On the origin of carbon dioxide released from rewetted soils

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Highlights

1. In soils rewetted after drying, the resultant flux of CO_2 was found to be extremely rapid with the peak efflux occurring in less than 6 minutes
2. Such CO_2 fluxes were prevented by autoclaving, suggesting an intrinsically biochemical or organismal origin to the source
3. Strong evidence for an extracellular oxidative pathway contributing to such CO_2 fluxes was found

Keywords:

Birch effect; dry:wet cycles; CO_2 flux; extracellular oxidative metabolism; soil sterilisation

Abstract

When dry soils are rewetted a pulse of CO_2 is invariably released, and whilst this phenomenon has been studied for decades, the precise origins of this CO_2 remain obscure. We postulate that it could be of chemical (i.e. via abiotic pathways), biochemical (via free enzymes) or biological (via intact cells) origin. To elucidate the relative contributions of the pathways, dry soils were either sterilised (double autoclaving) or treated with solutions of inhibitors (15% trichloroacetic acid or 1% silver nitrate) targeting the different modes. The rapidity of CO_2 release from the soils after the drying:rewetting (DRW) cycle was remarkable, with maximal rates of evolution within 6 minutes, and 41% of the total efflux over 96 h released within the first 24 h. The complete cessation of CO_2 flux following sterilisation showed there was no abiotic (dissolution of carbonates) contribution to the CO_2 release on rewetting, and clear evidence for an organismal or biochemical basis to the flush. Rehydration in the presence of inhibitors indicated that there were approximately equal contributions from biochemical (outside membranes) and organismal (inside membranes) sources within the first 24 h after rewetting. This suggests that some of the flux was derived from microbial respiration, whilst the remainder was a consequence of enzyme activity, possibly through remnant respiratory pathways in the debris of dead cells.

Rewetting of a dry soil invariably causes a large flux of carbon dioxide (CO_2) to be rapidly released, which is sometimes referred to as the Birch effect (Birch, 1958; 1960). This phenomenon has been observed both in laboratory incubations (Kieft et al., 1987; Unger et al., 2010; Shi and Marschner, 2014) and in field circumstances using closed chambers (Yan et al., 2014) or eddy covariance towers (Xu et al., 2004). These fluxes have been observed across a wide range of ecotypes (Jarvis et al., 2007; Thomas and Hoon, 2010; Sugihara et al., 2015), but are particularly significant in dryland and Mediterranean ecosystems where they can make up a significant proportion of soil C-emissions (Lee et al., 2004; Hunt et al.,

2004; Brito et al., 2013). These drying:rewetting (DRW) induced CO_2 efflux events can even significantly reduce the annual net C gain in Mediterranean forests (Jarvis et al., 2007).

Several theories have been proposed to explain this phenomenon including: (i) the exposure of physically-protected organic matter to microbial metabolism via aggregate dispersion on rewetting (Denef et al., 2001; Wu and Brookes, 2005; Xiang et al., 2008); (ii) microbial necromass increasing the supply of readily assimilable substrate to the surviving microbial populations (Kieft et al., 1987; Van Gestel et al., 1992; Blazewicz et al., 2013); (iii) increases in the supply of labile organic matter due to the rapid release, on rewetting, of intra-cellular solutes previously concentrated within microbial cells to maintain osmotic balance in response to dehydration (Halverson et al., 2000; Warren, 2014); and (iv) a supply of labile organic C is built up during the dry period prior to rewetting and subsequently quickly metabolised on rewetting. There is a known uncoupling of rates of CO_2 efflux and detectable microbial growth rates after a DRW cycle (Iovieno and Bååth, 2008; Meisner et al., 2015) and microbial populations in such circumstances show little change in their net size (Fierer and Schimel, 2002). However, recent work by Blazewicz et al., (2013) show that despite their unchanging size these populations turnover rapidly in response to a DRW cycle. They also suggest that more cellular derived organic-C is available in soil samples than is turned over in the initial phases after rewetting. This organic-C will contain cellular material including constituents of enzymatic pathways – remnant respiratory pathways – with the potential to carry out reactions leading to CO_2 efflux. Thus it is possible that CO_2 release from re-wetted soils is not exclusively derived from respiration pathways occurring in intact microbes. There are also reports of over-estimation of soil respiration rates due to contributions of CO_2 from dissolution of soil carbonates; however, reports are inconsistent and range from 1-2 % up to 74% of CO_2 efflux from soil being attributed to carbonate dissolution (Biasi et al., 2008; Ramnarine et al., 2012; Schindlbacher et al., 2015). It is as yet unclear how the DRW process may affect carbonate dissolution from soils although Tamir et al., (2011) found that in highly calcareous soils the rate of inorganic CO_2 production

76 was lower in drier samples. However, it is also known that increases in soil OM content can
77 alter the balance of pH, as a result of increased nitrification rates, leading to increase
78 dissolution of carbonates (Tamir et al., 2013). As such an increase in available OM as a
79 result of any of the 4 processes described above (aggregate dispersion, increased
80 necromass, release of intracellular-solutes, or accumulation of labile organic matter) could
81 potentially lead to this phenomenon on rewetting, and an abiotic route to CO_2 production
82 must also be considered.

