View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Bangor University Research Portal

PRIFYSGOL BANGOR / BANGOR UNIVERSITY

PRIFYSGOL
BANGOR
UNIVERSITY

Isotopic methods for non-destructive assessment of carbon dynamics in shrublands under long-term climate change manipulation

Andresen, Louise; Dominguez, Maria; Reinsch, Sabine; Smith, Andrew; Schmidt, Inger K. ; Ambus, Per; Beier, Claus ; Boeckx, Pascal; Bol, Roland; de Dato, Giovanbattista

Methods in Ecology and Evolution

DOI:
10.1111/2041-210X.12963

Published: 01/04/2018

Peer reviewed version

Cyswllt i'r cyhoeddiad / Link to publication

Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA):
Andresen, L., Dominguez, M., Reinsch, S., Smith, A., Schmidt, I. K., Ambus, P., Beier, C., Boeckx, P., Bol, R., & de Dato, G. (2018). Isotopic methods for non-destructive assessment of carbon dynamics in shrublands under long-term climate change manipulation. *Methods in Ecology and Evolution*, *9*(4), 866-880. https://doi.org/10.1111/2041-210X.12963

Hawliau Cyffredinol / General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal ?

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

09. Oct. 2020

1 **Isotopic methods for non-destructive assessment of carbon dynamics in shrublands**
2 **under long-term climate change manipulation**
3

4 L.C. Andresen[1*)], M.T. Domínguez[2 **)], S. Reinsch[2], A.R. Smith[4,2], I.K. Schmidt[3], P. Ambus[3],
5 C. Beier[3], P. Boeckx[6], R. Bol[7,1], G. de Dato[8**)], B.A. Emmett[2], M. Estiarte[11,12], M.H.
6 Garnett[13], G. Kröel-Dulay[14], S. Mason[1], C.S. Nielsen[15], J. Peñuelas[11,12], and A. Tietema[1].

7 **Running head:** Isotope techniques for ecosystem C science

8 ***Tweet:*** *The pros and cons of carbon assessment methods using isotopes across climate change*
9 *experiments in shrublands.*

10 Affiliations:

11 [1]University of Amsterdam, NL; [2]Centre for Ecology and Hydrology, Bangor, UK; [3]University
12 of Copenhagen, Dept. Geosciences and Natural Resource Management, DK; [4]School of
13 Environment, Natural Resources & Geography, Bangor University, Bangor, UK. [5]Institute of
14 Forest Ecology, BOKU, AU; [6]Isotope Bioscience Laboratory - ISOFYS, Ghent University,
15 BE; [7]Institute of Bio- and Geosciences, IBG-3: Agrosphere, Forschungszentrum Jülich, Jülich,
16 DE; [8]Department for Innovation in Biological, Agro-food and Forest systems, University of
17 Tuscia, Viterbo, IT; [9]Forest & Nature Lab, Ghent University, BE; [10]National Research
18 Council, Institute of Biometeorology, Sassari, IT; [11]CSIC, Global Ecology Unit CREAF-
19 CSIC-UAB, Cerdanyola del Vallès (Catalonia), SP; [12]CREAF, Cerdanyola del Vallès,
20 Barcelona, Catalonia, SP; [13]NERC Radiocarbon Facility, Scottish Enterprise Technology
21 Park, Rankine Avenue, East Kilbride, Scotland, UK; [14]Centre for Ecological Research,
22 Institute of Ecology and Botany, Hungarian Academy of Sciences, HU; [15]Department of
23 Forest Ecology and Management, Swedish University of Agricultural Sciences, SE.
24

25 *) Corresponding author´s current address: Department of Earth Sciences, University of Gothenburg, PO Box 460,
26 405 30 Göteborg, SE.

27 **) Current address: Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), SP.

28 ***) Current address: Council for Agricultural Research and Economics, Forestry Research Centre (CREA-SEL),
29 IT. (g.dedato@entecra.it)
30

Summary

1. Long-term climate change experiments are extremely valuable for studying ecosystem responses to environmental change. Examination of the vegetation and the soil should be non-destructive to guarantee long-term research. In this paper, we review novel field methods using isotope techniques for assessing carbon dynamics in the plant-soil-air continuum, based on recent field experience and examples from a European climate change manipulation network.
2. Eight European semi-natural shrubland ecosystems were exposed to controlled warming and drought manipulations. One field site was additionally exposed to elevated atmospheric CO_2. We evaluate the isotope methods that were used across the network to evaluate carbon fluxes and ecosystem responses: 1) analysis of the naturally rare isotopes of carbon (^{13}C and ^{14}C) and nitrogen (^{15}N); 2) tracing changes in isotopic signatures in ecosystem compartments *in-situ*, by using pulse labelling with $^{13}CO_2$, soil injections of ^{13}C- and ^{15}N-enriched substrates, or continuos labelling with ^{13}C-depleted CO_2 by Free Air Carbon dioxide Enrichment (FACE); and 3) manipulation and tracing the isotopic composition of soil substrates (^{14}C) in lab-based studies.
3. Questions related to long-term carbon turnover processes were investigated by natural ^{14}C signals, specifically ^{14}C signature of soil respiration gave insights into the decomposition of old soil carbon sources. Contrastingly, the stable isotopes ^{13}C and ^{15}N were used for shorter-term processes, as the residence time in a certain compartment of the stable isotope label signal is limited. ^{13}C-labelling techniques exert a minimal physical disturbance, however, the dilution of the applied isotopic signal can be challenging, and the contamination of the field site with released excess ^{13}C can be a problem for subsequent natural abundance (^{14}C and ^{13}C) or label studies.
4. Based on the experience with the experimental work, we provide recommendations for the application of the reviewed methods to study carbon fluxes in the plant-soil-air continuum in long-term, large-scale climate change experiments.

Key-words: warming; drought; bomb-C; FACE; pulse-labelling; stable isotopes; ^{14}C

60 # Inhalt

61 Isotopic methods for non-destructive assessment of carbon dynamics in shrublands under long-term
62 climate change manipulation 1
63 Summary 2
64 Introduction 3
65 Methodologies using natural abundance of carbon isotopes 6
66 1 Ecosystem processes reflected by stable isotope fractionation (^{13}C and ^{15}N) 6
67 2 Bomb-^{14}C technique to asses sources of soil respiration 8
68 Methods using *in-situ* ^{13}C labelling to study rhizodeposition utilisation 10
69 1^{13}C-CO_2 pulse labelling 11
70 2 Free Air Carbon dioxide Enrichment (FACE)-labelling 13
71 3 In situ injection of ^{13}C-enriched substrate solutions 14
72 Use of labelled carbon-compounds to analyse carbon mineralisation by soil microorganisms 16
73 Conclusions and recommendations 17
74 Acknowledgements 18
75 References 18
76 Table 30
77 Box 1. Isotopic signal of plant leaf responses to precipitation 32
78 Box 2. Impact of warming and drought on the ^{14}C signature of soil respiration 34
79 Box 3. Analysing rhizodeposit utilisation by microbes in the field 36
80 Box 4. Exponential decay kinetics for $^{14}CO_2$ evolution during microbial ^{14}C substrate mineralisation
81 38
82

83

84

85 # Introduction

86

87 Global climate change scenarios predict that increased greenhouse gas (e.g. CO_2, CH_4 and
88 N_2O) concentrations in the atmosphere will alter the periodicity and magnitude of drought
89 events and will increase mean global temperatures by approximately 0.2 °C per decade (IPCC
90 2013). For the European continent this will manifest as drier summers in the South and
91 increased precipitation in the North (IPCC 2013). Elucidating the consequences of such
92 atmospheric changes for biogenic carbon fluxes is one of the main challenges for the scientific

93 community. Some models have predicted a positive feedback to climate change, resulting from
94 higher increases in respiratory fluxes from ecosystems (e.g. carbon release through soil
95 respiration) than in net primary productivity, which would lead to further increases in
96 atmospheric CO_2 (Friedlingstein et al. 2006; Denman et al. 2007). To assess the likelihood of
97 positive feedback, experimental studies that analyse the long-term adaptations of ecosystem
98 carbon fluxes to climate change are critically needed. However, climate change experiments
99 are often conducted at short or medium time scales due to funding constraints, or due to the
100 limited life-span of the experimental plots, as repeated removal of samples often leads to
101 disturbances and experimental artefacts in the studied system. Hence, there is a necessity for
102 the maintenance of long-term experiments using non-destructive methods.

103 Carbon fluxes through the plant-soil-air continuum play a central role in soil carbon cycling.
104 Drought and warming alter the quantity and composition of carbon inputs to the soil by
105 changing plant available carbon and nitrogen sources (de Graaff et al. 2007; Leakey et al. 2009).
106 Microbial growth in soil is generally constrained by available carbon, therefore qualitative and
107 quantitative changes in rhizodeposition are likely to alter the activity of heterotrophic
108 microorganisms and the rates of soil organic matter (SOM) mineralisation (Zak et al. 2000;
109 Phillips et al. 2006). Consequently, aboveground to belowground fluxes might largely
110 determine carbon emissions from ecosystems under the different climate change scenarios
111 (Chapin et al. 2009).

112 Stable carbon isotope studies can give important insights into carbon fluxes through the plant-
113 soil-air continuum with the minimal disturbance to the system. The isotopic carbon composition
114 of compartments in this continuum is a result of the different isotope fractionation processes
115 along the pathway from CO_2 fixation by plants to carbon allocation to soil (reviewed in
116 Brüggemann et al. 2011). Thus, the analysis of the natural abundance of carbon isotopes in
117 these compartments can give information about some processes related to photosynthesis and
118 carbon losses through plant or soil respiration (Brüggemann et al. 2011). In addition, *in-situ*
119 pulse labelling with the heavy stable carbon isotope (^{13}C) is a powerful tool to analyse short-
120 term dynamics of carbon allocation to the soil with high resolution (Högberg et al. 2008; Epron
121 et al. 2012; Reinsch & Ambus 2013). The recent development of techniques for ^{13}C analyses in
122 specific compounds such as phospholipid fatty acids (PLFAs), amino-sugars, RNA and DNA,
123 constitutes a remarkable advance in the studying of carbon cycling. These analyses allow for
124 the examination of rhizodeposit utilisation by microbes or trophic interactions between
125 functional groups (Ostle et al. 2003; Jin & Evans 2010; Ruess & Chamberlain 2010). The

126 application of these isotopic methods can therefore provide unique information about
127 aboveground-belowground linkages and their alterations in response to climate changes.

128 In order to investigate long-term effects of climate change on shrubland ecosystems, an
129 experimental network was established across Europe (the INCREASE network). Studying the
130 response of shrublands to climate change is important, since they are representative ecosystems
131 in Mediterranean and North European countries, where they play an important ecological role
132 in preserving biodiversity (Verdú 2000; Wessel et al. 2004). In addition, land area covered by
133 shrublands has dramatically decreased in temperate Europe during the past century, due to land
134 use changes, increased pollution and eutrophication, and climate change (Fagúndez 2013). In
135 Mediterranean regions, however, shrublands have increased their extension due to land
136 abandonment (Fagúndez 2013).

137 Within the climate change network, common non-destructive methods were used across sites
138 to ensure the comparison of treatment effects across different climatic regions (Beier et al. 2004;
139 Mikkelsen et al. 2008). Evaluating the impact of climate change treatments on shrubland carbon
140 dynamics was one of the main objectives of this experimental network, and thus a range of
141 methodologies to quantify and trace distinct carbon pools and their fluxes have been applied
142 since 1999. Priority was given to those techniques that minimise disturbances to vegetation and
143 soil to guarantee long-term research.

