

# Re-visiting soil carbon and nitrogen stocks in a temperate heathland seven years after the termination of free air CO<sub>2</sub> enrichment (FACE)

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## ABSTRACT

The response of soil carbon to global climate change remains one of the largest uncertainties for future climate projection. In this study, we re-sampled the soil in a long-term, field-scale, multi-factorial climate experiment, CLIMAITE (Free Air CO<sub>2</sub> Enrichment (FACE), warming and drought in all combinations in a Danish heathland ecosystem) in 2020, seven years after the experiment was terminated. We aimed to study the dynamics of the soil carbon after the cessation of long-term multi-factorial climate manipulation, with special attention to the fate of the additional soil carbon (19% increase) that was sequestered in plots exposed to elevated CO<sub>2</sub> concentrations (eCO<sub>2</sub>). Soil carbon pools in former eCO<sub>2</sub> plots, as well as in drought and warming plots, had normalized again by 2020. However, the difference in soil isotopic composition between ambient and former eCO<sub>2</sub> plots remained, indicating similar loss fractions from older and newer soil carbon pools in the eCO<sub>2</sub> plots as well as stimulation of the decomposition of old soil carbon via priming. Throughout the study period, soil nitrogen dynamics tracked the changes in soil carbon, suggesting that nitrogen from deeper soil layers was transported upwards to meet increasing plant demand during eCO<sub>2</sub> but was lost again from the topsoil after termination of the FACE treatment. Our findings show that the soil carbon and nitrogen pools in this ecosystem are highly dynamic and may respond strongly and rapidly to changes in major ecosystem drivers, and that revisiting climate experiments after the cessation of treatments may provide valuable insights into the dynamics, stability and resilience of major element pools in ecosystems.

## 1. Introduction

Soil contains the largest organic carbon pool in terrestrial ecosystems (Raich & Potter, 1995; Davidson & Janssens, 2006) and plays an important role as carbon source or sink in response to global climate change (Schimel et al., 1994; Scharlemann et al., 2014; Köchy et al., 2015). A growing number of investigations have addressed the influence of elevated atmospheric CO<sub>2</sub> (eCO<sub>2</sub>) on soil carbon stocks by the application of the FACE (free-air CO<sub>2</sub> enrichment) technique (Reich et al., 2001; Jastrow et al., 2005; Sulman et al., 2014; Meeran et al., 2021), including combinations with other factors such as nitrogen availability, climate warming, or drought. However, there has been little attention to the soil carbon and nitrogen cycling and stock changes in these experiments after treatments have been terminated. Studying the

post-experimental changes in ecosystems could be a cost-effective way to investigate the resistance and resilience of ecosystems to climate change and to provide additional insights into important ecosystem functions, such as soil carbon and nitrogen dynamics, recalcitrance of soil carbon under future eCO<sub>2</sub> conditions and the overall, long-term ecosystem climate mitigation potential.

Growing evidence suggests that eCO<sub>2</sub> can lead to enhanced soil carbon input due to increased plant photosynthesis and growth (Dietzen et al., 2019; Briones et al., 2021) accompanied by increased below-ground biomass allocation (Adair et al., 2011; Arndal et al., 2018) aimed at acquiring additional nutrients (Terrer et al., 2021). In response to the additional labile soil carbon input, soil microbial activity may be accelerated resulting in higher levels of soil carbon decomposition, i.e. the 'priming effect' (Kuzyakova et al., 2000; Reinsch et al., 2013; Chen

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et al., 2019). Due to high variability across ecosystem types, interactions between eCO<sub>2</sub> and other climate drivers – as well as other potentially limiting factors for both plant growth and microbial decomposition, the combined net global effect of eCO<sub>2</sub> on soil carbon stocks remains uncertain.

Due to the close connection between eCO<sub>2</sub>, global warming, and changes in precipitation, as well as increasing frequency of extreme heat/drought events (IPCC, 2013) an increasing number of field-scale eCO<sub>2</sub> experiments in managed and non-managed ecosystems has been carried out, including combinations of eCO<sub>2</sub> with warming or drought treatments (Mikkelsen et al. 2008; Albert et al., 2011; Roy et al., 2016; Meeran et al., 2021). Warming has been demonstrated to stimulate photosynthesis directly by optimizing plant growth temperatures (Sage & Kubien, 2007; Coast et al., 2020) and indirectly via longer growing seasons or changing plant phenology (Walther et al., 2002; Penuelas et al., 2007; Piao et al., 2007). Meanwhile, warming can increase the frequency of heat waves and drought events, during which soil water availability can be reduced to a critically low level (Beier et al., 2004). Lower soil water availability limits both aboveground plant biomass production and belowground microbial processes (Emmett et al., 2004; Huxman et al., 2004; Blumenthal et al., 2018). However, Dieleman et al. (2012) found, that eCO<sub>2</sub> responses often dominated over warming responses in multi-factorial climate treatments, which is consistent with our previous findings at our experimental site (Dietzen et al., 2019) where a strong soil carbon response was found in the eCO<sub>2</sub> plots only. Therefore, here we focus on the soil carbon variations after the termination of eCO<sub>2</sub> the experiment.

