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Effects of Below Ground Controlled Injections of CO_2 on Microbial Respiration of Soil Planted With Wheat (*Triticum aestivum L.*)

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ABSTRACT: Below-ground carbon dioxide (CO_2) emissions occur naturally at CO_2 springs, but the risk of occurrence at other sites will increase as geologic CO_2 storage is implemented to help mitigate climate change. This investigation examines the effect of controlled injection of below ground CO_2 emission on wheat plant and soil microbial respiration where spring wheat (t*riticum aestivum* L.*)* was grown. The study involved setting up eight (8) experimental plots (each 2.5 x 2.5m) for the growth of spring wheat. The experimental plots labelled A1 - A8 (A1, A6 and A7 were gassed plots while A2, A3, A4, A5 and A8 were used as control plots). Soil CO_2 concentration was increased by the release of concentrated CO_2 gas from a source point 60 cm below the soil for eight (8) weeks at the rate of $1L^{-1}$ via tubes into the soil when wheat was grown. Five plots were used as control, while three plots were used as treatment plots with a regulated meter gas supply. The variability of CO_2 concentrations was determined by a revised 2D method known as Barholing using a geotechnical Instruments GA2000 gas analyser to map CO_2 at 30 cm depth across each plots. This was used to measure the dispersion of CO_2 throughout the plots. This method which produced contour plots of spatial variation shows the rate of dispersion of soil gas in the treated plots. The concentrations of CO_2 at the centre of the plots were different, showing values up to 70% CO_2 but reduced rapidly from the centre to plot borders. No significant changes in microbial biomass or carbon utilisation were observed (at $P>0.05$), but a trend towards reduced microbial respiration was apparent in the gassed plots.

Keywords: Carbon capture and storage (CCS), Elevated CO_2, Soil, Microbial respiration

Introduction

Carbon capture and storage in geologic formations has the potential to be an effective way to reduce atmospheric carbon dioxide levels (IPCC, 2005; Pierce and Sjogersten, 2009; West, *et al.,* 2009). CO_2 would be harvested from power plants and other industrial emissions, transported via pipe- lines and injected into saline aquifers, spent oil fields, and other suitable geologic storage sites (Bachu, 2000). CCS has the potential to reduce carbon dioxide emissions from power generation by 80 - 90 percent (IPCC, 2001). However, in order for this process to be effective, systems must ensure that leakage is minimised from both sudden releases and slow leaks. Well or pipe- line failures, over-pressurisation, or poor engineering could all lead to leakage during capture and transportation of CO_2; while migration in geologic media, large releases due to over-pressurisation, slow releases via faults and fractures could all occur in situ (Heinrich *et al.,* 2003). While these leakages would likely be limited by safety systems and proper site selection, it is important to understand what effects leaks could have on overlying ecosystems. In many cases, slow releases could go unnoticed due to CO_2 diffusion in the atmosphere (Heinrich *et al.,* 2003). However, even small leaks may increase soil CO_2 concentrations and atmospheric concentrations below plant canopies. Increased soil CO_2 levels could have significant effects on vegetation and soil fauna, but the effects of this sort of slow release of CO_2 in soil on plant and microbial communities are not yet well understood.

Carbon dioxide has been injected into the soil for various purposes; however, its long term storage is a new concept. The first commercial CCS project was in the year 2000 in Weyburn, Canada (Markels and Barber, 2002). Looking at the long term effect, the storage of CO_2 would have on the environment, it is therefore very necessary to understand the effects of CO_2 leakage on the overlying ecological unit (Heinrich *et al*., 2003). Literature on the effects of below ground diffusion of CO_2 in soil on plants and microbial communities is scanty despite the fact that the increase in soil CO_2 level could have a significant effect on vegetation and soil fauna (Bouma *et al*., 1997; Sowerby *et al*., 2000a). Toxic concentration of CO_2 in the soil could lead to the death of vegetation (Pierce and Sjogersten, 2009).

