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Title: **No evidence for increased loss of old carbon in a temperate organic soil after 13 years of simulated climatic warming despite increased CO_2 emissions**

Running head: Warming does not increase old soil C release

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No evidence for increased loss of old carbon in a temperate organic soil after 13 years of simulated climatic warming despite increased CO_2 emissions

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Abstract

Determining the temperature sensitivity of terrestrial C stores is an urgent priority for predicting future climate feedbacks. A key aspect to solve this long-standing research gap is to determine whether warmer temperatures will increase autotrophic activities leading to greater C storage or promote heterotrophic activities that will drive these systems to become C sources. We experimentally addressed this critical question by subjecting intact plant-soil systems in a UK upland ecosystem to simulated climate warming under natural field conditions. We report the results of a 13 year field-based climate manipulation experiment combining *in situ* respiration measurements with radiocarbon (^{14}C) analyses of respired CO_2, dissolved organic carbon (DOC), soil and the tissue contents of the dominant soil fauna (enchytraeids). We found that warming during the growing season produced the largely expected increases in ecosystem respiration (63%) and leaching of DOC (19%) with no evidence for thermal acclimation or substrate exhaustion over the whole 13 year experimental period. Contrary to expectations, we found no evidence to support an increased release of old soil C after more than a decade of simulated climatic change, and indeed, ^{14}C analyses indicated that warming caused a significant shift towards mineralisation of more recent plant-derived C inputs. Further support came from the radiocarbon analyses of the enchytraeid tissues which showed a greater assimilation of the more recent (plant-derived) C sources following warming. Therefore, in contrast to sub-arctic ecosystems, our results suggest that changes in C storage in this UK upland soil are strongly coupled to plant activities and that increasing temperatures will drive the turnover of organic material fixed only within recent years, without resulting in the loss of existing old carbon stores.

KEYWORDS bomb ^{14}C, carbon stores, climate change, ecosystem respiration, dissolved organic carbon, enchytraeids, peatlands, soil fauna.

1 INTRODUCTION

Concerns over the feedback responses of terrestrial ecosystems to elevated atmospheric temperatures, resulting from increasing atmospheric greenhouse gas concentrations, focused attention on the role of soils as a sink and potentially powerful source of CO_2 (e.g. Cox et al., 2000; Smith 2006; Oertel et al., 2016). These abiding concerns remain as key uncertainties in contemporary General Circulation Models (e.g. Friedlingstein, et al., 2014; Nishina et al., 2015; Shi et al., 2018; Bonan et al., 2019; Tharammal et al., 2019). Indeed, the estimated 3,000 Pg of carbon (C) stored in the world's soils (down to 3 m depth; Lal, 2018) could represent a significant potential global positive feedback source of atmospheric carbon dioxide (CO_2) under a changing climate (Crowther et al., 2016; Turesky et al., 2019). Organic soils from cold northern latitudes are of special concern because they show the strongest temperature sensitivity responses (Karhu et al., 2014; Koven et al., 2017; Gallego-Sala et al., 2018; Turesky et al., 2019; Qiu et al., 2020). In relation to this, increasing global temperatures have been controversially linked to net losses of 0.6% a^{-1} topsoil organic C (Bellamy et al., 2005) and to the estimated export of ca. 7 tonnes C km^{-2} a^{-1} to rivers as dissolved organic carbon (DOC) in the UK (Worrall and Burt, 2007; Smith et al., 2007). However, some models indicate that only 10-20% of these C losses could be attributed to warming (Smith et al., 2007).

As a result of the conflicts between observations and model calculations, the magnitude of the feedbacks between soils and climate remain uncertain and has become a hotly debated issue in the literature (e.g. Conant et al., 2011; Friedlingstein, et al., 2014; Shi et al., 2018). This led to an appeal by Schmidt et al. (2011) for the establishment of long-term experiments in which intact soil-plant systems should be subjected to climate manipulation, taking advantage of developing radiocarbon (^{14}C) technologies to identify underpinning C transfers. Although ^{14}C measurements of soil organic matter (SOM) have increased our understanding of the stability of the different C substrates present in the soils (Hopkins et al., 2012; Ahrens et al., 2015; Street et al., 2020), the relative contribution of different aged organic matter to C losses is still poorly quantified. For example, whilst a number of studies have concluded that most of the C respired and leached from soils is derived from recently-fixed plant matter (Evans et al., 2006; Marwick et al., 2015; Pries et al., 2015: Jia et al., 2019), others have demonstrated that older C contributions (fixed prior to AD1955) can constitute an important fraction of the total amount of C released from soils (Neff et al., 2006; Trumbore, 2009; Hartley et al., 2008, 2012; Hopkins et al., 2012; Cheng et al., 2017; Olid et al., 2020).

Another alarming aspect of reviews and models of soil C (Schmidt et al., 2011; Conant et al., 2011; Hopkins et al., 2012; Lehmann and Kleber, 2015; Shi et al., 2018; Luo et al., 2019; Wiesmeier et al., 2019; Woolf and Lehmann, 2019; Lehmann et al., 2020) is the total failure to consider the functional role of soil fauna, despite their critical and long-recognised role as major agents of soil C turnover. Besides the environmental and substrate quality constraints to SOM decomposition, biological activities (including plants, soil biota and their enzymatic systems) and their responses to climatic variations are key regulators of C dynamics (García-Palacios et al., 2013; Crowther et al., 2019; Street et al., 2020). One specific group of Oligochaeta worms (Enchytraeidae), which frequently contribute up to 70% of the total animal biomass in organic soils (Coulson and Whittaker, 1978), have a strong sensitivity to climate change and a critical impact on the C sink/source function of C rich soils (e.g. Briones et al., 2004, 2007, 2014; Carrera et al., 2009). Unfortunately, whilst vegetation, microbial and biogeochemical activities are becoming increasingly recognised in climate change and global C cycle models (e.g. Manzoni et al., 2012; Todd-Brown et al., 2012; Liang et al., 2017; Woolf and Lehmann, 2019; Lehmann et al., 2020; Guo et al., 2020), soil fauna are consistently excluded on the basis of the difficulties in assessing, and quantifying, their role at global scales (Wall et al., 2008).