In 2005–2013, the CLIMAITE experiment was conducted to explore the individual and combined effects of eCO<sub>2</sub>, warming and drought on a temperate heathland/grassland ecosystem in Denmark (Mikkelsen et al., 2008). After eight years of experimental treatments, total soil carbon stocks in the top 30 cm had increased by ca. 19 % in the former eCO<sub>2</sub> plots compared to the ambient plots, while warming and drought showed no or minor effects on the soil carbon stocks (Dietzen et al., 2019). Thaysen et al. (2017) found that the mean residence time of carbon was generally highest in the heavy fraction of soil organic matter and increased with soil depth, while the dynamics of the additional labile soil carbon stored in former eCO<sub>2</sub> plots, as well as the older, more recalcitrant carbon, remained unclear. Previous eCO<sub>2</sub> studies have provided contrasting evidence of the effects of the additional labile organic matter on the new and old carbon cycling (Van Kessel et al., 2000; Cardon et al., 2001; Van Groenigen et al., 2014). Cardon et al. (2001) reported that soil microorganisms tended to shift their consumption from older soil organic carbon sources to more easily degradable rhizodeposits when available, which resulted in increased retention of the old soil carbon, while Van Kessel et al. (2000) reported that the additional new carbon input stimulated the microbial priming effects and resulted in more decomposition of older soil carbon. Although the above studies were performed during the eCO<sub>2</sub> experimental period, these contrasting findings indirectly indicate that the long-term fate and dynamics of the ca. 19 % extra stored carbon at our experimental site is worth exploring further in the post-experimental period.

The use of stable isotopes is a powerful tool for detecting changes in soil carbon pools and fluxes and the ratio of <sup>13</sup>C to <sup>12</sup>C has been widely applied in eCO<sub>2</sub> experiments across a range of scales (Hungate et al., 1995; Cardon et al., 2001; Thaysen et al., 2017). The soil carbon ratio of <sup>13</sup>C to <sup>12</sup>C is influenced by the discrimination against the heavier <sup>13</sup>C isotope by both plant photosynthesis and processes involved in respiratory CO<sub>2</sub> production, i.e. root respiration and soil microbial decomposition activity (De Graaff et al., 2010; Lloyd et al., 1996). The distinctive isotopic signatures of different carbon sources provide the opportunity to use carbon isotopic tracers to detect the rate of incorporation of newly fixed carbon into the soil carbon pool (De Graaff et al., 2010; Ehleringer et al., 2000) as well as the dynamics of the new and old soil carbon pools. During the initial FACE experiment at the CLIMAITE

site, the ecosystem was effectively labelled in-situ for eight years because the CO<sub>2</sub> used for fumigation was depleted for <sup>13</sup>C in eCO<sub>2</sub> plots (-29 ‰ on long-term average) compared to ambient atmospheric air (-8‰) (Thaysen et al., 2017). The long-term stable isotopic labelling period provides an opportunity to use the changes in the <sup>13</sup>C to <sup>12</sup>C ratio over time to reveal information about the soil carbon dynamics in the ecosystem under different treatments.

It is well-known that the cycling of various elements in ecosystems, such as carbon and nitrogen in particular, are tightly coupled (Gruber & Galloway, 2008; Soussana and Lemaire, 2014). A range of previous experimental studies have shown the increased nitrogen uptake in response to FACE application (Finzi et al., 2007; Jiang et al., 2020) as well as reduced available soil nitrogen levels (Hu et al., 2001; Yao et al., 2021). As previously stated, the nitrogen cycle in ecosystems may track the carbon cycle (Cannell and Thornley, 1998). However, as long as the eCO<sub>2</sub> levels are not saturating and keep rising there will be a time lag between soil carbon enrichment and nitrogen increase (Thornley and Cannell, 2000). In addition, the increased soil carbon input may stimulate the sequestration and retention of nitrogen by perturbing the balance between immobilization and mineralization to very slowly reach a higher fertility equilibrium (Thornley and Cannell, 2000). At our experimental site, Larsen et al. (2011) reported decreased nitrogen leaching in response to eCO<sub>2</sub> after two years of treatments, indicating increased plant or microbial nitrogen demand, while Dietzen et al. (2019) reported changes for soil carbon only after six years of experimental treatment.

