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Loss of labile carbon following soil disturbance determined by measurement of respired $\partial^{13}CO_2$

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ABSTRACT

Soils are the largest pool of carbon (C) in terrestrial ecosystems with labile C being particularly vulnerable to loss. In this study we incubated a range of soils in both the short- (minutes) and long-term (months) to assess the loss of labile soil C by measuring the isotopic signature of soil respired CO_2 ($\partial^{13}CO_2$). Strong temporal trends in $\partial^{13}CO_2$ values were observed following soil disturbance: $\partial^{13}CO_2$ rapidly changed from a range of −22.5 to −23.9‰ to −25.8 to −27.5‰ during short-term incubations and reverted back to the initial values in long-term incubations. The shifts in $\partial^{13}CO_2$ over the course of soil incubations were consistent with changes in labile C substrate utilization following the disturbance of sampling the soil. An independent experimental approach which immobilised labile soil C onto allophane and included chemical extractions as a measure of extractable C in soils also confirmed this interpretation. Collectively, these results indicate that the isotopic analysis of respired CO_2 can be a powerful technique which enables us to probe mechanisms and examine the consequences of disturbance on the labile component of soil C.

1. Introduction

Soils are the largest pool of carbon (C) in terrestrial ecosystems, globally containing more than two-thirds of ecosystem total C (Amundson, 2001). Land-use change and any accompanying soil disturbance can be a major cause of loss of soil C, for example following deforestation (Cadisch et al., 1996; Guo and Gifford, 2002; Zingore et al., 2005), cultivation (Elliott, 1986) or cropping (Guo and Gifford, 2002). Labile, soil organic matter (SOM) is considered to have fast decomposition rates and short turnover times. It accounts for only about 5% of SOM, but is highly active as it consists of easily decomposable compounds (Townsend et al., 1997; Krull et al., 2003). It can be vulnerable to microbial degradation due to lack of stabilisation onto clay minerals or lack of physical protection by soil aggregates (Krull et al., 2003). Such protection occurs due to a range of factors, including reduced oxygen diffusion into soil aggregates and physical separation from soil microbes (Six et al., 2002). Systematic loss of soil C following mechanical soil disturbance is attributed to loss of physically protected SOM (Six et al., 2002). Microbial access to soil C is hypothesised to play a larger role in regulating SOM turnover than molecular recalcitrance (Dungait et al., 2012).

A range of methods have been used to measure labile soil C pools, relying on chemical, physical or biological separation from recalcitrant SOM (McLauchlan and Hobbie, 2004). While results from different methods are positively correlated, they can produce large differences in estimates of labile C pools (McLauchlan and Hobbie, 2004). Dissolved organic C (DOC) and hot water extractable C (HWEC) are common measures of chemically extractable C in soils which can be used to estimate the size of soluble labile soil C pools. Positive correlations have been observed between DOC and total soil C, HWEC and total soil C, as well as between DOC and HWEC (Ghani et al., 2003). HWEC is a small pool of labile C, generally representing 3–6% of total soil C, and has been shown to be a sensitive indicator of changes in organic matter due to soil management (Ghani et al., 2003).

A refinement to the fractionation of SOM for separation of labile from recalcitrant C pools has been the use of ^{13}C signatures to measure loss of recently assimilated C. This approach has relied upon inputs of C into soil with contrasting isotopic signatures, either through a transition between C_4 and C_3 vegetation (Cadisch

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et al., 1996; Zingore et al., 2005; Virto et al., 2010; Blagodatskaya et al., 2011) or supplying elevated carbon dioxide (CO_2) with a ^{13}C signature different from air thus providing a label (Pendall and King, 2007). In both cases, the distinct difference in ^{13}C signature between the inputs of newly fixed C and the SOM provides a way to differentiate relatively labile *versus* relatively recalcitrant C pools. Measurement of the isotopic signatures of whole soils (Zingore et al., 2005), isolated SOM fractions (Cadisch et al., 1996; Virto et al., 2010; Blagodatskaya et al., 2011), or respired CO_2 (Townsend et al., 1997; Crow et al., 2006; Pendall and King, 2007) has then been used to calculate the residence time of C.

In a study of the $\partial^{13}C$ signature of soil respiration, Millard et al. (2010) found a rapid depletion of $\partial^{13}CO_2$ within a few hours of soil incubation. These changes in $\partial^{13}CO_2$ were ascribed to disturbance (as a result of extracting soil cores and removal of roots), causing small but relatively labile soil C pools, previously protected from microbial activity, to become available as respiratory substrates (Millard et al. (2010). In a longer-term study Crow et al. (2006) found that, during incubation of different SOM fractions from a forest soil, there was an initial isotope depletion of the respired CO_2, which subsequently (over a 25 d period) became more enriched, more closely reflecting the value of the solid sample. Crow et al. (2006) suggested that one reason for these changes in the isotopic signature of respiration through time was loss of the more labile C pools in the soil that were relatively depleted in ^{13}C. The present study extends this approach, to test the hypothesis that shifts in $\partial^{13}CO_2$ over the course of soil incubations were due to changes in labile C substrate utilization.

