



## Accumulation of soil carbon under elevated CO<sub>2</sub> unaffected by warming and drought

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**Title: Accumulation of soil carbon under elevated CO<sub>2</sub> unaffected by warming and drought**

Running Title: Soil carbon under eCO<sub>2</sub>, warming, & drought

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## Abstract:

Elevated atmospheric CO<sub>2</sub> concentration (eCO<sub>2</sub>) and climate change may substantially alter soil carbon (C) dynamics and thus feedback to future climate. However, only very few field experiments world-wide have combined eCO<sub>2</sub> with both warming and changes in precipitation in order to study the potential combined effects of changes in these fundamental drivers of C cycling in ecosystems. We exposed a temperate heath/grassland to eCO<sub>2</sub>, warming, and drought, in all combinations for 8 years. At the end of the study, soil C stocks were on average 0.927 kg C m<sup>-2</sup> higher across all treatment combinations with eCO<sub>2</sub> compared to ambient CO<sub>2</sub> treatments (equal to an increase of 0.120 ± 0.043 kg C m<sup>-2</sup> y<sup>-1</sup>), and showed no sign of slowed accumulation over time. However, if observed pre-treatment differences in soil C are taken into account, the annual rate of increase caused by eCO<sub>2</sub> may be as high as 0.177 ± 0.070 kg C m<sup>-2</sup> y<sup>-1</sup>. Further, the response to eCO<sub>2</sub> was not affected by simultaneous exposure to warming and drought. The robust increase in soil C under eCO<sub>2</sub> observed here, even when combined with other climate change factors, suggests that there is continued and strong potential for enhanced soil carbon sequestration in some ecosystems to mitigate increasing atmospheric CO<sub>2</sub> concentrations under future climate conditions. The feedback between land C and climate remains one of the largest sources of uncertainty in future climate projections, yet experimental data under simulated future climate, and especially including combined changes, are still scarce. Globally coordinated and distributed experiments with long-term measurements of changes in soil C in response to the three major climate change-related global changes, eCO<sub>2</sub>, warming, and changes in precipitation patterns, are therefore urgently needed.

## Introduction

Soils contain the largest terrestrial carbon (C) pool (Jobbagy & Jackson, 2000) and changes in soil C stocks have the potential to serve as a strong positive or negative feedback to elevated CO<sub>2</sub> (eCO<sub>2</sub>) and thus to future climate change (Davidson & Janssens, 2006). How elevated atmospheric CO<sub>2</sub> and associated changes in climate will affect soil C depends on the balance between their effects on the rates of C accumulation through plant inputs and losses due to microbial decomposition of soil organic matter (Pendall et al., 2004). In order to accurately predict future changes in climate and their impacts on terrestrial ecosystems, it is critical to understand the role that soil C pools will play in the global C cycle under eCO<sub>2</sub> and changing climatic conditions.

The net effect of increasing atmospheric CO<sub>2</sub> concentrations on soil C stocks is still unclear. Elevated CO<sub>2</sub> can stimulate plant biomass production directly by increasing the availability of CO<sub>2</sub> for photosynthetic uptake or indirectly by improving plant water use efficiency (Ainsworth & Rogers, 2007). There is growing evidence that a substantial fraction of the additional biomass produced by plants will be allocated belowground to access nutrients necessary for increased plant growth, resulting in additional inputs to soil C pools (Dieleman et al., 2012). However, these additional inputs of labile organic C under eCO<sub>2</sub> may result in *priming*, i.e. additional loss of soil organic carbon (SOC) caused by the stimulation of microbial activity in response to the addition of easily decomposable organic substrates (Kuzyakov, Friedel, & Stahr, 2000).

Further, the climate change-associated increase in temperature and concurrent changes in precipitation may modulate the effects of elevated CO<sub>2</sub> on ecosystem processes. Plant growth and microbial activity are often stimulated by climatic warming (Wu, Dijkstra, Koch, Peñuelas, & Hungate, 2011), resulting in additional uncertainty regarding the direction of a soil C feedback under warmer conditions (Crowther et al., 2016; Kirschbaum, 2000; Van Gestel et al., 2018). By contrast, lower soil moisture under drought conditions is expected to

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decrease microbial activity and plant growth (Wu et al., 2011). As the effects of climate drivers such as elevated CO<sub>2</sub>, warming, and summer drought are rarely additive (Dieleman et al., 2012; Larsen et al., 2011), investigating their interactions is crucial to understanding the potential feedbacks between SOC and future atmospheric CO<sub>2</sub> concentrations.