In the current study, we re-sampled the soil at the CLIMAITE field site during the growing season of 2020, i.e. seven years after the termination of the long-term multifactorial experiment. We measured the soil carbon and nitrogen stocks at three different depths (0–10 cm, 10–30 cm, 30–50 cm), but in order to compare with the previous soil carbon and nitrogen datasets, here we pooled the first and second layers and report the results for 0–30 cm soil depths. Again to compare with previously reported data, we analyzed the δ<sup>13</sup>C values of the soil and roots in the top 0–10 cm soil depth to investigate the dynamics of the topsoil carbon and nitrogen pools after the FACE manipulation had ended. Additional data on soil carbon and nitrogen stocks and their stable isotopic signal in deeper soil layer (30–50 cm) are provided in the [supplementary information](#).

Based on results reported by Dietzen et al. (2019), we hypothesized that soil nitrogen tracked soil carbon changes closely in this ecosystem, but that most of the extra stored soil carbon and nitrogen during the FACE experiment was labile and that pools have decreased back towards the initial ambient level in the post-experimental period. We further hypothesized that these changes would be confirmed by a similar return towards ambient levels of the stable isotopic δ<sup>13</sup>C signal in the former eCO<sub>2</sub> plots.

## 2. Materials and methods

### 2.1. Site description and experimental design

The CLIMAITE site is situated at Brandbjerg around 50 km northwest of Copenhagen, Denmark (55°53'N, 11°58'E). The site is a temperate heathland/grassland ecosystem dominated by wavy hair grass (*Deschampsia flexuosa*, leaf coverage ca. 80 %) and the evergreen dwarf shrub common heather (*Calluna vulgaris*, leaf coverage ca. 40 %) (Kongstad et al. 2012; Tiiva et al., 2017). The annual mean precipitation is 583 mm, and the annual mean air temperature is 10.1 °C ([www.DMI.dk](#)), the mean wind speed of 4.2 m s<sup>-1</sup>.

The CLIMAITE experiment was originally designed to simulate a potential climate scenario of Denmark in the year 2075, and these treatments were active from 2005 to 2013. Twelve pair-wise octagons were laid out in six blocks, where one of the paired octagons was ambient CO<sub>2</sub> (A) and the other was eCO<sub>2</sub> (CO<sub>2</sub>) in a FACE setup with a target concentration of 510 ppm. All octagons were divided into four

equal-size plots, where the different plots acted as either controls or were exposed to warming (T), extended summer drought (D) or their combination. This setup allowed for six replicates of 8 different treatment combinations (A, D, T, TD, CO<sub>2</sub>, DCO<sub>2</sub>, TCO<sub>2</sub>, TDCO<sub>2</sub>) in a fully factorial experimental design (n = 48). Warming was achieved by passive nighttime warming using automatic curtains that increased the nighttime air temperatures by 0.6 to 1.3 °C during winter and summer, respectively. Summer drought periods of 4 to 8 weeks were manipulated by other automatic curtains that removed on average, 8 % of the annual precipitation. Refer to [Beier et al. \(2004\)](#), [Mikkelsen et al. \(2008\)](#) and [Dietzen et al. \(2019\)](#) for further experimental details. At the end of the experiment in September 2013, an attempt was made to create an extreme drought event by covering all octagons with a raised plastic cover. However, due to high precipitation levels prior to initiating the drought and low temperatures during the fall of 2013, the experiment failed and was stopped after two months with no observable effects on the ecosystem. No climate treatments were active until June 2016, when half of the original drought plots (i.e. D, TD, DCO<sub>2</sub> and TDCO<sub>2</sub> plots in three of the original six blocks) were covered by permanent DroughtNet-style (see <https://drought-net.colostate.edu/>) rainout shelters removing 40–66 % of annual precipitation to investigate potential thresholds for structural and functional changes in the ecosystem in response to water availability. The new treatments are not the focus of the analysis presented here but the results have been carefully analyzed with respect to potential biases caused by the new drought treatments.

