



**HAL**  
open science

## Increases in soil organic carbon sequestration can reduce the global warming potential of long-term liming to permanent grassland

Dario Fornara, Sibylle Steinbeiss, Niall Mcnamara, Gerd Gleixner, Simon Oakley, Paul Poulton, Andrew Macdonald, Richard D Bardgett

### ► To cite this version:

Dario Fornara, Sibylle Steinbeiss, Niall Mcnamara, Gerd Gleixner, Simon Oakley, et al.. Increases in soil organic carbon sequestration can reduce the global warming potential of long-term liming to permanent grassland. *Global Change Biology*, 2010, 17 (5), pp.1925. 10.1111/j.1365-2486.2010.02328.x . hal-00599529

**HAL Id: hal-00599529**

**<https://hal.science/hal-00599529v1>**

Submitted on 10 Jun 2011

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



**Increases in soil organic carbon sequestration can reduce  
the global warming potential of long-term liming to  
permanent grassland**

Journal:	<i>Global Change Biology</i>
Manuscript ID:	GCB-10-0540
Wiley - Manuscript type:	Primary Research Articles
Date Submitted by the Author:	30-Jun-2010
Complete List of Authors:	Fornara, Dario; University of Ulster, Environmental Sciences Steinbeiss, Sibylle; Institute of Groundwater Ecology, Helmholtz Centre Munich McNamara, Niall; CEH Gleixner, Gerd; Max-Planck-Institute for Biogeochemistry Oakley, Simon; CEH Poulton, Paul; Department of Soil Science, Rothamsted Research MacDonald, Andrew; Department of Soil Science, Rothamsted Research Bardgett, Richard D; Soil and Ecosystem Ecology Laboratory, Lancaster Environment Centre
Keywords:	agro-ecosystems, climate change mitigation, legumes, nitrogen fertilizer, soil density fractionation, soil microbial community
Abstract:	The application of calcium- and magnesium-rich materials to soil, known as liming, has long been a foundation of many agro-ecosystems worldwide because of its role in counteracting soil acidity. Although liming contributes to increased rates of respiration from soil thereby potentially reducing soils ability to act as a CO <sub>2</sub> sink, the long-term effects of liming on soil organic carbon (C <sub>org</sub> ) sequestration are largely unknown. Here, using data spanning 129 years of the Park Grass Experiment at Rothamsted (UK), we show net C <sub>org</sub> sequestration measured in the 0-23 cm layer at different time intervals since 1876 was 2 to 20 times greater in limed than in unlimed soils. The main cause of this large C <sub>org</sub> accrual was greater biological activity in limed soils, which despite increasing soil respiration rates, led to plant C inputs being processed and incorporated into resistant soil organo-mineral pools. Limed organo-mineral soils showed: (1) greater C <sub>org</sub> content for similar plant productivity levels (i.e. hay yields); (2) higher <sup>14</sup> C incorporation after 1950s atomic-bomb-testing; and (3) lower C:N ratios than unlimed organo-mineral soils, which also indicate higher microbial

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

	<p>processing of plant C. Our results show that greater Corg sequestration in limed soils strongly reduced the global warming potential (GWP) of long-term liming to permanent grassland suggesting the net contribution of agricultural liming to global warming could be lower than previously estimated. Our study demonstrates that liming might prove to be an effective mitigation strategy, especially because liming applications can be associated with a reduced use of nitrogen fertilizer which is a key cause for increased greenhouse gas emissions from agro-ecosystems.</p>



For Review Only

1  
2  
3 **Increases in soil organic carbon sequestration can reduce the global warming**  
4 **potential of long-term liming to permanent grassland**  
5  
6  
7  
8

9  
10 **Running title:** Liming effects on soil carbon sequestration  
11

12  
13 **Authors:** D. A. Fornara<sup>1,2</sup>, S. Steinbeiss<sup>3</sup>, N. P. McNamara<sup>4</sup>, G. Gleixner<sup>5</sup>, S. Oakley<sup>4</sup>, P.  
14 R. Poulton<sup>6</sup>, A. J. Macdonald<sup>6</sup>, and R. D. Bardgett<sup>2</sup>  
15

16  
17  
18 <sup>1</sup> Environmental Sciences Research Institute, University of Ulster, Coleraine, BT52 1SA,  
19 UK  
20  
21