144 Here, we review isotope methods that have been applied across this climate change
145 experimental network to study ecosystem carbon dynamics in the plant-soil-air continuum. In
146 particular, we focus on methodologies that: 1) analyse the abundance of naturally rare isotopes
147 of carbon (^{13}C and ^{14}C) and nitrogen (^{15}N) in the different ecosystem compartments to evaluate
148 their responses to climate change; and 2) trace experimentally-induced changes in the isotopic
149 signatures to assess rhizodeposition utilisation by soil biota, by using either $^{13}CO_2$ pulse
150 labelling, continuous labelling with ^{13}C-depleted CO_2 from Free Air Carbon dioxide
151 Enrichment (FACE), or injections of ^{13}C- and ^{15}N-enriched substrates in the field, and finally
152 3) manipulate and trace the isotopic composition of C-compounds to analyse C mineralisation
153 by soil microorganisms in laboratory studies. Along-side the methods, data from the field
154 studies are presented as accompanying illustrative boxes, and practical recommendations for
155 the applications of these methodologies at large-scale climate change experiments are outlined
156 in Table 1. The combination of the isotope methods with methods for *in-situ* quantification of

157 aboveground, root and fungal mycelia biomass will increase our understanding of climate
158 change effects on carbon dynamics with the least possible disturbance.
159

160 **The experimental climate change network INCREASE**

161 The experimental network for the study of climate change impacts on European shrublands
162 (INCREASE, 'An Integrated Network on Climate Research Activities on Shrubland
163 Ecosystems') was established in 1998. The network is comprised of eight shrublands situated
164 across a natural temperature gradient of mean annual temperature from c. 8 °C in the North to
165 c. 16 °C in the South, and a rainfall gradient ranging from 510 mm to 1741 mm from East to
166 West (see Fig. 1 in Reinsch et al. 2017). These sites represent Continental, Atlantic and
167 Mediterranean shrublands. At each site, whole-ecosystem warming and drought treatments
168 were applied in triplicates of 20 m^2 plots, by using automated retractable curtain constructions
169 (see Beier et al. 2004 and Mikkelsen et al. 2008 for a full description). At one of the Danish
170 sites (DK-BRA), a FACE treatment was installed, and combinations of the climate treatments
171 were established and resulted in a plot size of 9 m^2 (Mikkelsen et al. 2008). Climatic conditions
172 at the plot level (air temperature, humidity, soil temperature and moisture) were recorded in
173 half-hour or hourly intervals, and main carbon pools and fluxes have been periodically
174 monitored. Most frequent measurements include aboveground plant biomass and composition
175 (Kröel-Dulay et al. 2015), litter production, soil respiration and net ecosystem carbon exchange
176 (Beier et al. 2008; 2009; Lellei-Kovács et al. 2016). Measurements of litter decomposition, soil
177 nitrogen mineralisation (Emmett et al. 2004) and soil microbial biomass and activity (Sowerby
178 et al. 2005) have also been conducted with different periodicity.

179

180

Methodologies using natural abundance of carbon isotopes

181

182

1 Ecosystem processes reflected by stable isotope fractionation (^{13}C and ^{15}N)

183

184 The relative abundance of the rare and heavy stable isotopes of nitrogen (^{15}N) and carbon (^{13}C)
185 compared to the most abundant stable isotope, ^{14}N and ^{12}C respectively, is a signature that
186 reflects the isotopic discrimination associated with gain and loss processes of a given entity.
187 These signatures are expressed as the delta (δ) notation (e.g. $\delta^{13}C$ and $\delta^{15}N$ in ‰), which is the
188 deviation of the rare isotope abundance in the sample compared to a reference material (Brand

189 & Coplen 2014; Muccio & Jackson 2009). The naturally occurring background level is termed
190 'natural abundance' of the given rare stable isotope (Berglund & Wieser 2011). Most natural
191 processes (chemical, physical or enzymatically catalysed) discriminate against heavy isotopes
192 (e.g. ^{13}C, ^{15}N, ^{18}O), which in open systems results in an isotopically depleted product with
193 comparably smaller concentration of the heavy isotopes than its corresponding substrate
194 (Robinson 2001; Fry 2006). If the dominant process rate changes, or if the substrate is
195 exhausted, then the δ value of the product (such as the plant leaf) may significantly change, due
196 to the underlying fractionation. Delta notation is appropriate when dealing with natural samples
197 that are not labelled with excess heavy isotopes (see next sections), and variations at the level
198 of natural abundance are useful for evaluating natural discrimination processes. Importantly,
199 natural abundance cannot be studied if 'overlain' by a study of labelling with heavy isotopes.

200 Decreases in soil water availability due to drought can alter the isotope signature of both carbon
201 and nitrogen in the aboveground plant biomass. During drought stress, leaves reduce stomatal
202 opening to preserve water. As this happens, the space that confines the air as an immediate
203 source of CO_2 for photosynthesis (the sub-stomatal cavity) becomes a more closed system due
204 to the restriction of the renewal of CO_2, and as a result a higher proportion of the heavy ^{13}C in
205 CO_2 is fixed by Rubisco (C3 plants; Tcherkez et al. 2011). Hereby the discrimination against
206 the heavy ^{13}C isotope is decreased. As a consequence, in plants with a C3 photosynthetic
207 pathway a ^{13}C enrichment in the leaf occurs during drought stress (Cernusak et al. 2013).
208 Indeed, the ^{13}C enrichment at the leaf level is related to an increased intrinsic water use
209 efficiency (WUEi), the ratio of assimilation to stomatal conductance (Farquhar & Richards
210 1984; Donovan & Ehleringer 1994). Changes in soil water availability may also alter the leaf
211 nitrogen isotope signature by changing the nitrogen availability with soil depth, and thereby the
212 ^{15}N signature of the plant nitrogen source (Lloret et al. 2004). Since $\delta^{15}N$ is often analysed at
213 the same solid sample as $\delta^{13}C$ by IRMS (Isotope Ratio Mass Spectrometer), interpretation of
214 $\delta^{15}N$ can be a useful complement to understand the ecosystem processes. Nitrate and
215 ammonium sources of different origin or at different soil depths can vary in $\delta^{15}N$ signature (Xue
216 et al. 2009). Hence, if a drought event changes the vertical nitrogen availability in the soil, the
217 plant nitrogen source can shift to a different soil depth possibly causing a change in $\delta^{15}N$
218 signature in the leaves. In general, an increase in $\delta^{15}N$ signature in the leaves indicates a
219 progressive N saturation and/or N losses in the surrounding system because all major pathways
220 of N loss (denitrification, ammonia volatilization and nitrate leaching) cause $\delta^{15}N$ enrichment
221 of the remaining nitrogen (Peñuelas et al 2000). Interpretation of changes in leaf $\delta^{15}N$, however,

222 is not straightforward since leaf $\delta^{15}N$ signatures might largely depend on mycorrhizal
223 associations and shifts in nitrogen sources between organic and inorganic compounds (the
224 increase in plant δ15N values with aridity may also result from increasing reliance on recycled
225 organic N sources as opposed to new inputs) under a drought or warming could influence the
226 leaf $\delta^{15}N$ as well (Michelsen et al. 1998; Pardo et al. 2006; Andresen et al. 2009).

227 Across the field sites, the effects of warming and drought on plant ^{13}C and ^{15}N natural
228 abundance was monitored over four years, starting two years after onset of the climate
229 manipulations. Current year shoots or leaves were analysed for $\delta^{13}C$ and $\delta^{15}N$ immediately after
230 each artificially prolonged drought. Plant material was dried at 70°C and ground to a fine
231 powder before analysis for natural abundance values of $\delta^{13}C$ and $\delta^{15}N$ using isotope ratio mass
232 spectrometry (IRMS). We expected to find higher $\delta^{13}C$ values: i) in drought treated plants
233 (compared to control plots) and, ii) in plants growing at drier locations across the precipitation
234 gradient (within a given plant species). Furthermore, we expected iii) the $\delta^{15}N$ to change in
235 response to drought, as the nitrogen source (depth) is changed (at one location, within-species).
236 Some significant effects of the drought treatment were observed on plant tissue $\delta^{13}C$ and $\delta^{15}N$
237 (Box 1). Differences between years (effect of time) were more pronounced than the effect of
238 the drought treatment for *Populus alba* $\delta^{13}C$ (HU), *Erica multiflora* $\delta^{15}N$ (SP) and *Globularium*
239 *alypum* $\delta^{15}N$ and $\delta^{13}C$ (SP). Only *Calluna vulgaris* showed a significant response to the drought
240 treatment for $\delta^{13}C$ as hypothesized (Box 1A). For *C. vulgaris*, which was growing at several
241 locations (UK-CL, NL and DK-MOLS), the $\delta^{13}C$ was higher at drier locations, when compared
242 along the precipitation gradient, and also higher in the drought treatment, at the NL or UK-CL
243 sites (Box 1B). Finally, we found no response of leaf $\delta^{15}N$ to drought or warming, however, *P.*
244 *alba* had a much depleted $\delta^{15}N$ relative to the other species. We attribute these differences to
245 species specific utilization of different nitrogen sources (perhaps more dependent on nitrate at
246 the HU site), or different mycorrhizal associations with higher rates of isotopic fractionation
247 (e.g. ericoid mycorrhiza in *E. multiflora* and *C. vulgaris*; Michelsen et al. 1998; Pardo et al.
248 2006).
249

250 ## 2 Bomb-^{14}C technique to asses sources of soil respiration
251

252 The analysis of natural ^{13}C abundance in CO_2 can be used to distinguish between autotrophic
253 and heterotrophic components of soil respiration in some ecosystems where the sources of
254 respired substrates (i.e. recent photosynthates vs. SOM) have contrasting $\delta^{13}C$ values. Changes

255 in $\delta^{13}C$ values can be observed after vegetation changes, e.g. where C4 plants are introduced in
256 areas previously dominated by C3 vegetation, or vice versa, the so-called C3-C4 shift. In these
257 situations, the $\delta^{13}C$ signature of plants and soil carbon may differ by up to 10 ‰ (Hanson et al.
258 2000). The partitioning of soil respiration between historical SOM and recent photosynthates
259 can then be calculated using a linear isotope mixing models (Robinson & Scrimgeour 1995;
260 Hanson et al. 2000). However, such vegetation shifts are rare in natural ecosystems, and
261 therefore the use of ^{13}C natural abundance to differentiate between autotrophic and
262 heterotrophic respiration is limited (but see Millard et al. 2008).

263 As an alternative to stable carbon isotopes (^{12}C, ^{13}C), the natural radioactive ^{14}C abundance can
264 be used to identify sources of soil respiration. Radiocarbon signatures of recent and older carbon
265 are different as a result of the nuclear bomb tests in the atmosphere during the 1950/60s. These
266 tests led to an increase in the ^{14}C content in the atmospheric CO_2 in the Northern hemisphere,
267 which reached its maximum in 1963 ('bomb peak'). Ever since the subsequent atmospheric
268 nuclear test moratorium, the 'bomb-^{14}C' content has decreased due to the dilution with fossil
269 fuel-derived CO_2 in the atmosphere and its incorporation in ocean and terrestrial carbon pools
270 (Trumbore 2009). Through that incorporation, the radiocarbon analysis of ecosystem fluxes
271 provides information about the age of decomposed carbon substrates and can be used to
272 differentiate carbon sources within ecosystems: recently plant-assimilated carbon (autotrophic
273 component of soil respiration) should have a similar radiocarbon signature as the current
274 atmosphere, while the radiocarbon content of older carbon released through SOM
275 mineralisation (heterotrophic component) reflects the year of fixation of that carbon, again a
276 mixing model solution. Several studies have successfully achieved the separation of sources of
277 respiration across ecosystems using the 'bomb-^{14}C' method (Gaudinski et al. 2000; Cisneros-
278 Dozal et al. 2006; Czimczik et al. 2006; Schuur & Trumbore 2006; Subke et al. 2011). In these
279 studies, analysis of the ^{14}C-CO_2 signatures of roots and SOM was performed under controlled
280 conditions and collated with analyses of field gas efflux (the mixed pool). Radiocarbon analysis
281 of soil or ecosystem respiration has been used to evaluate the response of a range of ecosystems
282 to different factors of climate change, such as increasing temperatures, decreasing rainfall or
283 permafrost thaw (Borken et al. 2006; Muhr & Borken 2009; Muhr et al. 2009; Schuur et al.
284 2009).

285 We tested the effect of experimental warming and drought on the natural abundance of ^{14}C in
286 respired soil CO_2 at early stages of the climate manipulations at the Peaknaze field site (UK-
287 PK). Our hypothesis was that drought increased heterotrophic respiration more than warming

in this seasonally waterlogged soil, due to a greater responsiveness of old soil carbon to drought relative to temperature as a driver (Bol et al. 2003; Domínguez et al. 2015). Therefore, we expected the greatest ^{14}C-enrichment in the field-collected soil respiration samples from the drought plots. Soil efflux samples were collected in the late experimental drought period (September 2011), using a molecular sieve sampling system (Bol et al. 1995; Hardie et al. 2005; Hardie et al. 2009) attached to closed dark respiration chambers placed on the soil overnight. CO_2 was subsequently recovered from the molecular sieve traps for ^{14}C analysis by Accelerator Mass Spectrometry (AMS; Box 2). Soil and root samples were collected to conduct separate incubations to obtain the ^{14}C-signatures of the heterotrophic and autotrophic respiration, respectively. These incubations were performed in leak-tight glass jars with a connection to the molecular sieve sampling system. The results revealed a high heterogeneity of the ^{14}C signature of the soil efflux with no significant effect of the warming treatment, and a trend towards the release of older carbon from the drought plots (although not statistically significant). By comparison with the known record of post-bomb atmospheric ^{14}C-CO_2 concentration (Box 2), the carbon being released from the plots was estimated to have been fixed between six and eight years earlier (M. Dominguez, unpublished).