## 2.2. Soil sampling

In the middle of June and August 2020, soil cores were resampled using the same sampling scheme as in 2013 ([Dietzen et al., 2019](#)). Soil cores were collected at random positions in previously unsampled areas within each plot using a soil column cylinder auger (Eijkelkamp Agri-search Equipment BV, Giesbeek, The Netherlands) with an inner diameter of 87 mm attached to a gasoline-powered percussion hammer (Cobra Combi; Atlas Copco AB, Nacka, Sweden). All 48 original plots were sampled and the soil cores were separated in three depth intervals (0–10 cm, 10–30 cm and 30–50 cm). The 0–10 cm and 10–30 cm soil layer data were pooled into a single value for the 0–30 cm soil layer in order to compare these results with previously reported soil carbon and nitrogen datasets ([Dietzen et al., 2019](#)).

Soil samples were temporarily stored in a cooling room (4 °C) until further analysis. Soil samples were sieved through a 2 mm sieve. All the visible roots were then removed and handled separately. Soil samples were oven-dried at 55 °C to obtain dry weight and then homogenized by ball-milling ([Dietzen et al., 2019](#)).

The root samples were flushed with milli-Q water and separated from the litter in the surface layer. Then root samples were oven-dried at 55 °C for dry weight estimation and then homogenized by ball-milling similarly to the soil samples.

## 2.3. Sample analyses

The dry matter carbon and nitrogen concentrations (% C and % N) as well as the isotope ratios of <sup>13</sup>C/<sup>12</sup>C of soil and root samples were analyzed on an elemental analyzer (CE 1110, Thermo Electron, Milan, Italy) combined in continuous flow mode to a Finnigan MAT Delta PLUS isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany) in 2020. The carbon isotope compositions of soil and root samples were expressed in δ units (‰) ([Van Kessel et al., 2000](#)):

$$\delta^{13}\text{C} (\text{‰}) = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \quad (1)$$

where  $R = {}^{13}\text{C}/{}^{12}\text{C}$ . The  $\delta^{13}\text{C}$  values are expressed relative to the Vienna Pee Dee Belemnite standard ( $R_{\text{standard}} = {}^{13}\text{C}/{}^{12}\text{C} = 0.0112372$ ) and peach leaves (NIST 1547) were used as internal standards for %C, % N, and  $\delta^{13}\text{C}$ .

Because [Thaysen et al. \(2017\)](#) collected and analyzed soil samples at 0–5.1 cm and 5.1–12.3 cm, we transformed and unified the atom% of the different soil layers datasets to 0–10 cm by a mass balance approach together with the linear regression at midpoint of each depth in every plot in 2013 soil sample dataset:

$$\begin{aligned} \text{Atom\%} &= \frac{[{}^{13}\text{CCO}_2]}{[{}^{13}\text{CCO}_2] + [{}^{12}\text{CCO}_2]} \times 100 = \frac{[{}^{13}\text{CCO}_2]}{[\text{CCO}_2]} \times 100 \\ &= \frac{R_{\text{sample}}}{1 + R_{\text{sample}}} \times 100 \end{aligned} \quad (2)$$

## 2.4. Statistical analyses

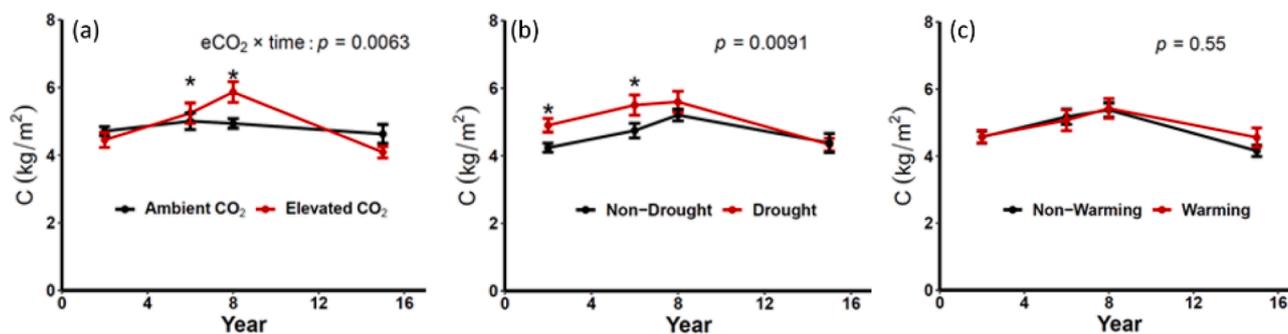
Statistical analysis and models were applied by following the same approach as ([Dietzen et al., 2019](#)) using R (Version 4.0.2). Linear mixed effects models ('lme' in the 'nlme' package) were applied to explore the effects of climate manipulations on soil carbon ([Suppl. Table S1, S1.1, S1.2](#)) and nitrogen stocks ([Suppl. Table S2, S2.1, S2.2](#)) and C:N ratios ([Suppl. Table S3, S3.1, S3.2](#)) of the top 30 cm soil profile. The main climate factors eCO<sub>2</sub>, T, D and time were included as fixed effects, while Plot was nested within Block as a random intercept term to account for the design of the experiment and repeated measures. Time was represented by month number since the start of the experiment and included as a categorical variable in the model. Pretreatment soil carbon stocks for each plot were included in the model as a covariate. A *t*-test was applied for the deeper soil layer (30–50 cm) to explore the legacy effects of eCO<sub>2</sub> on soil carbon and nitrogen stocks as well as stable isotopic values ([Suppl. Table S4–6](#)). As the period 2005–2013 was already reported ([Dietzen et al., 2019](#)), here we focus on the changes observed since the experimental manipulations were stopped in 2013. Soil nitrogen stocks and C:N ratios were likewise analyzed with pretreatment soil nitrogen stocks and C:N ratios included as covariates in their respective models ([Suppl. Fig. S1-2](#) and [Table S2-3](#)).