Quantifying the relative importance of the autotrophic and heterotrophic components to ecosystem respiration is central to making more accurate predictions of the vulnerability of these C stores to climate change. Indeed, partitioning studies have shown that in peatlands with mosses and in coniferous forests, heterotrophic respiration dominates soil efflux (Subke et al., 2006; Schuur and Trumbore, 2006; Pries et al., 2015), whereas in tundra and peatland systems dominated by graminoids, autotrophic respiration made the greatest contribution (Pries et al., 2015; Gatis et al., 2019). However, changes in water tables could reduce photosynthesis and increase heterotrophic respiration under vascular plants (Gatis et al., 2019), and mosses have been seen to contribute recently synthesized carbon to the peatland dissolved organic carbon pool (Fenner et al., 2004; Bell et al., 2018). Therefore, although non-vascular plants have an important role in accumulating C in peatlands through decreasing the proportion of C lost in gaseous form, they could increase C losses through leaching. Consequently, accounting for the DOC exported by water could reduce the estimates of C accumulation based on gas fluxes alone (Kindler et al., 2011; D’Acunha et al., 2019).

In this study, we addressed the critical question of whether long-term warming would result in sustained soil and ecosystem C losses by subjecting, under as natural conditions as possible, a UK upland system to simulated long-term global warming. We first investigated the long-term stability (13-year study, 2005-2018) of organic C in the field by combining *in situ* CO_2 respiration measurements and radiocarbon (^{14}C) analysis of soil C respired from the soil, leached as DOC and assimilated by key soil fauna (Enchytraeidae) using an experimental transplant approach. This allowed insights into the C fluxes in these complex systems (including plants, intact soil profile and associated biota) and how these were influenced by temperature change, against a backdrop of the normal seasonal and diurnal-nocturnal cycles occurring in nature (Ineson et al., 1998). Second, we used natural abundance radiocarbon to partition ecosystem respiration into its auto- and heterotrophic components at both sites to determine the balance between autotrophic (plant root contribution) and heterotrophic (microorganisms and soil animals) contributions to ecosystem respiration (R_{ECO}) in response to long-term warming.

2 MATERIALS AND METHODS

2.1 Transplant experiment

We used part of the long-term altitudinal transect of sites monitored during the NERC TIGER (Terrestrial Initiative in Global Environmental Research) project at the Moor House National Nature Reserve, Cumbria, UK (54º 68’ N, 2º 36’ W). Established in the 1990s, this site benefits from a considerable resource of background information (e.g. meteorological, plant and soil data) and meteorological measurements continue to be taken at the site (Environmental Change Network (ECN): http://www.ecn.ac.uk/measurements; Cosmic-ray Soil Moisture Monitoring Network (COSMOS-UK): https://cosmos.ceh.ac.uk/).

The soil selected for this study, typical for an upland soil in the UK, was a cambic stagnohumic gley, characterized by a high organic matter content (49% C and 2.5% N; Briones et al., 2014), clay loam soil texture (46% sand, 35% clay; Hornung, 1968) and low pH (<4.5; Adamson et al., 1995). The formation of a peaty top soil (Of and Oh), which is commonly thicker than 10 cm overlies approximately 4 cm of dark organic-rich mineral (Ah) soil and below 16 cm the soil is frequently waterlogged where the acid gley mineral horizon is present (Bol et al., 1996). Preliminary radiocarbon analyses of this soil showed that the bomb-^{14}C signal, when the experiment was established, was limited to the top 0-4 cm (Briones and Ineson, 2002). This allowed us to investigate, under field conditions, whether warming led to the release of old C over time, avoiding the large errors associated with following C mass balance changes which may only become apparent or detectable over longer time scales (Hopkins et al., 2012).

We collected 48 intact vegetated soil cores (PVC pipes, 10.3 cm internal diameter x 20.5 cm deep) from an area near the summit of Great Dun Fell (GDF) for transplanting on 23rd August, 2005. The vegetation is dominated by *Juncus squarrosus* L., with *Festuca ovina* L., *Deschampsia flexuosa* (L.) Trin. and *Polytrichum commune* L. Half of the cores were placed back at GDF (845 m; 54º 41' 03" N, 2º 27' 08" W) to act as controls, whilst the remaining half were transplanted to a lower altitude at Sink Beck (SNK, 480 m; 54º 39' 33" N, 2º 28' 04" W), resulting in an anticipated annual temperature increase at the SNK site, with the decreasing altitude raising air temperature by *ca.* +3°C (Ineson et al., 1998; see also Figs. S1 and S2). Despite these differences in temperature, the two sites are less than 3 km apart.

In contrast to temperature, total rainfall volumes at these sites have been found not to be linearly related to altitude due to strong seasonal and spatial variation (Ineson et al., 1998); however, the climatological observations during the investigated period support previous altitudinal rainfall gradients, with an overall mean annual total rainfall of 1470 mm at GDF and a reduction by ca. 350 mm (ca. 20%) of rain at SNK. Despite this difference in rainfall inputs, previous field rainfall manipulations across this transect have revealed no effect on soil processes and no significant relationships between rainfall and enchytraeid numbers for the GDF or SNK sites (Briones et al., 1997).