22  
23 <sup>2</sup> Soil and Ecosystem Ecology Laboratory, Lancaster Environment Centre, Lancaster  
24 University, Lancaster LA1 4YQ, UK  
25  
26

27  
28 <sup>3</sup> Institute of Groundwater Ecology, Helmholtz Centre Munich, 85764 Neuherberg,  
29 Germany  
30  
31

32  
33 <sup>4</sup> Centre for Ecology and Hydrology, Lancaster Environment Centre, Library Avenue,  
34 Bailrigg, Lancaster, LA1 4AP  
35  
36

37  
38 <sup>5</sup> Max-Planck-Institute for Biogeochemistry, 100164 – 07701 Jena, Germany  
39

40  
41 <sup>6</sup> Department of Soil Science, Rothamsted Research, Harpenden, AL5 2JQ, UK  
42  
43

44  
45 **Corresponding author:** D.A. Fornara, Environmental Sciences Research Institute,  
46 University of Ulster, Coleraine BT52 1SA, UK  
47  
48

49 Email: [d.fornara@ulster.ac.uk](mailto:d.fornara@ulster.ac.uk); Phone: +44 (0) 28 70324658; Fax: +44 (0) 2870 324911  
50  
51

52  
53  
54 **Key words:** agro-ecosystems; climate change mitigation; legumes; nitrogen fertilizer;  
55 Park Grass Experiment; soil density fractionation; soil microbial community  
56  
57  
58  
59  
60

**Abstract**

The application of calcium- and magnesium-rich materials to soil, known as liming, has long been a foundation of many agro-ecosystems worldwide because of its role in counteracting soil acidity. Although liming contributes to increased rates of respiration from soil thereby potentially reducing soils ability to act as a CO<sub>2</sub> sink, the long-term effects of liming on soil organic carbon (C<sub>org</sub>) sequestration are largely unknown. Here, using data spanning 129 years of the Park Grass Experiment at Rothamsted (UK), we show net C<sub>org</sub> sequestration measured in the 0-23 cm layer at different time intervals since 1876 was 2 to 20 times greater in limed than in unlimed soils. The main cause of this large C<sub>org</sub> accrual was greater biological activity in limed soils, which despite increasing soil respiration rates, led to plant C inputs being processed and incorporated into resistant soil organo-mineral pools. Limed organo-mineral soils showed: (1) greater C<sub>org</sub> content for similar plant productivity levels (i.e. hay yields); (2) higher <sup>14</sup>C incorporation after 1950s atomic-bomb-testing; and (3) lower C:N ratios than unlimed organo-mineral soils, which also indicate higher microbial processing of plant C. Our results show that greater C<sub>org</sub> sequestration in limed soils strongly reduced the global warming potential (GWP) of long-term liming to permanent grassland suggesting the net contribution of agricultural liming to global warming could be lower than previously estimated. Our study demonstrates that liming might prove to be an effective mitigation strategy, especially because liming applications can be associated with a reduced use of nitrogen fertilizer which is a key cause for increased greenhouse gas emissions from agro-ecosystems.

## Introduction

Global warming mitigation requires an effective strategy portfolio which includes reduction in CO<sub>2</sub> emissions, but also increased CO<sub>2</sub> sequestration in long-lived pools, such as world soils (Lal, 2004; Six *et al.*, 2004). Agricultural soils in particular have the potential to act as C sinks following changes in land-use from arable cropping to long-term grasslands, or after the introduction of no-tillage systems, and changes to lime and fertilizer inputs (Lal, 2004; Six *et al.*, 2004; West & McBride, 2005; Hamilton *et al.*, 2007; Biasi *et al.*, 2008). The application of calcium- and magnesium-rich materials to soil (liming), has long been a common agricultural practice worldwide, because it counteracts soil acidity and helps sustain and improve plant growth (Edmeades & Ridley, 1998; Norton & Chang, 2003). Lime applications are estimated to be ~ 30 Tg y<sup>-1</sup> in the US alone (Hamilton *et al.*, 2007) and globally are expected to increase threefold in the next 50 years due to the expansion of intensive agricultural practices (Tilman *et al.*, 2001).

