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Short communication

On the origin of carbon dioxide released from rewetted soils

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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 min, and 41% of the total efflux over 96 h released within the first 24 h. The complete cessation of CO_2 eflux 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

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

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

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

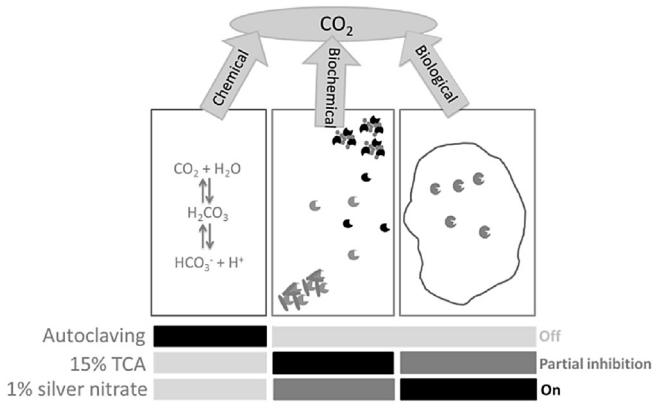

Fig. 1. Three potential sources of CO_2 to account for the flush on rewetting of dry soils and the treatments used to identify the respective contributions of these. Light grey bars in lower panel indicates which potential sources of CO_2 are uninhibited by each treatment, mid-grey shows which sources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

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

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