Thaysen, E.M.; Reinsch, S.; Larsen, K.S.; Ambus, P. 2017. **Decrease in heathland soil labile organic carbon under future atmospheric and climatic conditions**. *Biogeochemistry*, 133 (1). 17-36. 10.1007/s10533-017-0303-3

Contact CEH NORA team at
noraceh@ceh.ac.uk

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

2

3 E. M. Thaysen[1,2]*, S. Reinsch[3], K.S. Larsen[4], P. Ambus[5]

4 [1] Institute of Environmental Assessment and Water Research (IDAEA), CSIC, Jordi Girona 18-26, 08034

5 Barcelona, Catalonia, Spain**.**

6 [2] Associated Unit: Hydrogeology Group (UPC-CSIC), 08034 Barcelona, Catalonia, Spain

7 [3] Centre for Ecology & Hydrology, Environment Centre Wales, Deiniol Rd, Bangor, Gwynedd, LL57 2UW, United

8 Kingdom

9 [4] Department of Geosciences and Natural Resource Management, University of Copenhagen, Rolighedsvej 23,

10 1958, Denmark

11 [5] Center for Permafrost (CENPERM), Department of Geosciences and Natural Resource Management, University of

12 Copenhagen, Øster Voldgade 10, 1350, Copenhagen, Denmark

13
14

15 * Corresponding author: Email: eike.thaysen@idaea.csic.es

16

17

20

21 **Abstract** Characterization of the impacts of climate change on terrestrial carbon (C) cycling is important due to

22 possible feedback mechanisms to atmospheric CO_2 concentrations. We investigated soil organic matter (SOM)

23 dynamics in the A1 and A2 horizons (~0-5.1 cm and ~5.1-12.3 cm depth, respectively) of a shrubland grass

24 (*Deschampsia flexuosa*) after eight years of exposure to: elevated CO_2 (CO2), summer drought (D), warming (T)

25 and all combinations hereof, with TDCO2 simulating environmental conditions for Denmark in 2075. The mean C

26 residence time was highest in the heavy fraction (*HF*), followed by the occluded light fraction (*oLF*) and the free

27 light fraction (*fLF*), and it increased with soil depth, suggesting that C was stabilized on minerals at depth. A2-

28 horizon SOM was susceptible to climate change whereas A1 horizon SOM was largely unaffected. The A2 horizon

29 *fLF* and *HF* organic C stocks decreased by 43 % and 23 % in response to warming, respectively. Organic nitrogen

30 (N) stocks of the A2 horizon *fLF* and *HF* decreased by 50 % and 17 %, respectively. Drought decreased the A2

31 horizon *fLF* N stock by 38 %. Elevated CO_2 decreased the A2 horizon *fLF* C stock by 39 % and the *fLF* N stock by

32 50 %. Under TDCO2, A2 horizon *fLF* C and N stocks decreased by 22 % and 40 %, respectively. Overall, our

33 results indicate that shrubland SOM will be susceptible to increased turnover and associated net C and N losses in

34 the future.

35

36 **Introduction**

37 Climate change is accelerated by increasing atmospheric concentration of carbon dioxide (CO_2) (IPCC 2013). The

38 extent to which soil carbon (C) sequestration will counterbalance increasing atmospheric CO_2 concentrations

39 depends in part on soil organic matter (SOM) dynamics (Davidson & Janssens 2006; Hofmockel et al. 2011b;

40 Trumbore & Czimczik 2008). However, it is unclear how SOM will respond to climate change (Hofmockel et al.

41 2011b; Nie et al. 2014) because links and feedback mechanisms between SOM dynamics and climate are not fully

42 understood (Heimann & Reichstein 2008; Trumbore & Czimczik 2008). Changes in C and nitrogen (N) cycling

43 within SOM pools could drastically change long-term C sequestration and soil N availability (Hofmockel et al.

44 2011b).

45 SOM contains roughly 50 % C and 0.1-6 % N (Cotrofo & Gorissen 1997; Schnitzer & Khan 1978) and is mainly

46 derived from plants through exudates, symbiotic fungi and litter (Davidson & Janssens 2006; Trumbore & Czimczik

47 2008), and to a minor extent from mesofauna, fungi (Mehrabanian 2013) and bacteria/archaea. The incorporation of

48 OM into soil aggregates or sorption onto mineral or other organic surfaces slows SOM decomposition by microbes

49 and contributes to its stabilization in soil (Kleber et al. 2007). As changes in bulk SOM stocks can be difficult to

50 observe due to high spatial variability in most natural ecosystems, improved understanding of climate change effects

51 on SOM turnover and changes in soil C and N pools can be gained from SOM fractionation in combination with

52 climate manipulation experiments (Trumbore & Czimczik 2008).

53 The SOM fractionation approach is particularly valuable in climate change experiments because non-complexed

54 SOM pools often display more sensitive responses to environmental change than the bulk SOM pool (Christensen

55 2001). SOM fractionation techniques are based on the assumption that the extent and degree to which SOM is

56 adsorbed to mineral soil particles regulates SOM dynamics and function (Gregorich et al. 2006). Soil density

57 fractionation provides a mean to separate SOM inside and outside of aggregates (designated occluded light fraction,

58 *oLF*, and free light fraction, *fLF*, respectively, with densities <1.5 g cm^{-3}) from mineral-associated SOM (heavy

59 fraction, *HF*, with a density typically 2.5-3.0 g cm^{-3}). Particles that sink in heavy liquid are thought to be absorbed to

60 clay and sesquioxides, and contain variable amounts of humified SOM (Beare & Gregorich 2007; Kogel-Knabner et

61 al. 2008).

62 In general, the youngest, most labile and least ^{13}C enriched (=$\delta^{13}C$ most negative) SOM prevails as discrete particles

63 of plant origin (*fLF*) whereas older, most processed, recalcitrant and ^{13}C enriched SOM is associated with the *HF*

64 (Gunina & Kuzyakov 2014; Kogel-Knabner et al. 2008; Meyer & Leifeild 2013; Wagai et al. 2009). It is believed

65 that the *HF* can be formed from the *oLF* or directly from *fLF* material (Wagai et al. 2009). The *oLF* is thought to

66 originate from the *fLF* and may partially be more degraded and recalcitrant (Buurman & Roscoe 2011; Wagai et al.

67 2009). Stabilization of soil organic C (SOC) and soil organic N (SON) is typically connected to mineral association

68 in the *HF* (Bimüller et al. 2014; Marschner et al. 2008; Schrumpf et al. 2013). Organic C pesistence via selective

69 preservation of recalcitrant compounds such as melanoidins, black C, tannins or aliphatic structures in the *oLF*

70 (Mikutta et al. 2006; Poirier et al. 2003) is probably a less important stabilization mechanism (Marschner et al.

71 2008).

72 Climate change manipulation experiments have traditionally investigated single-factorial or combined effects of, in

73 particular, elevated atmospheric CO_2 concentrations and warming (reviewed in Dieleman et al. 2012). These

74 experiments, however, lack studying the effect of more severe future drought events (Dieleman et al. 2012) or

75 anticipated changed precipitation patterns in general (IPCC 2013), which may also influence soil C and N turnover.

76 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 the increased SOM turnover rates (Amundson & Davidson 1990; Kotroczo et al. 2008). Previous investigations from the heath 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 & 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

4

105   reduced when combined with drought (Andresen et al. 2010; Kongstad et al. 2012; Larsen et al. 2011;

106   Maraldo et al. 2010; Reinsch & Ambus 2013).

107

108   **Methods**

109

110   **Experimental field site**

111   Soil samples were collected at the site of the *CLIMAITE* experimental site, a temperate shrubland/grassland ca. 50

112   km north of Copenhagen, Denmark (55º53'N 11º58'E), matured on moraine deposits (Mikkelsen et al. 2008). The

113   soil is a coarse textured sandy Arenosol (FAO) / Entisol (US Soil Taxonomy) from the Weichsel glaciation with

114   only weak signs of podsolization, a relatively low Cation Exchange Capacity (CEC) and acidic pH (Table 1). The

115   dominating plant types are grasses (ca. 77 % coverage by *Deschampsia flexuosa*) and evergreen shrubs (ca. 23 %

116   coverage by *Calluna vulgaris*) (Kongstad et al. 2012). The experiment comprises twelve octagon-shaped plots

117   (6.8 m diameter) that have been exposed to multiple environmental treatments since October 2005. The octagons are

118   organized pair-wise in six blocks, where one of the paired octagons is exposed to ambient (A) atmospheric CO_2

119   concentration (390 ppm) and one is exposed to elevated CO_2 at 510 ppm (CO2) realized by Free-Air CO_2

120   Enrichment (FACE). All octagons are split into four equal-sized plots exposed to, in addition to ambient or elevated

121   CO_2, either no treatment (A), extended spring/summer droughts (D) via horizontally moving curtains (removing 8-

122   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

123   percentage points on average during the whole drought treatment periods and during the last 7 days of drought

124   treatments, respectively; Fig. 1a), to passively elevated night-time temperature (T) via a second set of horizontally

125   moving reflective curtains (annual mean temperature at 20 cm above soil surface and at 5 cm soil depth elevated by

126   0.3 °C and 0.4 °C, respectively, in T compared to A plots, ranging from 0.1 °C in both air and soil during winter to

127   0.5 °C and 0.7 °C, respectively, during spring/summer; Fig. 1b) or a combination of drought and warming (TD).