Plants grown at naturally high CO_2 levels have been shown to be affected in a variety of ways. Studies at Bossoleto and a natural CO_2 spring in Iceland have shown that exposed plants exhibit earlier leaf senescence and decreased photosynthetic capacity (Cook *et al.,* 1998; Miglietta *et al.,* 1998). Some studies suggest that leaf litter quality is changed by long-term exposure to elevated CO_2, but the results are not consistent (Gahrooee 1998, Cotrufo *et al.,* 1999, Cortufo and Ineson 2000, Sowerby *et al*., 2000a). According to Norby *et al.,* (2001), naturally senesced leaves grown at elevated CO_2 levels exhibit 7.1% lower nitrogen content and 6.5% greater lignin content. If litter quality is altered by exposure to increased CO_2, it is likely that microbial communities would alter in order to best utilise the changed leaf litter.

Every plant has a favourable environmental and soil condition within which their growth, survival and performance are optimal. Generally, all plant species share the same limiting factor, as the significance of increased climatic variability and limits on the growth and development of crop yield is a rising observation in the midst of our climatic environment (Wilks and Riha, 1996). Several decomposition studies have been undertaken at sites of natural CO_2 release and also in conditions of artificially elevated atmospheric CO_2. Some results have shown increased initial mineralisation (Sowerby *et al.,* 2000b), some reported decreased mineralisation (Gahrooee 1998), and some have reported no differences at all (Cotrufo *et al.,* 1999 and Cotrufo and Ineson 2000). None of these studies looked at the organisms that were actually decomposing the litter, but focused instead on litter quality, so there are no data on the microbial communities involved.

The effects of below ground CO_2 emissions, whether natural or anthropogenic, on plant and microbial communities are poorly understood. This study examines the effects of controlled injection of below ground CO_2 emissions on wheat plant and soil microbial respiration as examined by carbon source utilisation. It is hypothesised that increased soil CO_2 concentrations will result in reduced above and below ground vegetation biomass due to anoxic conditions at the roots, and reduced microbial biomass and/or activity due to decreased carbon inputs into the soil from the vegetation.

Materials and Methods

Description of study site

This study was carried out at the Artificial Soil Gassing and Response Detection (ASGARD) site, situated at the Sutton Bonington Campus of the University of Nottingham, UK. The University lies between 52.8 °N and 1.2 °W of Leicestershire, and is approximately 18 km south out of central Nottingham (West, *et al.,* 2009). The study area is located on flat open grassland which was formerly used for sheep grazing. The maximum temperature in January is approximately 6.9^{o} C and the minimum temperature is 1.2 °C, and in July, 21.3 °C and 11.4 °C respectively. Moreover, the mean annual rainfall of the area is 606 mm, which is distributed evenly all through the year (The University of Nottingham Sutton Bonington Metrological Site) (West, *et al.,* 2009).

The geology of ASGARD site is characterised by up to 1.5 m of overlying mudstones of the Mercia group, sand and gravel rich terrace deposits, surrounded by sheets of lithologically variable head (Ford, 2006). These sand and gravel deposits are dissected and highly degraded, as much of their material has been remobilised through periglacial processes and recent weathering (Ford, 2006). The resulting head deposits incorporate varying amounts of red clay from the Mercia Mudstone Group, and showing a wide range of grain sizes, degrees of sorting and levels of consolidation. A detailed geological description of the site and surrounding area is given in Ford (2006).

Experimental Site and plot layout

The ASGARD facility developed at the University of Nottingham Sutton Bonnington Campus, United Kingdom was used. Carbon dioxide was injected into soil at a depth of 60 cm, so that a range of response of the vegetation and soil ecosystem can be studied. This site is located in a field of permanent pasture. The site was chosen by the Asgard facilitators on the basis of reasonable uniformity of soil type down to a depth of 1m with good exposure, particularly towards the north, to give room for experimental analysis and for access to facilities (The Asgard Facility Resource Document for UKCCSC, 2007).

CO_2 gas was released from a source which is 60cm m below the centre of each 2.5 x 2.5 m plot. Eight plots (each 2.5 x 2.5m) were laid out within the experimental area to enable CO_2 to be delivered to different experimental plots, where spring wheat (*triticum aestivum* L.) has been grown. Carbon dioxide was delivered to three plots within the experimental site; the remaining five plots were controls and are distributed among the experimental plots, adjacent to gassed plots (Source : RISCS, 2010).