Methods using *in-situ* ^{13}C labelling to study rhizodeposition utilisation

In-situ pulse labelling with the stable carbon isotope (^{13}C) is an efficient method for evaluating the time lag between carbon assimilation and CO_2 release from soil (Kuzyakov & Gavrichkova 2010). It can be applied to investigate a wide range of processes in the plant-soil-atmosphere continuum (Högberg et al. 2008; Brüggemann et al. 2011; Epron et al. 2012). With this method, a concentrated pulse of ^{13}C-enriched or depleted substrate in the form of CO_2 or of a carbon-containing organic substrate, is released into the undisturbed ecosystem. Subsequently assimilation or heterotrophic consumption will transfer the labelled carbon, and the ^{13}C content of the product and organism will reflect the rate and the quantity of carbon transfer from one pool to another (Studer et al. 2014). Analysis of ^{13}C in specific compounds such as PLFAs is a specific tool to assess the utilisation of different carbon sources by different functional groups of soil biota. The development of ^{13}C labelling has increased the recognition of the central role that rhizodeposition plays in soil carbon cycling (Ostle et al. 2003; Jin & Evans 2010; Kuzyakov & Gavrichkova 2010; Dijkstra et al. 2013).

321 **1 $^{13}C-CO_2$ pulse labelling**
322

323 In $^{13}C-CO_2$ pulse labelling experiments, ^{13}C enriched CO_2 is released in closed, intact plant-
324 soil systems during daylight hours, typically for 1.5 to 6 hours, where it is assimilated by the
325 green plant biomass. Plant and soil samples are taken from unlabelled and labelled systems at
326 different time intervals, with a higher sampling frequency within the first 48 hours after the
327 labelling. The allocation of ^{13}C to belowground pools (roots, exudates, microbiota) is
328 subsequently analysed, which allows the determination of the fraction of recently fixed carbon
329 actively utilized by e.g. different microbial functional groups. Using $^{13}C-CO_2$ pulse labelling,
330 several authors demonstrated that the flux of recently photosynthesized carbon to soil microbes
331 occurs very fast, often within a few hours of $^{13}CO_2$ uptake (Rangel-Castro et al. 2005). The
332 maximum incorporation of ^{13}C into microbial RNA occurs within four to eight days after the
333 pulse (Ostle et al. 2003). Fungi typically show the greatest utilisation of plant-derived carbon
334 within the first 48 hours after plant labelling. Lower ^{13}C enrichment in bacterial biomarker
335 PLFAs indicates a delay in the utilisation of plant-derived carbon by bacteria, or a greater
336 dependence of bacteria on carbon sources different from recently-fixed carbon. Gram positive
337 bacteria and, in particular actinomycetes, are known to rely less on plant-derived carbon than
338 Gram negative bacteria (Butler et al. 2004; Treonis et al. 2004; Balasooriya et al. 2008; 2013;
339 2014). The levels of allocation of belowground fixed carbon and the subsequent utilisation by
340 microbes might be affected by a range of factors such as the seasonality of plant activity.
341 Usually, more carbon is allocated belowground towards the end of the growing season
342 (Högberg et al. 2010; Balasooriya et al. 2013), under exposure to elevated atmospheric CO_2
343 concentrations (Denef et al. 2007; Jin & Evans 2010; Reinsch et al. 2013), under drought
344 conditions (Fuchslueger et al. 2014) or in plants grown on fertile soils (Denef et al. 2009;
345 Paterson et al. 2011).

346 In the climate change network, several pulse-labelling experiments have been conducted in
347 combination with ^{13}C-PLFA analyses to study rhizodeposit utilisation by microbes. At the
348 Clocaenog site (UK-CL) we aimed to study the utilisation of rhizodeposits along a soil moisture
349 gradient, by applying a $^{13}C-CO_2$ pulse during the late growing-season (August 2011). We used
350 transparent domes of 50 cm diameter and 100 cm height, enclosing individual *C.*
351 *vulgaris* plants. We applied repeated pulses of $^{13}C-CO_2$ (99 atom% ^{13}C = 99% ^{13}C + 1% ^{12}C)
352 over eight hours (Box 3). The domes were sealed to a frame which was inserted into the ground
353 at least ten days before the pulse, and had several sealed septa to collect gas samples to estimate
354 the concentration of the ^{13}C-labelled CO_2. Plant leaves and soil from the rooting zone were

355 collected at different times after the labelling, using a higher sampling frequency during the
356 first hours after the pulse. Soils were freeze-dried, sieved to ≤ 5 mm and PLFAs were extracted.
357 Fatty acid methyl esters (FAMEs) were analysed by gas chromatography combustion-isotope
358 ratio mass spectrometry (GC-c-IRMS). The main challenge was the low recovery of ^{13}C label
359 in the belowground compartment, especially in individual FAMEs. Despite the applied ^{13}C
360 concentration of 99 atom%, the apparent low photosynthetic rates combined with the excessive
361 dilution of the ^{13}C label in the large carbon pools of unlabelled woody branches and root- and
362 microbial biomass resulted in an overall low level of ^{13}C enrichment in the FAMEs (Box 3).
363 Similar patterns have also been observed in other pulse labelling experiments (Griffith et al.
364 2004).

365 Three pulse-labelling events were conducted at the Brandbjerg site (DK-BRA,) between 2010
366 and 2013 (Box 3). The Brandbjerg experiment consists of drought and warming manipulations
367 in combination with ambient and elevated levels of CO_2 concentration. The developed
368 experimental setup for pulse-labelling aimed i) to be easily deployable in remote areas, ii) to
369 distribute labelled ^{13}C-CO_2 to as many plots at the same time as possible to ensure similar and
370 constant conditions for CO_2 uptake by the vegetation, and iii) to ensure constant CO_2
371 concentration available to the vegetation throughout the labelling period. Therefore, a mobile
372 flow-through system suitable for continuous ^{13}C-CO_2 delivery was developed (Box 3): A gas-
373 tight vinyl balloon (~3 m diameter) was filled with CO_2 free synthetic air and mixed with ^{13}C-
374 CO_2 (50 or 99 atom%) that supplied the transparent chambers enclosing the vegetation of
375 interest with air over the duration of the experiments ranging from 4 to 7.5 hours. Air was
376 pumped continuously through gas tight tubing via electric diaphragm pumps (Reinsch & Ambus
377 2013). The first experiment was conducted at the end of the growing season (October 2010),
378 when we observed the highest allocation of carbon belowground as measured by ^{13}C in soil
379 respiration (Reinsch et al. 2014). The second experiment was conducted in the spring (May
380 2011) and showed a major allocation of carbon to aboveground structures under elevated
381 atmospheric CO_2 concentration, but carbon allocation to belowground structures was higher in
382 drought plots than in untreated control plots. The allocation of recently-assimilated carbon
383 under warming conditions was similar to that under ambient conditions. The last experiment,
384 conducted in early season 2013 (June), was performed during a period with impeded
385 photosynthetic activity and indicated that labelling performance is poor when vegetation is
386 recovering from harsh winter conditions with bare frost or severe drought conditions (Box 3).

387 Thus, it is important that the vegetation of interest displays green, photosynthetically active
388 structures to facilitate CO_2 uptake and sufficient labelling of ecosystem carbon pools.

389 Our studies illustrate the complexity of controlling *in-situ* pulse-labelling experiments in
390 ecosystems dominated by woody plants, which is even more challenging with ^{13}C-CO_2 than
391 with ^{14}C-CO_2 because of their respective atmospheric backgrounds and detection limits (Epron
392 et al. 2012). Ideally, ^{13}C doses for *in-situ* use should be carefully tested in trials, considering
393 the nature of the studied vegetation and the compounds to be analysed. If e.g. specific
394 compounds of the soil microbial biomass are the main interest, then strong isotopic doses should
395 be applied, and it is advisable to deploy the ^{13}C pulse when plants naturally allocate carbon
396 belowground e.g. when preparing for winter. The ^{13}C signal can be increased by using highly
397 labelled ^{13}C-CO_2 (99 atom %). However, the usage of a highly enriched CO_2 can potentially
398 lead to blurry signals and has to be applied with caution (Watzinger 2015). Furthermore, ^{13}C-
399 CO_2 concentration inside the labelling chamber should be as close as possible to ambient
400 values, because unrealistic high CO_2 concentration will change plant CO_2 uptake. Repeated
401 moderated ^{13}C-CO_2 applications during longer exposure times might be more appropriate, but
402 inside closed transparent chambers, temperature and humidity may increase if the labelling
403 period is prolonged, which also affects photosynthetic processes (Epron et al. 2012). Losses of
404 ^{13}C due to physical diffusion and adsorption/desorption into the chamber and tubing material
405 should also be considered. In particular, the back-diffusion of the $^{13}CO_2$ from the soil to the
406 atmosphere which entered the soil pores during the labelling might confound the interpretation
407 of measured belowground respiration (Subke et al. 2009; Selsted et al. 2011). However, when
408 applied properly, the insights into terrestrial carbon allocation will be detailed and novel (Box
409 3).
410

411 ## 2 Free Air Carbon dioxide Enrichment (FACE)-labelling
412

413 An alternative method for ^{13}C pulse-labelling of vegetation and whole-ecosystems is to utilize
414 the ^{13}C-depleted CO_2 in already planned or ongoing FACE experiments. The FACE technique
415 has through decades been used within cropping systems (Kimball 2016), grasslands (Hovenden
416 et al. 2014; Reich et al. 2014; Mueller et al. 2016) and forests (Terrer et al. 2016) experiments,
417 with the primary goal of assessing potential carbon dynamics and enhancement of plant growth
418 (Andresen et al. 2016). As a side effect, the change in carbon isotopic composition of vegetation
419 exposed to FACE-treatment can be used to trace freshly assimilated carbon into soil microbial

420 biomass, fauna and organic carbon pools. This approach was used at the Brandbjerg site (DK-
421 BRA). The CO_2 used to elevate concentrations of atmospheric CO_2 to 510 ppm had $\delta^{13}C$ values
422 ranging from –3.0 to –36.7 ‰ throughout 8 years of experimental treatment, with and overall
423 mean of -26.1 ‰. The source of the CO_2 supplied by Air Liquide (Air Liquide, Denmark) was
424 most often a brewery surplus CO_2 as a chemically obtained side product. The FACE mixing of
425 the added CO_2 with ambient CO_2 in the moving air mass resulted in a ^{13}C depletion ranging
426 from -6.7 to -15.6 ‰. On average, this equals a depletion of CO_2 in FACE plots of -4.8 ‰
427 relative to the atmospheric -8 ‰ average. Ecosystem carbon pools became depleted
428 accordingly, and the FACE ^{13}C depletion acted as a long-term persistent isotope labelling. As
429 a result, soil fauna (Enchytraeids) sampled from each of the climate-treated plots was
430 significantly depleted in $\delta^{13}C$ by -0.5 to -2.0 ‰ in the CO_2 treatments (Andresen et al. 2011).
431 This was due to translocated ^{13}C substrate through the food web, starting with plant assimilation
432 of ^{13}C-depleted CO_2, followed by plant root exudation and microbial utilization of the ^{13}C
433 depleted substrate and eventual digestion of microbes by enchytraeids. Hereby the freshly
434 supplied carbon source was recognized to be transferred in the natural setting, within a given
435 time scale. Also microbial biomass and PLFAs had different baseline of ^{13}C content in ambient
436 (not-treated) plots compared to CO_2 treated plots (Andresen et al. 2014). This was used for the
437 calculation of ^{13}C enrichments for each PLFA biomarker individually, also illustrating the
438 pathway of newly-assimilated carbon into microbial biomass. A drawback of the ^{13}C-FACE
439 label is again contamination of the surroundings, as even short and small un-planned draft winds
440 can carry the depleted label onto 'ambient' plots, and these will most likely be 'contaminated'
441 with ^{13}C (though not markedly exposed to high CO_2 concentrations) after some years of FACE
442 activity. Therefore, one needs to collect reference material for the 'natural abundance' level
443 well away from the FACE experiment.
444

445 3 In situ injection of ^{13}C-enriched substrate solutions
446

447 As a much more localized approach to a specific area, *in-situ* addition of ^{13}C- and ^{15}N-enriched
448 substrates directly below the soil surface can be used to assess the competition for the substrate
449 between i) plants and soil microbes, ii) microbial groups, and iii) the effects of the climate
450 change treatments upon the competition for carbon or nitrogen substrates. Much research has
451 focused on the sharing of nitrogen sources between plant and microbes (Kuzyakov & Xu 2013)
452 using *in-situ* soil injections of ^{15}N labelled inorganic nitrogen (ammonium and nitrate) or

453 organic nitrogen (amino acids) (Bardgett et al., 2003; Sorensen et al., 2008). Once amino acids
454 with dual labelled compounds (^{15}N and ^{13}C) were available for experimental use, double-
455 labelled substrate was used to explore e.g. plant uptake of intact amino acids (Näsholm et al.
456 2009; Rasmussen et al. 2010), and microbial utilization of carbon substrates (Dungait et al.
457 2013; Rinnan & Baath 2009).