A range of soils were incubated in both short- and long-term experiments and $\partial^{13}CO_2$ measured in order to determine if: (1) rapid changes in $\partial^{13}CO_2$ following soil disturbance were due to release of labile C, (2) long-term incubations resulted in $\partial^{13}CO_2$ reverting back to original values as labile C pools were exhausted, (3) large scale soil perturbation had an effect on the time needed for $\partial^{13}CO_2$ to revert back in the long-term incubations. Additional soil was incubated with sand (as a control treatment) and allophane, which due to its large active surface area adsorbs SOM by forming Al-organic complexes (Baldock and Skjemstad, 2000; Yuan et al., 2000), in order to determine if $\partial^{13}CO_2$ can indicate the loss of labile soil C.

2. Materials and methods

2.1. Soils

2.1.1. Site 1: kānuka stand

Soil was sampled from a stand of kānuka (*Kunzea ericoides* (A. Rich) J. Thompson) trees 6 m high in Lincoln, New Zealand (43° 38′ S, 172° 29′ E, 11 m above sea level) which was planted in 1984 (Harris, 1996). Previously, the site was temperate grassland dominated by C_3 grass species. The soil was a Wakanui silt loam (Hewitt, 2010) (USDA classification, Aquic Haplustept), with a litter layer of variable depth (25–70 mm) and an H1 horizon to 180 mm depth. The soil is described in detail by Watt and Burgham (1992).

2.1.2. Site 2: Montane grassland

Soil was sampled from a tussock grassland with a mix of native tussocks (*Festuca novae-zelandiae (Hack.) Cockayne*) and pasture species (*Agrostis capillaris* L.) (Burrows, 1977) at the University of Canterbury Cass Field Station in the Central South Island, New Zealand (43° 02′ S, 171° 46′ E, 590 m above sea level). Soils at this site were classified as Acidic Allophanic Brown (Hewitt, 2010) (USDA classification, Typic Dystruchrept). Samples were taken from two different treatment areas at this site: 1) the perturbed plots of a soil warming experiment and 2) an adjacent undisturbed area of tussock grassland. Establishment of the soil warming experiment involved significant large scale soil perturbation: the top 300 mm of soil was excavated with heavy machinery in late 2008–January 2009 (one year prior to sampling), left at the site for several months and redistributed after installation of the heating cables. Samples were only taken from the plots, which received the soil perturbation, but were not subjected to warming.

2.1.3. Site 3: arable cropping

Soil was sampled in the Millennium Tillage Trial (MTT), a field site established by Plant and Food Research in November 2000 on a Wakanui silt loam (Hewitt, 2010) (USDA classification, Aquic Haplustept) near Lincoln, New Zealand (43° 40′ S, 172° 28′ E, 5 m above sea level). Soil was sampled from replicated no tillage plots sown with barley (*Hordeum vulgare* L.) and also plots which had been maintained under a permanent pasture of ryegrass (*Lolium perenne* L.) and clover (*Trifolium repens* L.).

2.1.4. Site 4: peatland

Soil was sampled from Middlemuir Moss, a former raised mire site in Scotland (57° 36′ N, 2° 9′ W, 110 m above sea level). The site has a long history of manual and mechanised peat cutting; up to 4 m of peat has been removed during mechanised harvesting operations between 1961 and 1995. The remaining peat is highly acidic (pH 3.0), humified and between 2 and 4 m deep. No restoration has been carried out and the site is unmanaged. Surface peat exposed after harvesting was used for the experiment and because of its original depth was probably several thousand years old. The vegetation was characterised by patchy spontaneous regeneration with typical mire species such as *Calluna vulgaris* L., *Eriophorum vaginatum* L. and *E. angustifolium Honck*., with scattered patches of *Sphagnum auriculatum Schimp*. and extensive areas of bare peat (Trinder, 2007; Trinder et al., 2008b). Soil at the site was classified as drained oligotrophic amorphous peat (Acid Humic Organic soil (Hewitt, 2010), USDA classification, Cryohemist). Soil was only sampled from areas containing no vegetation.

Site descriptions are summarised in Table 1.

2.2. Soil sampling and incubations

The protocol used for soil sampling was similar to the one described by Millard et al. (2010). At each site and for each treatment replicate 50 mm diameter soil cores taken to a depth of 250 mm were broken open and visible roots and any stones quickly (within minutes) removed by hand and discarded. All the remaining soil from each core was mixed and placed in a Tedlar® bag (Keika Ventures, Chapel Hill, NC, USA). The bag was sealed, and then quickly flushed with CO_2-free air repeatedly (typically 5–6 times) until less than 20 ppm CO_2 remained in the bag. Samples were incubated *in situ* at an ambient temperature and aliquots of gas were regularly removed to check the CO_2 concentration with a

Table 1
Site descriptions.

Site	Kānuka stand	Montane grassland	Arable	Peatland
Latitude, longitude	43° 38′ S 172° 29′ E	43°02′ S 171°46′ E	43°40′ S 172°28′ E	57° 36′ N 2° 9′ W
Altitude	11 m a.s.l.	590 m a.s.l.	5 m a.s.l.	110 m a.s.l.
Annual rainfall	684 mm	1300 mm	684 mm	748 mm
MAT	11.4 °C	9.0 °C	11.4 °C	8.8 °C
Soil type (NZ classification)	Wakanui silt loam	Acidic Allophanic Brown	Wakanui silt loam	Acid Humic Organic soil
Soil type (USDA)	Aquic Haplustept	Typic Dystruchrept	Aquic Haplustept	Cryohemist