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Depth-dependent response of soil aggregates and soil organic carbon content to long-term elevated CO_2 in a temperate grassland soil



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	Facing rising atmospheric CO_2 concentrations, subsoils may play an important role in the global carbon (C) cycle due to the presence of unsaturated mineral surfaces. Further, macroaggregation is considered a crucial process influencing C sequestration. However, analyses on subsoil aggregation and C retention processes under long- term elevated CO_2 (eCO ₂) are lacking. In this study we investigated the long-term effect of + 20% above ambient CO_2 concentration (corresponds to conditions reached 2035–2045) in a temperate grassland ecosystem at the Giessen Free Air CO ₂ Enrichment (Gi-FACE), Germany. A depth-dependent response of macroaggregation to eCO_2 was observed: While in subsoil (15–45 cm depth) macroaggregation increased under eCO ₂ , no CO ₂ induced change in macroaggregation was detected in topsoil (0–15 cm). Increased macroaggregation in subsoil coincided with higher SOC content of large macroaggregates (LM). Mean residence time (MRT) of SOC in aggregate-size classes were not different among each other under eCO ₂ . However, macroaggregates and bulk soil differed in their MRT between soil depths. Despite increased macroaggregation and an estimated high SOC sequestration					

potential in subsoil we could not observe an increase in SOC content of bulk soil.

1. Introduction

Since soil organic carbon (SOC) presents the largest terrestrial pool of C (Amundson, 2001), its potential to store additional C from the atmosphere has been widely discussed in the scientific literature (Stockmann et al., 2013). Accordingly, the 4 per mille initiative considers SOC sequestration as a contribution to mitigate climate change (Minasny et al., 2017) and calls out for accounting the rate of SOC sequestration and to identify mechanisms increasing SOC stocks.

It is widely accepted that SOC sequestration depends on the distribution of soil organic matter (SOM) in soil aggregates. The potential to physically protect certain SOM fractions from decomposition varies with aggregate-size class, which governs their residence time in soil (Tisdall and Oades, 1982; Van Veen and Kuikman, 1990; Jastrow et al., 1996). Further, subsoils may play an important role in the global C cycle due to their high mean residence times (MRT) relative to topsoil (Rumpel and Kögel-Knabner, 2011) and the presence of unsaturated mineral surfaces which was shown to be related to the formation of macroaggregates and C accrual (Kaiser and Guggenberger, 2003; Poirier et al., 2014).

However, in view of rising atmospheric CO₂ concentrations, it remains unclear how elevated CO2 (eCO2) affects the distribution of SOC to soil aggregate-size classes in different soil depths, the associated MRT and the resulting SOC content. For effective C sequestration, it is relevant that additional C is allocated to pools with long-term stabilization and not fast cycling pools.

It has been reported that eCO₂ may alter many factors known to influence the distribution of soil aggregate-size classes (Díaz, 1995; Eviner and Chapin, 2002). For example, eCO₂ can alter the vegetation community composition and related fungal biomass which was shown to affect aggregate stability (Rillig et al., 2002). Six et al. (2001) showed that eCO₂ changed the quality of residue inputs and enhanced the proportion of recently photosynthesized C with increasing aggregate size. They concluded that the quantity and quality of residues, which was altered by eCO₂, determined the turnover time of macroaggregates. Furthermore, it was reported that eCO₂ enhanced rhizodeposition which may stimulate fungal biomass (Phillips et al., 2006) that may serve as a binding-agent for macroaggregates (Tisdall and Oades, 1982).

Free-Air CO₂ Enrichment (FACE) experiments proofed to be a powerful approach to examine ecosystem responses to eCO₂ (Ainsworth and Long, 2005). FACE experiments allow the investigation of intact ecosystems which are exposed in-situ to eCO2 concentration without enclosure. Nine FACE studies that investigated the effect of eCO2 on the

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distribution of soil aggregate-size classes across a variety of ecosystems showed contrasting results (Table S1). Eight out of nine FACE studies reported results after short-term enrichments (< 10 years of CO₂ enrichment) which may not be representative of long-term dynamics. Not all of the studies incorporated measurements of SOC-content and some focused on microbial responses within aggregates (Dorodnikov et al., 2009; Nie et al., 2014) or the influence of arbuscular mycorrhizal fungi to aggregation changes (Rillig et al., 2001). In five of the FACE studies, assessment of aggregate-size class distribution was limited to the topsoil, while two studies analyzed pooled samples of top- and subsoil, consequently losing any depth-dependent information. As a result, only very limited information is available on how the distribution of soil aggregate-size classes responds to soil depth under long-term eCO_2 .

To our knowledge only one other FACE study (Hofmockel et al., 2011) exists to date that investigated long-term effects (> 10 years) of eCO₂ on the distribution of soil aggregate-size classes and SOC-content. Hofmockel et al. (2011) demonstrated that eCO₂ changed C turnover of different particle-size classes in a forest soil suggesting a eCO₂ induced priming of older, relatively stable SOC.

Thus our main objective was to quantify long-term and depth-dependent effects of eCO_2 on the abundance of soil aggregate-size classes and soil C dynamics in a FACE-experiment which, to our knowledge, has not been investigated in detail so far. Since the Gi-FACE is located on temperate managed grassland our study complements the results from the long-term forest FACE study (Hofmockel et al., 2011).

In this study we investigated if eCO_2 (1) affected the distribution of soil aggregate-size classes at different soil depths; (2) induced a change in aggregate and bulk SOC content at different soil depths and (3) affected the mean residence time (MRT) and distribution of newly sequestered C (C_{new}) in soil aggregates and bulk soil at different depths.