128   Hence the experimental design allows for the test of eight treatments (A, T, D, CO2, TD, TCO2, DCO2, TDCO2),

129   each replicated six times. The full factorial treatment, TDCO2, simulates as closely as possible a likely Danish

130   climate scenario in 2075, as predicted by the Danish Meteorological Institute (www.DMI.dk). For more details, see

131   Mikkelsen et al. (2008) and Scherber et al. (2013).

132

133 **Soil sampling and sample pre-treatment**

134 Four to five soil cores (Ø 2 cm, depth 12.3±0.3 cm corresponding to the approximate depth of the A horizon) were

135 collected randomly beneath *D. flexuosa* from the outer periphery of each experimental plot in December 2013. Soil

136 cores were divided into an A1 horizon (0-5.1±0.2 cm) and an A2 horizon (5.1±0.2 cm to 12.3±0.3 cm) using color-

137 and density differences. Any litter fraction was removed from the samples. The soil was air-dried and large

138 aggregates were gently crushed to pass a 2 mm sieve. The fraction >2 mm was removed by dry sieving.

139 Subsequently, roots and visible plant remains were removed from the samples and the soil was homogenized using

140 the cone and quarter technique (Raab et al. 1990). Three subsamples of 5 g were weighed into 50 mL Falcon tubes

141 (BD Biosciences, DK) for density fractionation, bulk (non-fractionated) soil analysis and pH measurement,

142 respectively. Roots were dried at 70 °C and analyzed as described below.

143

144 **Soil fractionation**

145 Soil density fractionation was carried out following protocols of Schrumpf et al. (2013) using sodium polytungstate

146 (SPT, Sigma Aldrich No. 71913, Denmark) at a density of 1.6 g mL^{-1}. After addition of 25 mL SPT to the soil

147 samples, the Falcon tubes were shaken gently by hand to release the free light fraction (*fLF*). Suspensions were left

148 to settle for ~1 hr prior to 30 min of centrifugation at 4000 *g*. The floating *fLF* and SPT supernatant were pipetted

149 onto glass fibre filters (porosity 4, DUAN, Schott, Germany) and filtered under vacuum. The filtered SPT was

150 checked for density changes and poured back into the Falcon tubes. Density changes were not observed in the

151 current experiment. The *fLF* on the glass fibre filters was washed with milli-Q water to a conductivity of the rinsing

152 water <50 μS. The occluded light fraction (*oLF*) was obtained by treating the re-suspended SPT-soil solution with

153 ultrasound at 26 J mL^{-1}. Calorimetrical calibration of the sonicator (Digital Sonifier No. 450, Branson, USA) was

154 performed according to Schmidt et al. (1999) to provide an estimate for the applied energy. The applied energy level

155 was based on 1) a strong discoloration of the SPT at energy levels higher than 26 J m L^{-1} that indicated reallocation

156 of C (SI Fig. S1) and 2) tests on the effect of different levels of sonication energy on the amount and the C

157 concentration of the *oLF* and *HF* (Schmidt et al. 1999) (results not shown). Complete disruption of aggregates was

158 assumed when no further *oLF* was released (i.e. the mass of *oLF* increased) at the next sonication step. After

159 sonication, samples were centrifuged (4000 *g*, 30 min) and the floating *oLF* and SPT were pipetted onto quartz fibre

160 filters and filtered under vacuum. The *oLF* was washed with milli-Q water to a conductivity of the rinsing water <50

161 μS. The settled *HF* was transferred onto glass microfiber filters (GF/C, Whatman, DK) and washed with milli-Q

162 water to a conductivity of <50 μS of the rinsing water. The density separated soil fractions were transferred

163 quantitatively onto tin trays, dried at 60 °C and weighed.

164 The recovery of soil mass was calculated from the sum of the mass in the density fractions and the initial bulk soil

165 sample weight. Recovery of soil C was calculated from the sum of the C in the density fractions, the SPT solution

166 and the rinse water versus the amount of C contained in the bulk soil sample. Recovery of soil N was calculated

167 from the sum of the N in the density fractions versus the amount of N contained in the bulk soil sample. Average

168 soil mass, C and N recoveries were 99.1 %, 111.7 % and 87.9 %, respectively (Table SI3).

169

170 **Soil solution pH**

171 A soil subsample was gently suspended in milli-Q water (5:25 w:vol) and allowed to stand for 10 min. Soil solution

172 pH was measured using a Radiometer Copenhagen PHM92 Laboratory pH meter.

173

174 **C loss to fractionation medium and rinsing water**

175 Water soluble components of the SOC pool may easily be lost during SPT suspension and rinsing. In order to

176 quantify this C loss, SPT solutions and collected rinsing water samples were filtered through 0.45 μm nylon filters

177 (Minisart, DK) and analyzed for dissolved organic C (DOC) on a TOC_V CPH Analyzer (Shimadzu Suzhou

178 Instruments, JP). Loss of C to the SPT solution and to the rinse water during density fractionation accounted for

179 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

180 were freeze-dried and the precipitate was analyzed for total C and the $^{13}C/^{12}C$ isotope ratio.

181

182 **Total C, N and stable isotope analyses**

183 For analysis of the dry matter C and N concentrations (% C and % N) and isotopic ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$,

184 duplicates of finely ball-milled samples were weighed into tin capsules, using 10, 0.1-1, 20, 20 and 10 mg of the

185 *fLF*, *oLF*, *HF*, bulk soil and root mass, respectively. Samples were measured by Dumas combustion (1020 ºC) on an

186 elemental analyzer (CE 1110, Thermo Electron, Milan, Italy) coupled in continuous flow mode to a Finnigan MAT

187 Delta PLUS isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany). The isotope ratios are reported

188   by the delta notation ($\delta^{13}C$ and $\delta^{15}N$), i.e. the change in isotopic ratio relative to international reference materials, *i.e.*

189   Pee Dee Belemnite (PDB) and atmospheric air for C and N, respectively.

190

191   **Newly-assimilated C and C residence time**

192   The concentrated CO_2 used for the FACE treatment had a distinctly lower ^{13}C isotopic value (signature of the added

193   CO_2, $\delta^{13}CO_{2FACE}$ = -29 ‰; Reinsch and Ambus 2013) than ambient air ($\delta^{13}CO_{2AIR}$ = -8 ‰), and newly assimilated C

194   (C_{new}) in plots subjected to elevated CO_2 was subsequently tracked into the SOM fractions according to the equation

195   proposed by Bock et al. (2007):

196

197   Eq. (1): $\%C_{new} = 100 \times \dfrac{\delta^{13}C_{SOM.CO2} - \delta^{13}C_{SOM.A}}{\delta^{13}C_{root} - \delta^{13}C_{SOM.A}}$

198

199   where $\delta^{13}C_{SOM.CO2}$= $\delta^{13}C$ of the SOM fraction in the CO2 treatment, $\delta^{13}C_{SOM.A}$ = $\delta^{13}C$ of the SOM fraction in the A

200   treatment, and $\delta^{13}C_{root}$ = $\delta^{13}C$ of the root material in the CO2 treatment. The calculation assumes an instantaneous

201   change in $\delta^{13}C_{root}$, a temporal persistent value of $\delta^{13}C_{root}$, and a negligible impact of aboveground litter on SOM

202   formation, assumptions that are a simplification of the reality. The $\delta^{13}C$ values of collected root materials are

203   presented in supplementary Table SI1.

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

205

206   Eq. (2): $MRT_c = 1/k$

207

208   where k = -ln(proportion of old C) / (years elapsed since the start of the experiment). A negative C_{new} was observed

209   for 7.5 % of the samples. Because k requires a positive value for C_{new} to be meaningful, the calculation of k was

210   based on a plot average C_{new} (n=6). Mean turnover rates for C were calculated across treatments by multiplying C_{new}

211   with the grams of C in a given fraction, followed by division with the fraction dry weight and eight years of elevated

212   CO_2 treatment.

213

214   **Statistical analyses**

215　Results are presented as means ± standard error (n=6) unless indicated otherwise. 182 Outliers (i.e. values lower or

216　higher than the quartile ± interquartile range*1.5) corresponding to 4.5 % of the values were removed from the

217　dataset. Statistical analyses of treatment effects were conducted with a linear mixed effect model (lmer, $p<0.05$) (R

218　Core Team 2014). Data were divided into A1 and A2 horizon samples since almost all variables within the *fLF*, *oLF*

219　and *HF* showed a significant difference between the horizons in Welch's t-test (Welch 1947) (Table SI2). The same

220　statistical model was used for all variables, with all main climate factors (T, D, CO2) and their interactions included.

221　The model included a random statement that accounted for the experimental design (block, octagon octagon×D,

222　octagon×T; the CO2 treatment is accounted for in the octagon as CO_2 is manipulated at octagon level). P-values

223　<0.05 were considered significant, and trends in treatment effects ($p<0.1$) are indicated.

224

225　**Results**

226

227　**Distribution and characteristics of density fractions**

228　The *HF* constituted at least 96 % and 98 % of the total soil mass in the A1 and A2 horizons, respectively. The *HF*

229　was associated with high mineral contents as reflected by lower total soil C and N concentrations than in the bulk

230　soil (Table 2). In contrast to the total mass, the light fractions constituted important reservoirs of OC and ON in both

231　soil horizons (10-24 % of the total C and 3-21 % of the total N each; Table 2).

