On the origin of carbon dioxide released from rewetted soils

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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

23   Abstract

24   When dry soils are rewetted a pulse of CO_2 is invariably released, and whilst this

25   phenomenon has been studied for decades, the precise origins of this CO_2 remain obscure.

26   We postulate that it could be of chemical (i.e. via abiotic pathways), biochemical (via free

27   enzymes) or biological (via intact cells) origin. To elucidate the relative contributions of the

28   pathways, dry soils were either sterilised (double autoclaving) or treated with solutions of

29   inhibitors (15% trichloroacetic acid or 1% silver nitrate) targeting the different modes. The

30   rapidity of CO_2 release from the soils after the drying:rewetting (DRW) cycle was

31   remarkable, with maximal rates of evolution within 6 minutes, and 41% of the total efflux

32   over 96 h released within the first 24 h. The complete cessation of CO_2 flux following

33   sterilisation showed there was no abiotic (dissolution of carbonates) contribution to the CO_2

34   release on rewetting, and clear evidence for an organismal or biochemical basis to the flush.

35   Rehydration in the presence of inhibitors indicated that there were approximately equal

36   contributions from biochemical (outside membranes) and organismal (inside membranes)

37   sources within the first 24 h after rewetting. This suggests that some of the flux was derived

38   from microbial respiration, whilst the remainder was a consequence of enzyme activity,

39   possibly through remnant respiratory pathways in the debris of dead cells.

40

41   Rewetting of a dry soil invariably causes a large flux of carbon dioxide (CO_2) to be rapidly

42   released, which is sometimes referred to as the Birch effect (Birch, 1958; 1960). This

43   phenomenon has been observed both in laboratory incubations (Kieft et al., 1987; Unger et

44   al., 2010; Shi and Marschner, 2014) and in field circumstances using closed chambers (Yan

45   et al., 2014) or eddy covariance towers (Xu et al., 2004). These fluxes have been observed

46   across a wide range of ecotypes (Jarvis et al., 2007; Thomas and Hoon, 2010; Sugihara et

47   al., 2015), but are particularly significant in dryland and Mediterranean ecosystems where

48   they can make up a significant proportion of soil C-emissions (Lee et al., 2004; Hunt et al.,

49   2004; Brito et al., 2013). These drying:rewetting (DRW) induced CO_2 efflux events can even

50   significantly reduce the annual net C gain in Mediterranean forests (Jarvis et al., 2007).

51   Several theories have been proposed to explain this phenomenon including: (i) the exposure

52   of physically-protected organic matter to microbial metabolism via aggregate dispersion on

53   rewetting (Denef et al., 2001; Wu and Brookes, 2005; Xiang et al., 2008); (ii) microbial

54   necromass increasing the supply of readily assimilable substrate to the surviving microbial

55   populations (Kieft et al., 1987; Van Gestel et al., 1992; Blazewicz et al., 2013); (iii)

56   increases in the supply of labile organic matter due to the rapid release, on rewetting, of

57   intra-cellular solutes previously concentrated within microbial cells to maintain osmotic

58   balance in response to dehydration (Halverson et al., 2000; Warren, 2014); and (iv) a supply

59   of labile organic C is built up during the dry period prior to rewetting and subsequently

60   quickly metabolised on rewetting. There is a known uncoupling of rates of CO_2 efflux and

61   detectable microbial growth rates after a DRW cycle (Iovieno and Bååth, 2008; Meisner et

62   al., 2015) and microbial populations in such circumstances show little change in their net

63   size (Fierer and Schimel, 2002). However, recent work by Blazewicz et al., (2013) show that

64   despite their unchanging size these populations turnover rapidly in response to a DRW

65   cycle. They also suggest that more cellular derived organic-C is available in soil samples

66   than is turned over in the initial phases after rewetting. This organic-C will contain cellular

67   material including constituents of enzymatic pathways – remnant respiratory pathways – with

68   the potential to carry out reactions leading to CO_2 efflux. Thus it is possible that CO_2 release

69   from re-wetted soils is not exclusively derived from respiration pathways occurring in intact

70   microbes. There are also reports of over-estimation of soil respiration rates due to

71   contributions of CO_2 from dissolution of soil carbonates; however, reports are inconsistent

72   and range from 1-2 % up to 74% of CO_2 efflux from soil being attributed to carbonate

73   dissolution (Biasi et al., 2008; Ramnarine et al., 2012; Schindlbacher et al., 2015). It is as

74   yet unclear how the DRW process may affect carbonate dissolution from soils although

75   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

4

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

130   processes and membranes/cell walls therefore $AgNO_3$ may also affect some extracellular

131   enzymes (e.g. thiol-proteases). This treatment is designed to primarily inhibit the organismal

132   pathway but is likely to have a lesser effect on biochemical mechanisms – i.e. extracellular

133   enzymes (Fig. 1). Whilst the extent to which these inhibitors operate exclusively on these

134   pathways is unknown (and may be impossible to precisely establish), the rationale is that

135   they will be at least partly informative. However, autoclaving twice unequivocally sterilises

136   soil.

137

138   The rapidity of CO_2 release from the soils after the DRW cycle was remarkable, in that we

139   detected maximal rates of evolution after 6 minutes, and never captured the actual peak as

140   such, only a downward trend from a presumed peak (Fig. 2). Within the first hour following

141   wet-up an average of 5% of the total CO_2 efflux over 96 h was observed and of this

142   approximately 24% occurred within the first 12 minutes (Fig. 2a - d). Of the total CO_2 efflux

143   measured over 96 h after rewetting, an average of 41% was measured in the first 24 h (Fig.

144   2e – h); this consistency of effect with – where the same proportion of CO_2 was measured in

145   the first 24 h after each of a series of rewetting events - was also observed by Birch (1958).

146   A large difference in CO_2 release on rewetting between the wet control and the standard

147   response to DRW was manifest (Fig. 3a). This is likely because the 3-day drying period

148   resulted in different amounts of moisture loss between treatments; those exposed to the

149   prescribed DRW cycle lost 34% of their mass on average over the 3 days of drying,

150   however, the moist controls lost only 16% of their mass on average. This shows that soil

151   dried to a greater extent will give a larger flush of CO_2 on rewetting than a sample of the

152   same soil dried less severely (Kieft et al., 1987; Fierer and Schimel, 2002; Unger et al.,

153   2010; Meisner et al., 2015). Those samples treated with 15% TCA and 1% $AgNO_3$ dried to a

154   greater extent over 3 days than the moist controls (21 and 28% mass loss respectively) and

155   those that were autoclaved lost 45% of their mass on average. Despite these large

156   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.

211 Our results demonstrate the apparent immediacy of the Birch effect, and go some way to

212 explaining the pathways by which the CO_2 is evolved, *viz.* primarily organismal but with a

213 potentially large contribution from the biochemical pathways. We note that for our

214 experiments, these are roughly equivalent in magnitude. Thus we reject the hypothesis that

215 the origin of the CO_2 released following rehydration is predominantly organismal. We have

216 shown that in these temperate soils, unlike in more calcareous, arid systems, there is no

217 contribution of carbonate dissolution even when the intrinsic concentration of $CaCO_3$ is high.

218 This means that this effectively instantaneous release of CO_2 is governed by the soil biota.

219 We have shown evidence that not only are intact microbial cells apparently capable of

220 reinstating their high rates of respiration within minutes following rehydration after 3 days of

221 drying, but also that there is a potentially extensive contribution of CO_2 from remnant

222 enzymatic pathways outside of cell membranes.

223 Acknowledgements

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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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