Interactions between climate change drivers are currently not well incorporated into coupled carbon-climate models, which are often parameterized based on responses to single factor experiments that are the source of most data on the impacts of changing climatic conditions (Dieleman et al., 2012). As feedbacks between land C and climate are one of the largest sources of uncertainty in future climate projections (Arora et al., 2013; Todd-Brown et al., 2014), direct measurements of changes in soil C stocks in response to multi-factor studies are needed (Bradford et al., 2016). Yet, only few studies have investigated the combined impacts of multiple interacting global change drivers on soil C (Ni et al., 2017; Yue et al., 2017). In fact, soil C stocks have often been neglected in experimental investigations of the effects of climate change as sampling is both expensive and time consuming (Ni et al., 2017). When soil C was measured, the study length was often too short to detect significant changes (Jastrow et al., 2005). The current lack of empirical observations of interactive effects from long-term climate experiments on soil C limits our ability to parameterize and validate the soil C component of Earth System Models (Bradford et al., 2016; Crowther et al., 2016).

The CLIMAITE experiment was designed to examine the effects of climate change on a mixed heath and grassland ecosystem using a unique, multi-factor approach that allowed for the determination of the effects of eCO<sub>2</sub>, warming (T), and drought (D), both individually and in all possible combinations (Mikkelsen et al., 2008). Previous results from the experiment have shown significant differences between the effects of single vs. combined factor manipulations on root growth (Arndal et al., 2013; Arndal, Schmidt, Kongstad, Beier, & Michelsen, 2014; Arndal, Tolver, Larsen, Beier, & Schmidt, 2018), aboveground biomass

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production (Kongstad et al., 2012), photosynthetic activity (Albert, Ro-Poulsen, et al., 2011b; Albert, Mikkelsen, Michelsen, Ro-Poulsen, & van der Linden, 2011), C and nitrogen (N) cycling (Larsen et al., 2011; Thaysen, Reinsch, Larsen, & Ambus, 2017), microbial abundance and growth (Andresen, Michelsen, Ambus, & Beier, 2010; Haugwitz et al., 2014), and soil respiration (Selsted et al., 2012). In most cases, treatment combinations have dampened rather than enhanced single-factor responses, and simple additive responses were rare (Larsen et al., 2011).

Here, we used long-term soil C data from the unique experimental setup of this multifactorial experiment to investigate the effects of elevated atmospheric CO<sub>2</sub>, warming and extended drought on soil C stocks to a depth of 30 cm over an experimental period of 8 years. We focused particularly on determining the effect of eCO<sub>2</sub> on soil C stocks and how its effect may be altered by simultaneous changes in temperature (warming) or soil water (drought).

## Materials and Methods

### *Site Description*

This research was conducted at the CLIMAITE experimental site at Brandbjerg, located approximately 50 km NW of Copenhagen, Denmark (55°53' N, 11°58' E). The site is a dry heath/grassland ecosystem dominated by two perennial species, a grass (*Deschampsia flexuosa* (L.), c. 70% cover) and an evergreen dwarf shrub (*Calluna vulgaris* (L.), c. 30% cover). The experimental plots are situated on a sandy moraine from the Weichsel glaciation. Soils at the site are Cambic Arenosols with relatively low cation exchange capacity, weak signs of podsolization, and a pH<sub>CaCl2</sub> in the topsoil of 3.3 increasing to 4.5 in the B-horizon. These well-drained soils are 71.5% sand, 20.5% coarse sand, 5.8% silt and 2.2% clay (Nielsen, Andresen, Michelsen, Schmidt, & Kongstad, 2009). The well-defined O-horizon above the mineral soil is approximately 2-5 cm thick. The site receives on average 613 mm of

rainfall annually, and the mean annual air temperature is 8 °C (Danish Meteorological Institute, 2009, <http://www.dmi.dk>). Bulk atmospheric N deposition at the site is relatively low (Larsen et al., 2011); in 2007 the site received  $1.35 \pm 0.04 \text{ g N m}^{-2} \text{ y}^{-1}$ .