232　^{13}C abundance under ambient CO_2 decreased in the order *oLF*≥ leaf litter and roots≥ bulk soil≥ *HF*> *fLF* and under

233　elevated CO_2 in the order *oLF* and *HF* ≥ bulk soil> *fLF*> roots> leaf litter in both horizons (Tables 2 and SI1). ^{15}N

234　abundance decreased in the order *HF*> bulk soil and *oLF*> *fLF*, leaf litter and roots in the A1 horizon. In the A2

235　horizon, ^{15}N-enrichment decreased in the order *HF*> bulk soil> *oLF* and *fLF*> roots> leaf litter (Tables 2 and SI1).

236

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

238　Effects of climate treatments on plant and soil C and N concentrations, and total C and N pools (OC and ON) were

239　investigated (Fig. 2). In general, treatment effects appeared more frequently in the A2 horizon than in the A1

240　horizon (Table 3). An exception to this was $\delta^{13}C$, which was decreased by elevated CO_2 in both horizons in all

241　measured C pools (Table 2; Table 3; Table SI1). Likewise, root material $\delta^{13}C$ was markedly reduced in all plots

242　exposed to elevated CO_2, ranging from -27.2±0.1 ‰ to -35.0±0.5 ‰, independent of soil depth (Table SI1). Samples

243   generally showed large variability, and hence some of the statistical results have to be interpreted with reservation. It

244   is worth mentioning that all climate treatments reduced the A2 horizon *fLF* N stock relative to the ambient

245   treatment, while treatments hardly differed from each other (Fig. 3m, Table 3). This could indicate that the

246   treatments are non-additive but it could also reflect that the higher *fLF* N stock of the ambient treatment was caused

247   by high variability between replicates.

248

249   *Responses to warming*

250   Across all treatment combinations, warming (T) significantly decreased soil C and N stocks in the A2 horizon *fLF*,

251   *HF* and the bulk soil (Fig. 2m and 3m, 2o and 3o, and 2p and 3p, respectively; Table 3). When combined with CO2

252   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}

253   (Fig. 2p, Table 3), which was linked to a decreased C concentration (Fig. 2h, Table 3). The dominant source of C

254   loss was associated with the *HF* (-272 g C m^{-2}), and to lesser extent with the *fLF* (-74 g C m^{-2}).

255   Much in parallel to the reduction in soil C (C/N ratio remained unchanged, data not shown), the N pool decreased in

256   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

257   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

258   bulk soil showed a substantial 17 g N m^{-2} (19 %) decrease of the A2 horizon N pool in response to warming (Fig.

259   3p, Table 3).

260

261   *Responses to drought*

262   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

263   non-significant decreases in the N concentration, the *fLF* weight fraction, and the soil bulk density. Drought also

264   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

265   (significant DCO2 interaction; Table 3, Table SI1). Drought responses often acted in combination with CO_2 and/or

266   warming (Table 3). A noticeable example is the temperature-driven loss of N from the *HF* in the A2 horizon. The

267   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}

268   (Fig. 3o, Table 3).

269

270   *Responses to elevated CO_2*

271   With respect to elevated CO_2 as a driver for soil C and N stocks in this ecosystem, we observed responses in the A2

272   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

273   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 %

274   (Fig. 2e, Table 3). A concurrent reduction of the relative weight proportion of the A2 horizon *fLF* from

275   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

276   warming (significant antagonistic TCO2 interaction, Table 3; data not shown).

277   The loss of C under elevated CO_2 was lower in combinations with both warming and drought (Fig. 2m). The A2

278   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

279   for C in the *fLF*, the elevated CO_2-induced loss of N was reduced by significant interactions with both, warming and

280   drought.

281

282   A change in N concentration was not observed for any of the density fractions. However elevated CO_2 decreased the

283   bulk A2 horizon soil N concentration from 0.06±0.003 % to 0.05±0.003 %, but only when not combined with

284   warming (significant TCO2 interaction; Table 3).

285

286   *Responses to future environmental conditions*

287   The combination of all three imposed climate drivers (TDCO2), i.e. the simulation of future climate scenario,

288   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);

289   this decrease was observed in spite of the increase in relative C concentration (Fig. 2e, Table 3). In contrast, the

290   relative C concentration in the A2 horizon *oLF* decreased in the combined treatment (Fig. 2f, Table 3), but this was

291   not accompanied by a concurrent decrease of the C stock (Fig. 2n). The full treatment combination also tended to

292   decrease the C stock of the A2 horizon bulk soil and the *HF* (p<0.1; Table 3, Fig. 2p and o, respectively).

293   Furthermore, the full treatment combination caused a 40 % reduction in N from the A2 horizon *fLF*, from 4.2±0.7 g

294   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

295   smaller *fLF* weight fraction or by a lower soil bulk density alone (Table 3) but was probably caused by a

296   combination of non-significant decreases in these variables.

New C and mean C residence time in SOM

299   The specific $^{13}C/^{12}C$ isotopic composition of the atmospheric CO_2 in experimental plots exposed to elevated CO_2

300   enabled the calculation of C_{new} into the two soil horizon SOM fractions. The C_{new} generally decreased in the order

301   *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

302   horizon *HF* (Fig. 4a-c). None of the treatments affected the formation of new C, although the drought treatment

303   tended to decrease C_{new} formation in the *oLF* of the A2 horizon (Fig. 4b; Table 3). The incorporation of new C

304   during the eight years of the experiment in relation to the current C stock further enabled an assessment of the

305   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

306   years), independently of the applied treatments and horizons (Fig. 4d-f).

307

Effect of soil depth on soil C and N

309   With increasing soil depth, i.e. the transition from the A1 to the A2 horizon, the pool of bulk soil C decreased from

310   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

311   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

312   (Fig. 2e, i and m, Table 3). DOC followed the same pattern and decreased with depth, as indicated by the DOC

313   concentration in the SPT solution (p<0.001; Fig. SI2a). The $\delta^{13}C$ of the *fLF, HF* and bulk soil increased with depth

314   for ambient CO_2 (0.3 ‰) and elevated CO_2 (0.8 ‰) treatments (Table 2; Table SI2; Fig. SI3). In parallel to the

315   depth-related distribution of C, the N concentrations and N pools generally also decreased with depth in the SOM

316   fractions and bulk soil (Table 2, Fig. 3). The C:N ratio was generally higher in the deeper soil layer, most

317   pronounced in the *fLF* where A2 horizon C:N>50 (Table 2). Similarly, the $\delta^{15}N$ generally increased with soil depth,

318   up to 3.8 ‰ for the bulk soil (Table 2; Fig. SI3). Newly assimilated C in the *fLF* and *HF* decreased with soil depth

319   (p<0.001 and p<0.01, respectively; Fig. 4a and c) and correspondingly, the MRT_C of the *fLF* increased with depth

320   (p<0.001 and p<0.01, respectively; Fig. 4d and f).

321

Discussion

323

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

This section examines the origins of the *oLF* and *HF* under ambient CO_2 concentration using the indicators C:N ratio, $\delta^{13}C$, and $\delta^{15}N$, and localizes the stabilization of SOC. Considering the general pattern of enrichment in $\delta^{13}C$ of SOM with age, the observed higher ^{13}C enrichment of the *oLF* relative to the *HF* 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 & Epstein 1961) could provide 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*. However, also here chemical analysis of the SOM fractions is needed to confirm that the *fLF* is the predominant source of C for the *oLF*. The differences in $\delta^{15}N$ 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 & 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 & 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 & 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 & 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) suggests 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 &

351 Franzluebbers 2003), as shown previously (Roscoe et al. 2004). However, due to the high C concentration in the

352 *oLF*, C storage within aggregates at intermediate MRT_Cs was considerable in our ecosystem (4-12 % of total C).

353 The isotopic composition of the *oLF* was low in $\delta^{15}N$ (Hofmockel et al. 2011a) and high in $\delta^{13}C$ (Biasi et al. 2005;

354 Cheng et al. 2007) relative to the *HF*. This, combined with a smaller MRT_C of the *oLF* compared to the *HF*,

355 suggests that the *oLF* contained a substantial amount of easily degradable organic substances, presumably with a

356 relatively high concentration of compounds such as cellulose, starch or hemicellulose that are isotopically enriched

357 in ^{13}C (Badeck et al. 2005; Benner et al. 1987; Park & Epstein 1961).

358

Effect of soil depth on SOM turnover

360 The increases in $\delta^{15}N$ and $\delta^{13}C$ in the SOM fractions and the bulk soil with soil depth (except the $\delta^{13}C$ of the *oLF*)

361 were presumably caused by isotopic discrimination by the microbial community, and suggest that SOM age

362 increases with depth, in accordance with the general conceptual understanding of SOM formation and turnover

363 (Brunn et al. 2014; Schrumpf et al. 2013). The relatively higher C input to the A1 horizon reflected a substantial

364 contribution from aboveground litter to C_{new}, as also indicated by the different $\delta^{13}C$ signatures of the *fLF* and the

365 roots (Table 2). Decreases in C and N concentration with depth have been reported previously (e.g., Johnsen et al.