Gas delivery and instrumentation details

The Asgard facility is a purposed-built field facility for the study of ecosystem responses to elevated soil gas concentrations. It was established in during winter 2005 to spring 2006, and is designed to investigate the impacts of leakage from underground CO_2 storage. The gas flow rate to each plot is normally 1 litre per minute. (Source: The Asgard Facility Resource Document for UKCCSC, 2007).

Prior to the field experiment of this study, a 25-mm screw auger was used to drill the initial hole into the soil and then the tubes were pushed into the resulting hole. Soil was tamped around the tubing to ensure that it was a good fit and that gas would not leak back up along the tube edges. The tubing is closed at the end, and has twenty-six 5-mm openings drilled at the end of each 21 cm of the tube to release the gas. Vertical plastic sampling tubes (100 mm long, 19mm internal diameter) are installed permanently into the plots to enable measurements of soil gas concentration to be taken, (Figure 1) (RISCS 2010).

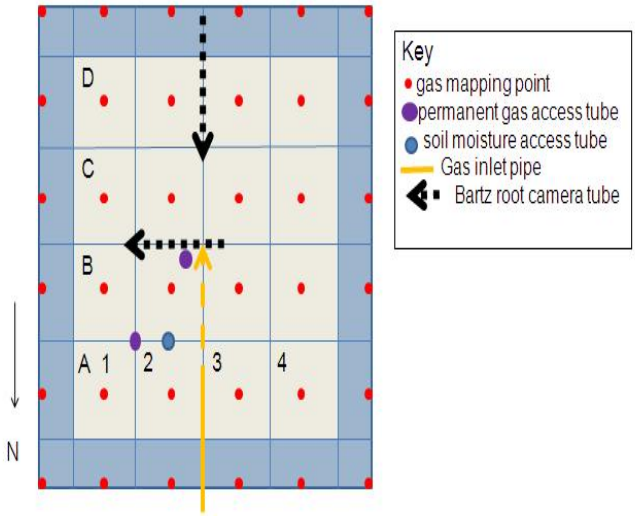

Figure 1: Diagram illustrating the position of plot infrastructure. (Source : RISCS, 2010)

The topmost end of the tube is sealed with a bung containing a plastic on/off valve. Two tubes are installed at 15cm and 70 cm from the centre of each gassed plot on a diagonal line from the centre and towards the North East of each plot. One tube is installed at 15 cm from the centre of each control plot. One soil moisture access tube is installed in each plot and Bartz mini rhyzotrons were also installed in each plot. Two tubes were installed in gassed plots and one tube in control plots. The depth of gas injection was restricted to 60 cm or less, in order to mitigate the effects that this variation may have on gas migration (West *et al.*, 2009).

Plants and treatments

The experimental plots labelled A1 - A8 on plate 1 were prepared in summer 2010 by the Asgard facilitators and were covered with black plastic to prevent weed growth until ready for sowing in spring 2011. Spring wheat was sown on the 23rd and 24th of March, 2011 at a rate of 350 seeds m^{-2} into eight plots. All experimental beds received an initial application of Nitram seed bed fertilizer, which was applied to the plot at the rate of 72 g per plot. The wheat seeds germinated on the 5th of April, 2011. When they had reached the three leaf stage, a further application of Nitram seed bed fertiliser was added at the rate of 308 g per plot. CO_2 was delivered to three plots on the 23rd of May 2011 at a nominal flow rate of 1 Lmin^{-1} and switched off on the 15th of July, 2011. Five plots were used as control.

Field measurements and Sample Preparation

2D measurement of CO_2

This determines the amount of CO_2 in the experimental plots. A narrow hollow stainless steel probe of 8mm with a sacrificial tip was steadily driven into the soil to a depth of 30, 50 and 70 cm at each sampling point. A Geotechnical Instruments GA2000 gas analyser is connected via tubing to the soil gas probe and soil gas is drawn through small holes near the base of the soil probe. This method ensures a good seal at the ground surface so that soil gas is drawn directly from the measurement depth without significant influence from atmospheric air. Soil gas is pumped through the instrument until a stable reading is recorded with CO_2 percentage being measured by infrared absorption

Plate 1 and 2 shows the experimental plots which enabled CO_2 to be delivered where spring wheat (*Triticum aestivum L.*) was planted.