The Danish Meteorological Institute has predicted that in the future, Denmark will experience warmer air temperatures and more frequent drought events in the summer in response to globally elevated atmospheric CO<sub>2</sub> (Danish Meteorological Institute, 2009, <http://www.dmi.dk>). The experimental treatments implemented at this site were designed to mimic these predicted climatic changes. The implementation of the eCO<sub>2</sub> and warming treatments began in October 2005, and the first prolonged summer drought was imposed in July 2006. Free air CO<sub>2</sub> enrichment (FACE) was used to increase atmospheric CO<sub>2</sub> concentrations in the eCO<sub>2</sub> treatment plots to a target value of 510 ppm. The FACE treatments were employed from dawn until dusk and switched off overnight and during periods of complete snow cover.

Passive nighttime warming was achieved by the use of curtains that reflected infrared radiation back to the soil surface and vegetation. These were employed from dusk until dawn throughout the year, but removed during periods of rain, high winds, and severe frost. The warming treatments increased nighttime air temperatures by 0.6°C and 1.3°C in the summer and winter months, respectively. Nighttime soil temperatures at a depth of 5 cm were increased by 0.7°C in the summer and 0.3°C in the winter. Averaged across night and daytime measurements, the warming treatments increased soil temperatures by 0.4°C in the summer and 0.2°C in the winter. The warming treatment also had the effect of increasing the growing season by two weeks in the spring (Kongstad et al., 2012) and reducing soil moisture relative to the control.

Droughts were induced once or twice a year in the spring or summer by use of rainfall exclusion curtains during rain events, removing on average  $59\pm 6$  mm of rainfall per year (8% of annual precipitation). Drought periods continued until the soil water content fell below 5% in the upper 20 cm of the soil profile as determined by TDR probes, at which point re-wetting was allowed in order to maintain soil moisture slightly above the wilting point of vegetation at the site. Drought periods typically lasted between 1-5 weeks, which is within the range of naturally occurring summer droughts at the site. Mean annual soil moisture was significantly reduced by the drought treatment over the course of the experiment. However, because re-wetting was allowed, the significant drying effect observed when the treatment was applied was not always persistent throughout the growing season (Selsted et al., 2012).

### *Experimental Design*

Treatments at the site consisted of a full factorial combination of eCO<sub>2</sub>, warming and summer drought (Figure S1). Six blocks contained pairwise combinations of 12 octagons. In each block one octagon received eCO<sub>2</sub> (CO<sub>2</sub>), whereas the other did not. Each octagon was 6.8 m in diameter and divided into four subplots: control, warming (T), summer drought (D), and combined warming and drought (TD). There were six replicates of each of the three individual treatments (T, D, CO<sub>2</sub>), their combinations (TD, TCO<sub>2</sub>, DCO<sub>2</sub>, TDCO<sub>2</sub>), and a non-treated control (A) resulting in a total of 48 treatment plots. The full factorial treatment (TDCO<sub>2</sub>) simulates the predicted future climatic conditions at the site. A full description of the experimental setup can be found in Mikkelsen et al. (2008).



### *Sampling and Analyses*

Soil samples were collected three times over the course of 8 years from the upper 30 cm of the soil profile. Samples were taken in all experimental plots, for a total of 48 samples per depth interval. As these samples were collected for a variety of experimental purposes, soil-sampling intervals varied. A full description of the sampling depth intervals and sampling dates can be found in Table S1.

In July 2007, soil samples were taken during the installation of minirhizotrons (Arndal et al., 2018). These samples were taken by augering at a 45° angle. Fourteen samples were lost from this dataset in the 0-5 cm depth interval, but these missing samples were fairly evenly distributed across treatments. One entire warmed profile was removed from the July 2007 data since plant material was suspected to have contaminated the samples from this plot during the sampling process. In November 2011 soil cores were taken as part of a  $^{13}\text{C}$  labeling experiment, and in June 2013, soil samples were taken using a soil column cylinder auger (Eijkelkamp Agrisearch 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 samples were sieved to 2 mm and visible roots passing the sieve were removed before drying and grinding. Samples were oven dried at 55° C and homogenized by ball-milling. C concentrations of the samples from 2007 and 2013 were determined using an EA Flash 2000 elemental analyzer (Thermo Fisher Scientific) and the samples from 2011 were analyzed using a Eurovector CN elemental analyzer.