Care was taken in inserting the cores into freshly dug placement holes, with cores showing signs of horizon disturbance being rejected. Intact cores were rested directly on the underlying soil in the placement holes, allowing the ground water to circulate freely beneath them; the immediate surrounding area was infilled with soil and turf from the excavation area to the same level as that in each core. This comparison of the behaviour of intact vegetated cores, either placed back at the 'source' site (control; GDF) or transplanted to the warmer site (warmed; SNK), meant that all the aspects of within-year seasonality and climatic factors on plant growth and soil activity of the sites were maintained.

2.2 Climatological data

Meteorological information for both sites was obtained from the available weather stations. The UK Met Office (https://www.metoffice.gov.uk/) provided information for Great Dun Fell (2005-2018) while the Environmental Change Network (http://www.ecn.ac.uk/measurements) and the Cosmic-ray Soil Moisture Monitoring Network (https://cosmos.ceh.ac.uk) produced data for Sink Beck using automatic weather stations (2005-2010 and 2014-2018, respectively).

2.3 Sampling

Although there is no permafrost at either of the two sites (GDF or SNK), the soils were often covered by snow at either site for several months of the year. Because of the restricted growth season, we undertook two sampling campaigns per year, one at the peak of the growing season (Summer-S) and another at the end of the growing season (Autumn-A) during thirteen consecutive years from the initial establishment of the experiment, starting with the initial summer sampling in 2005 (two weeks after establishment of the experiment) and ending with the final sampling in 2018, also in the summer (August 2018).

2.4 Ecosystem respiration

At both sites and on each sampling occasion CO_2 production rate was measured in the field from all cores using a LI-8100 automated soil CO_2 flux system (LI-COR Biosciences, Lincoln, Nebraska, USA) connected to a 10 cm survey chamber. Respiration fluxes were calculated using linear regression over the initial 180 seconds.

Samples of respired CO_2 were taken in the field for $^{14}CO_2$ analysis from three random replicate cores collected during the summer samplings of 2006, 2007, 2008, 2010 and 2018 (i.e. after 1, 2, 3, 5 and 13 years since the start of the experiment; Fig. S1). This was achieved by using the molecular sieve sampling system developed at the NERC Radiocarbon Facility (Hardie et al., 2005). In brief, the system was attached to a dark chamber (10.3 cm internal diameter x 20.5 cm deep) and the headspace air circulated (500 ml/min) through a cartridge containing soda lime to remove all atmospheric CO_2 inside the chamber headspace. The chamber was then left for approximately 4-6 hours to allow respired CO_2 to build up. The sampling system was then re-attached to the chamber and this time the air circulated through a cartridge containing type 13X zeolite molecular sieve (1/16″ pellets, BDH Laboratory supplies, UK) and then back to the chamber in a closed loop. As the air passed through the cartridge, CO_2 was trapped onto the molecular sieve.

2.5 DOC concentrations

On summer samplings (Fig. S1), after taking the $^{14}CO_2$ samples, the three selected 'destructive' cores from both sites were collected and placed in separate plastic bags and then transported back to the laboratory in a cool box. DOC was obtained by leaching the top 10 cm of the soil core with deionised water (Briones et al., 1998) and stored at 4ºC in acid-washed bottles prior to analysis.

DOC concentrations in the leachates were determined from a sub-sample (50 cm^3) which was filtered through a 0.45 μm filter before analysis. Samples were acidified with 3M HCl and sparged with zero grade air to remove inorganic carbon as CO_2. The remaining organic carbon was determined using a high temperature combustion method with an infra-red detector (Formacs^{HT} Skalar Analytical B.V. Breda, the Netherlands). Another subsample was acidified to pH 4, sparged using N_2, neutralised to pH 7 or below and then dried to solids by rotary evaporation and freeze-drying in preparation for radiocarbon analyses.

2.6 Enchytraeid population numbers

After DOC samples were collected, the three soil cores were vertically split into halves, with one half used for estimating the enchytraeid population sizes and the other half retained for subsequent soil analyses (see below; Fig. S1). Animals were extracted into deionised distilled water from five 2-cm horizontal layers (approximately 40 cm^2 each one) to a depth of 10 cm using a wet extractor (O'Connor, 1955) and then counted alive and their tissues freeze-dried in preparation for radiocarbon analyses (Briones and Ineson, 2002).

2.7 Radiocarbon analyses

$^{14}CO_2$ samples trapped in the molecular sieves were recovered at the NERC Radiocarbon Facility by heating (500°C) and cryogenically purified (Hardie et al., 2005), whereas the dried DO^{14}C samples were combusted in sealed quartz tubes or an elemental analyser and the pure CO_2 cryogenically recovered.

Soil samples for ^{14}C analyses were obtained from the core halves resulting from destructive sampling (Fig. S1), freeze-dried and the living plant material and roots removed prior to analyses by sieving through a 4 mm mesh. Several grams of the soil were combusted in a high pressure combustion bomb in an atmosphere of pure O_2, and the CO_2 cryogenically recovered.