Based on studies reporting higher C sequestration potential in subthan topsoil (Kaiser and Guggenberger, 2003; Poirier et al., 2014) we hypothesized that (i) topsoil will be close to C saturation and will show small increases in SOC content under long-term eCO_2 and (ii) subsoil will have a higher C saturation deficit and will therefore increase to a higher extent in SOC relative to topsoil under eCO_2 .

2. Materials and methods

2.1. Study site and design

The Giessen Free Air Carbon Enrichment (Gi-FACE) experiment, is located on permanent semi-natural grassland. It is situated near Giessen, Germany ($50^{\circ}32'N$ and $8^{\circ}41.3'E$) at an elevation of 172 m above sea level.

The set-up and performance of the Gi-FACE system has been described in detail by Jäger et al. (2003) and Andresen et al. (2017). In brief, from May 1998 until present, atmospheric CO2 concentrations were enriched by 20% above ambient, all-year-round during daylight hours. From May 1998 to June 2004 the δ^{13} C signature of the CO₂ used for enrichment was -25% (compared to ambient atmospheric CO₂ (aCO₂): -8%). From July 2004 onwards the δ^{13} C signature of the CO₂ was changed to -48% without altering the CO₂ concentration. The CO2 enrichment was applied in three rings, each eight meter in diameter (E plots). Three equally sized control plots were maintained at aCO₂ levels (A plots). The experimental design was a randomized block design. A block consisted of two plots to which ambient and eCO₂ treatments were randomly assigned. A characteristic attribute of the study site is a soil moisture gradient, resulting from a gradual terrain slope (2-3°) and varying depths of a subsoil clay layer. Within each of the three blocks, soil moisture conditions were relatively homogeneous (Jäger et al., 2003). The soil of the study site is classified as a Fluvic Gleysol (FAO classification). The soil texture and the depth of the clay layer is presented in Table 1.

The vegetation is an Arrhenatheretum elatioris Br.Bl. Filipendula ulmaria subcommunity, dominated by Arrhenaterum elatium, Galium Table 1

Soil texture in the soil profile of each ring pair at the Gi-FACE study site according to Lenhart (2008).

Horizon	Lower horizon boundary	Sampling depth	Depth of clay layer	Sand	Silt	Clay	Silt and clay		
	(cm)			(%)					
Ring pair 1									
Ah	10	2–7	128-155	43.25	39.00	17.75	56.75		
Μ	32	12-17		40.89	42.13	16.97	59.10		
SwM	78	40-45		48.10	51.90	nd	51.90		
Ring pair 2									
Ah	12	2–7	48-110	59.26	20.89	19.85	40.74		
MSw	42	15-20		34.52	40.50	24.98	65.48		
GoSw	65	50–55		35.34	52.33	12.33	64.66		
Ring pair 3									
Ah	12	2–7	65–135	9.98	58.13	31.89	90.02		
М	20	15-20		9.78	55.56	34.66	90.22		
MSw	50	40–45		14.94	50.56	34.50	85.06		

nd: not determined.

album and Geranium pratense. At least 12 grass species, 15 non-leguminous herbs and 2 legumes are present within a single ring. For at least 100 years, the grassland has not been ploughed. Since at least 60 years, it was managed as a hay meadow with two cuts per year, and fertilized at the rate of $50-100 \text{ kg N} \text{ ha}^{-1} \text{ yr}^{-1}$. From 1996, fertilizer was applied in mid-April with granular mineral calcium-ammonium-nitrate fertilizer at the rate of $40 \text{ kg N} \text{ ha}^{-1} \text{ yr}^{-1}$ (Kammann et al., 2008).

2.2. Soil sampling

Soil samples were taken at nine sampling dates (April 1998, June 2004, December 2004, July 2005, December 2005, June 2006, June 2007, November 2011 and December 2015) in 0–7.5 cm depth. After six (June 2004), nine (June 2007) and 13 years (November 2011) of CO_2 enrichment soil samples were taken in 0–7.5, 7.5–15, 15–30 and 30–45 cm depth (soil sampler: Ejkelkamp, Giesbeek, The Netherlands) with three sub-samples per plot in each depth. Soils were passed through an 8 mm sieve and air-dried. Subsequently, roots were picked out with tweezers until all visible roots were removed. The soil samples were used for the wet sieving procedure to separate soil aggregate-size classes.

2.3. Soil aggregate fractionation

Soil samples were separated into four aggregate-size classes by wet sieving of 80 g of soil according to a method adapted from Cambardella and Elliott (1993). Soil samples were submerged for 2 min in deionized water on top of the 2000 μ m sieve and subsequently a series of three sieves (2000 μ m, 250 μ m and 53 μ m) was used to obtain the four aggregate-size classes: > 2000 μ m (large macroaggregates (LM)), 250–2000 μ m (small macroaggregates (SM)), 53–250 μ m (microaggregates (MIC)) and < 53 μ m (silt and clay (SC)). The separation of water-stable aggregates was achieved by manually moving the sieve up and down with 50 repetitions during a 2 min period. Each aggregate-size class was transferred into aluminum pans and dried at 60 °C until a constant weight was reached.

2.4. Carbon analysis

All solid samples were ground with a ball mill (Retsch, type MM). 15–20 mg of bulk soil and of isolated soil aggregates were placed into tin capsules to determine stable carbon (δ^{13} C) isotope composition, as well as C and N contents. The same procedure was applied with two milligrams of roots for each depth on composite samples. Stable carbon

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