Emissions of CO<sub>2</sub> from lime are currently calculated with the mass fraction of C (12%) in either limestone (CaCO<sub>3</sub>) or dolomite (MgCa(CO<sub>3</sub>)<sub>2</sub>) assuming an emission factor of 100%, whereby all C in lime is released into the atmosphere (IPCC, 1997, 2006). Liming is expected to increase soil C losses, mainly because of lime-induced increases in soil biological activity and therefore rates of soil CO<sub>2</sub> respiration (Zelles *et al.*, 1987; Badalucco *et al.*, 1992; Brady & Weil 2002; Biasi *et al.*, 2008). Recent studies, however, suggest at least two potential pathways through which lime could enhance CO<sub>2</sub> sink capacity of agricultural soils. First, lime dissolution could increase carbonic acid (HCO<sub>3</sub><sup>-</sup>) concentrations in soil water solutions which could sequester 25-50% of lime C

1  
2  
3 in moderately acid soils (Hamilton *et al.*, 2007). Second, long-term liming and  
4  
5 fertilization treatments may contribute to redistribute  $C_{\text{org}}$  from labile to more humified  
6  
7 soil pools which are resistant to decomposition (Manna *et al.*, 2007). This may occur  
8  
9 through positive calcium (Ca)-induced effects on soil aggregate stability (Grant *et al.*,  
10  
11 1992) and on the physical protection of soil  $C_{\text{org}}$  (Clough & Skjemstad, 2000), which  
12  
13 ultimately contribute to enhanced soil organic matter stabilization (Morris *et al.*, 2007).  
14  
15

16  
17  
18       Despite the evidence of positive lime-induced effects on the soil  $C_{\text{org}}$  pool, no  
19  
20 studies have yet quantified the effects of long-term liming on net changes in  $C_{\text{org}}$   
21  
22 sequestration in humified soils. Such data is essential to estimate the net contribution of  
23  
24 liming to soil C dynamics and to quantify the global warming potential (GWP) of liming  
25  
26 in agro-ecosystems. In particular, it is important to know how different agricultural  
27  
28 practices contribute to net greenhouse gas emissions if we are to fully understand their  
29  
30 impact at the global scale (Robertson *et al.*, 2000; Smith *et al.*, 2008).  
31  
32

33  
34       Here we examine long-term data collected from the Park Grass Continuous Hay  
35  
36 Experiment at Rothamsted (UK). Park Grass was established in 1856 (Lawes & Gilbert,  
37  
38 1859) and is the oldest ungrazed permanent grassland experiment the world. The data  
39  
40 was used to: (1) determine whether long-term liming has influenced net changes in soil  
41  
42  $C_{\text{org}}$  sequestration across experimental plots which have received four different long-term  
43  
44 fertilization treatments (see Methods); (2) examine aboveground-belowground  
45  
46 interactions that could influence long-term lime-induced effects on soil  $C_{\text{org}}$   
47  
48 sequestration; (3) estimate the global warming potential (GWP) of agricultural liming on  
49  
50 the basis of its impact on changes in soil  $C_{\text{org}}$  sequestration and  $\text{CO}_2$  fluxes associated  
51  
52 with liming and fertilization treatments on the Park Grass Experiment.  
53  
54  
55  
56  
57  
58  
59  
60

