}$ g N m^{-2} y^{-1}; Carter et al. (2011)] were affected by CO_2 levels, and the increase in root mass under elevated CO_2 was not accompanied by a proportional increase in root N uptake (Arndal et al. 2013). Emissions of dinitrogen (N_2) were not quantified, but as nitrate levels at the experimental site are low ($\ll$1 mM; Larsen et al. (2011)) the production of N_2 as the end product of denitrification is favored. As such, N_2 emission may have been the pathway for the loss of *fLF* N.

The apparent persistence of organic C and N stocks of the bulk soil and the *HF* in response to elevated CO_2 indicates that stabilization of C and N does not change under elevated CO_2 alone after eight treatments years. Our findings contrast those by Van Groenigen et al. (2014) who used a two-pool model to simulate equal increases in the turnover rate of old and new C under elevated CO_2.

Similarly to the observed effects of elevated CO_2 on organic C and N stocks, increases in soil C concentration were only observed for the A2 horizon *fLF*, and were probably caused by higher plant C concentrations under elevated CO_2 (reviewed in Dieleman et al. 2012). Nitrogen concentrations of the bulk A2 horizon soil decreased under elevated CO_2, in line with previous reports on enhanced organic N mineralization to support increased primary production under elevated CO_2 (Hofmockel et al. 2011a).

Changes in organic C and N stocks in a future climate

In accordance with the SOM response under elevated CO_2 only, the full treatment combination, simulating a future climate scenario, decreased the A2 horizon *fLF* C and N stocks and tended to decrease the A2 horizon *HF* C and bulk C stocks. Net photosynthesis (Albert et al. 2011) and R_s (Selsted et al. 2012) were increased under the full treatment combination, however neither aboveground (Kongstad et al. 2012) nor belowground biomass (Arndal et al. 2013) changed significantly relative to ambient conditions. Hence, with unchanged litter inputs to the ecosystem across treatments, the decline of the *fLF* C and N stocks suggest a faster SOM turnover under future environmental conditions. Contrary to our observations under elevated CO_2 alone, future conditions tended to reduce SOM stabilization. Our findings contrast previous short-term observations on unchanged plant biomass, SOM turnover and soil fauna at the experimental site in the three-factorial treatment (hypothesis 4) and indicate different responses of ecosystem C turnover in the short- and longer term.

The increase in R_s of 140–150 g C m^{-2} y^{-1} under the full treatment combination (Selsted et al. 2012) by far exceeded the annual C loss from the *fLF* C pool (5 g C m^{-2} y^{-1}), in analogy to the conditions under elevated CO_2 only. Reasons for the deviation between the increase in R_s and the observed SOC losses are similar to ones stated in the previous section, but can further result from a decline in the SOC stocks of the *HF* and bulk soil under the full treatment combination.

Few studies have investigated the combined controls of atmospheric CO_2, warming and drought on SOM dynamics. In a replanted, N-poor old-field ecosystem (seven plant species including two N_2-fixers), moderate increases of the labile SOC stock were reported (Garten et al. 2009) after four years with experimental factors similar to the current work. Contrasting changes in SOM stocks in response to similar experimental conditions are possible for several reasons: (1) differences in the magnitude of the applied climate treatments. In the old-field experiment (Garten et al. 2009), the imposed temperature and CO_2 increases were 1.5 °C and 180 ppm higher, respectively, relative to our experiment; (2) differences in the plant succession, geological material and ecosystem at the experimental sites; (3) adaptable effects of climate change on different plant species (Albert et al. 2011; Andresen et al. 2010). The relative allocation of C to soluble low molecular weight compounds and insoluble lipids differs among plant types, potentially affecting litter decay rates and C stabilization (Cotrofo et al. 2013); (4) different timescales of investigations. Short-term ecosystem responses to climate change may increase (Kröel-Dulay et al. 2015) or decrease (Boesgaard 2013) in the long term or may be reversed (Suttle et al. 2007); and (5) recent disturbance of the ecosystem equilibrium in Garten et al. (2009). According to Kröel-Dulay et al. (2015) the dynamic state of an ecosystem may determine its responsiveness to climate change with recently disturbed ecosystems being more sensitive than ecosystems that are in equilibrium.

Conclusions

Soil organic matter beneath the shrubland species *Deschampsia flexuosa* was older in the A2 horizon than in the overlying A1 horizon, and within each horizon, SOM was oldest in the mineral-associated, more recalcitrant soil fraction, indicating C stabilization on minerals at depth. A2 horizon SOM was susceptible to environmental change whereas A1 horizon SOM was largely unaffected; in the A2 horizon, significant decreases of the *fLF* (labile) C and N stocks (precursor to *HF* (stable) SOM) were observed under warming, elevated CO_2 and the three-factorial treatment, *i.e.* the 2075 climate scenario for Denmark. These results suggest reduced C stabilization in this heathland soil under future climatic conditions. Combined with previous reports of increased net photosynthesis and soil respiration at the experimental site, our results further provide evidence to the hypothesis that shrubland SOM will be susceptible to increased C and N turnover, increased N mineralization, and increased associated net C losses in the future.

Danish shrublands have hitherto been anticipated to be CO_2 neutral (Gyldenkærne et al. 2005). Extrapolating our results on 98.000 ha shrubland in Denmark (or 2.3% of the country's area; Gyldenkærne et al. 2005), under the assumption of an unchanged plant cover of 77% *D. flexuosa* with time (Kongstad et al. 2012) and a linear decrease of the *fLF* C stock, our results imply a release of 14 Gg CO_2 y^{-1} to the atmosphere. This corresponds to only ~0.5% of the CO_2 emissions from land use and land use change in Denmark (2600 Gg CO_2 equivalents y^{-1}, 2003 figures; Gyldenkærne et al. (2005)), and a decline in Danish shrubland topsoil OC stocks is hence not expected to contribute substantially to the national greenhouse gas budget. In countries with larger shrubland cover, however, a future C loss in this ecosystem type could have a much higher significance.

Based on our results we suggest that future research efforts should be centered around the characterization of potential long-term effects of climate change on SOC and SON dynamics beneath different shrubland plant species with augmented focus on the detailed examination of the ingoing and outgoing C and nutrient fluxes.

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