All recovered CO_2 from respiration, DOC, soil and enchytraeid tissue samples were split into aliquots for ^{13}C and ^{14}C analysis. One aliquot of CO_2 was used for ^{13}C measurement using isotope ratio mass spectrometry (VG Optima, Micromass, UK or Delta V, Thermo-Fisher, Germany) with results expressed as δ^{13}C (^{13}C/^{12}C ratio in ‰ units relative to the Vienna Pee Dee Belemnite standard). A second aliquot of recovered CO_2 was converted to graphite by Fe/Zn reduction (Slota et al., 1987) and analysed for ^{14}C at the Scottish Universities Environmental Research Centre Accelerator Mass Spectrometry Laboratory. Radiocarbon concentrations are expressed as %modern after normalisation of the measured ^{14}C enrichment of each sample relative to δ^{13}C of -25‰ (Stuiver and Polach, 1977). The average age of the respired CO_2, DOC and enchytraeid carbon since fixation was determined by comparing the bomb-^{14}C contents to an annual record of recent atmospheric ^{14}C concentration (Levin and Kromer, 2004; see Fig. S3).

2.8 Partitioning incubation experiment

Short-term incubations were used to measure the contribution of autotrophic (R_{AUTO}) and heterotrophic respiration (R_{HETERO}) to ecosystem respiration (R_{ECO}) following the methods outlined in Pries et al. (2013, 2015) using the final set of samples remaining at both sites (3 cores per site) that had been in the field for 13 years (2005-2018). Briefly, plant material (living roots and rhizomes, rinsed free of soil and shaken dry) and root-free soil were collected from each soil core (GDF-control and SNK-warm treatment), and placed in air-tight glass jars. The jar headspace was scrubbed to remove atmospheric CO_2 using soda lime and then left in the dark to respire. When enough CO_2 had accumulated for ^{14}C analysis it was collected using the RCF molecular sieve sampling system, as described above.

We used the ^{14}C concentration of the field-collected ecosystem respiration, and that of the auto- and heterotrophic components from the incubations, to partition ecosystem respiration using:

$$FR_{RECO} \times {}^{14}CR_{ECO} = FR_{AUTO} \times {}^{14}CR_{AUTO} + FR_{HETERO} \times {}^{14}CR_{HETERO} \quad (1)$$

$$FR_{HETERO} = ({}^{14}CR_{ECO} - {}^{14}CR_{AUTO}) / ({}^{14}CR_{HETERO} - {}^{14}CR_{AUTO}) \quad (2)$$

where F is the fractional contribution, and ^{14}C the radiocarbon content (in %modern) of the autotrophic (R_{AUTO}) and heterotrophic (R_{HETERO}) components of ecosystem respiration (R_{RECO}).

2.9 Statistical analyses

Data were previously checked for normality and homogeneity of variances and transformed accordingly to ensure that the assumptions of ANOVA were met. Mean CO_2 production rates from cores incubated in the field at each site on every sampling occasion (S and A campaigns during 13 consecutive years) were compared by repeated-measures ANOVA, with the results being interpreted using the multivariate output of repeated measures ANOVA. One-way ANOVA was applied to test for significant differences in cumulative and averaged seasonal respiration values, DOC concentrations and enchytraeid numbers between sites. One-way ANOVA was also used to compare independent field measurements of ^{14}C concentrations of the respired CO_2, DOC, soils and enchytraeid tissues between sites and sampled years. Separation of means was determined using Tukey's Studentized range (HSD) test ($\alpha = 0.05$).

Linear correlations (Pearson correlation coefficient) were used to investigate the interdependence of CO_2 and DOC values and their respective ^{14}C signals ($^{14}CO_2$ and $DO^{14}C$). In addition, linear regressions were used to analyse the temporal variations in the ^{14}C concentrations of the respired, leached and assimilated C observed in response to the treatments (control and warmed).

All statistical analyses were performed using the SAS system (version 9.0).

3 RESULTS

3.1 Effects of warming on the rate and age of ecosystem respiration

Over the thirteen investigated years, R_{ECO} flux was significantly higher at the warmer treatment than at the control site (repeated measures ANOVA: site, $F_{1,353} = 36.58$, $P = 0.0263$; time, $F_{12,342} = 14.73$, $P < 0.0001$, time*site, $F_{12,342} = 3.67$, $P = 0.0032$; Fig. 1a), with an average increase of 63% in the summer and 49% in the autumn (Fig. 1b). Furthermore, despite the marked seasonal and inter-annual variation observed, the warming effect was found at the majority of samplings (Fig. 1a) and resulted in 2.5 times more CO_2 being emitted from the warmer site than from the control treatment (Fig. 1c).

All ^{14}C analyses of the respired CO_2 samples collected during the summer samplings of 2006, 2007, 2008, 2010 and 2018 were slightly enriched relative to the contemporary atmosphere (Fig. 2a), which indicates that the emitted CO_2 resulted from a mix of contemporary plant-respired and older (post-bomb; Fig. S2) soil-respired C fixed from the atmosphere within the last few years. Although there was a significant decline of the ^{14}C value over the sampled years at both sites (ANOVA: time, $F_{4,24} = 76.72$, $P < 0.0001$), it paralleled the atmospheric values (Fig. 2a; see also Fig. S2), suggesting that contemporary carbon fixation was a consistent and major component of the soil respiration flux. The positive relationship observed between the amount of CO_2 being respired from these soils and the ^{14}C enrichment of this C fraction (Pearson correlation coefficient $r = 0.4828$; $P = 0.0080$; $n = 29$) confirmed that during the growing season, increasing temperatures promoted higher plant metabolic rates and more recent plant-derived C was respired from these soils.