Post-hoc comparisons to interpret significant ( $\alpha = 0.05$ ) interactions and inspect treatment effects at specific time points were conducted using differences in estimated marginal means ('emmeans' in R). Results are presented as mean ± standard error of the mean (SEM).

## 3. Results

### 3.1. Responses of soil carbon stock to climate manipulations

Soil carbon stocks in the 0–30 cm soil layer increased in the former eCO<sub>2</sub> plots especially during year six to eight of the experiment ending at  $5.87 \pm 0.31 \text{ kg C m}^{-2}$  in 2013 compared to  $4.94 \pm 0.14 \text{ kg C m}^{-2}$  in the ambient CO<sub>2</sub> plots ([Dietzen et al., 2019](#)). However, when revisiting the same plots in 2020, seven years after the termination of the FACE treatment, the former eCO<sub>2</sub> plots no longer showed higher C stocks ([Fig. 1a](#); [Suppl. Table S1.1](#),  $P = 0.58$ ). In fact, by 2020, the soil carbon in the former eCO<sub>2</sub> plots ( $4.09 \pm 0.17 \text{ kg C m}^{-2}$ ) tended to be slightly lower than the ambient CO<sub>2</sub> plots ( $4.63 \pm 0.28 \text{ kg C m}^{-2}$ ), i.e. similar to the tendency observed in 2007. Although the soil carbon stock in the drought plots was significantly higher than the non-drought plots during the experimental period, likely due to pretreatment differences as suggested by [Dietzen et al. \(2019\)](#), soil carbon stocks in drought and non-drought plots had converged by 2020 ([Fig. 1b](#)). Similarly, and as also observed in 2013, there was no significant difference in soil carbon stock in the warmed plots when re-sampled in 2020 ([Fig. 1c](#) and [Suppl. Table S1](#)). Finally, no differences in soil carbon stocks between ambient and eCO<sub>2</sub> treatments were found for the 30–50 cm soil layer in 2020 ([Suppl. Fig. S3](#) and [Table S5](#)) and since this layer was not adequately sampled in the previous period no analysis of changes over time for this layer was possible.



**Fig. 1.** Mean soil C stocks in 0–30 cm soil depth averaged across all treatment combinations with ( $n = 24$ ) and without ( $n = 24$ ) elevated CO<sub>2</sub> (a), extended summer drought (b), and warming (c) treatments (i.e., main factor effects). Year 2 is 2007, year 6 is 2011, year 8 is 2013, and year 15 is 2020. Error bars indicate standard error of the mean. Significant main factor effects ( $p < 0.05$ ) are indicated by an asterisk at that time point.

### 3.2. Stable isotopic values of the soil and roots samples

As a result of the fumigation with <sup>13</sup>C depleted CO<sub>2</sub> from 2005 to 2013, the mean  $\delta^{13}\text{C}$  value of the soil layer 0–10 cm in eCO<sub>2</sub> plots was  $0.78 \pm 0.07$   $\delta$  units lower than that of ambient plots in 2013. This difference remained almost unchanged at  $0.76 \pm 0.16$   $\delta$  units difference in 2020 (Fig. 2). In contrast, the root  $\delta^{13}\text{C}$  value in former eCO<sub>2</sub> plots, which was 7.6  $\delta$  units lower than ambient in 2013, converged toward the value observed in ambient plots in the period, although still being 2.5  $\delta$  units lower in 2020 (Fig. 2). Except for the roots in the former eCO<sub>2</sub> plots, we observed a downward trend in  $\delta^{13}\text{C}$  value from 2013 to 2020, likely in response to the continuously decreasing atmospheric background signal. For the deepest soil layer (30–50 cm), following the trend from the upper soil layer, we found significant differences of both soil and root  $\delta^{13}\text{C}$  values between former eCO<sub>2</sub> and ambient plots in 2020 although the differences were smaller in magnitude (Suppl. Table S4 and Table S6).