458 In a labelling experiment at the DK-BRA site, amino acid injections into the soil were
459 conducted to analyse the impact of the climate treatments on the uptake of free amino acid
460 nitrogen by plants and soil microbes. Dual-labelled glycine ($^{13}C_2{}^{15}N$-glycine: 99 atom% ^{13}C -
461 of both carbon atoms - and 99 atom% ^{15}N) was added to 20×20 cm^2 sub-plots (Andresen et al.
462 2009). Each sub-plot received 0.1 L of re-demineralised water labelled with 0.027 g glycine,
463 corresponding to 687 mg glycine m^{-2} (223 mg C m^{-2} or 0.016 mg glycine g^{-1} dry weight soil).
464 The label was injected into the soil just below the soil surface with a syringe moved among 16
465 evenly spaced points of a template, placed on top of the vegetation (Andresen et al. 2009). One
466 day (c. 24 h) after labelling with glycine, soil cores were sampled from the soil surface to 15
467 cm depth, for determining the relative uptake of the amino acid in plant roots (IRMS solid
468 sample) and soil microbes. As in many other soil labelling experiments, the largest label
469 recovery (measured by ^{15}N recovery since respiratory losses of ^{13}C remain unknown) was found
470 in the total microbial biomass compared to total plant biomass (Kuzyakov & Xu 2013). A
471 subsample of fresh soil was extracted with re-demineralised water, and another set of
472 subsamples was first vacuum-incubated with chloroform for 24 hours to release microbial
473 carbon and nitrogen (Joergensen & Mueller 1996; Brookes et al. 1985), before extraction with
474 re-demineralised water. A third subsample of soil was freeze dried (lyophilized) and later used
475 for PLFA extractions. The ^{13}C enrichment in marker PLFAs thus indicated the activity (vitality)
476 of the specific microbial group (Watzinger 2015). We found that bacteria opportunistically
477 utilised the freshly added glycine substrate, i.e. incorporated ^{13}C, whereas fungi showed only
478 minor or no glycine derived ^{13}C-enrichment (Andresen et al. 2014). In comparison, ^{13}C traced
479 into the microbial community via the ^{13}C-CO_2 pulse label at the same site (DK-BRA) also
480 reached the bacterial community first. Bacteria showed high ^{13}C enrichment compared to fungal
481 groups (Reinsch et al. 2014). This suggests that *in-situ* injection of ^{13}C substrates might be a
482 plausible alternative to mimic rhizodeposition effects. With the direct addition of ^{13}C label to
483 the soil a strong labelling of the microbial community was more easily achieved than with the
484 indirect ^{13}C labelling of microbes via plant assimilated ^{13}C-CO_2 (Box 3).
485

Use of labelled carbon-compounds to analyse carbon mineralisation by soil microorganisms

Since soil microorganisms have an important role in controlling the availability of nutrients via mineralisation of SOM, our understanding of how microbial functioning in the ecosystem is altered by global change must be improved (Grayston et al. 1997). Microbial catabolic diversity of a soil is directly related to the carbon decomposition function within a soil and potentially provides a sensitive and ecologically relevant measure of the microbial community structure (Garland & Mills 1991). Subsequently, multiple assays have been developed to generate community level physiological profiles (CLPP) that can act as fingerprints of microbial function. Three approaches for measuring CLPP in soils are reported in the literature: (i) Biolog (Garland & Mills 1991); (ii) a substrate-induced respiration (SIR) technique (Degens & Harris 1997); and (iii) MicroResp (Campbell et al. 2003). These methods are all based on quantifying CO_2 respired during the mineralisation of organic carbon compounds that vary in size, charge and structural complexity. The first approach, Biolog MicroPlateTM (Biolog), assesses the catabolic diversity of soil organisms using a microtitre plate by incubating a soil culture in the presence of nutrients and 95 different carbon substrates; respired CO_2 is used to reduce a tetrazolium violet salt, which results in a colour change that can be quantified colorimetrically (Garland & Mills 1991). This approach, however, has been criticized for bias towards fast growing organisms that thrive in culture (Preston-Mafham et al. 2002). In response to the criticism of the Biolog method, Degens & Harris (1997) developed a method based on SIR where individual substrates are added to intact soil and evolved CO_2 is sampled and quantified. Finally, Campbell et al. (2003) combined aspects of both methods (MicroRespTM) where the response to carbon substrate addition to soil is measured colorimetrically using a cresol red indicator dye in a microtitre plate format.

Community level physiological profiling of soils samples collected from all treatments across the network was conducted to determine the catabolic utilisation profile, turnover and pool allocation of low molecular weight (LMW) carbon compounds by using a selection of ^{14}C-labelled substrates. This method enabled the attribution of respired CO_2 to specific metabolic processes that facilitates the quantification and qualification of microbial mineralisation kinetics of substrates varying in structural complexity and recalcitrance. The kinetics of microbial $^{14}C\text{-}CO_2$ evolution can be described using a first order exponential decay model (Box 4). The number of terms used in the exponential decay model can be used to explain how microbial kinetics relates time, substrate complexity and carbon pool allocation to, for example,

520 rapidly cycled labile soil solution carbon, microbial structural carbon and recalcitrant
521 extracellular soil organic carbon (Kuzyakov & Demin 1998; Nguyen & Guckert 2001; Boddy
522 et al. 2007). Attribution of modelled carbon pool sizes and turnover rates to biological function
523 are not only time and substrate dependent. Therefore, soil physical, biological and chemical
524 interactions may be miss attributed to biological function. Indeed, the lack of knowledge and
525 techniques available to examine the interaction between discrete carbon pools (Glanville et al,
526 2016). Using the half-life of ^{14}C labelled carbon in soil solution we were able to examine the
527 environmental gradient of the warming treatment across the climate change network and
528 identified that temperature becomes rate limiting for microbial uptake of carbon from the soil
529 solution pool at < 10.5 °C. We also showed that experimentally manipulated warming simply
530 speeds up the catabolic utilisation of labile LMW carbon in a predictable pattern (Box 4).
531

532 Conclusions and recommendations
533

534 Stable isotope studies provide insightful information about carbon (and nitrogen) fluxes through
535 the plant-soil-atmosphere continuum with minimal disturbance to the system, and contribute to
536 advance our understanding of climate change impacts on aboveground-belowground linkages.
537 However, their application is not exempt from difficulties and disadvantages. To keep a high
538 caution and avoid mistakes, our collective recommendations for applying the described
539 methods are provided and addressed in Table 1.

540 *In-situ* pulse-labelling studies are powerful to analyse short-term carbon fluxes in the plant-soil
541 system, but there are major seasonality constraints to the distribution of the label throughout
542 the ecosystem compartments, *i.e.* the seasonality of carbon allocation belowground due to
543 changing plant activity, or the plant health status which determines the amount of tracer entering
544 the system. A significant challenge was the achievement of sufficient ^{13}C enrichment in
545 microbial biomass in *in-situ* ^{13}C-CO_2 pulse-labelling studies, where the pools of background
546 carbon in the studied compartments were high and hence diluted the ^{13}C signal. This was less
547 of a problem for ^{14}C studies, due to more sensitive analysis methods.

548 Importantly, field plots previously 'contaminated' by highly enriched isotope labelling should
549 be considered potentially inoperable for further scientific isotope studies using the natural
550 abundance approach. However, plant and soil structures remain largely undisturbed. In outlook
551 for setting up a large-scale climate manipulation, areas that have not been previously used for
552 experimental work with isotopes should consequently be selected. In particular, the 'bomb-C'

553 method is very sensitive to the contamination of soil or plant samples with ^{14}C-enriched
554 material, and thus its application should be limited to sites and facilities where no ^{14}C-labelling
555 work has been conducted. Additionally, it should be noted that any history of fertilization might
556 also alter the natural isotope abundance of ecosystem compartments (in particular ^{15}N
557 signatures), potentially confounding experimental results. Furthermore, military training
558 grounds, public recreational activity and vicinity of traffic are known to potentially contaminate
559 the soil with 'artificial compounds' which might interfere with delicate measurements on HPLC
560 and GC-MS systems.

561 Incubation studies with isotope labelled carbon compounds *in-situ* or *in-vitro* are relatively fast
562 to conduct and produce insightful data. The rapid utilisation of labelled substrates by soil
563 microorganisms, occurring immediately upon application, is a controlling factor for the timing
564 of the experimental work both at field and lab conditions. Hence, a sampling scheme needs to
565 be carefully planned before experiment initiation. Furthermore, pre-obtained knowledge of site
566 specific plant and microbial activity, substrate affinity and natural substrate concentrations is
567 important for planning any tracer application experiment (Table 1).

568

569 **Acknowledgements**

570

571 This paper is a WP3 and WP15 of the INCREASE EU-infrastructure. Financial support by the
572 European Commission (FTP7-ICT-2008-1, Grant Agreement no. 227628) is gratefully
573 acknowledged. LCA, IKS, PA and CB received support from the VKR foundation through the
574 CLIMAITE project. ME and JP acknowledge support from the Spanish Government grant
575 CGL2016-79835-P, the Catalan Government grant SGR2014- 274, and the European Research
576 Council grant ERC-SyG-610028 IMBALANCE-P. GKD was supported by the Hungarian
577 Scientific Research Fund (OTKA K112576) and by the János Bolyai Research Scholarship of
578 the Hungarian Academy of Sciences. The ClimMani (ESSEM COST Action ES1308, Climate
579 Change Manipulation Experiments in Terrestrial) is acknowledeged.

580

581 **References**

582

583 Andresen, L.C., Michelsen, A., Jonasson, S., Beier, C. & Ambus, P. (2009) Glycine uptake in
584 heath plants and soil microbes s responds to elevated temperature, CO_2 and drought. *Acta*
585 *OEcologica*, 313, 283-295.

586 Andresen, L.C., Konestabo, H.S., Maraldo, K., Holmstrup, M., Ambus, P., Beier, C. &
587 Michelsen, A. (2011) Organic matter flow in the food web at a temperate heath under
588 multifactorial climate change. *Rapid Communications in Mass Spectrometry*, 25, 1485-1496.

589 Andresen, L.C., Dungait, J.A.J., Bol, R., Selsted, M.B., Ambus, P. & Michelsen, A. (2014)
590 Bacteria and Fungi Respond Differently to Multifactorial Climate Change in a Temperate
591 Heathland, Traced with ^{13}C-Glycine and FACE CO_2. *PlosOne*, 9, e85070.

592 Balasooriya, W.K., Denef, K., Peters, J., Verhoest, N.E.C. & Boeckx, P. (2008) Vegetation
593 composition and soil microbial community structural changes along a wetland hydrological
594 gradient. *Hydrology and Earth System Sciences*, 12, 277-291.

595 Balasooriya, W., Huygens, D., Denef, K., Roobroeck, D., Verhoest, N.C. & Boeckx, P. (2013)
596 Temporal variation of rhizodeposit-C assimilating microbial communities in a natural wetland.
597 *Biology and Fertility of Soils*, 49, 333-341.

598 Balasooriya, W., Denef, K., Huygens, D. & Boeckx, P. (2014) Translocation and turnover of
599 rhizodeposit carbon within soil microbial communities of an extensive grassland ecosystem.
600 *Plant and Soil*, 376, 61-73

601 Beier, C., Emmett, B.A., Gundersen, P., Tietema, A., Peñuelas, J., Estiarte, M., Gordon, C.
602 Gorissen, A., Llorens, L., Roda, F. & Williams, D. (2004) Novel approaches to study climate
603 change effects on terrestrial ecosystems in the field: drought and passive night-time warming.
604 *Ecosystems*, 7, 583-597.

605 Beier, C., Emmett, B.A., Peñuelas, J., Schmidt, I.K., Tietema, A., Estiare, M., Gundersen, P.,
606 Llorens, L., Riis-Nielsen, T., Sowerby, A. & Gorissen, A. (2008) Carbon and nitrogen cycles
607 in European ecosystems respond differently to global warming. *Science of the Total*
608 *Environment*, 407, 692-697.

609 Beier, C., Emmett, B.A., Tietema, A., Schmidt, I.K., Peñuelas, J., Kovács-Lang, E., Duce, P.,
610 De Angelis, P. Gorissen, A., Estiare, M., de Dato, G.D., Sowerby, A., Kröel-Dulay, G., Lellei-
611 Kovács, E., Kull, O., Mänd, P., Petersen, H., Gjelstrup, P. & Spano, D. (2009) Carbon and
612 nitrogen balances for six shrublands across Europe. *Global Biogeochemical Cycles*, 23, Art.
613 No. GB4008.