83 On this basis we posit that there are three potential sources of CO_2, all of which could
84 contribute to the efflux on rewetting: (i) *abiotic* via carbonate dissolution (Shanhun et al.,
85 2012); (ii) *biochemical*, involving the release of CO_2 from organic matter outside cell
86 membranes and mediated by free or residually-bound enzymes (Maire et al., 2013)
87 (Blankinship et al., 2014); (iii) *organismal*, i.e. microbial respiration via the Krebs cycle
88 carried out within intact organelles or cells (Fig. 1). One potential way to determine the
89 relative contribution of these sources is to probe the phenomenon in soils treated in various
90 ways to block certain of the pathways involved, such as via complete sterilization (i.e. any
91 form of biochemical or organismal pathway), or to spike the rehydration water with various
92 forms of metabolic inhibitors (i.e. to distinguish biochemical from organismal). We
93 hypothesised that i) the majority of CO_2 released is derived from an organismal source, and
94 hence that CO_2 efflux upon rehydration would be curtailed where organismal pathways were
95 blocked and ii) there would be no significant contribution to the total CO_2 efflux of CO_2 from
96 an abiotic source.

97 Soils were collected from the top 15 cm of 4 long-term grassland sites in May 2015 (soil
98 parameters shown in Table 1); all soils were sieved to pass a 2 mm mesh, adjusted to 45%
99 water holding capacity (WHC) and pre-incubated at 25°C for 7 days. Aliquots of the soils (1
100 g; 3 replicates of each soil) were then exposed to 4 DRW cycles over 28 days, where each
101 cycle consisted of 3 days drying followed by rewetting to 45% WHC using sterile, deionised
102 water. Drying was standardised by locating the soils in a sealed container in the presence of

103 silica gel. Aliquots of 1.0 g of soil were adopted in order to ensure that penetration of water
104 throughout the soil volume would be rapid. The time-course of CO_2 evolution at 6-minute
105 intervals following rewetting was determined independently for each replicate using an
106 automated multi-channel conductimetric respirometer (RABIT, Don Whitley, Shipley, UK;
107 (Butler et al., 2012), for 5 days. To account for any background variation in CO_2 efflux
108 blanks were run alongside soil samples; this involved measuring the signal from empty,
109 sealed cells.

110 Another set of three replicates was subjected to a further range of treatments, *viz*. (i) 'Live
111 controls' - involving no sterilisation, DRW as described above; (ii) 'Moist controls' – also
112 unsterilized but with 0.2 mL sterile, deionised water added prior to exposure to DRW – this is
113 a procedural control to account for the fact that liquid was added to the sample prior to
114 drying as described above; (iii) 'Autoclaved', where samples were autoclaved twice at 121°C
115 at 3.1 bar for 20 minutes with a 24 hour pause between (Systec 3150 EL, Linden, Germany);
116 (iv) 'TCA', with 0.2 mL of 15% trichloroacetic acid (TCA) addition; (v) '$AgNO_3$', with 0.2 mL of
117 1% silver nitrate addition. All amendments and autoclaving were carried out prior to the
118 DRW process described above. The rationale for these treatments (Fig.1) is that
119 autoclaving would prevent all organismal or biochemical activity by denaturing all proteins –
120 in this circumstance any CO_2 produced would be via abiotic pathways. TCA (15%) would
121 precipitate proteins, including extracellular enzymes (Ladd and Butler, 1972) and as such
122 remove any biochemical source of CO_2. The mechanism of protein precipitation by TCA is
123 unclear but is likely to be due to protein unfolding (Rajalingam et al., 2009) and as such may
124 also affect microbial membranes. $AgNO_3$ is a known antiseptic and so kills microbes; the
125 precise mode of action is surprisingly poorly understood but the Ag^+ ions are known to cause
126 physical damage to cells and DNA – separation of cytoplasmic membranes from cell walls
127 and condensing of DNA in both *Escherichia coli* and *Staphylococcus aureus* (Feng et al.,
128 2000). Silver and other heavy metals are also known to bind to thiol groups in proteins
129 resulting in their inactivation (Liau et al., 1997). They also interfere with intra-cellular