### 3.3. Responses of soil nitrogen stock to climate manipulations

Compared to the ambient plots, soil nitrogen stocks in 0–30 cm depth increased significantly in the former eCO<sub>2</sub> plots throughout the CO<sub>2</sub> fumigation experiment from  $0.26 \pm 0.011$  kg m<sup>-2</sup> in 2007 to  $0.34 \pm 0.014$  kg m<sup>-2</sup> (Fig. 3a) in 2013. However, after the CO<sub>2</sub> fumigation was terminated, the nitrogen stocks decreased again in the former eCO<sub>2</sub> plots to a level of  $0.26 \pm 0.011$  kg m<sup>-2</sup> in 2020, which is similar to the soil nitrogen content of ambient plots:  $0.29 \pm 0.018$  kg m<sup>-2</sup> (Suppl. Table S2.1,  $P = 0.4326$ ). For the drought treatments, the pretreatment bias as reported by Dietzen et al. (2019) was still evident, which could explain the higher values of nitrogen stocks in the drought treatments

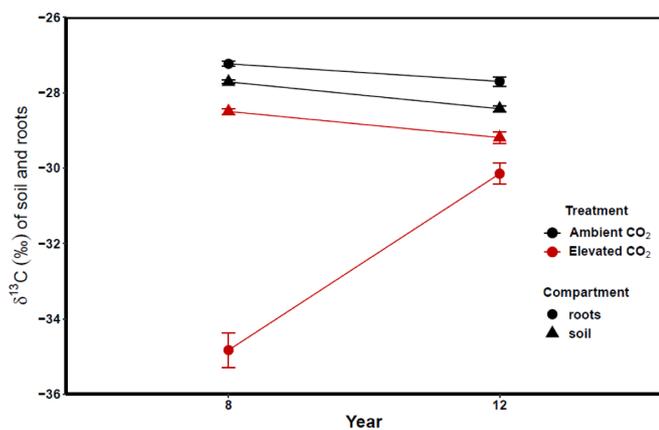
than in the ambient plots (Fig. 3b). In both the ambient and drought plots, the nitrogen stocks increased about  $0.06$  kg m<sup>-2</sup> and  $0.04$  kg m<sup>-2</sup> respectively, from 2007 to 2013. However, seven years after the experiment terminated, the nitrogen stocks were back to the initial level in 2007. For the warming and non-warming treatments, the soil nitrogen stocks changed from around  $0.27$  kg m<sup>-2</sup> to  $0.32$  kg m<sup>-2</sup> during 2007–2013 (Fig. 3c). After that, the nitrogen stock returned to  $0.26 \pm 0.01$  kg m<sup>-2</sup> in the non-warming plots, while in the warming plots the values decreased to  $0.29 \pm 0.02$  kg m<sup>-2</sup>. Compared to soil carbon, the soil nitrogen stocks in the 30–50 cm layer (Suppl. Fig. S3 and Table S5) did not differ significantly in 2020.

### 3.4. Responses of C:N ratio to climate manipulations

Despite the tight C–N coupling observed, there was a significant decrease in the C:N ratio (Suppl. Fig. S1 and Table S3,  $P < 0.01$ ) over the course of the study, from 17.0 in the second year of the experiment (2007) to 15.7 after 15 years (2020) across all plots. Significant differences were observed between ambient and eCO<sub>2</sub> plots in 2013 (Suppl. Fig. S2 and Table S3.1,  $P < 0.05$ ).