614 Boddy, E., Hill, P.W., Farrar, J.F. & Jones, D.L. (2007) Fast turnover of low molecular weight
615 components of the dissolved organic carbon pool of temperate grassland field soils. *Soil Biology*
616 *and Biochemistry*, 39, 827-835.

617 Bol, R. & Harkness, D.D. (1995) The use of zeolite molecular sieves for trapping low
618 concentrations of CO_2 from environmental atmospheres. *Radiocarbon*, 37, 643-647

619 Bol, R., Bolger, T., Cully, R. & Little, D. (2003) Recalcitrant soil organic material mineralise
620 more efficiently at higher temperatures. *Journal of Plant Nutrition and Soil Science*, 166, 300-
621 307.

622 Borken, W., Savage, K., Davidson, E.A. & Trumbore, S.E. (2006) Effects of experimental
623 drought on soil respiration and radiocarbon efflux from a temperate forest soil. *Global Change*
624 *Biology*, 12, 177-193.
625 Brand, W.A. & Coplen, T.B. (2014) Stable isotope deltas: tiny, yet robust signatures in nature.
626 *Isotopes in Environmental and Health Studies*, 48, 393-409;

627 Brookes, P.C., Landman, A., Pruden, G., & Jenkinson, D.S. (1985) Chloroform fumigation and
628 the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass
629 nitrogen in soil. *Soil Biology and Biochemistry*, 17, 837-842.

630 Brüggemann, N., Gessler, A., Kayler, Z., Keel, S.G., Badeck, F., Barthel, M., Boeckx, P.,
631 Buchmann, N., Brugnoli, E., Esperschuetz, J., Gavrichkova, O., Ghashghaie, J., Gomez-
632 Casanovas, N., Keitel, C., Knohl, A., Kuptz, D., Palacio, S., Salmon, Y., Uchida, Y. & Bahn,
633 M. (2011) Carbon allocation and carbon isotope fluxes in the plant-soil-atmosphere continuum:
634 a review. *Biogeosciences*, 8, 3457-3489.

635 Butler, J.L., Bottomley, P.J., Griffith, S.M. & Myrold, D.D. (2004) Distribution and turnover
636 of recently fixed photosynthate in ryegrass rhizospheres. *Soil Biology and Biochemistry*, 36,
637 371-382.

638 Campbell, C.D., Chapman, S.J., Cameron, C.D., Davidson, M.S. & Potts, J.M., (2003) A rapid
639 microtitre plate method to measure carbon dioxide evolved from carbon substrate amendments
640 so as to determine the physiological profiles of soil microbial communities by using whole soil.
641 *Applied and Environmental Microbiology*, 69, 3593-3599.

642 Cernusak, L.A., Ubierna, N., Winter, K., Holtum, J.A.M., Marshall, J.D. & Farquhar, G.D.
643 (2013) Environmental and physiological determinants of carbon isotope discrimination in
644 terrestrial plants. *New Phytologist*, 200, 950–965.

645 Chapin, F.S., McFarland, J., David McGuire, A., Euskirchen, E. S., Ruess, R. W., & Kielland,
646 K. (2009) The changing global carbon cycle: linking plant-soil carbon dynamics to global
647 consequences. *Journal of Ecology*, 97, 840–850.

648 Cisneros-Dozal, L.M., Trumbore, S. & Hanson, P.J. (2006) Partitioning sources of soil-respired
649 CO_2 and their seasonal variation using a unique radiocarbon tracer. *Global Change Biology*,
650 12, 194-204.
651 Czimczik, C.I., Trumbore, S.E., Carbone, M.S. & Winston, G.C. (2006) Changing sources of
652 soil respiration with time since fire in a boreal forest. *Global Change Biology*, 12, 957-971.

653 de Graaff, M.A., Six, J., & van Kessel, C. (2007) Elevated CO_2 increases nitrogen
654 rhizodeposition and microbial immobilization of root-derived nitrogen. *New Phytologist*, 173,
655 778-786.

656 Degens, B.P. & Harris, J.A. (1997) Development of a physiological approach to measuring the
657 catabolic diversity of soil microbial communities. *Soil Biology and Biochemistry*, 29: 1309-
658 1320.

659 Denman, K.L., Brasseur, G., Chidthaisong, A., Ciais, P., Cox, P.M., Dickinson, R.E.,
660 Hauglustaine, D., Heinze, C., Holland, E., Jacob, D., Lohmann, U., Ramachandran, S., da Silva
661 Dias, P.L., Wofsy, S.C., & Zhang, X. (2007) Couplings between changes in the climate system
662 and biogeochemistry. In: Solomon, S., Qin, D., Manning, M., Chen, M.Z., Marquis, M., Averyt,
663 K.B., Tignor, M. & Miller, H.L. (eds.) Climate change 2007: the physical science basis.
664 Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental
665 Panel on Climate Change. Cambridge University Press, Cambridge, pp 500-587.

666 Denef, K., Bubenheim, H., Lenhart, K., Vermeulen, J., Van Cleemput, O., Boeckx, P. & Müller,
667 C. (2007) Community shifts and carbon translocation within metabolically-active rhizosphere
668 microorganisms in grasslands under elevated CO_2. *Biogeosciences*, 4, 769-779.

669 Denef, K., Roobroeck, D., Manimel Wadu, M.C.W., Lootens, P. & Boeckx, P. (2009) Microbial
670 community composition and rhizodeposit-carbon assimilation in differently managed
671 temperate grassland soils. *Soil Biology and Biochemistry*, 41, 144-153.

672 Dijkstra, F.A., Carrillo, Y., Pendall, E. & Morgan, J.A. (2013) Rhizosphere priming: a nutrient
673 perspective. *Frontiers in Microbiology*, 4, 216.

674 Domínguez, M.T, Sowerby, A., Smith, A., Robinson, D., Van Baarsel, S., Mills, R.E., Marshall,
675 M., Koller, E., Lebron, I., Hall, J., & Emmett, B. (2015) Sustained impact of drought on wet
676 shrublands mediated by soil physical changes. *Biogeochemistry*, 122, 151-163.

677 Donovan, L.A. & Ehleringer, J.R. (1994) Carbon isotope discrimination, water-use efficiency,
678 growth, and mortality in a natural shrub population. *Oecologia*, 100, 347-354.

679 Dungait, J.A.J., Kemmitt, S.J., Michallon, L., Guo, S., Wen, Q., Brookes, P.C. & Evershed,
680 R.P. (2013) The variable response of soil microorganisms to trace concentrations of low
681 molecular weight organic substrates of increasing complexity. *Soil Biology & Biochemistry*, 64,
682 57–64.

683 Emmett, B.A., Beier, C., Estiarte, M., Tietema, A., Kristensen, H.L., Williams, D., Peñuelas,
684 J., Schmidt, I. & Sowerby, A. (2004) The response of soil processes to climate change: results
685 from manipulation studies of shrublands across and environmental gradient. *Ecosystems*, 7,
686 625-637.

687 Epron, D., Bahn, M., Derrien, D., Lattanzi, F.A., Pumpanen, J., Gessler, A., Hörgberg, P.,
688 Maillard, P., Dannoura, M., Gérant, D. & Buchmann, N. (2012) Pulse-labelling trees to study
689 carbon allocation dynamics: a review of methods, current knowledge and future prospects. *Tree*
690 *Physiology*, 32, 776-798.

691 Farquhar, G.D. & Richards, R.A. (1984) Isotopic composition of plant carbon correlates with
692 water-use efficiency of wheat genotypes. *Australian Journal of Plant Physiology*, 11, 539-552.

693 Friedlingstein, P., Cox, P., Betts, R., Bopp, L., von Bloh, W., Brovkin, V., Cadule, P., Doney,
694 S., Eby, M., Fung, I, Bala, G., John, J., Jones, C., Joos, F., Kato, T., Kawamiya, M., Knorr, W.,
695 Lindsay, K., Matthews, H.D., Raddatz, T., Rayner, P., Reick, C., Roeckner, E., Schnitzler, K.G.,
696 Schnur, R., Strassmann, K., Weaver, A.J., Yoshikawa, C., & Zeng, N. (2006) Climate–carbon
697 cycle feedback analysis: results from the C4MIP model intercomparison. *Journal of Climate,*
698 19, 3337-3353

699 Fry. B. (2006) Stable Isotope Ecology. Springer, New York.

700 Fuchslueger, L., Bahn, M., Fritz, K., Hasibeder, R. & Richter, A. (2014) Experimental drought
701 reduces the transfer of recently fixed plant carbon to soil microbes and alters the bacterial
702 community composition in a mountain meadow. *New Phytologist*, 201, 916-927.

703 Gaudinski, J.B., Trumbore, S.E., Davidson, E.A. & Shuhui, Z. (2000) Soil carbon cycling in a
704 temperate forest: radiocarbon-based estimates of residence times, sequestration rates and
705 partitioning of fluxes. *Biogeochemistry*, 51, 33-69.

706 Garland, J.L. & Mills, A.L. (1991) Classification and characterisation of heterotrophic
707 microbial communities on the basis of patterns of community-level sole-carbon-source
708 utilisation. *Applied and Environmental Microbiology*, 57, 2351-2359.

709 Glanville, H.C., Hill, P.W., Schnepf, A., Oburger, E., Jones. D.L. (2016) Combined use of
710 empirical data and mathematical modelling to better estimate the microbial turnover of
711 isotopically labelled carbon substrates in soil. *Soil Biology and Biogeochemistry*, 94, 154-168.

712 Grayston, S.J., Vaughan, D. & Jones, D. (1997) Rhizosphere carbon flow in trees, in
713 comparison with annual plants: the importance of root exudation and its impact on microbial
714 activity and nutrient availability. *Applied Soil Ecology*, 5, 29-56.

715 Gunina, A. & Kuzyakov, Y. (2014) Pathways of litter C by formation of aggregates and SOM
716 density fractions: Implications from ^{13}C natural abundance. *Soil Biology and Biogeochemistry*,
717 71, 95-104.

718 Hanson, P.J., Edwards, N.T., Garten, C.T. & Andrews, J.A. (2000) Separating root and soil
719 microbial contributions to soil respiration: a review of methods and observations.
720 *Biogeochemistry*, 48, 115-146.

721 Hardie, S.M.L., Garnett, M.H., Fallick, A.E., Rowland, A.P. & Ostle, N.J. (2005) Carbon
722 dioxide capture using a zeolite molecular sieve sampling system for isotopic studies ^{13}C and
723 ^{14}C of respiration. *Radiocarbon*, 47, 441-451.

724 Hardie, S.M.L., Garnett, M.H., Fallick, A.E., Ostle, N.J. & Rowland, A.P. (2009) Bomb-^{14}C
725 analysis of ecosystem respiration reveals that peatland vegetation facilitates release of old
726 carbon. *Geoderma*, 153, 393-401.

727 Hovenden, M.J., Newton P.C.D. & Wills, K.E. Seasonal not annual rainfall determines
728 grassland biomass response to carbon dioxide. *Nature*, 511, 583-586.

729 Högberg, P., Högberg, M.N., Göttlicher, S.G., Betson, N.R., Keel, S.G., Metcalfe, D.B.,
730 Campbell, C., Schindelbacher, A., Hurry, V., Lundmark, T., Linder, S. & Näsholm, T. (2008)
731 High temporal resolution tracing of photosynthate carbon from the tree canopy to forest soil
732 microorganisms. *New Phytologist*, 177, 220-228.

733 Högberg, M.N. & Högberg, P. (2002) Extramatrical ectomycorrhizal mycelium contributes
734 one-third of microbial biomass and produces, together with associated roots, half the dissolved
735 organic carbon in a forest soil. *New Phytologist*, 154, 791-795.

736 Högberg, M.N., Briones, M.J.I., Keel, S.G., Metcalfe, D.B., Campbell, C., Midwood, A.J.,
737 Thornton, B., Hurry, V., Linder, S., Näsholm, T. & Högberg, P. (2010) Quantification of effects
738 of season and nitrogen supply on tree below-ground carbon transfer to ectomycorrhizal fungi
739 and other soil organisms in a boreal pine forest. *New Phytologist*, 187, 485-493.

740 IPCC Intergovernmental Panel on Climate Change (2013) Working group I contribution to the
741 IPCC fifth assessment report (AR5), Climate Change 2013: The Physical Science Basis.

742 Jin, V.L. & Evans, R.D.E. (2010) Microbial ^{13}C utilisation patterns via stable isotope probing
743 of phospholipid biomarkers in Mojave Desert soils exposed to ambient and elevated
744 atmospheric CO_2. *Global Change Biology*, 16, 2334-2344.

745 Joergensen, R.G. & Mueller, T. (1996) The fumigation-extraction method to estimate soil
746 microbial biomass: calibration of the ken value. *Soil Biology and Biochemistry*, 28, 33–37.