processes and membranes/cell walls therefore $AgNO_3$ may also affect some extracellular enzymes (e.g. thiol-proteases). This treatment is designed to primarily inhibit the organismal pathway but is likely to have a lesser effect on biochemical mechanisms – i.e. extracellular enzymes (Fig. 1). Whilst the extent to which these inhibitors operate exclusively on these pathways is unknown (and may be impossible to precisely establish), the rationale is that they will be at least partly informative. However, autoclaving twice unequivocally sterilises soil.

The rapidity of CO_2 release from the soils after the DRW cycle was remarkable, in that we detected maximal rates of evolution after 6 minutes, and never captured the actual peak as such, only a downward trend from a presumed peak (Fig. 2). Within the first hour following wet-up an average of 5% of the total CO_2 efflux over 96 h was observed and of this approximately 24% occurred within the first 12 minutes (Fig. 2a - d). Of the total CO_2 efflux measured over 96 h after rewetting, an average of 41% was measured in the first 24 h (Fig. 2e – h); this consistency of effect with – where the same proportion of CO_2 was measured in the first 24 h after each of a series of rewetting events - was also observed by Birch (1958).

A large difference in CO_2 release on rewetting between the wet control and the standard response to DRW was manifest (Fig. 3a). This is likely because the 3-day drying period resulted in different amounts of moisture loss between treatments; those exposed to the prescribed DRW cycle lost 34% of their mass on average over the 3 days of drying, however, the moist controls lost only 16% of their mass on average. This shows that soil dried to a greater extent will give a larger flush of CO_2 on rewetting than a sample of the same soil dried less severely (Kieft et al., 1987; Fierer and Schimel, 2002; Unger et al., 2010; Meisner et al., 2015). Those samples treated with 15% TCA and 1% $AgNO_3$ dried to a greater extent over 3 days than the moist controls (21 and 28% mass loss respectively) and those that were autoclaved lost 45% of their mass on average. Despite these large differences in moisture loss between the moist controls and the inhibitor treated samples

157 (both TCA and $AgNO_3$) the effect of moisture loss on total CO_2 efflux was found to be non-
158 significant using an analysis of covariance (ANCOVA; $p = 0.71$), nor was there a significant
159 interaction between inhibitor treatment and moisture loss ($p = 0.25$). As such, the main effect
160 of inhibitor treatment can be interpreted directly.

161 Hereafter, responses of inhibitor-treated samples to DRW are compared to that of the moist
162 controls (Fig. 3b). Autoclaving effectively 'switched off' CO_2 production after a rewetting
163 event (total CO_2 efflux over 24 h was significantly different between water controls and
164 autoclaved samples and autoclaved totals were not significantly different from blanks ($p =$
165 0.01, $p = 0.99$ respectively, Fig. 3). A preliminary experiment using soil with higher $CaCO_3$
166 contents (0.93 % compared to 0.48 % on average for soils listed in Table 1) showed the
167 same lack of activity after autoclaving and a DRW event (data not presented). These results
168 show that there was effectively no chemical contribution to the CO_2 flush observed after
169 rewetting in these soils. This is in contrast to observations made in some calcareous, arid
170 soils where CO_2 derived from inorganic–C has been observed to account for 30-75% of the
171 total soil CO_2 efflux (Tamir et al., 2011; Shanhun et al., 2012). As previously stated, these
172 observations have also been made in temperate soils but results are scarce and inconsistent
173 with ranges of 1-2% (Schindlbacher et al., 2015), to 50% (Biasi et al., 2008) all the way up to
174 74% (Ramnarine et al., 2012) of the total CO_2 flux attributable to inorganic C sources.
175 Notably, none of these studies examined the response to a DRW event although Biasi et al.
176 (2008) noted an effect of water addition in the laboratory. The effect of autoclaving observed
177 in our study is therefore strong evidence for an organismal and/or biochemical origin for the
178 evolved CO_2

179 Treating soils with either 15% TCA or 1% $AgNO_3$ substantially reduced but did not eliminate
180 CO_2 production, compared to the moist control, following a DRW event (Fig. 3b). Inhibition
181 of CO_2 evolution by AgNO3 was greater than by TCA for the latter half of the measurement
182 period (Fig. 3), although the accumulated total release was not statistically significant in the
183 case of these two inhibitors ($p = 0.98$). This suggests that a greater portion of the CO_2