366 2013; Ostrowska & Porębska 2012) and are probably due to a lower SOM input (lower C_{new}) in the A2 horizon

367 combined with a different quality of the SOM entering the soil (Bowden et al. 2014). The increases in C:N ratios of

368 the *oLF* and *fLF* with depth were probably due to concurrent increases in the C:N ratio of the roots but could also

369 originate from higher concentration of recalcitrant compounds (Brunn et al. 2014). The higher MRT_Cs of the *fLF*

370 and *HF* in the A2 horizon compared to the A1 horizon suggest increased C stabilization with depth.

371

Effect of climate treatments on SOM cycling

373 The different patterns of $\delta^{13}C$ signatures between SOM fractions and the plant roots under elevated CO_2 and ambient

374 CO_2, respectively, indicate that the ecosystem had not yet established a new equilibrium in terms of C allocation

375 after eight years of continuous exposure to ^{13}C depleted CO_2. The percentage of C_{new} in the SOM fractions of the

376 elevated CO_2 plots peaked at around 50 % in the A1 horizon *fLF* and confirmed an ecosystem in transition. Hence

377 the reported changes in C and N allocation to SOM pools under elevated CO_2 have to be interpreted with this

378 reservation.

14

379

Depth-dependent responses to climate treatments

While ^{13}C labelling of the SOC occurred in both horizons, the majority of all changes in response to climate treatments were observed in the A2 horizon (Fig. 2 and 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 relation 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} yr^{-1} was similar to the increase in R$_s$ induced by warming of 56-58 g m^{-2} yr^{-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} yr^{-1} in response to 2 ℃ warming (Luo et al. 2009) but slightly higher than the estimated decrease in OC at temperature increase of 3 ℃ in a range of grassland soils (19 g C m^{-2} yr^{-1}; Follett et al. 2012), however in the latter study only C stocks from 0-10 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.

15

407

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.

417

Elevated CO₂

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

16

435   al. 2011), may have reduced ecosystem N availabilty after eight years of treatment and may have contributed to the

436   decreases in the *fLF* C and N stocks. Changes in more stable SOM (*HF*) and in the bulk soil C and N stocks under

437   elevated CO_2 were not detected, perhaps due to longer turnover times of SOM within the *HF*.

438   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

439   study) was much smaller than the increase in R_s (124–146 g C m^{-2} y^{-1}) during the initial three treatment years

440   (Selsted et al. 2012). This suggests a substantial increase in root respiration and/or flux of labile organic compounds

441   such as root exudates rapidly utilized and respired by the soil microbial community, but also potentially additional

442   losses of C from deeper soil layers than those sampled in this study (the average sampling depth was 17.4 cm).

443

444   The loss of N from the *fLF* under elevated CO_2 averaged ca. 0.25 g N m^{-2} yr^{-1}. However, neither N-leaching (0.1-0.6

445   g N m^{-2} yr^{-1}; Larsen et al. (2011)) nor nitrous oxide (N_2O) degassing (<$8.8*10^{-4}$ g N m^{-2} yr^{-1}; Carter et al. (2011))

446   were affected by CO_2 levels, and the increase in root mass under elevated CO_2 was not accompanied by a

447   proportional increase in root N uptake (Arndal et al. 2013). Emissions of dinitrogen (N_2) were not quantified, but as

448   nitrate levels at the experimental site are low («1 mM; Larsen et al. (2011)) the production of N_2 as the end product

449   of denitrification is favored. As such, N_2 emission may have been the pathway for the loss of *fLF* N.

450

451   The apparent persistence of organic C and N stocks of the bulk soil and the *HF* in response to elevated CO_2 indicates

452   that stabilization of C and N does not change under elevated CO_2 alone after eight treatments years. Our findings

453   contrast those by Van Groenigen et al. (2014) who used a simplified two-pool model to simulate equal increases in

454   the turnover rate of old and new C under elevated CO_2.

455   Similarly to the observed effects of elevated CO_2 on organic C and N stocks, increases in soil C concentration were

456   only observed for the A2 horizon *fLF*, and were probably caused by higher plant C concentrations under elevated

457   CO_2 (reviewed in Dieleman et al. 2012). Nitrogen concentrations of the bulk A2 horizon soil decreased under

458   elevated CO_2, in line with previous reports on enhanced organic N mineralization to support increased primary

459   production under elevated CO_2 (Hofmockel et al., 2011a).

460

17

461 *Changes in organic C and N stocks in a future climate*

462 In accordance with the SOM response under elevated CO_2 only, the full treatment combination, simulating a future

463 climate scenario, decreased the A2 horizon *fLF* C and N stocks and tended to decrease the A2 horizon *HF* C and

464 bulk C stocks. Net photosynthesis (Albert et al. 2011) and R_s (Selsted et al. 2012) were increased under the full

465 treatment combination, however neither aboveground (Kongstad et al. 2012) nor belowground biomass (Arndal et

466 al. 2013) changed significantly relative to ambient conditions. Hence, with unchanged litter inputs to the ecosystem

467 across treatments, the decline of the *fLF* C and N stocks suggest a faster SOM turnover under future environmental

468 conditions. Contrary to our observations under elevated CO_2 alone, future conditions tended to reduce SOM

469 stabilization. Our findings contrast previous short-term observations on unchanged plant biomass, SOM turnover

470 and soil fauna at the experimental site in the three-factorial treatment (hypothesis 4) and indicate different responses

471 of ecosystem C turnover in the short- and longer term.

472 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

473 the annual C loss from the *fLF* C pool (5 g C $m^{-2} yr^{-1}$), in analogy to the conditions under elevated CO_2 only.

474 Reasons for the deviation between the increase in R_s and the observed SOC losses are similar to ones stated in the

475 previous section, but can further result from a decline in the SOC stocks of the *HF* and bulk soil under the full

476 treatment combination.

477 Few studies have investigated the combined controls of atmospheric CO_2, warming and drought on SOM dynamics.

478 In a replanted, N-poor old-field ecosystem (seven plant species including two N_2-fixers), moderate increases of the

479 labile SOC stock were reported (Garten et al. 2009) after four years with experimental factors similar to the current

480 work. Contrasting changes in SOM stocks in response to similar experimental conditions are possible for several

481 reasons: 1) differences in the magnitude of the applied climate treatments. In the old-field experiment (Garten et al.

482 2009), the imposed temperature and CO_2 increases were 1.5 ºC and 180 ppm higher, respectively, relative to our

483 experiment; 2) differences in the plant succession, geological material and ecosystem at the experimental sites; 3)

484 adaptable effects of climate change on different plant species (Albert et al. 2011; Andresen et al. 2010). The relative

485 allocation of C to soluble low molecular weight compounds and insoluble lipids differs among plant types,

486 potentially affecting litter decay rates and C stabilization (Cotrofo et al. 2013); 4) different timescales of

487 investigations. Short-term ecosystem responses to climate change may increase (Kröel-Dulay et al. 2015) or

488 decrease (Boesgaard 2013) in the long term or may be reversed (Suttle et al. 2007); and 5) recent disturbance of the

489 ecosystem equilibrium in Garten et al. (2009). According to Kröel-Dulay et al. (2015) the dynamic state of an

490 ecosystem may determine its responsiveness to climate change with recently disturbed ecosystems being more

491 sensitive than ecosystems that are in equilibrium.

492

493 **Conclusions**

494 Soil organic matter beneath the shrubland species *Deschampsia flexuosa* was older in the A2 horizon than in the

495 overlying A1 horizon, and within each horizon, SOM was oldest in the mineral-associated, more recalcitrant soil

496 fraction, indicating C stabilization on minerals at depth. A2 horizon SOM was susceptible to environmental change

497 whereas A1 horizon SOM was largely unaffected; in the A2 horizon, significant decreases of the *fLF* (labile) C and

498 N stocks (precursor to *HF* (stable) SOM) were observed under warming, elevated CO_2 and the three-factorial

499 treatment, *i.e.* the 2075 climate scenario for Denmark. These results suggest reduced C stabilization in this heathland

500 soil under future climatic conditions. Combined with previous reports of increased net photosynthesis and soil

501 respiration at the experimental site, our results further provide evidence to the hypothesis that shrubland SOM will

502 be susceptible to increased C and N turnover, increased N mineralization, and increased associated net C losses in

503 the future.

504 Danish shrublands have hitherto been anticipated to be CO_2 neutral (Gyldenkærne et al. 2005). Extrapolating our

505 results on 98.000 ha shrubland in Denmark (or 2.3 % of the country´s area; Gyldenkærne et al. 2005), under the

506 assumption of an unchanged plant cover of 77 % *D. flexuosa* with time (Kongstad et al. 2012) and a linear decrease

507 of the *fLF* C stock, our results imply a release of 14 Gg CO_2 yr^{-1} to the atmosphere. This corresponds to only ~0.5 %

508 of the CO_2 emissions from land use and land use change in Denmark (2600 Gg CO_2 equivalents yr^{-1}, 2003 figures;

509 (Gyldenkærne et al. 2005)), and a decline in Danish shrubland topsoil OC stocks is hence not expected to contribute

510 substantially to the national greenhouse gas budget. In countries with larger shrubland cover, however, a future C

511 loss in this ecosystem type could have a much higher significance.

512 Based on our results we suggest that future research efforts should be centered around the characterization of

513 potential long-term effects of climate change on SOC and SON dynamics beneath different shrubland plant species

514 with augmented focus on the detailed examination of the ingoing and outgoing C and nutrient fluxes.