747 Kimball, B.A. (2016) Crop responses to elevated CO_2 and interactions with H_2O, N, and
748 temperature. *Current Opinion in Plant Biology*, 31, 36-43.

749 Kuzyakov, Y. & Demin, V. (1998) CO_2 efflux by rapid decomposition of low
750 molecular organic substances in soils. *Sciences of Soils,* 3, 11–22.

751 Kuzyakov, Y. & Gavrichkova, O. (2010) Time lag between photosynthesis and carbon dioxide
752 efflux from soil: a review of mechanisms and controls. *Global Change Biology*, 16, 3386-3406.

753 Kuzyakov, Y. & Xu, X. (2013) Competition between roots and microorganisms for nitrogen:
754 mechanisms and ecological relevance. *New Phytologist*, 198, 656–669.

755 Kröel-Dulay, G., Ransijn, J., Schmidt, I.K., Beier, C., De Angelis, P., de Dato, G., Dukes, J.S.,
756 Emmett, B., Estiarte, M., Garadnai, J., Kongstad, J., Kovacs-Lang, E., Larsen, K.S., Liberati,
757 D., Ogaya, R., Riis-Nielsen, T., Smith, A.R., Sowerby, A., Tietema, A. & Peñuelas, J. (2015)
758 Increased sensitivity to climate change in disturbed ecosystems. *Nature communications* 6,
759 6682.

760 Leakey, A.D.B., Ainsworth, E.A., Bernacchi, C.J., Rogers, A., Long, S.P. & Ort, D.R. (2009)
761 Elevated CO_2 effects on plant carbon, nitrogen and water relations: six important lessons from
762 FACE. *Journal of Experimental Botany*, 60, 2859-2876.

763 Lellei-Kovács, E., Botta-Dukat, Z., de Dato, G., Estiarte, M., Guidolotti, G., Kopittke, G.R.,
764 Kovacs-Lang, E., Kroel-Dulay, G., Larsen, K.S., Penuelas, J., Smith, A.R., Sowerby, A.,
765 Tietema, A. & Schmidt I.K. (2016) Temperature dependence of soil respiration modulated by
766 threholds in soil water availability across European shrubland ecosystems. *Ecosystems*, 19,
767 1460–1477.

768 Lichter, J., Barron, S.H., Bevacqua, C.E., Finzi, A.C. Irving, K.E., Stemmler, E.A. &
769 Schlesinger, W.H. (2005) Soil carbon sequestration and turnover in a pine forest after six years
770 of atmospheric CO_2 enrichment. *Ecology*, 86, 1835:1847.

771 Lloret, F., Siscart, D., & Dalmases, C. (2004). Canopy recovery after
772 drought dieback in holm-oak Mediterranean forests of Catalonia (NE
773 Spain). *Global Change Biology*, 10, 2092–2099.

774 Michelsen, A., Quarmby, C., Sleep, D et al.. (1998) Vascular plant N-15 natural abundance in
775 heath and forest tundra ecosystems is closely correlated with presence and type of mycorrhizal
776 fungi in roots. *Oecologia*, 115, 406-418.

777 Millard, P., Midwood, A.J., Hunt, J.E., Whitehead, D. & Boutton, T.W. (2008) Partitioning soil
778 surface CO_2 efflux into autotrophic and heterotrophic components, using natural gradients in
779 soil $\delta^{13}C$ in an undisturbed savannah soil. *Soil Biology and Biochemistry*, 40, 1575–1582.

780 Mikkelsen, T.N., Beier, C., Jonasson, S., Holmstrup, M., Schmidt, I.K., Ambus, P., Pilegaard,
781 K., Michelsen, A., Albert, K., Andresen, L.C., Arndal, M.F., Bruun, N., Christensen, S.,
782 Danbæk, S., Gundersen, P., Jørgensen, P., Linden, L.G., Kongstad, J., Maraldo, K., Priemé, A.,
783 Riis-Nielsen, T., Ro-Poulsen, H., Stevnbak, K., Selsted, M.B., Sørensen, P., Larsen, K.S.,
784 Carter, M.S., Ibrom, A., Martinussen, T., Miglietta, F. & Sverdrup, H. (2008) Experimental
785 design of multifactor climate change experiments with elevated CO_2, warming and drought:
786 the CLIMAITE project. *Functional Ecology*, 22, 185-195.

787 Muccio, Z. & Jackson, G.P. (2009) Isotope ratio mass spectrometry. *Analyst*, 134, 213–222.
788 Mueller, K.E., Blumenthal, D.M., Pendall, E., Carrillo, Y., Dijkstra, F.A., Williams, D.G.,
789 Follett, R.F. & Morgan, J.A. (2016). Impacts of warming and elevated CO_2 on a semi-arid
790 grassland are non-additive, shift with precipitation, and reverse over time. *Ecology Letters*, 19,
791 956–966.

792 Muhr, J.A.N., Borken, W. & Matzner, E. (2009) Effects of soil frost on soil respiration and its
793 radiocarbon signature in a Norway spruce forest soil. *Global Change Biology*, 15, 782-793.

794 Muhr, J. & Borken, W. (2009) Delayed recovery of soil respiration after wetting of dry soil
795 further reduces C losses from a Norway spruce forest soil. *Journal of Geophysical Research-*
796 *Biogeosciences*, 114, G04023.

797 Nguyen, C. & Guckert, A. (2001) Short-term utilisation of ^{14}C-[U]glucose by soil
798 microorganisms in relation to carbon availability. *Soil Biology and Biochemistry*, 33, 53-60.

799 Ostle, N., Whiteley, A.S., Bailey, M.J., Sleep, D., Ineson, P. & Manefield, M. (2003) Active
800 microbial RNA turnover in a grassland soil estimated using a $^{13}CO_2$ spike. *Soil Biology and*
801 *Biochemistry*, 35, 877-885.

802 Näsholm, T., Kielland, K. & Ganeteg, U. (2009) Uptake of Organic Nitrogen by Plants *The*
803 *New Phytologist*, 182, 31-48.

804 Paterson, E., Neilson, R., Midwood, A.J., Osborne, S.M., Sim, A., Thornton, B. & Millard, P.
805 (2011) Altered food web structure and C-flux pathways associated with mineralisation of
806 organic amendments to agricultural soil. *Applied Soil Ecology*, 48, 107-116.

807 Pardo, L.H., Templer, P.H., Goodale, C.L., Duke, S., Groffman, P.M., Adams, M.B. Boeckx,
808 P., Goggs, J., Campbell, J., Colman, B., Compton, J., Emmett, B., Gundersen, P., Kjönaas, J.,
809 Lovett, G., Mack, M., Magill, A., Mbila, M., Mitchell, M.J., McGee, G., McNulty, S.,
810 Nadelhoffer, K., Ollinger, S., Ross, D., Rueth, H., Rustad, L., Schaberg, P., Schiff, S., Schlepp,
811 P., Phillips, DA, Fox, TC & Six J (2006) Root exudation (net efflux of amino acids) may
812 increase rhizodepostion under elevated CO_2. *Global Change Biology*, 12, 561-567.

813 Phillips, D. A., Fox, T. C., & SIX, J. (2006) Root exudation (net efflux of amino acids) may
814 increase rhizodeposition under elevated CO_2. *Global Change Biology*, 12, 561–567.

815 Preston-Mafham, J., Boddy, L. & Randerson, P.F., (2002) Analysis of microbial community
816 functional diversity using sole-carbon source utilisation profiles—a critique. *FEMS*
817 *Microbiology Ecology*, 42, 1–14.

818 Rangel-Castro, J.I., Killham, K., Ostle, N., Nicol, G.W., Anderson, I.C., Scrimgeour, C.M.,
819 Ineson, P., Meharg, A. & Prosser, J.I. (2005) Stable isotope probing analysis of the influence
820 of liming on root exudate utilisation by soil microorganisms. *Environmental Microbiology*, 7,
821 828-838.

822 Rasmussen, J., Sauheitl, L., Eriksen, J. & Kuzyakov, Y. (2010) Plant uptake of dual-labeled
823 organic N biased by inorganic C uptake: Results of a triple labeling study. *Soil Biology &*
824 *Biochemistry*, 42, 524e527

825 Reich, P.B., Hobbie, S.E. & Lee, T.D. (2014) Plant growth enhancement by elevated CO_2
826 eliminated by joint water and nitrogen limitation. *Nature Geoscience*, 7, 920–924.

827 Reinsch, S., Arndal, M.F., Schmidt, I.K., Domínguez, M.T., Beier C., de Dato, G., Devan S.,
828 Emmett, B.A., Estiarte, M., Godbold, D.L., Sandén, H., Kröel-Dulay, G., Larsen, K.S., Lellei-
829 Kovács, E., Mason, S., Peñuelas, J., Riis-Nielsen, T., Tietema, A., and Andresen, L.C. Methods
830 for non-destructive assessment of terrestrial carbon pools and fluxes in shrublands in long-term
831 climate change experiments. *Submitted companion paper to Methods in Ecology and Evolution.*

832 Reinsch, S., Michelsen, A., Sárossy, Z., Egsgaard, H., Schmidt, I.K., Jakobsen, I. & Ambus, P.
833 (2014) Short-term utilisation of carbon by the soil microbial community under future climatic
834 conditions in a temperate heathland. *Soil Biology and Biochemistry*, 68, 9-19.

835 Reinsch, S. & Ambus, P. (2013) In situ $^{13}CO_2$ pulse-labeling in a temperate heathland
836 development of a mobile multi-plot field setup. *Rapid Communications in Mass Spectrometry*,
837 27, 1417-1428.

838 Rinnan, R. & Baaaath, E. (2009) Differential utilization of carbon substrates by bacteria and
839 fungi in tundra soil. *Applied and Environmental Microbiology*, 75, 3611–3620.

840 Robinson, D. (2001) $\delta^{15}N$ as an integrator of the nitrogen cycle. *TRENDS in Ecology and*
841 *Evolution*, 16, 153-162.

842 Robinson, D. & Scrimgeour, C.M. (1995) The contribution of plant C to soil CO_2 measured
843 using ^{13}C. *Soil Biology and Biochemistry*, 27, 1653–1656.

844 Ruess, L., & Chamberlain, P. M. (2010). The fat that matters: Soil food web analysis using fatty
845 acids and their carbon stable isotope signature. *Soil Biology and Biochemistry*, 42, 1898–1910.

846 Schuur, E.A. & Trumbore, S.E. (2006) Partitioning sources of soil respiration in boreal black
847 spruce forest using radiocarbon. *Global Change Biology*, 12, 165-176.

848 Schuur, E.A.G., Vogel, J.G., Crummer, K.G., Lee, H., Sickman, J.O. & Osterkamp, T.E. (2009)
849 The effect of permafrost thaw on old carbon release and net carbon exchange from tundra.
850 *Nature*, 459, 556-559.

851 Schrumpf, M., Kaiser, K., Guggenberger, G., Persson, T., Kogel-Knabner, I.& Schulze, E.D.
852 (2013) Storage and stability of organic carbon in soils as related to depth, occlusion within
853 aggregates, and attachment to minerals. *Biogeosciences*, 10, 1675-1691.

854 Selsted, M. B., Ambus, P., Michelsen, A., van der Linden, L., Larsen, K. S., Pilegaard, K.,
855 Mikkelsen, T. N. & Beier, C. (2011) Measurement of carbon dioxide fluxes in a free-air carbon
856 dioxide enrichment experiment using the closed flux chamber technique. *Atmospheric*
857 *Environment*, 45, 208-214.

858 Sorensen, P.L., Michelsen, A., & Jonasson, S. (2008) Nitrogen uptake during one year in
859 subarctic plant functional groups and in microbes after long-term warming and fertilization.
860 *Ecosystems*, 11, 1223–1233.

861 Sowerby, A., Emmett, B., Beier, C., Tietema, A., Peñuelas, J., Estiarte, M., Van Meeteren,
862 M.J.M., Hughes, S. & Freeman, C. (2005) Microbial community changes in heathland soil

863 communities along a geographical gradient: interaction with climate change manipulations. *Soil*
864 *Biology and Biochemistry*, 37: 1805-1813.

865 Sowerby, A., B.A. Emmett, A. Tietema & C. Beier (2008) Contrasting effects of repeated
866 summer drought on soil carbon efflux in hydric and mesic heathland soils. *Global Change*
867 *Biology*, 14, 2388-2404.

868 Studer, M.S., Siegwolf, R.T.W. & Abiven, S. (2014) Carbon transfer, partitioning and residence
869 time in the plant-soil system: a comparison of two $^{13}CO_2$ labelling techniques. *Biogeosciences*,
870 11, 1637-1648.