184 measured after a DRW event is derived from the organismal pathway. This effect appeared
185 to increase over time with the amount of CO_2 produced hourly by $AgNO_3$ treated soils
186 decreasing more rapidly over the first 24 hours than it did for TCA treated soils this is
187 exemplified by the increasing gap between the confidence bands for $AgNO_3$ and TCA
188 treated soils after approximately 13 hours of incubation in Fig. 3. It is commonly assumed
189 that the majority of CO_2 measured after a DRW event is derived from the organismal
190 pathway, and the effect of $AgNO_3$ would certainly suggest this. There was also a substantial
191 reduction in CO_2, compared to the moist control, due to the addition of TCA, which suggests
192 that an additional contribution to the CO_2 flux after the DRW event was via the biochemical
193 route. This is consistent with the findings of Maire et al. (2013) who report a 16-48%
194 contribution of an extracellular oxidative metabolism pathway, termed 'EXOMET', to soil CO_2
195 flux. Blankinship et al., (2014) found only a 26-47% reduction in CO_2 emission from
196 intermediates in the TCA cycle after sterilisation suggesting that these enzymes are still
197 active when cells are dead but not completely dispersed, again noting that neither of these
198 two studies were in response to DRW events. It is known that many enzymes are stable in
199 the soil environment on a long term basis (Burns et al., 2013). Such stability is generally
200 achieved by adsorption onto soil colloids or incorporation with humic complexes (Nannipieri
201 et al., 1996). The effects of adsorption or humic complexing can include inhibition and steric
202 hindrance which can cause a reduction in potential activity of this sizeable enzyme pool by
203 up to 90% (Quiquampoix et al., 2002). If even a small proportion of these enzymes were to
204 be brought into solution after rewetting this could have a large effect on the levels of activity
205 in soils (Stursova and Sinsabaugh, 2008). Significant increases in rates of enzyme activity
206 have been recorded in soils exposed to DRW both during laboratory preparation (Kandeler
207 and Gerber, 1988) and as a result of environmental conditions (Hinojosa et al., 2004)
208 suggesting that portions of the adsorbed enzyme pool are solubilised by the process of
209 rewetting after drying increasing the potential for a biochemically driven response in DRW
210 soils.

Our results demonstrate the apparent immediacy of the Birch effect, and go some way to explaining the pathways by which the CO_2 is evolved, *viz*. primarily organismal but with a potentially large contribution from the biochemical pathways. We note that for our experiments, these are roughly equivalent in magnitude. Thus we reject the hypothesis that the origin of the CO_2 released following rehydration is predominantly organismal. We have shown that in these temperate soils, unlike in more calcareous, arid systems, there is no contribution of carbonate dissolution even when the intrinsic concentration of $CaCO_3$ is high. This means that this effectively instantaneous release of CO_2 is governed by the soil biota. We have shown evidence that not only are intact microbial cells apparently capable of reinstating their high rates of respiration within minutes following rehydration after 3 days of drying, but also that there is a potentially extensive contribution of CO_2 from remnant enzymatic pathways outside of cell membranes.

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355 Figure captions

356 Fig. 1: Three potential sources of CO_2 to account for the flush on rewetting of dry soils and
357 the treatments used to identify the respective contributions of these. Light grey bars in lower
358 panel indicates which potential sources of CO_2 are uninhibited by each treatment, mid-grey
359 shows which sources are potentially inhibited, and dark grey shows those that are 'switched
360 off' by the different treatments.

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362 Fig. 2: CO_2 release profiles from unsterilized grassland soil exposed to 4 repeated DRW
363 events (Cycles 1 – 4); (a-d) CO_2 release measured at 6 minute intervals in the first hour after
364 rewetting, (e-h) hourly CO_2 release over the first 24 h after rewetting, (I – l) hourly CO_2
365 release over the entire 94 hour wet period. Means (n = 3) indicated by black line surrounded
366 by confidence bands of ± 1 standard error.
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370 Fig. 3: CO_2 efflux rates following rewetting of a dry soil with various solutions; (a) live soil
371 (green) exposed to a DRW cycle compared to all other treatments including a moist control
372 (blue), area outlined in red is shown in greater detail in (b); (b) amplification of y-axis from
373 (a), i.e. CO_2 efflux following a DRW cycle from the moist control (blue), blanks (no soil -
374 brown), autoclaved (orange), 15% TCA (purple) and 1% silver nitrate (grey) treated soils.
375 Lines show mean rates of CO_2 efflux (n=12 (3 reps each of 4 soils)) surrounded by
376 confidence bands of ± 1 standard error.

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