515

516

523

524 **References**

525 Albert KR, Ro-Poulsen H, Mikkelsen TN, Michelsen A, Van der Linden L, Beier C (2011) Interactive effects of
526 elevated CO_2, warming, and drought on photosynthesis of Deschampsia flexuossa in a temperate heath ecosystem. J
527 Exp Bot 62(12): 4253-4266

528 Amundson RG, Davidson EA (1990) Carbon dioxide and nitrogenous gases in the soil atmosphere. J Geochem
529 Explor 38(1-2): 13-41

530 Andresen LC, Michelsen A, Jonasson S, Schmidt IK, Mikkelsen T, Ambus P, Beier C (2010) Plant nutrient
531 mobilization in temperate heatland responds to elevated CO_2, temperature and drought. Plant Soil 328(1): 381-396

532 Arndal MF, Schmidt IK, Kongstad J, Beier C, Michelsen A (2013) Root growth and N dynamics in response to
533 multi-year experimental warming, summer drought and elevated CO_2 in a mixed heathland-grass ecosystem. Funct
534 Plant Biol 42(1): 1-10

535 Badeck FW, Tcherke G, Nogue S, Piel C, Ghashghaie J (2005) Post-photosynthetic fractionation of stable carbon
536 isotopes between plant organs—a widespread phenomenon. Rapid Commun Mass Spectronom 19(11): 1381-1391

537 Baisden WT, Amundson R, Cook AC, Brenner DL (2002) Turnover and storage of C and N in five density fractions
538 from California annual grassland surface soils. Global Biogeochem Cy 16(4): 1117-1132

539 Beare MH, Gregorich EG (2007) Physically uncomplexed organic matter. In: Soil sampling and methods of
540 analysis, Second Edition. CRC Press.

541 Beier C, Emmett BA, Tietema A, Schmidt IK, Penuelas J, Kovács Láng E, Duce P, De Angelis P, Gorissen A,
542 Estiarte M, D. de Dato G, Sowerby A, Kröel-Dulay G, Lellei-Kovács E, Kull O, Mand P, Petersen H, Gjelstrup P,
543 Spano D (2009) Carbon and nitrogen balances for six shrublands across Europe. Global Biogeochem Cy 23(4): 1-13

544 Benner R, Fogel ML, Sprague EK, Hodson RE (1987) Depletion of ^{13}C in lignin and its implications for stable
545 carbon isotope studies. Nature 329(22): 708-710

546 Biasi C, Rusalimova O, Meyer H, Kaiser C, Wanek W, Barsukov P, Junger H, Richter A (2005) Temperature-
547 dependent shift from labile to recalcitrant carbon sources of artic heterotrophs. Rapid Commun Mass Spectronom
548 19: 1401-1408

549 Bimüller C, Dannenmann M, Tejedor J, von Lützow M, Buegger F, Meier R, Haug S, Schroll R, Kögel-Knabner I
550 (2014) Prolonged summer droughts retard soil N processing and stabilization in organo-mineral fractions. Soil Biol
551 Biochem 68: 241-251

552 Bock M, Glaser B, Millar N (2007): Sequestration and turnover of plant- and microbially derived sugars in a
553 temperate grassland soil during 7 years exposed to elevated atmospheric pCO_2. Global Change Biol 13: 478-490

554 Boesgaard K (2013) Long-term ecophysiological responses to climate change. In., Kgs. Lyngby: Technical
555 University of Denmark. http://orbit.dtu.dk/files/74245683/Thesis_FINAL_kboe..PDF.

556 Bowden RD, Deemb L, Plantec AF, Peltre C, Nadelhoffer K, Lajtha K (2014) Litter input controls on soil carbon in
557 a temperate deciduous forest. Soil Sci Soc Am J 78: S66-S75

558 Brunn M, Spielvogel S, Sauer T, Oelmann Y (2014) Temperature and precipitation effects on $\delta^{13}C$ depth profiles in
559 SOM under temperate beech forests. Geoderma 235-236: 146-153

560 Buurman P, Roscoe R (2011) Different chemical composition of free light, occluded light and extractable SOM
561 fractions in soils of Cerrado and tilled and untilled fields, Minas Gerais, Brazil: a pyrolysis-GC/MS study. Eur J Soil
562 Sci 62: 253-266

563 Carney K, Hungate B, Drake B, Megonigal J (2007) Altered soil microbial community at elevated CO_2 leads to loss
564 of soil carbon. P Natl Acad Sci USA 104(12): 4990-4995

565 Carter MS, Ambus P, Albert K, Larsen KS, Anderson M, Prieme A, Van der Linden L, Beier C (2011) Effects of
566 elevated atmospheric CO_2, prolonged summer drought and temperature increase on N_2O and CH_4 fluxes in a
567 temperate heathland. Soil Biol Biochem 43(8): 1660-1670

568 Carter MS, Larsen KS, Emmett B, Estiarte M, Field C, Leith ID, Lund M, Meijide A, Mills RTE, Niinemets Ü,
569 Peñuelas J, Portillo-Estrada M, Schmidt IK, Selsted MB, Sheppard LJ, Sowerby A, Tietema A, Beier C (2012)
570 Synthesizing greenhouse gas fluxes across nine European peatlands and shrublands – responses to climatic and
571 environmental changes. Biogeosciences 9: 3739–3755.

572 Cheng L, Leavitt SW, Kimball BA, Pinter PJ, Ottmane MJ, Matthias A, Wall GW, Brooks T, Williams DG,
573 Thompson TL (2007) Dynamics of labile and recalcitrant soil carbon pools in a sorghum free-air CO_2 enrichment
574 (FACE) agroecosystem. Soil Biol Biochem 39(9): 2250-2263

575 Christensen BT (2001) Physical fractionation of soil and structural and functional complexity in organic matter
576 turnover. Eur J Soil Sci 52(3): 345-353

577 Cotrofo MF, Gorissen A (1997) Elevated CO_2 enhances below-ground C allocation in three perennial grass species
578 at different levels of N availability. New Phytol 137: 421-431

579 Cotrofo MF, Wallenstein MD, Boot CM, Denef K, Paul E (2013) The microbial efficiency-matrix stabilization
580 (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs
581 form stable soil organic matter? Global Change Biol 19(4): 988–995

582 Davidson EA, Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate
583 change. Nature 440(9): 165-173

584 Dieleman WIJ, Vicca S, Dijkstra FA, Hagedorn F, Hovenden MJ, Larsen KS, Morgan JA, Volder A, Beier C, Dukes
585 JS, King J, Leuzinger S, Linder S, Luo YQ, Oren R, de Angelis P, Tingey D, Hoosbeek MR, Janssens IA (2012)
586 Simple additive effects are rare: a quantitative review of plant biomass and soil process responses to combined
587 manipulations of CO_2 and temperature. Global Change Biol 18(9): 2681-2693

588 Follett RF, Stewart CE, Preuessner EG, Kimble JM (2012) Effects of climate change on soil carbon and nitrogen
589 storage in the US Great Plains. J Soil Water Conserv 67(5): 331-342

590 Garten C, Classen AT, Norby RJ (2009) Soil moisture surpasses elevated CO_2 and temperature as a control on soil
591 carbon dynamics in a multi-factor climate change experiment. Plant Soil 319(1): 85-94

592 Gregorich EG, Beare MH, Mckim UF, Skjemstad JO (2006) Chemical and biological characteristics of physically
593 uncomplexed organic matter. Soil Sci Soc Am J 70(3): 975-985

594 Gunina A, Kuzyakov Y (2014) Pathways of litter C by formation of aggregates and SOM density fractions:
595 Implications from ^{13}C natural abundance. Soil Biol Biochem 71: 95-104

596 Gyldenkærne S, Münier B, Olsen J, Elsnab Olesen S, Petersen B, Christensen B (2005) Opgørelse af CO_2-
597 emissioner fra arealanvendelse og ændringer i arealanvendelse. Arbejdsrapport fra DMU, nr. 213. In. Danmarks
598 Miljøundersøgelser. Miljøministeriet.