Bulk density values for each sampling interval at each time point were interpolated and, when necessary, extrapolated from bulk density measurements taken at the last sampling point in 2013 and pre-treatment measurements of bulk density conducted in 2004 to account for the decrease in bulk density over the course of the experiment, particularly in the plots

exposed to eCO<sub>2</sub> (Supplementary Methods, Figure S2). This allowed for the calculation of soil C stocks to 30 cm depth on an areal basis. Missing data points from the upper 0-5 cm interval of Month 22 were point filled for pool calculation by averaging across the other plots from that treatment combination.

### *Statistical Analyses*

Linear mixed effects models (“lme” in the “nlme” package in R Version 3.2.3) were used to test for climate treatment effects on soil C stocks to 30 cm over time (Table S3) (Pinheiro & Bates, 2000). Time and main factors, eCO<sub>2</sub>, T, and D, were included in the model as fixed effects in a full-factorial statement. Pretreatment soil C stocks for each octagon were included in the model as a covariate. A random intercept term, with Plot nested within Block, was used to account for the experimental design and repeated measures. Time was represented by the number of months passed since the implementation of the treatments (Table S1), and was included as a continuous variable.

Differences of least square means (“lsmeans” in R) were used to interpret significant interactions ( $\alpha = 0.05$ ) (Tables S2.1, S2.2) and to compute the slope of significant changes over time (Table S2.3). Results are presented as mean C stocks  $\pm$  standard error of the mean (SEM).

A linear mixed effects model was used to test differences in the C:N ratio of C and N stocks in both the entire 30 cm profile and the top 10 cm individually. This model included the main factors, eCO<sub>2</sub>, T and D, as fixed effects in a full-factorial statement and block as a random factor.

## Results

Soil C stocks increased under eCO<sub>2</sub> over time ( $p = 0.001$ , Table S2.2) but remained unchanged under ambient CO<sub>2</sub>, causing a significant interaction between eCO<sub>2</sub> and time (eCO<sub>2</sub> x time,  $p = 0.013$ , Figure 1a, Table S2). Though there was no significant eCO<sub>2</sub> effect after two years of experimental treatments, soil C was significantly increased by eCO<sub>2</sub> at both of the following measurement points ( $p = 0.001$ , Figures 1a & 2, Table S2.1). The response was consistent across all treatments combinations with elevated CO<sub>2</sub> (Figure 2b). After 8 years of treatments, eCO<sub>2</sub> had increased soil C stocks on average from  $4.94 \pm 0.14 \text{ kg C m}^{-2}$  under ambient CO<sub>2</sub> ( $n=24$ ) to  $5.87 \pm 0.31 \text{ kg C m}^{-2}$  in all treatment combinations with eCO<sub>2</sub> ( $n=24$ ), equal to a mean annual increase of  $0.120 \pm 0.043 \text{ kg C m}^{-2} \text{ yr}^{-1}$ . However, pre-treatment data showed that the eCO<sub>2</sub> plots initially contained substantially less C ( $3.91 \pm 0.19 \text{ kg C m}^{-2}$ ) than the ambient plots ( $5.06 \pm 0.24 \text{ kg C m}^{-2}$ ). When this pretreatment difference is taken into account by computing the annual increment from the difference in the slopes of the two CO<sub>2</sub> treatments over time (Table S2.3), the mean annual C accumulation rate was as high as  $0.177 \pm 0.070 \text{ kg C m}^{-2} \text{ y}^{-1}$  under eCO<sub>2</sub>.

Drought significantly increased soil C stocks ( $p = 0.002$ , Table S2) as a main effect (Figure 1b), whereas warming had no effect on soil C stocks (Figure 1c). There were no significant interactions between any of the three main factors.

The C:N ratio of the soil profile (0-30 cm) was unaffected by eCO<sub>2</sub>. However, there was a significant increase in C:N from  $17.2 \pm 0.3$  under ambient CO<sub>2</sub> to  $18.2 \pm 0.3$  with eCO<sub>2</sub> in the top 10 cm of the profile ( $p = 0.039$ ).