The radiocarbon analyses used to estimate the proportional contributions of autotrophic and heterotrophic sources to R_{ECO} (equations 1 and 2 described in the material and methods section) corroborated that 13 years of warming did not significantly alter the average age of the C respired when compared with the control soil, and at both sites approximately two-thirds of CO_2 emissions were autotrophic. Warming resulted in a slightly increased contribution from autotrophic sources, with 68% of CO_2 emissions being plant-derived at SNK, compared to 66% at the control, however, the differences were not statistically significant (Fig. 2b).

3.2 Effects of warming on the age of soil C and DOC production

Bulk ^{14}C analyses of the soil samples showed quite variable ages with no significant differences between bulk samples taken from the systems held at the two sites. Furthermore, with only few exceptions, ^{14}C values were usually lower than the respective contemporary atmospheric value (Fig. 3a). Interestingly, the samples collected at the control site (GDF) showed a gradual decline in ^{14}C enrichment over time, confirming the presence of substantial amounts of pre-bomb C (Fig. 3a).

Despite warming leading to a significant progressive increase in DOC production (Fig. 3b; ANOVA: time*site, $F_{4,25} = 2.59$, $P = 0.0401$), and representing an overall increase of 19.3% when compared to the equivalent amount leached from the control site, the overall averaged differences between sites were not significant (Fig. 3b inset). However, these observed increases in DOC concentrations at the warmed site (SNK) were positively correlated with the ^{14}C content present in this soluble C fraction (Pearson correlation coefficient r = 0.5206; $P = 0.0466$; n = 15), resulting in the DO^{14}C values at the warmed site becoming more ^{14}C enriched and almost identical to the contemporary atmosphere (Fig. 3c). In contrast, at the control site, a significant decline in DO^{14}C values was observed over time (Fig. 3c). This indicates that warming either reduced the amount of old (pre-bomb) carbon being lost as DOC, or that old DOC loss was extremely small compared to the production of modern DOC at the warmed site.

3.3 Effects of warming on the population size and the ^{14}C age of the C assimilated by dominating enchytraeid populations

There was considerable inter-annual variation in enchytraeid abundances during the investigated period, and population differences between sites were significant in the summer of 2007 only (ANOVA: site, $F_{1,28}= 8.94$, $P = 0.0403$), when 1.7 times more enchytraeids were recorded at SNK-warm treatment than at the control site (Fig. 4a). Despite the high temporal variability, on three sampling occasions, their numbers were higher at the warmed site than at the control site (Fig. 4a), which resulted in an overall difference of 23% between the two sites, albeit not significant (Fig. 4a inset). Furthermore, with the exception of year 2006, the enchytraeid populations were significantly concentrated in the upper soil layers at both sites (ANOVA: depth, $F_{4,145} = 4.22$, $P = 0.0032$; Fig. S4) and, on average, 72% and 65% of the total population remained in the top 0-4 cm at the control (GDF) and warm (SNK) sites, respectively (Fig. 4b), where they could access a wide range of C sources.

Indeed, the radiocarbon analyses of the enchytraeid tissues showed they were consistently ^{14}C-enriched relative to the contemporary atmosphere (Fig. 4c) and, during the first five years, the samples from SNK-warm treatment were slightly ^{14}C-depleted relative to the GDF control (Fig. 4c). But the situation reversed after 2010 and, by the end of the investigated period, the ^{14}C content of the animal tissues was lower at the control site (Fig. 4c). Despite these temporal variations in the ages of the C assimilated by enchytraeids (ANOVA: year, $F_{4,22} = 11.03$, $P < 0.0001$), on average, the ^{14}C concentrations in the enchytraeids tissues were significantly lower at the warmed site than those from the control populations (ANOVA: site, $F_{1,25} = 4.48$, $P = 0.0049$). This indicates a greater assimilation of the more recent (plant-derived) C sources (on average ~9 yrs old) at the warmer site than under control conditions (on average ~14 yrs old).

4 DISCUSSION

Over the thirteen years of the transplant study, CO_2 flux showed a consistent positive response to transplantation to the warmer site, reflecting an apparent strong link between ambient temperatures and overall C mineralisation. Although the rainfall inputs at the two sites differ by 20%, previous rainfall manipulation experiments across this climatic transect have shown that water is not a limiting factor, and that differences in thermal regimes dominate soil responses to transplantation (Ineson et al., 1998). Importantly, we did not find any evidence for microbial thermal acclimation (Crowther and Bradford, 2013; Ye et al., 2019; Guo et al., 2020) nor labile substrate depletion (Hartley et al., 2008, 2012; Chen et al., 2020), which are the main explanations for previously reported progressive declines of CO_2 emissions with time after temperature increases. Unlike these previous experiments, we subjected, under natural conditions, plant-soil intact systems to simulated climatic warming over a long time period and thus, allowed for the natural variations in the amount and composition of C forms that the plants return to soil (De Deyn et al., 2008). Furthermore, the soil under study contained large amounts of C which can be gradually mobilized, with microorganisms and their extracellular enzymes remaining active even after readily available substrates are exhausted and other (more recalcitrant) C sources become available (Kuzyakov, 2010). Despite the marked seasonal and inter-annual variation observed during the investigated years, the warming effect on soil respiration was found at the majority of samplings, confirming this long-lasting stimulating effect of warming on CO_2 release from these organic-rich systems, similar to those reported for sub-arctic systems (Dorrepaal et al., 2009; Koven et al., 2017).