599 Haugwitz MS, Bergmark L, Prieme A, Christensen S, Beier C, Michelsen A (2014) Soil microorganisms respond to
600 five years of climate change manipulations and elevated atmospheric CO_2 in a temperate heath ecosystem. Plant Soil
601 374: 211-222

602 He Z, Xu M, Deng Y, Kang S, Kellogg L, Wu L, Van Nostrand J, Hobbie S, Reich P, Zhou J (2010) Metagenomic
603 analysis reveals a marked divergence in the structure of belowground microbial communities at elevated CO_2. Ecol
604 Lett 13(5): 564-575

Heimann M, Reichstein M (2008) Terrestrial ecosystem carbon dynamics and climate feedbacks. Nature 451(7176): 289-292

Henry H, Juarez J, Field C, Vitousek P (2005) Interactive effects of elevated CO_2, N deposition and climate change on extracellular enzyme activity and soil density fractionation in a California annual grassland. Global Change Biol 11(10): 1808-1815

Hofmockel KS, Gallet-Budynek A, McCarthy HR, Currie WS, Jackson RB, Finzi A (2011a) Sources of increased N uptake in forest trees growing under elevated CO_2: results of a large-scale ^{15}N study. Global Change Biol 17: 3338–3350

Hofmockel KS, Zak DR, Moran KK, Jastrow JD (2011b) Changes in forest soil organic matter pools after a decade of elevated CO_2 and O_3. Soil Biol Biochem 43(7): 1518-1527

Hungate BA, Johnson DW, Dijkstra FA, Hymus G, Stiling P, Megonigal JP, Pagel AL, Moan JL, Day F, Li J, Hinkle R, Drake BG (2006) Nitrogen cycling during seven years of atmospheric CO_2 enrichment in a shrub oak woodland. Ecology 87(1): 26-40

IPCC (2013) Summary for Policymakers. In: Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V & Midgle PM (eds) Climate change 2013: The physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

John B, Yamashita T, Ludwig B, Flessa H (2005) Storage of organic carbon in aggregate and density fractions of silty soils under different types of land use. Geoderma 128(1-2): 63-79

Johnsen KH, Samuelson LJ, Sanchez FG, Eaton RJ (2013) Soil carbon and nitrogen content and stabilization in mid-rotation, intensively managed sweetgum and loblolly pine stands. Forest Ecol Manag 302: 144-153

Juo ASR, Franzluebbers K (2003) Tropical soils. Properties and management for sustainable agriculture. Oxford university press, New York, USA

Kleber M, Sollins P, Sutton R (2007) A conceptual model of organo-mineral interactions in soils: self-assembly of organic molecular fragments into zonal structures on mineral surfaces. Biogeochemistry 85(1): 9-24

Kogel-Knabner I, Guggenberger G, Kleber M, Kandeler E, Kalbitz K, Scheu S, Eusterhues K, Leinweber P (2008) Organo-mineral associations in temperate soils: Integrating biology, mineralogy, and organic matter chemistry. J Plant Nutr Soil Sci 171(1): 61-82

Kongstad J, Schmidt IK, Riis-Nielsen T, Arndal MF, Mikkelsen TN, Beier C (2012) High resilience in heathland plants to changes in temperature, drought, and CO_2 in combination: Results from the CLIMAITE experiment. Ecosystems 15(2): 269-283

Kotroczo Z, Fekete I, Toth JA, Tothmeresz B, Balazsy S (2008) Effect of leaf- and root-litter manipulation for carbon-dioxide efflux in forest soil. Cereal Res Commun 36: 663-666

Kröel-Dulay G, Ransijn J, Schmidt IK, Beier C, De Angelis P, de Dato G, Dukes JS, Emmett B, Estiarte M, Garadnai J, Kongstad J, Kovacs-Lang E, Larsen KS, Liberati D, Ogaya R, Riis-Nielsen T, Smith AR, Sowerby A, Tietema A, Penuelas J (2015) Increased sensitivity to climate change in disturbed ecosystems. Nat Commun 6: 1-6

Larsen KS, Andresen LC, Beier C, Jonasson S, Albert KR, Ambus P, Arndal MF, Carter MS, Christensen S, Holmstrup M, Ibrom A, Kongstad J, van der Linden L, Maraldo K, Michelsen A, Mikkelsen TN, Pilegaard K, Prieme A, Ro-Poulsen H, Schmidt IK, Selsted MB, Stevnbak K (2011) Reduced N cycling in response to elevated CO_2, warming, and drought in a Danish heathland: Synthesizing results of the CLIMAITE project after two years of treatments. Global Change Biol 17(5): 1884–1899

Leifeld J, Bassin S, Conen F, Hajdas I, Egli M, Fuhrer J (2013) Control of soil pH on turnover of belowground organic matter in subalpine grassland. Biogeochemistry 112: 59-69

Linn DM, Doran JW (1984) Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. Soil Sci Soc Am J 48: 1268-1272

Luo Y, Sherry R, Zhou X, Wan S (2009) Terrestrial carbon-cycle feedback to climate warming: experimental evidence on plant regulation and impacts of biofuel feedstock harvest. Global Change Biol 1(1): 62-74

652 Maraldo K, Krogh PH, van der Linden L, Christensen BT, Mikkelsen TN, Beier C, Holmstrup M (2010) The
653 counteracting effects of elevated atmospheric CO_2 concentrations and drought episodes: Studies of enchytraeid
654 communities in a dry heathland. Soil Biol Biochem 42(11): 1958-1966

655 Marschner B, Brodowski S, Dreves A, Gleixner G, Gude A, Grootes PM, Hamer U, Heim A, Jandl G, Ji R, Kaiser
656 K, Kalbitz K, Kramer C, Leinweber P, Rethemeyer J, Schäffer A, Schmidt MWI, Schwark L, Wiesenberg GLB
657 (2008) How relevant is recalcitrance for the stabilization of organic matter. J Plant Nutr Soil Sci 171: 91-110

658 Mehrabanian M (2013) Molecular geochemistry of soil organic matter by pyrolysis gas chromatography/mass
659 spectrometry (GC/MS) technique: A review. J Soil Sci Environ Manag 4(2): 11-16

660 Meyer S, Leifeild J (2013) Concurrent increase in ^{15}N and radiocarbon age in soil density fractions. J Plant Nutr Soil
661 Sci 176: 505-508

662 Mikkelsen TN, Beier C, Holmstrup M, Schmidt IK, Ambus P, Pilegaard K, Michelsen A, Albert K, Andresen LC,
663 Arndal MF, Bruun N, Christensen S, Danbæk S, Gundersen P, Jørgensen P, L. G. Linden, Kongstad J, Maraldo K,
664 Priemé A, Riis-Nielsen T, Ro-Poulsen H, K. Stevnbak, Selsted MB, Sørensen P, Larsen KS, Carter MS, Ibrom A,
665 Martinussen T, Miglietta F, Sverdrup H (2008) Experimental design of multifactor climate change experiments with
666 elevated CO_2, warming and drought: the CLIMAITE project. Funct Ecol 22: 185-195

667 Mikutta R, Kleber M, Torn MS, Jahn R (2006) Stabilization of soil organic matter: association with minerals or
668 chemical recalcitrance? Biogeochemistry 77: 25-56

669 Munné Bosch S (2004) Die and let live: leaf senescence contributes to plant survival under drought stress. Funct
670 Plant Biol 31(3): 203-216

671 Nie M, Pendall E, Bell C, Wallenstein MD (2014) Soil aggregate size distribution mediates microbial climate
672 change feedbacks. Soil Biol Biochem 68: 357-365

673 Ostrowska A, Porębska G (2012) Assement of TOC-SOM and SOM-TOC conversion in forest soil. P J Environ
674 Stud 21(6): 1767-1775

675 Park R, Epstein S (1961) Metabolic fractionation of C^{13} and C^{12} in plants. Plant Physiol 36(2): 133-138

676 Poirier N, Derenne S, Balesdent J, Mariotti A, Massiot D, Largeau C (2003) Isolation and analysis of the non-
677 hydrolysable fraction of a forest soil and an arable soil (Lacadee, southwest France). Eur J Soil Sci 54(2): 243-255

678 R Core Team (2014) R: A language and environment for statistical computing. In. R Foundation for statistical
679 computing, Vienna, Austria.

680 Raab GA, Bartling MH, Stapanian MA, Cole WH, Tidwell RL, Cappo KA (1990) The homogenization of
681 environmental soil samples in bulk. In. M.S. Simmons, editor, Hazardous waste measurements. CRC Press, Boca
682 Raton, FL, USA

683 Reinsch S, Ambus P (2013) In situ $^{13}CO_2$ pulse-labeling in a temperate heathland – development of a mobile multi-
684 plot field setup. Rapid Commun Mass Spectronom 27: 1417-1428

685 Roscoe R, Buurman P, van Lagen B, Velhorst E (2004) Transformation in occluded light fraction organic matter in a
686 clayey oxisol; evidence from ^{13}C-CPMAS-NMR and $d^{13}C$ signature. Rev Bras Cienc Solo 28: 811-818

687 Scherber C, Gladbach DJ, Stevnbak K, Karsten RJ, Schmidt IK, Michelsen A, Albert KR, Larsen KS, Mikkelsen
688 TN, Beier C, Christensen S (2013) Multi-factor climate change effects on insect herbivore performance. Ecol Evol
689 3(6): 1449-1460

690 Schlesinger WH, Reynolds JF, Cunningham GL, Huenneke LF, Jarrel WM, Virginia RA, Whitford WG (1990)
691 Biological feedbacks in global desertification. Sci Total Environ 247(4946): 1043-1048

692 Schmidt MWI, Rumpel C, Kogel-Knabner I (1999) Evaluation of an ultrasonic dispersion procedure to isolate
693 primary organomineral complexes from soils. Eur J Soil Sci 50(1): 87-94

694 Schnitzer M, Khan SU (1978) Soil organic matter. Developments in soil science 8. Elsevier science publishers B.V.,
695 Amsterdam, The Netherlands

696 Schrumpf M, Kaiser K, Guggenberger G, Persson T, Kogel-Knabner I, Schulze ED (2013) Storage and stability of
697 organic carbon in soils as related to depth, occlusion within aggregates, and attachment to minerals. Biogeosciences
698 10(3): 1675-1691