871 Subke, J.A., Vallack, H.M., Tord, M., Keel, S.G., Metcalfe, D.B., Högberg, P. & Ineson, P.
872 (2009) Short-term dynamics of abiotic and biotic soil $^{13}CO_2$ effluxes after in situ $^{13}CO_2$ pulse-
873 labelling of a boreal pine forest. *New Phytologist*, 183, 349–357.

874 Subke, J.A., Voke, N.R., Leronni, V., Garnett, M.H. & Ineson, P. (2011) Dynamics and
875 pathways of autotrophic and heterotrophic soil CO_2 efflux revealed by forest girdling. *Journal*
876 *of Ecology*, 99, 186-193.
877 Tcherkez, G., Mahe, A., & Hodges M. (2011) $^{12}C/^{13}C$ fractionations in plant primary
878 metabolism. *Trends in Plant Science*, 16, 499-506.

879 Terrer, C., Vicca, S., Hungate, B.A., Phillips, R.P. & Prentice, I.C., 2016. Mycorrhizal
880 association as a primary control of the CO_2 fertilization effect. *Science*, 353, 72–74.

881 Treonis, A.M., Ostle, N.J., Stott, A.W., Primrose, R., Grayston, S.J. & Ineson, P. (2004)
882 Identification of groups of metabolically-active rhizosphere microorganisms by stable isotope
883 probing of PLFAs. *Soil Biology and Biochemistry*, 36, 533-537

884 Trumbore, S.E. (2009) Radiocarbon and Soil Carbon Dynamics. *Annual Reviews on Earth*
885 *Planetary Sciences*, 37, 47–66.

886 Verdú, M. (2000) Ecological and Evolutionary Differences between Mediterranean Seeders and
887 Resprouters. *Journal of Vegetation Science*, 11, 265–268.

888 Watzinger, A. (2015) Microbial phospholipid biomarkers and stable isotope methods help
889 reveal soil functions. *Soil Biology and Biochemistry*, 86, 98-107.

890 Wessel, W.W., Tietema, A., Beier, C., Emmett, B.A., Peñuelas, J., & Riis–Nielsen, T. (2004)
891 A Qualitative Ecosystem Assessment for Different Shrublands in Western Europe under Impact
892 of Climate Change. *Ecosystems*, 7, 662–671.

893 Xue, D., Botte, J., De Baets, B., Accoe, F., Nestler, A., Taylor, P., Van Cleemput, O., Berglund,
894 M. & Boeckx, P. (2009) Present limitations and future prospects of stable isotope methods for
895 nitrate source identification in surface- and groundwater. *Water research*, 43, 1159-1170.

896 Zak, D.R., Pregitzer, K.S., King, J.S. & Holmes, W.E. (2000) Elevated atmospheric CO_2, fine
897 roots and the response of soil micro-organisms: a review and hypothesis. *New Phytologist*, 147,
898 201-222.

899

900 ## Table

901 **Table 1.** Suggestions and advice to consider when applying isotopic methods for the study of

902 carbon fluxes in the plant-soil system.

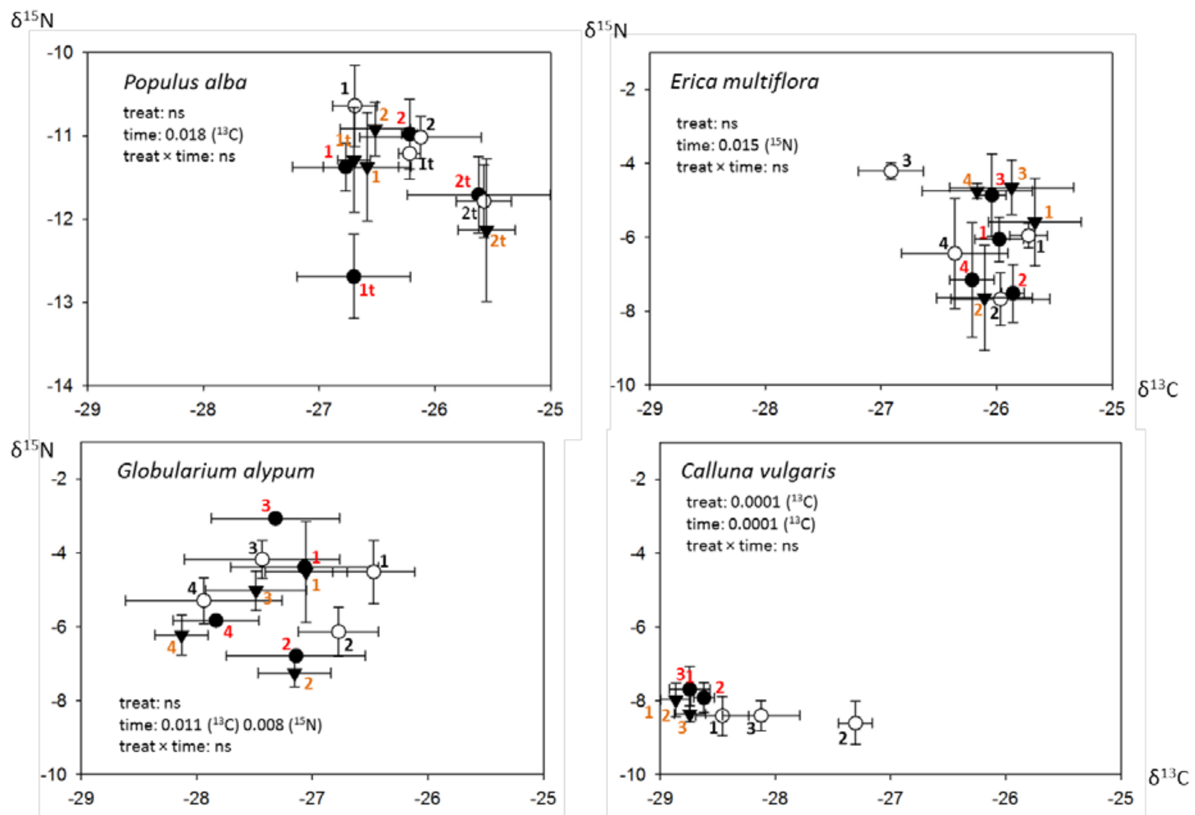
Method	Expenses (cost)	Advice (do's and don'ts)	Before you start	Data analysis hint	Time spent
Bomb-C (natural ^{14}C abundance)	High (AMS analysis); Equipment for CO_2 sampling is relatively cheap (closed chambers, carbon free pump, batteries, and molecular sieve system). An IRGA is also required.	- Avoid materials and labs with possible ^{14}C contamination - If soil CO_2 is to be analysed in the field, long incubation times are required to get sufficient CO_2 for AMS analysis (typically >1 ml). - Think carefully about the soil depths to be analysed, and take the sample consistently. ^{14}C signatures might vary strongly along few cm in the soil. - If bulk soil ^{14}C is to be analysed, try to remove the roots as much as possible, because of their contrasted ^{14}C signature.	- Discuss your experimental setup and objectives with the Radiocarbon facility staff. - If you are not sure about potential ^{14}C contamination in your lab, use another lab or make a swipe test. - Plan carefully the minimum number of samples required, as AMS analysis are expensive. - Make previous trials to assess the incubation times required to get a sufficient CO_2 sample - Go through the whole process of sample preparation with a trial sample.	- Discuss your results with the Radiocarbon facility staff.	- Processing time depends on the type of sample, although is usually low; determination by AMS may take several months depending on the facility.
In situ ^{13}C pulse-labelling	^{13}C- enriched compounds used for labelling and as standards are usually expensive; ^{13}C determination by IRMS is much cheaper than ^{14}C by AMS, although more expensive than ^{14}C by liquid scintillation (^{14}C labelling).	- Consider the target pools to be analysed and plan your doses consequently. Take the potential dilution of the label by the unlabelled root system or soil carbon pool into account. - Think about the trade-off between faster and stronger or weaker and longer ^{13}C pulses. - If your study requires a high ^{13}C enrichment, mind the potential risk of contaminating the site. - Avoid above ambient CO_2 concentrations in the chamber. - If you need to monitor CO_2 during your pulse, remember that IRGAs are rather insensitive to $^{13}CO_2$. - High sampling frequency immediately after the pulse application is recommended.	- Test your chamber and tubing materials for adsorption / desorption effects, and ensure these are without carbon content (use PTFE (Teflon) tape, not gluing paper-based). - Make a previous trial if possible and go through the whole process of sample preparation.	Report the label addition per area: g $^{13}C\ m^{-2}$.	- Pulse labelling experiments are usually short, but intensive. High sampling frequency after the pulse is very time-consuming. - Sample processing depends on the type of sample and number of replicates. - Experiments requiring root washing or microbial compound extraction are time consuming.
Natural abundance of isotopes (^{13}C and ^{15}N)	Rather cheap IRMS analysis.	- Make sure the history of sampling site is known (previous labelling experiments?)	- Avoid sample contamination. - Be aware that FACE can dilute the isotopic signal, most CO_2 enriched systems use ^{13}C depleted sources,	- Make sure the experiment is feasible with sufficient $\delta^{13}C$ shift and fractionation expected to be strong enough to	- Sampling time and grinding / weighing of sample. - Analysis usually done at dedicated

			because this is cheaper.	measure, base this on known discrimination at certain points in the carbon and nutrient cycle.	natural abundance facility.
^{14}C-substrates	Analysis of the trapped ^{14}C-CO_2 is relatively cheap.	- High risk of contaminating lab equipment.	- You need to work in a dedicated ^{14}C lab safely away from the natural abundance facility.		- Continue sampling until decline in emission is level, this ensures better model fit.
^{13}C-injection *in situ*	IRMS of dry matter plant material and soil cheap. The GC-c-MS of PLFAs for determination of ^{13}C-enrichment of individual PLFAs requires a specialised lab.	- Contamination risk of ^{13}C leaching is present, but smaller to our judgement than from ^{13}C-CO_2 experiments. - Do not use areas dedicated to natural abundance work.	- Labelling intended for soil microbial components is more intense from ^{13}C liquid substrate *in-situ* injection than from ^{13}C-CO_2 pulse labelling.		- Soil sampling is destructive, consider to have several parallel plots to harvest an undisturbed plot at each sampling event. - Sample handling from field work until the extraction takes a few days so plan only one sampling event per week if possible.

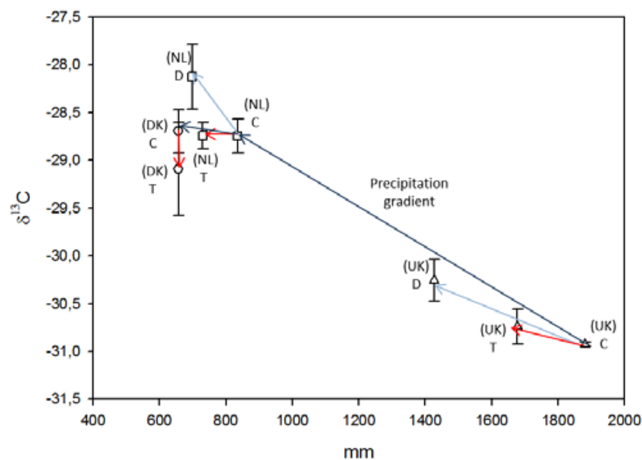
903

Box 1. Isotopic signal of plant leaf responses to precipitation

A



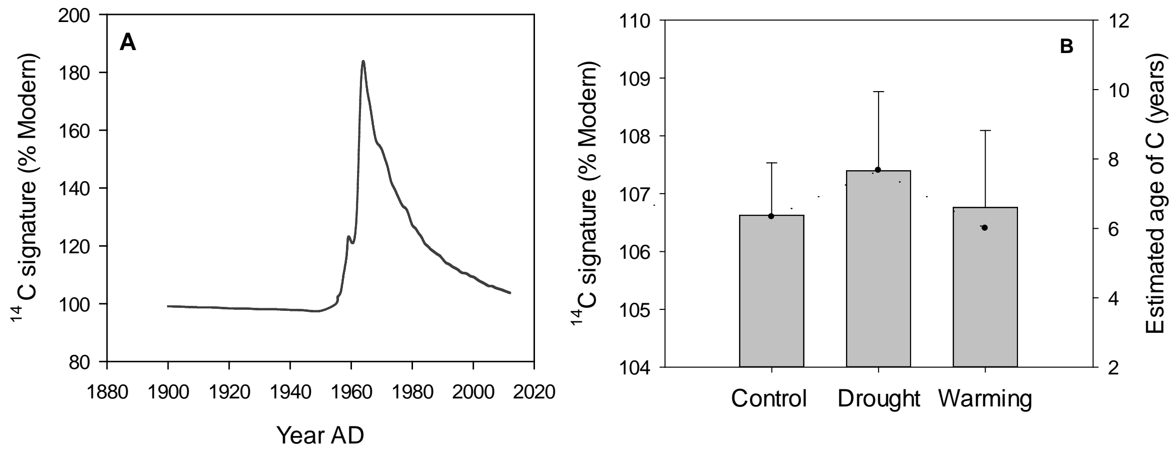
B

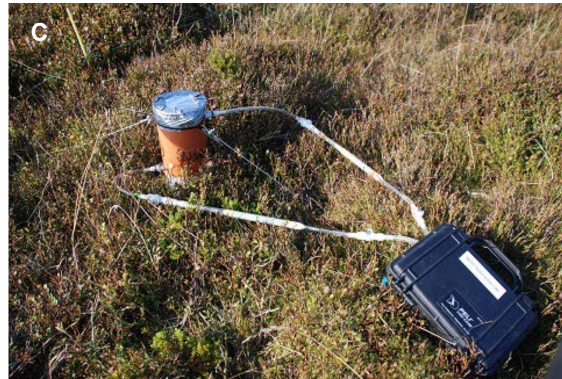


Stable isotopes in aboveground plant material: $\delta^{13}C$ and $\delta^{15}N$ from isotopic ratio mass spectrometry (IRMS) analysis of solid samples. **A:** Leaves and twigs (t) from *P. alba* (HU), *E. multiflora L.* (SP), *G. alypum L.* (SP) and *C. vulgaris* (NL); filled circle ● is control, open circle ○ is drought treatment, ▼ is warming treatment. P-values indicate effects of treatment, year, and the interaction of these factors on ^{13}C or ^{15}N, analysed by two-way ANOVA; ns is non-