## Discussion

Elevated CO<sub>2</sub> had a positive effect on soil C stocks in the studied temperate heath-grassland, and this response was unaffected by drought and warming. Drought, which has

previously been shown to reduce soil respiration rates at the site (Selsted et al., 2012), appeared to increase soil C stocks. However, any potential increase in soil C resulting from a reduction in decomposition was likely compensated for by the concomitant negative effects of drought on plant photosynthesis (Albert, Ro-Poulsen, et al., 2011b), aboveground biomass (Kongstad et al., 2012), and root growth (Arndal et al., 2014), resulting in reduced inputs to the soil C pool. In light of the fact that the observed increase in soil C with drought was consistent throughout the experiment, we therefore believe this difference to be due to random pretreatment differences in soil C caused by initial differences in plant community composition and associated differences in litter input and quality. As higher levels of soil organic matter are found under the dwarf shrub (*Calluna vulgaris*) than under the dominant grass species (*Deschampsia flexuosa*) at the site (Nielsen et al., 2009), the fact that shrubs happened to be more prevalent in the drought plots compared to the non-drought plots at the beginning of the experiment (Table S3) likely resulted in larger initial SOC pools. Many other studies have found drought to have no effect on soil C (Yue et al., 2017), though others have observed small yet significant increases with drought (Zhou et al., 2016). Regardless, the 15.6% increase in soil C with drought at our site observed after less than 2 years is many times greater than the 1.45% mean increase found by Zhou et al.'s (2016) meta-analysis, suggesting that such an increase was likely not a treatment, but likely a pre-treatment effect.

Warming did not change the soil C stock during our experiment, which was surprising as warming has previously been shown to stimulate microbial abundance and growth at the site (Haugwitz et al., 2014) and also to induce a small, yet consistent increase in soil respiration (Selsted et al., 2012). Additionally, standing root biomass was observed to be lower in the warmed plots that did not receive eCO<sub>2</sub> (Arndal et al., 2014, 2018), which may be a response to more easily accessible N resulting from increased turnover under higher temperatures (Larsen et al., 2011). However, as the warming treatment also lengthened the

growing season (Albert, Ro-Poulsen, et al., 2011a), it may be that the potential for plant growth over a longer period of time has compensated for the increased C loss caused by the higher microbial activity. Warming has similarly been found to have no net effect on soil C stocks in some meta-analyses (Dieleman et al., 2012; Ni et al., 2017; Van Gestel et al., 2018), whereas other meta-analyses have predicted soil C stocks across the globe to decrease with increasing temperatures (Crowther et al., 2016; Yue et al., 2017).

Elevated atmospheric CO<sub>2</sub> concentration was the only treatment that changed the soil C stock over time in our experiment. CO<sub>2</sub> fertilization of plant growth, especially belowground (Arndal et al., 2013), may be the primary cause of the observed increase in soil C under eCO<sub>2</sub>. Previous results from the experiment have shown that eCO<sub>2</sub> increased photosynthetic activity in both dominant plant species (Albert, Ro-Poulsen, et al., 2011b; Albert, Mikkelsen, et al., 2011) and resulted in seasonal increases in aboveground biomass production (Kongstad et al., 2012). Most notably, belowground biomass production increased in response to the greater demand for nutrients to accommodate increased aboveground plant growth (Arndal et al., 2013). The eCO<sub>2</sub> treatment induced the greatest increase in root growth (Arndal et al., 2014, 2018), particularly in the deeper soil horizons (30-70 cm), where eCO<sub>2</sub> increased root biomass by 57% (Arndal et al., 2018). The increase in belowground C inputs therefore outweighed losses due to increased soil respiration (+38%) under eCO<sub>2</sub> (Selsted et al., 2012). Pretreatment measurements also indicated that soil C stocks in the eCO<sub>2</sub> plots were initially 23% lower than in the ambient CO<sub>2</sub> plots, presumably due to the higher prevalence of *Calluna* in the ambient plots (Kongstad et al., 2012). These pretreatment differences suggest that the effect of eCO<sub>2</sub> on soil C may have been even larger than measured at the end of the experiment as the increase with eCO<sub>2</sub> also had to compensate for the initially lower soil C stocks.