Radiocarbon analyses showed that, at both sites, as well as contemporary plant-respired C, older (post-bomb) soil-respired C was also emitted from these soils, although still resulting in ^{14}C signals that were only slightly enriched relative to the contemporary atmosphere. Previous studies have also shown that, although most respired CO_2 is derived from recently deposited plant matter (Evans et al., 2006; Marwick et al., 2015; Pries et al., 2015; Jia et al., 2019), pre-bomb contributions can occur (Neff et al., 2006; Trumbore, 2009; Hartley et al., 2008, 2012; Hopkins et al., 2012; Cheng et al., 2017; Olid et al., 2020). However, since at both sites the rate of ^{14}C decline in the respired CO_2 paralleled closely the rate of ^{14}C decline in atmospheric CO_2, we can conclude that warming did not stimulate the release of old C and the autotrophic component dominated the contributions to the CO_2 efflux at both sites. In support of this, the radiocarbon partitioning results confirmed that the proportion of respiration coming from the younger plant-derived sources increased relative to the proportion of respiration coming from the heterotrophic component in response to warming. A similar shift in R_{ECO} towards the autotrophic component has been reported previously for permafrost ecosystems, more noticeable if the dominant vegetation was composed of vascular plants (Pries et al., 2013, 2015; Peng et al., 2015). Since our transplanted intact cores initially contained identical soil and vegetation, the increased contribution of autotrophic respiration observed here can only be attributed to warming, but the dominance of a vascular plant community in our systems was probably responsible for the high contributions of root respiration (> 65%) compared to those systems dominated by non-vascular plants such as mosses (Pries et al., 2013, 2015). Indeed, in these UK upland systems, the relationships between warming and net primary productivity have been well studied, with demonstrations of the importance of increased nutrient mineralisation in controlling plant productivity (Rawes and Welch, 1969); accelerated turnover of SOM due to warming causes increased release of nutrients and in turn, promotes plant growth (Ineson et al., 1998; Shaver et al., 2000). The peaty gley source site in this study is dominated by fast growing graminoids, including *Juncus squarrosus* which has been reported to be twice as productive as any other grasses present in the area (Rawes and Welch, 1969). This group of plants exhibit specific traits that not only allow them to promote C sequestration through high root and rhizome biomass, but also to stimulate C loss through high rates of root exudation, high litter quality and air channels in roots and stems (Cornelissen et al., 2007).

Bulk ^{14}C analyses of the soil samples, although showing great variability, indicated a trend towards a greater contribution of pre-bomb C over time at the control site when compared to the warmed site. This supports the suggestion that relatively less new C (e.g. root exudates, litter, etc.) was entering the soil and, consequently, the amount of young C being mineralised was not being replaced at the same rate at which it was being lost. This contrasted with the increase in the contemporary C contribution to C exports observed at the warmed site, which resulted, at the end of the experiment, in the age of the DOC from the warmed system overlapping with that of the atmosphere. These findings are also consistent with previous ^{14}C data for the nearby Moor House site (Tipping et al., 2010). These authors showed that DOC in stream waters at Moor House was dominated by a ‘fast pool’ of around 5 years in age, but also included a contribution from a ‘slow pool’ with a mean residence time in the soil of ~20 years. Indeed, the progressive increase in DOC production rates at the warmed site with time, and its positive correlation with the evolution of the ^{14}C content present in this soluble C fraction at SNK-warm treatment, confirms previous results using the same altitudinal transect that DOC release in this area is strongly linked to contemporary plant production (Harrison et al., 2008). This regulating effect of primary productivity on the age of the C mobilised from these soils also explains why at the control site less modern DOC was produced (due to lower biological activities), resulting in the observed decline of DO^{14}C values over time.

Although plants play an important role in controlling C dynamics in peatlands, increases in SOM turnover in these soils as a response to warming have also been shown to be directly related to the activities and vertical distribution of enchytraeids (Briones et al., 2010). Here, the greater enchytraeid population sizes observed at the warmed site, when compared with the control, confirms the positive influence of warmer temperatures on enchytraeid reproduction rates (Briones et al., 1997). However, while warmer temperatures promote enchytraeid reproduction, decreases in soil water content force their downward migration through the soil profile (Briones et al., 1997). Nevertheless, on nearly all sampling occasions, the enchytraeid populations were significantly concentrated in the upper soil layers at both sites, and thus we can conclude that moisture conditions remained favourable for their activities at both sites during the whole investigated period (Briones et al., 1997). Critically, in the upper layers of this peaty gley soil, enchytraeids can access a wide range of C sources (ranging from recently fixed carbon to decades-old material; Huang et al., 1996) and the radiocarbon analyses of enchytraeid tissues showed a greater assimilation of C that had resided in the ecosystem for more than a decade (on average ~14 yrs old) at the control site. The results from the partitioning study showing a lower contribution of the autotrophic component to R_{ECO} at the control site confirmed that the more limited supply of fresh labile plant-derived C substrates entering the soil was also reflected in the animal feeding activities by showing a greater assimilation of older C sources. This feeding flexibility exhibited by enchytraeids in response to changes in resource availability and quality has been observed in previous plant-free laboratory incubations of this soil (Briones et al., 2010). This implies that, in stark contrast to some previous results with mineral soils (Fontaine et al., 2007), a reduced supply of fresh plant-derived C does not necessarily prevent biological access to more recalcitrant organic pools in soils but may, importantly, have the reverse effect when the feeding habits of the dominant soil fauna are taken into consideration.