914 significant effect. Number indicates year (2001=1, 2002=2, 2003=3 or 2004=4). Species (site)
915 differences and annual differences are stronger than treatment effects. **B:** $\delta^{13}C$ of *C. vulgaris*
916 versus annual precipitation of the previous year. Within each site, leaf $\delta^{13}C$ was higher in the
917 drought treatment in comparison to control. Across sites, plants from drier sites (lower
918 precipitation) show higher leaf $\delta^{13}C$ values. The response to the drought treatment is the same
919 as moving to a drier site.
920

Box 2. Impact of warming and drought on the ^{14}C signature of soil respiration





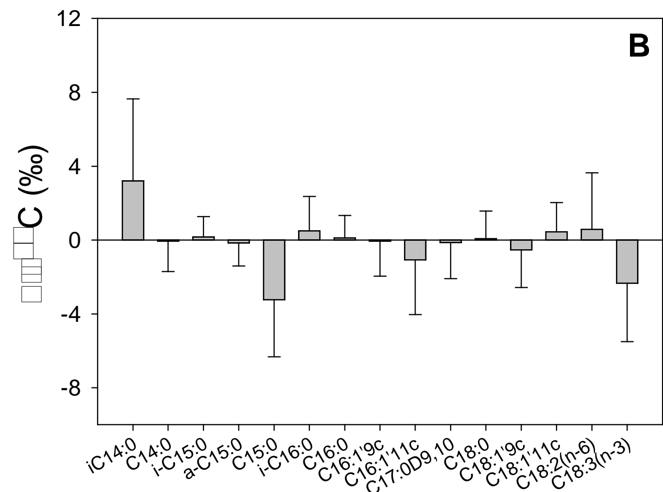
Closed dark respiration chambers and a molecular sieve sampling system was used in the field (UK-PK) to collect CO_2 of the soil efflux for the analysis of its ^{14}C signature by Accelerator Mass Spectrometry (AMS). **A:** Recent and older carbon sources have contrasting radiocarbon (^{14}C) signatures as a result of the nuclear bomb tests in the atmosphere during the 1950/60s. These tests led to a global increase in the ^{14}C content in the atmospheric CO_2, which reached its maximum in ~AD1963. The unit for ^{14}C signature (% Modern) is a measurement of the deviation of the $^{14}C/^{12}C$ ratio of a sample from the "Modern" standard, which is defined as 95% of the radiocarbon concentration (in AD 1950) of a reference material (NBS Oxalic Acid I, SRM 4990B), adjusted to a $\delta^{13}C$ reference value of –19 ‰. **B:** The ^{14}C signature of the soil efflux measured at the site (bars, left axis) was highly heterogeneous (ranging from 105.49 to 110.13 % Modern; values of > 100 % Modern suggest that a substantial component (and potentially all) of the carbon was trapped by photosynthesis during the post-bomb era i.e. since ~AD 1955), with no significant effect of the warming treatment, and a trend towards the release

938 of older carbon in the drought plots. On average, the carbon being released from the plots had
939 been fixed from the atmosphere between six and eight years earlier (line, right axis). Incubations
940 confirmed that the carbon respired by roots (mostly of *V. myrtillus L.*) was recently fixed
941 (similar ^{14}C signature as the atmosphere at the time of sampling), while the carbon released
942 from root-free soil samples (heterotrophic component) showed a variable range of ages, with
943 substantial components of pre-bomb carbon (carbon fixed before AD 1955). **C:** Detail of a
944 closed static chamber used to collect CO_2 from the soil efflux. Air is pumped in a closed loop
945 from the chamber through a quartz glass cartridge containing a zeolite molecular sieve, which
946 traps the CO_2 allowing it to be returned to the laboratory, recovered by heating, and analysed
947 by AMS.

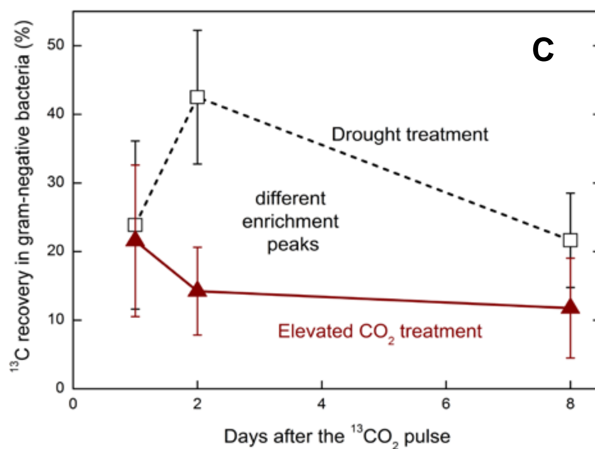
948

949 ## Box 3. Analysing rhizodeposit utilisation by microbes in the field

950
951
952
953
954
955
956
957



958
959
960
961
962
963
964

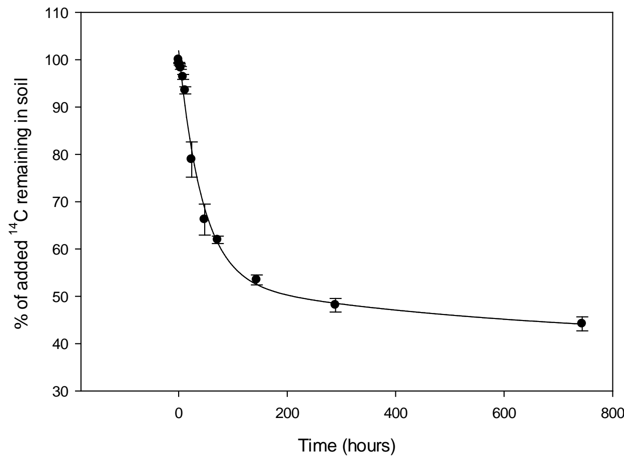


965 *In-situ* pulse-labelling experiments adding ^{13}C-CO_2 to closed transparent chambers were used
966 to study the translocation of the recently-fixed carbon belowground and the rhizodeposition
967 utilization by microbes, e.g. by measuring ^{13}C incorporation into microbial biomarkers. **A:** At
968 the Clocaenog site (UK-CL) this technique was applied along a peat layer gradient. Repeated
969 pulses of ^{13}C-CO_2 were applied during eight hours to *C. vulgaris* using sealed domes attached
970 to a core inserted into the ground. **B:** The incorporation of ^{13}C into soil microbial PLFAs was
971 analysed. Despite a high applied dose of ^{13}C (99 atom %), the dilution of the tracer within the
972 large pool of unlabelled root biomass was remarkable, and as a consequence most of the
973 analysed PLFAs showed no ^{13}C enrichment. **C:** ^{13}C recovery in Gram negative bacteria after a
974 ^{13}C-CO_2 pulse at the Brandbjerg site (DK-BRA). The enrichment pattern in PLFAs attributed
975 to Gram negative bacteria in soils exposed to drought and elevated CO_2 concentration (+120

976 ppm) for 8 years show different carbon utilization patterns and magnitudes under imposed
977 climatic conditions implying changed carbon cycle dynamics. **D:** Flow-through pulse-labelling
978 equipment showing the gas reservoir containing ^{13}C-CO_2 for up to eight hours of labelling
979 connected to transparent Plexiglas chambers via tubing.
980

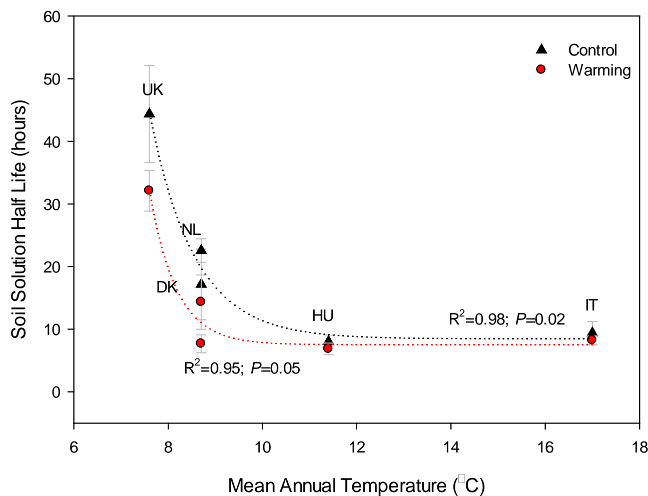
981 ## Box 4. Exponential decay kinetics for $^{14}CO_2$ evolution during microbial ^{14}C
982 ## substrate mineralisation

983 **A**



984

985 **B**



986

987 The catabolic utilisation profile, turnover and pool allocation of low molecular weight (LMW)
988 carbon substrates was determined in soils collected across the experimental network. A
989 selection of sixteen ^{14}C labelled amino acids and sugars varying in structural complexity and
990 recalcitrance were used in a multiple substrate induced respiration (SIR) assay on soil. Evolved
991 CO_2 was collected using NaOH traps and absorbed $^{14}CO_2$ was measured with a scintillation
992 counter (Perkin Elmer). The rate at which radiolabelled substrates were metabolised by the
993 microbial community was used to determine microbial uptake kinetics and turnover in the

994 absence of plants (no autotrophic input). A double term first order decay model with an
995 asymptote described our data best;

996 $$f = y_0 + a_1 e^{-k_1 t} + a_2 e^{-k_2 t} \qquad \text{eqn. 1.}$$

997 where f describes the amount of ^{14}C-labelled substrate or metabolites remaining in the soil at
998 time t, the asymptote y_0 explains the ^{14}C labelled material adsorbed to unavailable soil
999 complexes or metabolites partitioned into unavailable recalcitrant material, the exponential
1000 coefficient k_1 describes the initial rapid uptake and respiration of ^{14}C labelled substrate by the
1001 microbial community immediately following carbon substrate application, and k_2 describes a
1002 slower secondary mineralisation phase which we ascribed to immobilisation in microbial
1003 biomass and transformation of organic metabolites (microbial turnover). The terms a_1 and a_2
1004 relate to the proportion of ^{14}C labelled substrate that is associated with each exponential
1005 coefficient at time (t).

1006 The mean residence time (MRT) or substrate half-life ($t_{1/2}$) can be calculated according to

1007 $$t_{1/2} = \frac{\ln(2)}{k_n} \qquad \text{eqn. 2.}$$

1008 The turnover of each pool can then be calculated as the inverse of the MRT (1/MRT).

1009

1010 **A:** For substrate mineralisation the equation: y = 40.3766 + $48.3216^{0.0230x}$ + $13.1812^{0.0017x}$ fitted
1011 the data with an r^2 of 0.99. Using the coefficients (k_n) from the fitted equation, the half-life of
1012 the substrate can be calculated using eqn. 2. Substrate half-life was in the first phase (soil
1013 solution uptake) 30 h, and the second slower phase (microbial turnover) 408 h. Approximately
1014 40 % of the substrate was immobilised in the soil, 48.3 % respired during the first phase, and
1015 13.2 % respired during the slower second phase. **B:** Half-life of the substrate in the soil solution
1016 versus mean annual temperature, in control (triangle) and warming (circle) treatments, data
1017 points are mean ± SE (n=3). Warming treatment and relative warmer site, simply increases the
1018 catabolic utilisation of labile LMW-carbon until a threshold mean annual temperature of 11.5
1019 ºC.

1020