## Methods

### *Experimental design and data*

The Park Grass Experiment is located at Rothamsted, Harpenden, Hertfordshire, in the south-east of England (51°48'N and 0°22'W). It was established in 1856 on 2.8 ha of land that had been permanent pasture for at least 100 years (Lawes & Gilbert 1900; Richardson, 1938; Thurston *et al.*, 1976). Four plots with contrasting fertilizer treatments (see Table 3 in Supporting Information 1), whose effects on soil properties and plant community composition had been documented since the beginning of the experiment (Richardson, 1938; Thurston *et al.*, 1976; Sohi *et al.*, 2001; Crawley *et al.*, 2005; Hopkins *et al.*, 2009), were selected for this study. The whole experiment received a small amount of lime in the 1880s; 4.0 tCaCO<sub>3</sub>ha<sup>-1</sup> on the plots chosen for this study. A test of liming started on half-plots in 1903 (1920 on plot 14) with 4.0 tCaCO<sub>3</sub> ha<sup>-1</sup> applied every four years. A new liming scheme was introduced in 1965; depending on the fertilizer treatment different amounts of lime were applied with the aim of maintaining soil pH at 7 in one of four quarter-plots within each treatment, whilst one sub-plot remained unlimed (see Table 3 in Supporting Information 1). By 2005 soil pH values of sub-plots given the largest lime dressings within plots 7, 14, 9 and 3 were 7.0, 7.0, 7.2 and 7.3 respectively, whereas pH values of the corresponding unlimed sub-plots were: 4.9, 6.1, 3.6 and 5.3. The lack of treatment replication in the Park Grass Experiment (see also Data analysis) was overcome to some extent by using soil samples (0-23 cm) taken from the same treatments over an extended period of time. In particular, measurements of soil carbon were done on archived soil samples collected in 1870, 1876, 1959, 1971, 1976, 1984, 1991, 1998, 2002 and 2005. Soils for 1971 and 1976 were available only for limed and



1  
2  
3 unlimed plots respectively (Table 3 in Supporting Information 1). The unlimed sub-plot  
4  
5 given  $(\text{NH}_4)_2\text{SO}_4$  (plot 9) has developed through time a thick mat of undecomposed  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

unlimed plots respectively (Table 3 in Supporting Information 1). The unlimed sub-plot given  $(\text{NH}_4)_2\text{SO}_4$  (plot 9) has developed through time a thick mat of undecomposed vegetation which was kept separate when soil sampling (i.e. we sampled within c. 3-23 cm of mineral soil). Soil C values for 1932 were taken from literature (Richardson, 1938) and were measured in soil samples collected to a depth of 20 cm. Because some soils were ground (e.g. in 1959) they could not be used for density fraction analyses. We used soil weights measured in 1876 and 1959 to estimate tonnes of organic C per hectare which did not change substantially between 1959 and 2005 (Hopkins *et al.*, 2009).

Finally, we used long-term data of plant aboveground productivity (i.e. hay yields) which are available from the online Electronic Rothamsted Archive (ERA: <http://www.rothamsted.bbsrc.ac.uk/eRA/>).

#### *Soil organic matter fractionation*

In 1870 and 1876, soils were sampled with an open-ended metal box (30 x 30 x 23cm deep); one hole was sampled in 1870 and three in 1876. In later years, 15 to 20 soil cores were taken with a 2 or 3cm diameter auger from each sub-plot and bulked together. Archived soils from different years (Table 3 in Supporting Information 1) were sieved to remove roots, dried at 40 °C for 5 days and stored in glass vials. We separated soil organic matter fractions by floatation and sedimentation using a sodium iodide (NaI) solution at a density of 1.80 g cm<sup>-3</sup> following a detailed protocol (Sohi *et al.*, 2001; see [http://www.rothamsted.bbsrc.ac.uk/aen/CarbonCycling/pdf/Fractionation\\_manual.pdf](http://www.rothamsted.bbsrc.ac.uk/aen/CarbonCycling/pdf/Fractionation_manual.pdf)). This method distinguishes between: (a) a free-organo-mineral fraction (FR-SOM) at density < 1.80 g cm<sup>-3</sup> which represents discrete free organic particles located between

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

stable soil aggregates; (b) an intra-aggregate organo-mineral fraction (IA-SOM) also at density  $< 1.80 \text{ g cm}^{-3}$  which represents discrete organic particles located within stable soil aggregates; and (c) a residual heavy organo-mineral fraction at densities  $> 1.80 \text{ g cm}^{-3}$  which is considered more recalcitrant than the FR-SOM and IA-SOM fractions (Sohi *et al.*, 2001). Both soil bulk samples and soil fractions were analyzed for total C and N by combustion and gas chromatography (COSTECH Analytical ECS 4010 instrument). To determine organic C concentration, bulk soil subsamples were burned for 16 hours at  $550^\circ\text{C}$  in a muffle furnace. Organic carbon was then calculated as the difference between total soil C and inorganic C measured in the residue ashes after furnace burning (we repeated the same procedure using organo-mineral soil fractions). Total C in soils sampled in 2002 was determined by measuring  $\text{CO}_2$  produced following high temperature oxidation using a LECO CNS-2000 elemental analyser.