In conclusion, our findings suggest that vegetation exerts a crucial control on ecosystem C turnover in these organic rich-soils, and despite 13 years of simulated warming of soils containing C aged up to hundreds of years old, warming did not lead to a significant release of old C. Indeed, C turnover did not acclimate to temperature, with warming resulting in higher CO_2 emissions and

DOC release, which shows the sensitivity of contemporary C turnover to climate change. Therefore, as long as these autothrophic responses can be maintained in the long term these thick organic soils could be preserved. However, recent evidence indicates that changes in hydrology (Tiang et al., 2020) and/or in the composition of the plant (Street et al., 2020) and faunal communities (Juan-Ovejero et al., 2020) might compromise the C sink capacity of these northern peatlands. Clearly, the long-term temperature sensitivity of SOC turnover in these systems will strongly depend on the balance between the environmental constraints on SOM decomposition (soil physical and chemical characteristics), plant community composition and the biological accessibility of labile and recalcitrant pools, which is also driven by climatic variations (temperature and precipitation); these interactive effects need to be adequately incorporated into soil C models for them to make accurate predictions of future soil C stores.

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Figure legends

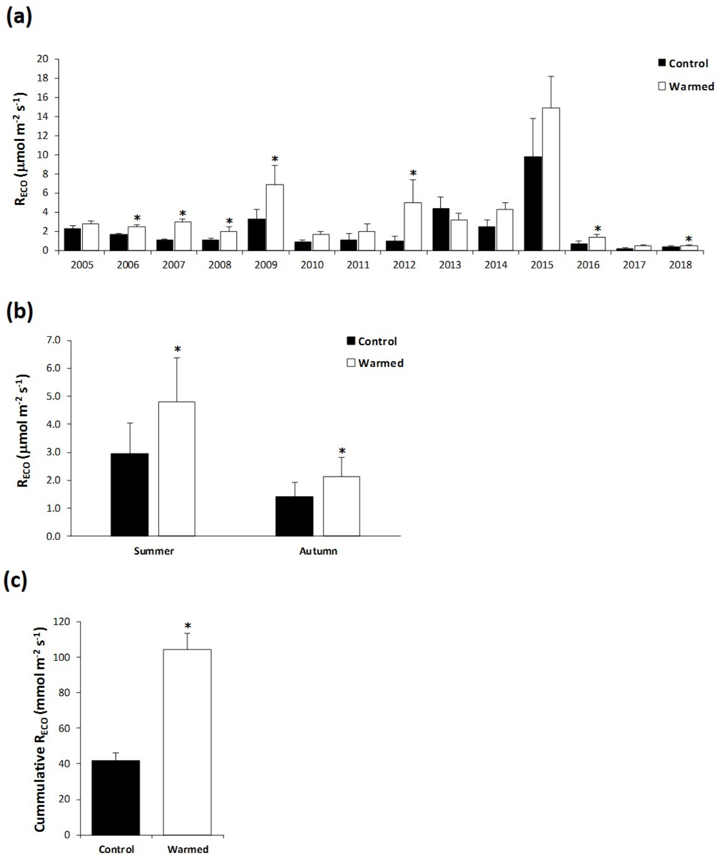
Figure 1. Effects of long-term climate change manipulations on CO_2 emissions at GDF (control treatment) and SNK (warmed treatment). **(a)** Averaged ecosystem respiration (R_{ECO}) at GDF and SNK for every full sampled year (averaged values of the two seasonal samplings (2005-2017)) and the final summer values measured in August 2018. **(b)** Averaged seasonal R_{ECO} during the whole investigated period (2015-2018) at each site. **(c)** Cumulative R_{ECO} values during the whole investigated period (2005-2018) at each site. Error bars represent standard errors (SE) and asterisks show significant differences between treatments per sampling time ($P < 0.05$).

Figure 2. Effects of long-term climate change manipulations on the age of the respired CO_2 at GDF (control treatment) and SNK (warmed treatment). **(a)** Linear regressions of the ^{14}C concentrations (%modern) of respired CO_2 for samples collected in August 2006, 2007, 2008, 2010 and 2018 in the control and warmed treatments ($n = 15$) together with the atmospheric value at the time of the sampling. **(b)** R_{ECO} partitioning into autotrophs and heterotrophic contributions; error bars represent standard errors (SE) and asterisks show significant differences between sites ($P < 0.05$; $n = 3$).

Fig. 3. Effects of long-term climate change manipulations on soil C and DOC leached at GDF (control treatment) and SNK (warmed treatment). **(a)** Linear regressions of the ^{14}C concentrations (%modern) of soil samples collected in August 2006, 2007, 2008, 2010 and 2018 ($n = 15$) in the control and warmed treatments together with the atmospheric value at the time of the sampling. (**b**) DOC concentrations (mean +/- SE; $n = 3$) in the leachates collected in August 2006, 2007, 2008, 2010 and 2018 in the control and warmed treatments together with the overall mean per site (inset). (**c**) Linear regressions of the ^{14}C concentrations (%modern) of DOC collected in August 2006, 2007, 2008, 2010 and 2018 in the control and warmed treatments ($n = 15$) together the atmospheric value at the time of the sampling.

Fig. 4. Effects of long-term climate change manipulations on abundances, vertical distribution and C assimilation of enchytraeids collected at GDF (control treatment) and SNK (warmed treatment). **(a)** Averaged abundances of enchytraeids (individuals m^{-2}) recorded in August 2006, 2007, 2008, 2010 and 2018 ($n = 3$) together with the overall mean per site (inset). (b) Vertical distribution of enchytraeids (individuals m^{-2}) along the soil profile (0-2, 2-4, 4-6, 6-8 and 8-10 cm) during the whole investigated period. Error bars represent standard errors (SE) and asterisks show significant differences between sites (insets) and between sites per sampling date ($P < 0.05$; $n = 3$). **(c)** Linear regressions of the ^{14}C concentrations (%modern) of enchytraeid tissue samples collected in in

August 2006, 2007, 2008, 2010 and 2018 in the control and warmed treatments (n = 15) together the atmospheric value at the time of the sampling.