699 Selsted MB, van der Linden L, Ibrom A, Michelsen A, Larsen KS, Pedersen JK, Mikkelsen T, Pilegaard K, Beier C,
700 Ambus P (2012) Soil respiration is stimulated by elevated CO_2 and reduced by summer drought: three years of
701 measurements in a multifactor ecosystem manipulation experiment in a temperate heathland (CLIMAITE). Global
702 Change Biol 18: 1216-1230

703 Skopp J, Jawson MD, Doran JW (1990) Steady-state aerobic microbial activity as a function of soil water content.
704 Soil Sci Soc Am J 54(6): 1619-1625

705 Suseela V, Tharayil N, Xing B, Dukes JS (2013) Labile compounds in plant litter reduced the sensitivity of
706 decomposition to warming and altered precipitation. New Phytol 200: 122-133

707 iKB, Thomsen MA, Power ME (2007) Species interactions reverse grassland responses to changing climate. Science
708 315(5812): 6640-6642

709 Thockmorton HM, Bird JA, Dane L, Firestone MK, Horwarth WR (2012) The source of microbial C has little
710 impact on soil organic matter stabilisation in forest ecosystems. Ecol Lett 15: 1257-1265

711 Trumbore SE, Czimczik CI (2008) Geology - An uncertain future for soil carbon. Science 321(5895): 1455-1456

712 Van Groenigen K, Qi X, Osenberg C, Luo Y, Hungate B (2014) Faster decomposition under increased atmospheric
713 CO_2 limits soil carbon storage. Science 344(6183): 508-509

714 Wagai R, Mayer LM, Kitayama K (2009) Nature of the "occluded" low-density fraction in soil organic matter
715 studies: A critical review. Soil Sci Plant Nutr 55(1): 13-25

716 Welch BL (1947) The generalization of "Student's" problem when several different population variances are
717 involved. Biometrika 34(1-2): 28–35

718 Xu M, Lou Y, Sun X, Wang W, Baniyamuddin M, Zhao K (2011) Soil organic carbon active fractions as early
719 indicators for total carbon change under straw incorporation. Biol Fert Soils 47: 745-752
720

721   Table 1. Soil physical and chemical characteristics. Soil was collected in 2004 (pre-treatment) adjacent to each

722   experimental octagon (n=12) for Cation Exchange Capacity (CEC) and pH; four locations (n=4) were randomly

723   selected in 2004 for textural analysis.

Soil depth	Sand	Silt	Clay	CEC	pH
(cm)		(% wt)		(meq 100 g^{-1})	(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[§]	4.2±0.06[§]

724   §: data for 10-30 cm soil depth.

725

726   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

727   composition ($\delta^{13}C$ and $\delta^{15}N$), pH and bulk density. Selected variables are displayed for roots and leaf litter. Data are means of observations across all treatments in 2013

728   (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

729   are from Boesgaard (2013).

Soil profile	Fraction	Mass proportion	Total C	Fraction of C	Total N	Fraction of N	C:N	$\delta^{13}C$		$\delta^{15}N$	pH	Bulk density
		(% of total)	(% of dry matter)	(% of total)	(% of dry matter)	(% of total)		(‰ vs. PDB)		(‰ vs air)	(H_2O)	(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)	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)	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

Table 3: Effects of climate treatments (D = drought, T = warming, CO2 = elevated CO_2, and combinations) on $\delta^{13}C$, $\delta^{15}N$, 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. Effects of climate treatments are assessed using a linear mixed effect model (lmer). *= 5% level, **= 1% level, ***= 0.1% level, § = $p<0.1$, n.s.= non-significant. Arrows indicate an increase or decrease due to the climate treatment.

Variable	Horizon	Sample	Significant and near-significant treatments and direction of movement
Weight fraction	A1	fLF	CO2**↓
		oLF	n.s.
		HF	n.s.
	A2	fLF	T§↓, CO2*↓, TCO2*, DCO2§
		oLF	n.s.
		HF	n.s.
%C	A1	Bulk	n.s.
		fLF	D§
		oLF	n.s.
		HF	n.s.
		Roots	n.s.
	A2	Bulk	T*↓, TCO2*, DCO2§
		fLF	CO2*↑, DCO2**, TCO2§, TDCO2*
		oLF	T§↓, TD§, TCO2§, TDCO2*
		HF	T*↓
		Roots	n.s.
%N	A1	Bulk	n.s.
		fLF	n.s.
		oLF	n.s.
		HF	T§
		Roots	n.s.
	A2	Bulk	T*↓, CO2*↓, TCO2*, TDCO2**
		fLF	n.s.
		oLF	n.s.
		HF	T*↓, TD§, TCO2§
		Roots	n.s.
C stock	A1	Bulk	n.s.
		fLF	CO2§
		oLF	TD§
		HF	n.s.
	A2	Bulk	T*↓, TD§, TCO2§, TDCO2§
		fLF	T**↓, CO2*↓, TD*, TCO2*, DCO2*, TDCO2*
		oLF	n.s.
		HF	T*↓
N stock	A1	Bulk	n.s.
		fLF	CO2§
		oLF	TD§
		HF	T*↑, TCO2§
	A2	Bulk	T*↓, TD§
		fLF	T**↓, D**↓, CO2**↓, TD**, TCO2*, DCO2*, TDCO2*
		oLF	n.s.
		HF	T*↓, TD*
$\delta^{13}C$	A1	Bulk	CO2***↓
		fLF	CO2***↓, DCO2*
		oLF	CO2*↓, T§
		HF	CO2*↓, TCO2*

		Roots	CO2***↓, TCO2*
		SPT	CO2§↓
	A2	Bulk	CO2*↓, TCO2**
		fLF	CO2**↓, TCO2§
		oLF	CO2*↓, TCO2*
		HF	CO2*↓, TCO2§
		Roots	CO2***↓, TD§, TCO2§, DCO2§
		SPT	CO2**↓
$\delta^{15}N$	A1	Bulk	n.s.
		fLF	n.s.
		oLF	n.s.
		HF	T*↓, TD*, TCO2§
		Roots	n.s.
	A2	Bulk	DCO2§
		fLF	TD§
		oLF	D**↑, DCO2***, TDCO2*
		HF	n.s.
		Roots	DCO2*, TCO2§
C_{new}	A1	fLF	n.s.
		oLF	n.s.
		HF	n.s.
	A2	fLF	n.s.
		oLF	D§↓
		HF	TD§
Bulk density	A1	Bulk	TD*
	A2	Bulk	n.s.
pH	A1	Bulk	CO2§, TCO2§
	A2	Bulk	T**↑, TCO2**

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. SWC were decreased compared to ambient plots on average by 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 curve 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 °C and 0.4 °C, respectively, ranging from differences of 0.1 °C during winter to 0.5 °C and 0.7 °C, in air and soil respectively, during spring/summer.

Fig. 2: Boxplots of effects of the climate treatments in soil A1 and A2 horizons (5.1±0.2 cm and 5.1±0.2 cm 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. 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.

Fig. 3: Boxplots of effects of the climate treatments in soil A1 and A2 horizons (5.1±0.2 cm and 5.1±0.2 cm to12.3±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. 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.