#### *Soil respiration analyses*

Sixteen soil samples (6 cm diameter 10 cm depth) were randomly collected in each of the 8 sub-plots in 2008 stored in cooler box for 6 hours and then in a fridge at  $4^\circ\text{C}$  for one day. Four soil samples from each sub-plot were then allocated to each of four controlled environment rooms operated at 4, 10, 15 and  $20^\circ\text{C}$ . Soil  $\text{CO}_2$  flux measurements were made using a static chamber approach where each core was sealed in a gas tight incubation chamber (1.8 L) with a rubber septa in the lid and 8 ml gas samples were taken at 0, 60, 120 and 240 minutes after chamber closure. Samples were stored in Exetainer gas vials (Labco Ltd, UK) and  $\text{CO}_2$  concentrations were analysed using a Perkin Elmer Autosystem XL Gas Chromatograph fitted with a flame ionisation detector

1  
2  
3 and methaniser. Results were calibrated against a certified gas standard comprising 541  
4 ppm CO<sub>2</sub> (BOC, UK). CO<sub>2</sub> fluxes rates per gram dry weight of soil were calculated using  
5  
6 a standard approach (Holland *et al.*, 1999) after applying linear regression to the CO<sub>2</sub>  
7  
8 concentrations versus time data for each replicate.  
9  
10

11  
12 The Q<sub>10</sub> values were then calculated from an exponential model fitted to the CO<sub>2</sub>  
13 flux data for the four incubation temperatures. The Q<sub>10</sub> is the proportional increase in  
14  
15 respiration as the temperature increases by 10°C where the  $Q_{10} = (R_2/R_1)^{10/(T_2-T_1)}$  with R1  
16  
17 and R2 being the respiration rates at two given temperatures (T1, T2) separated by 10°C.  
18  
19  
20  
21  
22  
23

#### 24 *Microbial analyses*

25  
26 Phospholipid fatty acid (PLFA) analysis was used to gain insight into the effects of  
27  
28 fertilization and liming treatments on microbial community composition. Four fresh soil  
29  
30 samples were collected in June 2008 from each subplot 0-10 cm soil depth, stored in a  
31  
32 cooler box for 16 hours, and freeze-dried. Microbial lipids were extracted from 2 g of  
33  
34 each freeze-dried soil with a solvent system that included methanol, chloroform, and a  
35  
36 phosphate buffer. Total extracted lipids collected in the organic phase were fractionated  
37  
38 into neutral, glyco-, and polar lipids with chloroform, acetone, and methanol using silicic  
39  
40 acid chromatography. We used i15:0, a15:0, i16:0, i17:0 and a17:0 PFLAs as indicative  
41  
42 of Gram + bacteria whereas 16:1ω7, 16:1ω9, 16:1ω5 and 18:1ω7 PFLAs were indicative  
43  
44 of Gram – bacteria (Frostegård *et al.*, 1993; Pawlett *et al.*, 2000; Kramer & Gleixner,  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60 2006).

### *<sup>14</sup>C measurements*

Radiocarbon measurements were performed at the Max-Planck-Institute for Biogeochemistry in Jena, Germany. Samples were weighed into tin capsules and combusted by an elemental analyzer. The evolved CO<sub>2</sub> was transferred into a glass tube cooled with liquid nitrogen containing the iron catalyst necessary for reduction. The CO<sub>2</sub> was reduced to graphite at 600°C with an excess of hydrogen gas. The resulting graphite was analyzed by <sup>14</sup>C AMS. We dated the organo-mineral soil fraction only because this is a large and relatively stable soil carbon pool, and is likely to give reliable information on the potential accumulation of <sup>14</sup>C after nuclear bombs testing in the 1950s.

### *Global Warming Potential (GWP) of agricultural liming*

We estimated the global warming potential (GWP) for each fertilization and liming treatments applied to the Park Grass plots between 1984 and 2005 based on contributions of individual gases (CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O-N; see Supporting Information 2). GWP gives an estimate of the cumulative radiative forcing of individual greenhouse gases relative to some reference gas, usually CO<sub>2</sub>, over a specific time span, here 21 years (see also Robertson *et al.*, 2000). We estimated the net CO<sub>2</sub> fluxes taking into account: (1) net soil CO<sub>2</sub> changes between 1984 and 2005; (2) liming contribution to GWP through increased soil CO<sub>2</sub> emissions using IPCC factors (IPCC, 1997, 2006); (3) liming contributions to GWP due to the production and transport of lime; (4) GWP associated with the production and transport of fertilizers; (5) GWP associated with the emissions of N<sub>2</sub>O and CH<sub>4</sub> from soil; and (6) GWP associated with machinery emissions during field operations (i.e. liming, fertilization and hay cutting activities; see Supporting Information 2).