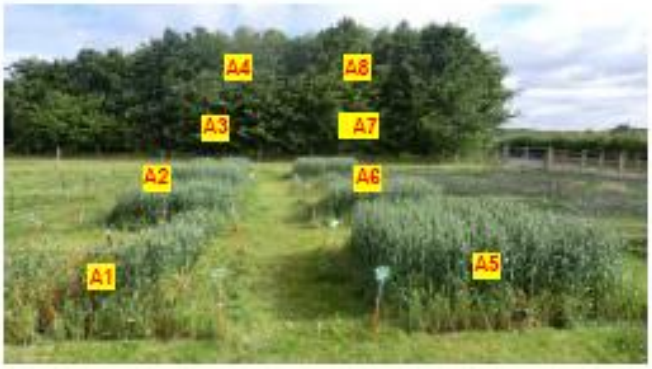

Plate 1: Shows eight experimental plot of wheat. Picture 1 taken on the 17/07/2011

Plate 2: Shows cleared plots after harvest of wheat. Pictures 2 taken on 14/7/2011

Determination of Microbial respiration in soil

Microbial activities in the soil were determined by biological oxygen demand method of Rowell (1994). This was measured for a duration of five days. This involved measuring of the microbial respiration of the soil using OxiTop® systems. The result was used to evaluate the biodegradable substance in the soil. It offers unique, modular and mercury-free instrument for measuring the activities of respiring organisms in a soil.

Data collected from this study were subjected to statistical analysis using Microsoft Excel worksheet.

Results

Results for field experimental measurements

Barholing measurement

Figure 2 shows a wireframe map of CO_2 concentration for plots A1 – A8 below, measured at 30 cm depth at 36 intersection points across each plot. A revised 2-D method known as Barholing was used to map CO_2 at 30 cm depth across the plots. This was used to measure the dispersion of CO_2 throughout the plots.

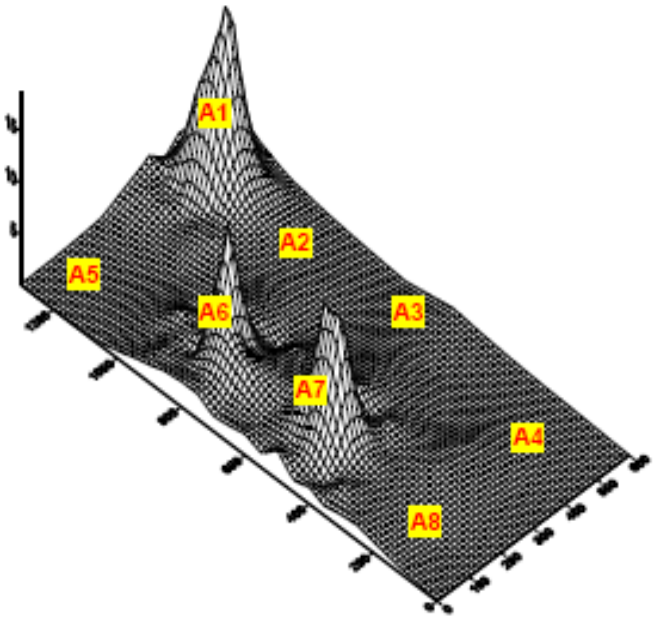

Figure 2: Wireframe map illustrating CO_2 distribution in plots A1-A8

Microbial activity in the soil

Table 2 shows the measurement of microbial respiration of the soil using OxiTop® systems. This was measured in duration of five days using biological oxygen demand method (Rowell, 1994). This was used to evaluate the biodegradable substance in the soil. It offers unique, modular and mercury-free instrument for measuring the activities of respiring organisms in a soil. Data collected was subjected to statistical analysis using Microsoft Excel worksheet.

Table 2: Microbial respiration or activities in the soil of plot A1-8 for five days

Number of days	Pre-Control (no injection of CO_2)	Post-Control (no injection of CO_2)
1	5.333	6.667
2	5.8	7.333
3	6.667	7.667
4	7	8
5	7.333	8.333
Number of days	Pre- High CO_2 concentration	Post-high CO_2 concentration
1	7	5.667
2	7.333	6.333
3	8	7
4	8.3	7.2
5	8.667	7.667
Number of days	Pre-Low CO_2 concentration	Post-low CO_2 concentration
1	8	6.333
2	8.667	7.333
3	9	7.8
4	9.6	8.333
5	10.667	9