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 standard error.
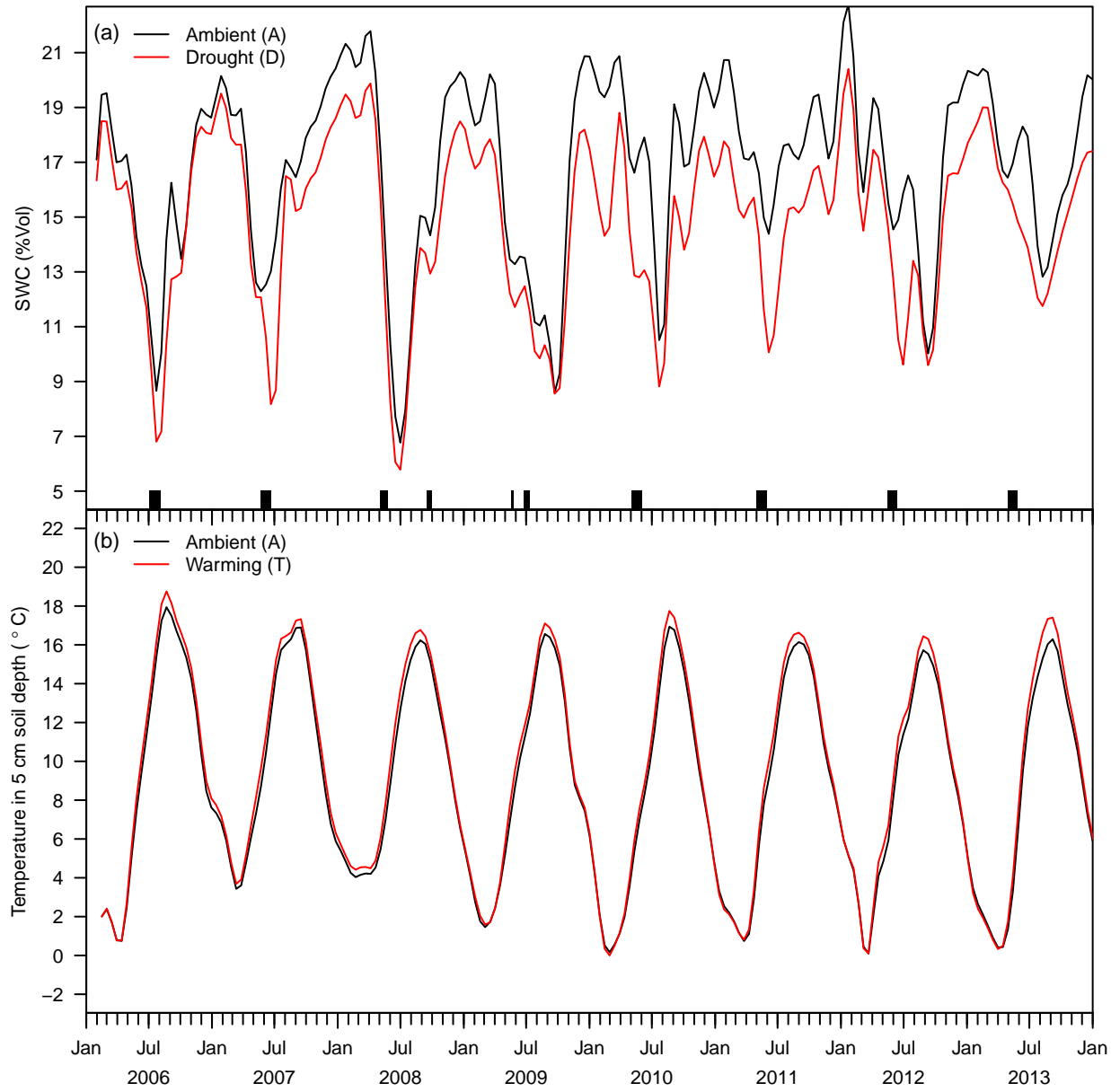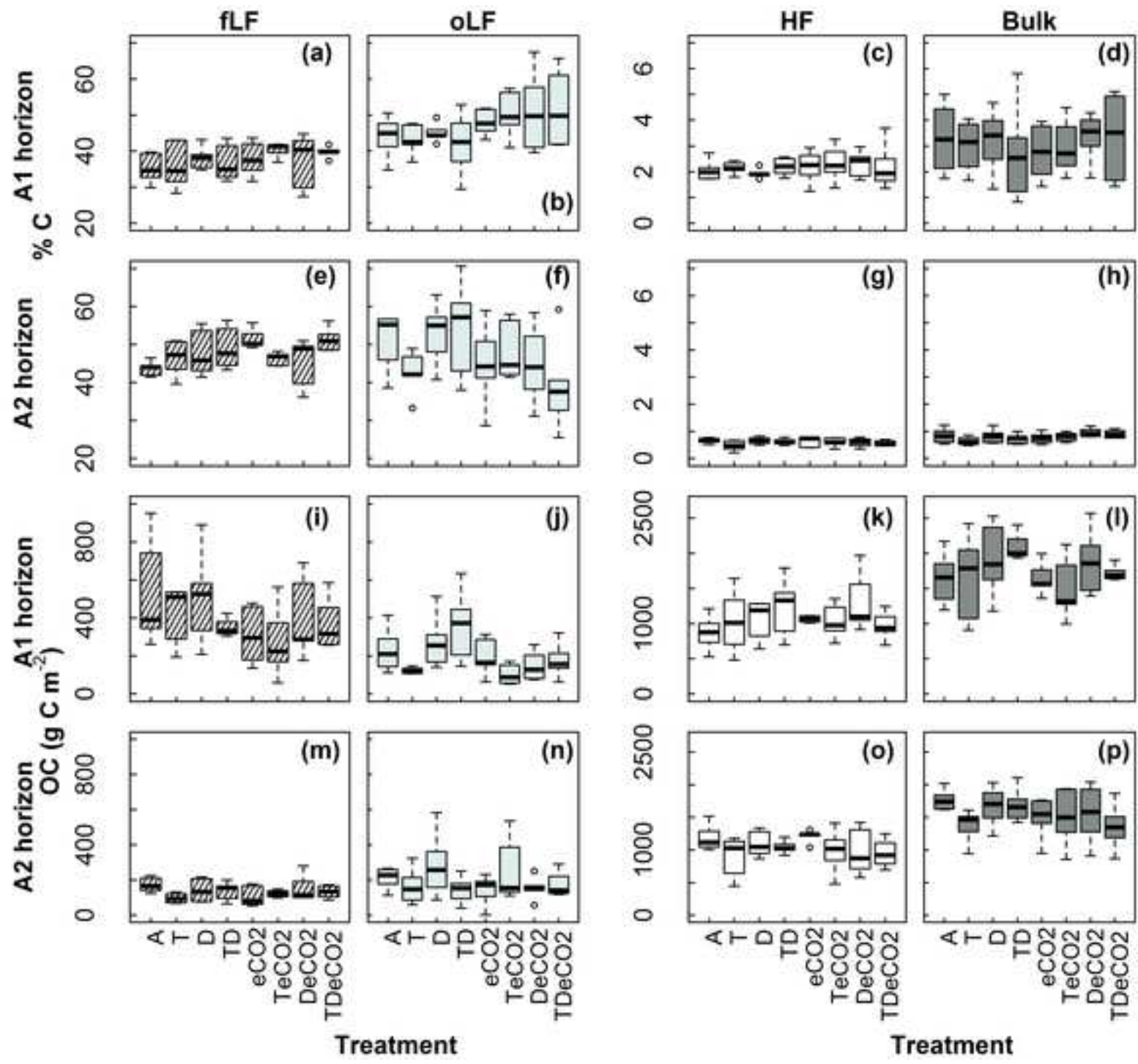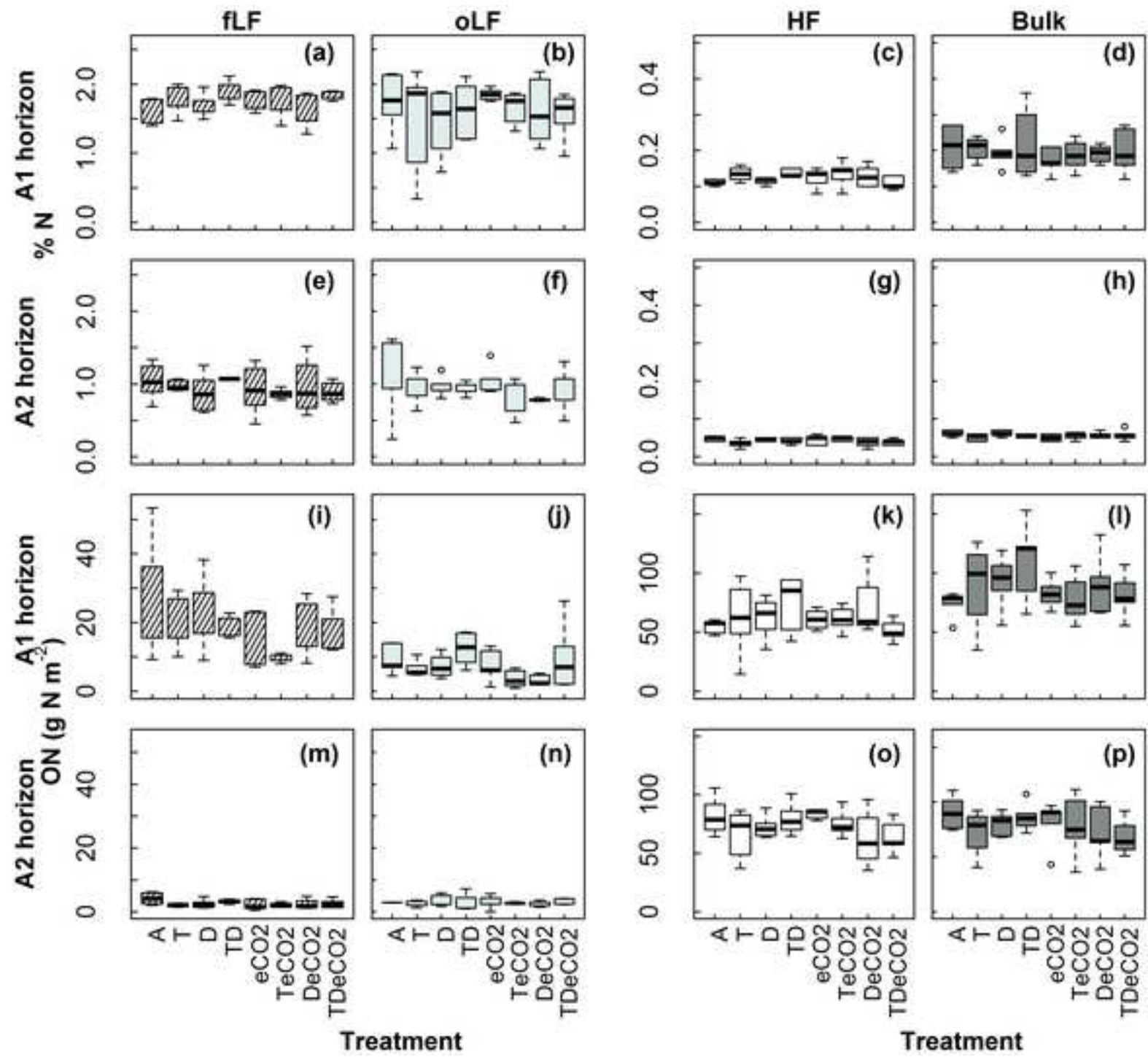
Figure 1

Figure 2

Figure 3

Figure 4