### Data analysis

The Park Grass Experiment was designed before modern statistical ideas about replication and randomization had been developed (Crawley *et al.*, 2005). However, the size of the experimental plots (>100 m<sup>2</sup>) and the number of soil samples collected within each plot (15 to 20), together with the repeated samplings over time, go some way to compensate for the lack of replication, particularly because this grassland was reasonably uniform before the experiment began (Lawes & Gilbert, 1859). Our main goal was to address whether and why net changes in soil C<sub>org</sub> sequestration might occur across years depending on different liming and fertilization treatments rather than to compare the total soil C<sub>org</sub> content of different experimental plots. To determine potential liming and fertilization effects on soil C<sub>org</sub> sequestration across years we used repeated measures MANOVA. The distribution of soil C and N values for each year met the preconditions of a normal distribution ( $\alpha = 0.01$ ,  $p > 0.55$  for multiple Shapiro-Wilk tests).

Linear and multiple regression analyses were performed to address potential relationships between different soil and plant variables. To quantify how soil C<sub>org</sub> content of the organo-mineral fraction changed in limed and unlimed plots between 1984 and 2005 under different fertilization treatments, we calculated the annual rate of change in C<sub>org</sub> (r). To do this, we used the C<sub>org</sub> content of each sub-plot in 1984 and in 2005, with r being:

$r = \text{Log}_e[(C_{\text{org } 1984}/C_{\text{org } 2005}]/t$ , where t, time, is 21 years. This gives, for each sub-plot, the rate of change in the C<sub>org</sub> content of the organo-mineral fraction with units of yr<sup>-1</sup>.

## Results

### *Soil C<sub>org</sub> sequestration*

We found that the C<sub>org</sub> sequestration to 23 cm soil depth since 1876, and averaged across the four different fertilization treatments, was significantly greater in limed than in unlimed soils (MANOVA,  $F_{1,6} = 10.8$ ,  $P = 0.015$ ; Fig. 1a). In particular, the net C<sub>org</sub> increase in limed soils since 1876 was 2 times and 20 times greater in 1932 and 1991 than in unlimed soils, respectively. Also, the total C<sub>org</sub> content of limed soils, averaged for four recent years (i.e., 1991, 1998, 2002 and 2005), was  $16.2 \pm 2.2 \text{ t ha}^{-1}$  greater than in unlimed soils.

Soils that have not received lime (except for the small amount applied in the 1880s) show no significant changes in C<sub>org</sub> sequestration between 1876 and 2005 ( $P = 0.34$ , Fig. 1a). We found the increased C<sub>org</sub> content of limed soils was mainly due to increases in C<sub>org</sub> in the organo-mineral soil fraction ( $F_{1,5} = 29.9$ ,  $P = 0.002$ ; Fig. 1b). Liming however did not affect the C<sub>org</sub> content of more labile soil fractions (Table 1; Table 4 in Supporting Information 1). Increased soil C<sub>org</sub> sequestration in the limed plots occurred while air temperatures at Rothamsted also increased  $> 1.0 \text{ }^\circ\text{C}$  between 1876 and 2005 (Fig. 1b).