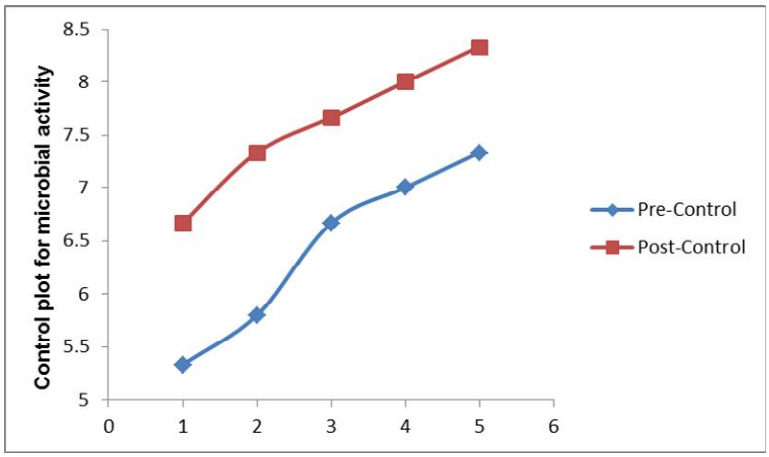

Figure 3: Change in Control plot (A2, A3, A4, A5, and A8) of microbial respiration in the soil

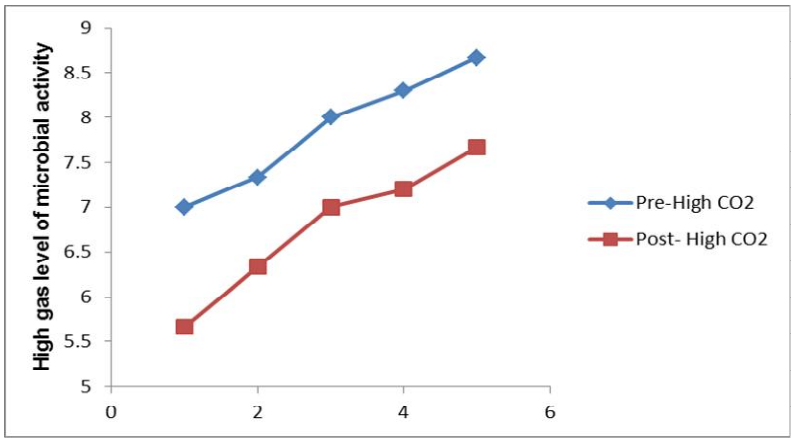

Figure 4: soil microbial respiration in high gas (CO_2 concentration) zone, at 75 cm from the centre of the plot

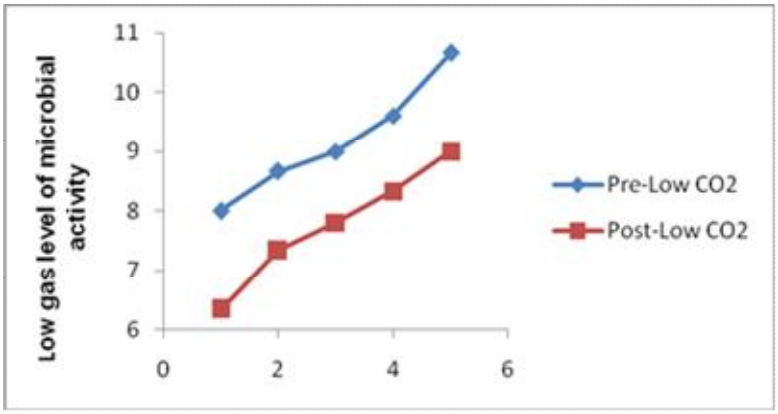

Figure 5: soil microbial respiration at low gas (CO_2 concentration) zone, at the edge of the plot.

Discussion

The gassing strategy aims to achieve a maximal value at the centre (Fig 2). The gas is released at a single point beneath the centre of the plot, this therefore indicates CO_2 gradient across each plot. The gradient derived is used as a means to investigate the dose response relationships. Gassing at a rate of $1L^{-1}$ is sufficient to generate values of 50-80% in the plot centre varying with weather, and to some extent, with the individual plots decreasing towards normal soil concentrations (-1%) at the plot edge (RISCS, 2010).