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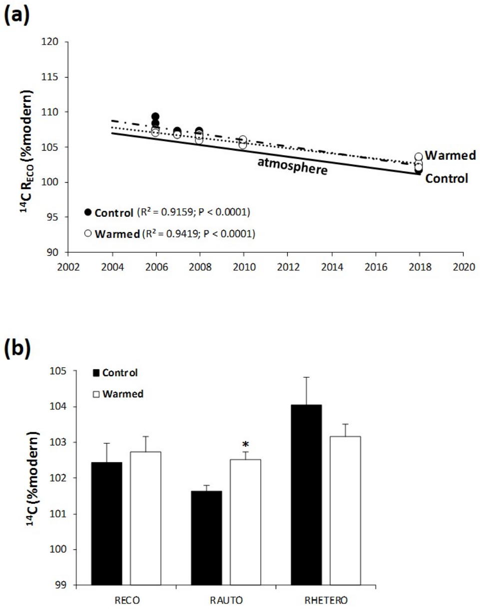

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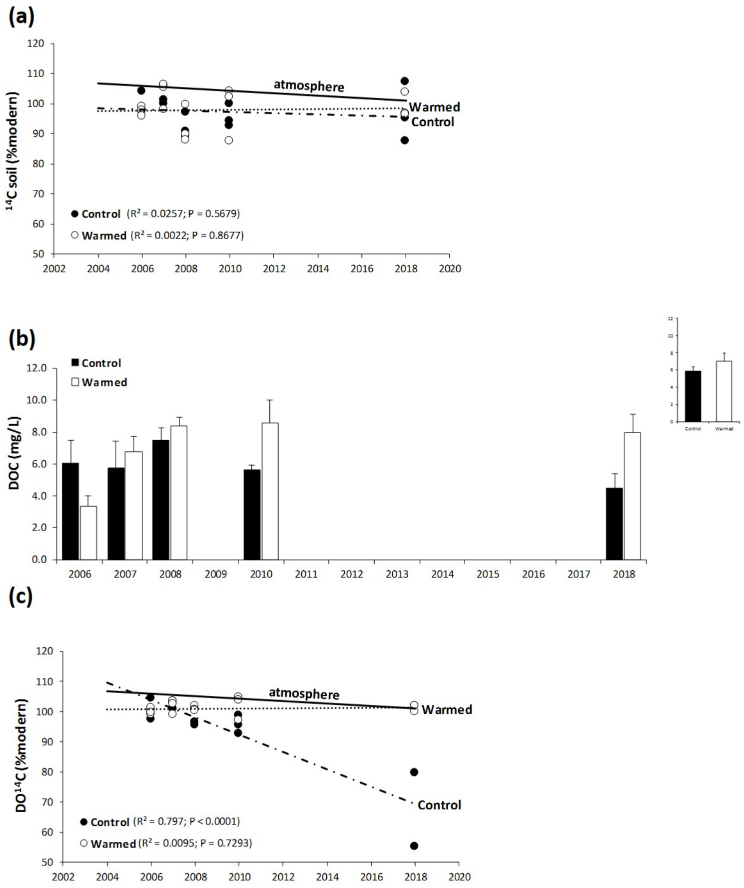

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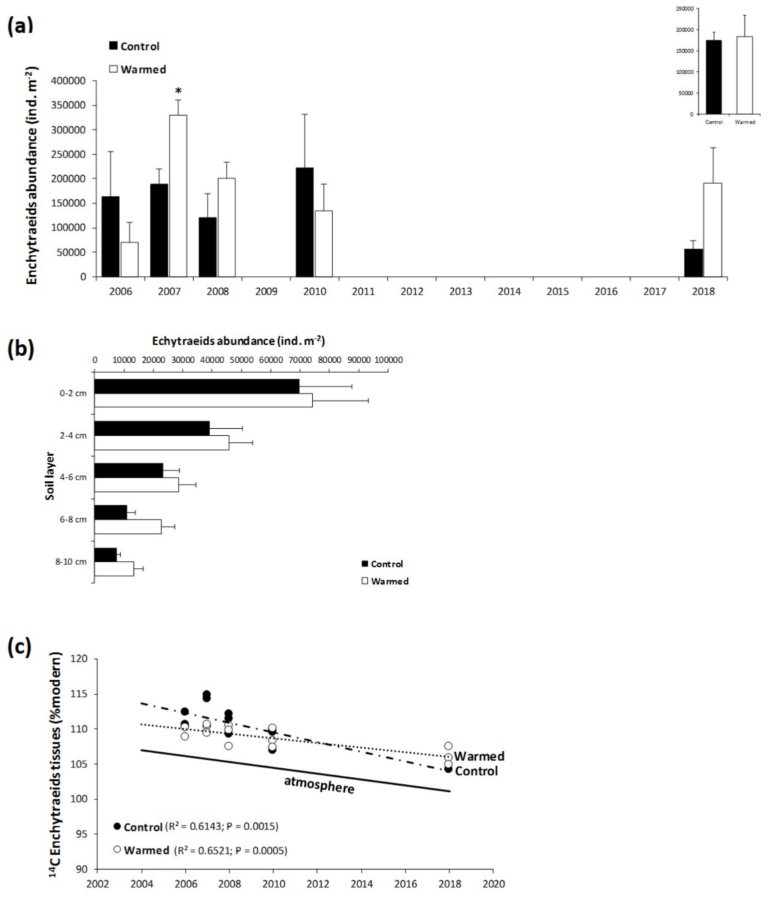

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