The field-scale application of eCO<sub>2</sub> to a variety of ecosystem types, including forests (Drake et al., 2011; Hofmockel, Zak, Moran, & Jastrow, 2011), grasslands (Dijkstra, Hobbie, Reich, & Knops, 2005; Reid, Adair, Hobbie, & Reich, 2012), and agro-ecosystems (Dorodnikov, Kuzyakov, Fangmeier, & Wiesenberg, 2011), has rarely been shown to impact soil C. In contrast to a few studies that observed a reduction in soil C due to a priming effect under eCO<sub>2</sub> (Butterly et al., 2016; Carney, Hungate, Drake, & Megonigal, 2007), meta-analyses have shown that overall eCO<sub>2</sub> increases soil C stocks (De Graaff, van Groenigen, Six, Hungate, & van Kessel, 2006; Hungate et al., 2009; Jastrow et al., 2005; Luo, Hui, & Zhang, 2006; van Groenigen et al., 2006; Yue et al., 2017). However, these studies did not examine combinations of eCO<sub>2</sub> with other climate variables that will change simultaneously with elevated CO<sub>2</sub> in the future. Only recently has enough data from multi-factor studies become available for drought or warming to be included as additional factors in meta-analyses of effects of eCO<sub>2</sub> on soil C, though the number of these combined experiments is still very low (6 studies included warming; 3 included drought), and three-factor studies are still lacking (Yue et al., 2017). Our study is the first field trial to confirm with long-term observational data that the increase in soil C under eCO<sub>2</sub> persists even in combination with both drought and warming, thus validating many current Earth System Models that project increased C storage over the 21<sup>st</sup> century (Todd-Brown et al., 2014).

The fact that a response to eCO<sub>2</sub> was not detected in most other experiments may be in part due to the experimental design. Despite the fact that the effects of eCO<sub>2</sub> can vary over time (Bradford et al., 2008; Luo et al., 2004), the majority of studies report only a single measurement point at the end of the experiment when examining differences in soil C under these treatments. Additionally, the strong interannual variability of rapidly cycling C pools may skew results when single measurements are used (Pendall, Osanai, Williams, & Hovenden, 2011). Finally, it may take years before treatment effects are large enough to

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become detectable against relatively large and inherently variable pre-existing soil C pools (Jastrow et al., 2005). Results are therefore highly dependent on the duration of the experiment, which on average among eCO<sub>2</sub> experiment is less than 4 years (Andresen et al., 2016; De Graaff et al., 2006; van Groenigen et al., 2006). Longer study periods, as in our case, are necessary to account for the effects on soil C pools with slower turnover rates, which may result in more permanent shifts in SOC. The few studies that did use multiple measurements similarly observed that the magnitude of the effect of eCO<sub>2</sub> on soil C increased over time (Hoosbeek, Li, & Scarascia-Mugnozza, 2006; Jastrow et al., 2005; Ross, Newton, Tate, & Luo, 2013).

However, the magnitude of increase in soil C stocks in the eCO<sub>2</sub> plots in our experiment was greater than that in other studies that also observed a significant SOC gain with eCO<sub>2</sub>. The higher of our two estimates of the annual C accumulation rate under eCO<sub>2</sub> ( $0.177 \pm 0.070 \text{ kg C m}^{-2} \text{ yr}^{-1}$ ), which accounts for the pretreatment differences in soil carbon pools, may partly be the product of initial differences in the plant community composition. It is possible that lower starting point in terms of *Calluna* biomass in the eCO<sub>2</sub> plots may have allowed for an overall faster growth rate relative to the ambient plots. This rate may therefore overestimate the potential response to eCO<sub>2</sub> at our experimental site. Yet, even the lower estimate based on the difference between C pools under ambient vs. elevated CO<sub>2</sub> at the final sampling point ( $0.120 \pm 0.043 \text{ kg C m}^{-2} \text{ yr}^{-1}$ ) is still substantially higher than the 0.079 and 0.059 kg C m<sup>-2</sup> yr<sup>-1</sup> increases observed with eCO<sub>2</sub> in a sweetgum plantation in eastern Tennessee (Iversen, Keller, Garten, Charles, & Norby, 2012) and a Kansas grassland (Jastrow et al., 2005), respectively.