The effects of CO_2 injection on soil CO_2 concentration/distribution is shown in Figure 2. Although the gas was released at a single point beneath the centre of the plot, there was significant CO_2 gradient across each gassed plot

relative to control plots. The high amount of CO_2 in some control plot suggests some movement of CO_2 from gassed plots diffusing along plot borders. Variation across the soil structure was a critical factor and was the central cause for flow variability. The soil comprises of a 30 cm deep sandy clay loam horizon that was similar across the plots. However, due to improved method of gas distribution, the flow rate of soil gas was improved which flows at a rate of $1L^{-1}$, which was achieved in most plots. The presence of wheat plant on a gassed plot can both enhance and reduce gas escape from the soil. Tangled roots can hold soil particles and therefore are seen to close the soil pores to reduce emission; large roots offer corridors for CO_2 leak (Boltze *et al.,* 1997; Smith *et. al.,* 2005). The vegetation of the soil in this study may have had an influence on the flow of CO_2 through the soil, which has been mechanically cultivated before the wheat was sown. The variations observed in the patterns of dispersal can be attributed to the fluctuations in the water content of soil opening and closing corridors or the movement of soil microbial organism (Christophersen and Kjedsen, 2001; Ravi *et al.,* 2010).

The injection of CO_2 into the soil at a rate of I litre per minute over 8 weeks had impact on the soil, variations observed in soil microbial activities and the health of crops growing at the surface. However, these variations were more in areas with high CO_2 concentration in the experimental plot. Elevated concentrations of CO_2 in the soil caused stress of wheat, which was detected by visual symptoms of wheat plant. These noticeable symptoms manifested were yellowing of the leaves, reduction in plant growth and decrease in chlorophyll content. Although, the presence of elevated levels of soil gas was detected, the symptoms were believed to be a generic response to soil oxygen depletion. Results from this study confirmed the findings of other researchers like Hutsch (2001) who noted that different plants have different sensitivity to natural gas zones (Hutsch, 2001). It has been reported that plants grown on naturally high CO_2 levels is affected in a variety of ways (Pierce and Sjogersten, 2009).

In this study, microbial respiration was higher in the analysed soil sample collected prior to gassing of the experimental plots and after gassing (pre and post injection) under control plots than gassed plots and within gassed plots. However, it decreased with increase in CO_2 concentration (low gas zones/areas). West *et al.* (2009) also found a decrease in microbial activity in high gas zones of the plots and also noted that microbes present within the gas zones were not biologically active at CO_2 concentration of 87 %. There was a significance difference between the gassed and control plots. Hoeks (1972) noted that the oxidation of microorganisms and or bacteria beneath the soil can lead to a reduction in soil gas concentration. This can be attributed to the low gas levels in the soil. Hoeks (1972) also observed that, microorganisms in the soil such as bacteria, make use of CO_2 at a very significant level and this was linked to the high rate of O_2 depleted in the surrounding area of gas leak. The rates at which O_2 was consumed were 50 times higher than normal soil.

Jones and Nedwell (1993) reported that respiring organisms that can use methane as its only source of carbon, depends upon the presence of sufficiently high CO_2 and O_2 concentrations. Without this, microorganisms have been observed to be confined within their habitat with limited distribution and downward diffusion of atmospheric O_2 and the upward diffusion of CO_2 (Jones and Nedwell, 1993). Studies carried out on the controlled injection of CO_2 into the soil have reported an existing relationship between CO_2 and O_2 concentration (Smith *et al.,* 2005; Pierce and Sjogersten, 2009, West *et al.,* 2009) which was measured using the CO_2 gas analyser via fixed tubes at 75 cm from the centre of the plot. This can be attributed directly to the high amount of CO_2 concentration at the centre of the plot, thereby displacing oxygen. This was in line with the study of Hutsch (2001) who noted that bacteria are ever-present in aerobic soils and therefore offer a substantial sink for atmospheric CO_2.

Conclusion and Recommendations

Low level increases in soil CO_2 concentrations can negatively impact vegetation, causing significant decreases in above and below ground vegetation biomass overtime and reduction in microbial respiration. In this study, there was no significant difference or changes in carbon utilisation or microbial biomass between plots injected with CO_2 and those not injected but a trend towards reduced microbial respiration was apparent in the gassed plots.

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