## 4. Discussion

As previously reported, soil carbon stocks increased by ca. 19 % in the upper 30 cm of the soil profile during the eight years of free-air carbon dioxide enrichment (Dietzen et al., 2019). The effects of eCO<sub>2</sub> were independent of combined treatments with warming and/or drought. The individual or combined effects of eCO<sub>2</sub>, warming, and drought on a variety of ecosystem types, including forests (Rasmussen et al., 2002), grasslands (Adair et al., 2011; Roy et al., 2016; Blumenthal et al., 2018; Meeran et al., 2021), agricultural (Wu et al., 2020) or multiple ecosystems (Terrer et al., 2021), have been explored over various time scales by a number of studies. Similar to our observations, other long-term studies have also shown an increase of soil carbon with eCO<sub>2</sub> (Hebeisen et al., 1997; Jastrow et al., 2005; Dieleman et al., 2012). However, the stability of the accumulated soil carbon under eCO<sub>2</sub> is highly uncertain. To our knowledge, it has never previously been reported how soil carbon dynamics changes after the long-term climate change manipulations have been terminated. Here, we report that seven years after the long-term FACE treatment was terminated, there were no significant differences between eCO<sub>2</sub> plots and ambient plots in either topsoil or deeper soil layer carbon stocks. The soil carbon stock in the former eCO<sub>2</sub> plots had declined again to a level similar to the ambient plots, revealing a highly dynamic soil carbon pool capable of responding strongly and quickly to changes in atmospheric CO<sub>2</sub> concentration. It must be noted that in the original experiment, we observed a dominant and strong effect of eCO<sub>2</sub> but limited effects of warming and drought, which may be because these treatments were applied at a moderate level (Larsen et al., 2011; Selsted et al., 2012; Dietzen et al., 2019).



**Fig. 2.**  $\delta^{13}\text{C}$  values of soil and roots in 0–10 cm soil depth across the treatments with ( $n = 24$ ) and without ( $n = 24$ ) elevated CO<sub>2</sub>. Year 8 is 2013 and year 15 is 2020. Error bars indicate standard error of the mean.

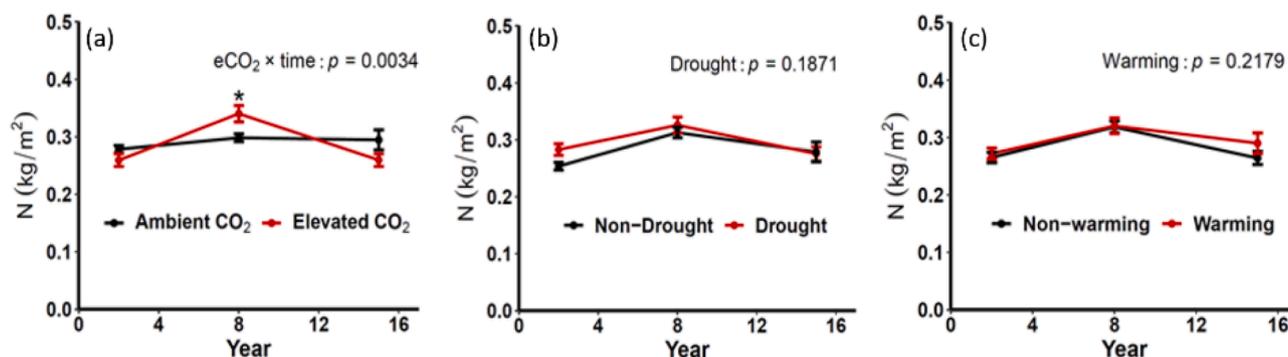


Fig. 3. Mean soil N stocks in 0–30 cm soil depth averaged across all treatment combinations with ( $n = 24$ ) and without ( $n = 24$ ) elevated CO<sub>2</sub> (a), extended summer drought (b), and warming (c) treatments (i.e., main factor effects). Year 2 is 2007, year 6 is 2011, year 8 is 2013, and year 15 is 2020. Error bars indicate standard error of the mean. Significant main factor effects ( $p < 0.05$ ) are indicated by an asterisk at that time point.

While the soil carbon pool in former eCO<sub>2</sub> plots had fallen again in 2020, the differences of soil  $\delta^{13}\text{C}$  observed between ambient and former eCO<sub>2</sub> plots were quite stable and consistent from 2013 to 2020 and was even still significantly different in the 30–50 cm soil depth in 2020, indicating that a substantial fraction of the extra new soil carbon sequestered still remained in the soil carbon pool. As the total soil carbon pool declined from 2013 to 2020, a simultaneous priming effect by the newly added carbon on the decomposition of older soil carbon fractions must have taken place (Fontaine et al., 2007; Finzi et al., 2015; Murphy et al., 2015; Bernal et al., 2016). Our finding of stabilized new carbon is supported by Jastrow et al. (2005), who found that 55 % of the accrued carbon in prairie soil was incorporated into microaggregates to be stabilized in longer-lived pools. Our results also support the concept of a complex and interwoven, flow-based soil carbon cycle as proposed by Dynarski et al. (2020), who suggested that soil organic matter is simultaneously cycled through microbial activities and stored via interactions with soil minerals that effectively introduce friction into the flow of carbon through the soil profile. The interwoven, complex, flow-based soil carbon cycle, where many different pools are more or less affected by addition of extra carbon during the eCO<sub>2</sub> experiment may also explain why a significant amount of older soil carbon fractions may be lost upon the termination of eCO<sub>2</sub> as indicated by the isotopic data in our experiment. Dynarski et al. (2020) further pointed out that the translocation of plant-derived carbon from topsoil into deeper soil layers likely helps facilitate long-term carbon sequestration. Arndal et al. (2013, 2018) found significantly increased root length in deeper soil layers under eCO<sub>2</sub> at our experimental site, indicating that the extra photosynthetically fixed carbon from 2005 to 2013 was at least partially transported to deeper soil layers.