While there is evidence that the impact of eCO<sub>2</sub> on soil C stocks is greater when high N availability is maintained, as in fertilized agricultural systems (De Graaff et al., 2006; Hungate et al., 2009; Jastrow et al., 2005; Luo et al., 2006; van Groenigen et al., 2006; Yue et

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al., 2017), progressive N limitation is likely to restrict the potential for continued stimulation of plant inputs with eCO<sub>2</sub> in natural ecosystems (Luo et al., 2004). The strong response of soil C to eCO<sub>2</sub> in our study may be partly facilitated by the association between *Calluna vulgaris* and ericoid mycorrhizal fungi, as the fungal symbiont is able to scavenge organic N and thereby may help to reduce potential N limitation of growth (Orwin, Kirschbaum, St John, & Dickie, 2011). Furthermore, it appears that N limitation has thus far been avoided by an increase in the C:N ratio of leaves (Albert, Ro-Poulsen, et al., 2011b), roots (Arndal et al., 2014), and soil organic matter, along with additional exploitation of N pools in deeper soil horizons. The increase in root biomass, particularly in deep horizons below 30 cm depth (Arndal et al., 2014, 2018), likely allowed for the upward transportation of N from deeper soil layers to meet increased plant nitrogen requirements under eCO<sub>2</sub>. Nonetheless, in the future, progressive N limitation may eventually limit plant growth and thus soil C accumulation under eCO<sub>2</sub> as deeper N pools are depleted. However, additional growth of the soil C pool seems likely as the rate of accumulation of soil C showed no sign of slowing over the course of 8 years of experimental treatment.

Changes in soil C pools have the potential to either exacerbate or alleviate rising atmospheric CO<sub>2</sub> concentrations and the resulting detrimental changes in climate. Over the 8 years of our study, we observed that the increase in soil C due to the stimulating effect of eCO<sub>2</sub> on belowground plant growth was not diminished by either drought or warming, signifying that eCO<sub>2</sub> had a stronger effect on soil C than either of these climatic variables. Though previous research has indicated that soil C is likely to increase under eCO<sub>2</sub>, the fact that neither warming nor drought – or their combination – significantly modified the effect of eCO<sub>2</sub> on soil C stocks is an observation that is unique to our study. Given the lack of significant interactions between treatments, our results suggest that moderate changes in warming and drought are unlikely to modify the rate of increase in soil C stocks under eCO<sub>2</sub>.



This site is therefore expected to store more C under future climate conditions, serving as a negative feedback to elevated atmospheric CO<sub>2</sub> concentrations.

Our findings also suggest that failing to account for the effects of eCO<sub>2</sub> may invalidate climate model parameters based on studies of warming or other climate change drivers alone. For example, Crowther et al. (2016) extrapolated global soil C stock changes from a range of warming experiments, including ours, and projected substantial losses of soil C in the future in response to higher temperatures. However, the large increase in soil C stocks with eCO<sub>2</sub> observed in our study, including in the plots with warming, indicates that eCO<sub>2</sub> may potentially counterbalance any losses that could theoretically occur with warming alone. As warming in the absence of eCO<sub>2</sub> is not a realistic future climate scenario, our results highlight the importance of including eCO<sub>2</sub> in combination with climate drivers in future climate experiments.

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## Figure Captions

Figure 1: Mean soil C stocks 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). Error bars indicate 1 SEM. Significant main factor effects ( $p < 0.05$ ) are indicated by an asterisk at that time point.

Figure 2: Mean soil C stocks for each of the 8 individual treatments ( $n = 6$ ) under ambient CO<sub>2</sub> (a) and elevated CO<sub>2</sub> (b). Error bars indicate 1 SEM. Treatments: Ambient control (A), drought (D), warming (T), warming + drought (TD), elevated CO<sub>2</sub> (CO<sub>2</sub>), drought + elevated CO<sub>2</sub> (DCO<sub>2</sub>), warming + elevated CO<sub>2</sub> (TCO<sub>2</sub>), and warming + drought + elevated CO<sub>2</sub> (TDCO<sub>2</sub>).