It is remarkable to observe how the soil carbon pools in 2020 had returned to levels very close to the initial values at the beginning of the original experiment indicating a “normalization” of carbon fluxes and an “equilibrium” carbon pool size for the ecosystem. This high plasticity indicates that if climate drivers, such as eCO<sub>2</sub>, warming and drought, are changed, soil carbon pools in ecosystem types like heathlands may increase or decrease significantly within time scales of years to decades. Our results show that this ecosystem has a highly dynamic carbon cycling, yet still is also a highly resilient system that buffers both inter- and intra-annual variation in major drivers to maintain an equilibrium of the soil carbon pool.

While there is a strong focus on changes in soil carbon in ecosystems because of its direct interaction with increasing atmospheric CO<sub>2</sub> levels and climate, knowledge on long-term influence of climate drivers on nitrogen cycling and pools is also important because of the tight coupling between carbon and nitrogen cycling in most ecosystems (Gruber & Galloway, 2008). Changes in overall soil nitrogen pools from this experiment have only previously been reported for the short-term treatment period of two years (Larsen et al., 2011), when above- and

belowground plant nitrogen pools remained unchanged under former eCO<sub>2</sub> plots. However, a decrease in concentrations of organic and inorganic nitrogen in leachate water provided an early indicator of increased root nitrogen uptake under eCO<sub>2</sub> (Larsen et al., 2011), which was confirmed later in the study by observations of increased root growth and root nitrogen uptake (Arndal et al., 2013; Arndal et al., 2018).

Over a total study period of 14 years in our experiment, soil nitrogen pools tracked soil carbon pools closely, both during the eCO<sub>2</sub> period with increasing soil carbon and during the post-experimental period of decreasing soil carbon. These observations suggest a very tight coupling between ecosystem carbon and nitrogen dynamics as well as sufficient flexibility of soil nitrogen as also observed in other experiments reported by Jastrow et al. (2005).

Thornley and Cannell (2000) suggested that eCO<sub>2</sub> increased symbiotic and non-symbiotic N<sub>2</sub> fixation and that the extra carbon fixed under eCO<sub>2</sub> is used to capture and retain more nitrogen. However, nitrogen fixation at rates high enough to explain the observed increase in soil nitrogen pools observed in the current study seems unlikely. As the soil C:N ratio over the study period was relatively stable and even tends to decrease slightly over the full study period, there is also no current indication of progressive nitrogen limitation in the ecosystem (Luo et al., 2004). The increase in the soil nitrogen pool during the FACE period is therefore best explained by a combination of increased retention of the atmospheric deposition of nitrogen as reported by Larsen et al. (2011), combined with a large, upward transport of nitrogen from deeper soil layers as originally suggested by Dietzen et al. (2019) based on the observation of increased root growth in deeper soil layers (Arndal et al., 2013; Arndal et al., 2018).

Revisiting a climate experiment seven years after the termination of the FACE treatment provided several insights. The soil carbon and nitrogen pools in the studied ecosystem were highly dynamic both in situations of increasing and decreasing carbon input. In periods with increased carbon sequestration, substantial fractions of newly sequestered carbon may be stabilized in the soil for longer time periods. However, when plant carbon inputs are weakened, the newly added carbon may cause priming of the decomposition of older soil carbon pools (e.g. Van Groenigen et al., 2014). We found a very tight coupling between carbon and nitrogen cycling and the strong response to eCO<sub>2</sub> observed was possible because plants were able to mobilize nitrogen resources from deeper soil layers to meet increasing plant demands. The results are overall supportive of a flow-based, interwoven soil carbon cycle as proposed by Dynarski et al. (2020). In conclusion, revisiting climate experiments after cessation of treatments may provide valuable insights into the dynamics, stability, and resilience of major element pools in ecosystems.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.geoderma.2022.116185>.

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