### *Soil C<sub>org</sub> and aboveground plant productivity*

We tested whether soil C<sub>org</sub> accrual could result from greater plant productivity in limed grasslands (i.e. increased C inputs to limed soils). Aboveground plant biomass (averaged for 25 years; see Fig. 4c in Supporting Information 1) was significantly higher ( $P < 0.0001$ ) in limed ( $6.1 \pm 0.4 \text{ t ha}^{-1}$ ) than unlimed plots ( $4.9 \pm 0.3 \text{ t ha}^{-1}$ ), and was

1  
2  
3 significantly lower ( $P < 0.0001$ ) in unfertilized ( $3.2 \pm 0.1 \text{ t ha}^{-1}$ ) than fertilized plots ( $6.3$   
4  $\pm 0.2 \text{ t ha}^{-1}$ ; see Fig. 4d in Supporting Information 1). However, we could not detect any  
5  
6 significant relationship between plant aboveground productivity and total soil  $C_{\text{org}}$  in our  
7  
8 plots ( $F_{1,22} = 0.21$ ,  $P = 0.65$ ), or between plant productivity and net changes in soil  $C_{\text{org}}$   
9  
10 between 1984 and 2005 ( $F_{1,7} = 2.08$ ,  $P = 0.2$ ). Instead, we found that for similar hay  
11  
12 yields across years, the organo-mineral fraction of limed soils had ~137% more  $C_{\text{org}}$  than  
13  
14 the same fraction of unlimed soils (see Fig. 5 in Supporting Information 1). We also  
15  
16 found that in both unlimed and limed plots fertilized with PKNaMg (but not N) the rates  
17  
18 of soil  $C_{\text{org}}$  accrual since 1984 increased significantly ( $P < 0.04$ ) with the proportion of  
19  
20 legume biomass measured in a previous study (Crawley *et al.*, 2005).  
21  
22  
23  
24  
25  
26

27 To identify potential belowground mechanisms responsible for increased  $C_{\text{org}}$  in  
28  
29 limed organo-mineral soils, we performed a linear regression which included soil pH as  
30  
31 predictor variable. This analysis ( $R^2 = 0.6$ ,  $F_{1,40} = 19.3$ ,  $P < 0.0001$ ) shows that the  $C_{\text{org}}$  of  
32  
33 the organo-mineral soil pool strongly increased with soil pH (Fig. 2a). We did not find  
34  
35 any significant fertilization treatment effect on total soil  $C_{\text{org}}$  of the organo-mineral pool  
36  
37 (ANOVA,  $F_{3,40} = 0.77$ ,  $P > 0.51$ ). Also we could not find any significant fertilization  
38  
39 effect on soil  $C_{\text{org}}$  of the organo-mineral pool across years (MANOVA,  $F_{3,4} = 0.12$ ,  $P =$   
40  
41  $0.94$ ). However, we found that rates of soil  $C_{\text{org}}$  change in the organo-mineral pool  
42  
43 between 1984 and 2005 were 5.4 times greater in limed than unlimed plots under the  
44  
45  $(\text{NH}_4)_2\text{SO}_4$  treatment (see blue bars in the small bars plot framed in Fig. 2a), and 9.2  
46  
47 times greater in unfertilized limed soils (red bars).  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

### *Changes in soil microbial community composition and soil respiration rates*

We tested whether potential liming and fertilization effects on soil  $C_{\text{org}}$  content were associated with changes in soil biology. We did not find any difference in microbial biomass between limed and unlimed soils ( $F_{1,7} = 0.0008$ ,  $P = 0.97$ ), but we found that soil pH exerted a strong negative effect on the ratio of Gram positive to Gram negative bacteria ( $G^+:G^-$ ;  $P < 0.0001$ ; Fig. 2b). Lower  $G^+:G^-$  ratios were associated with a higher temperature sensitivity of soil respiration ( $Q_{10}$ ) (Estimate = -0.78, t-ratio = -11.3,  $P < 0.0001$ ), with greater soil respiration rates, and with higher microbial metabolic quotients (a measure of the microbial respiration per unit biomass; see Table 5 in Supporting Information 1). Liming also had positive significant effects on soil respiration rates at room temperatures of soil incubation of 20°C ( $F_{1,30} = 5.9$ ,  $P = 0.02$ ), but not at incubation temperatures of 4°C, 10°C or 15°C. Finally, low  $G^+:G^-$  ratios in limed plots were associated with greater soil  $C_{\text{org}}$  of the organo mineral fraction (Fig. 6 in Supporting Information 1).

### *Radiocarbon measurements and C:N ratios of the organo-mineral fraction*

We found that  $^{14}\text{C}$  incorporation (expressed as percent of modern C (pMC)) into organo-mineral fractions after atomic bomb-testing in the 1950s was significantly higher in limed soils ( $R^2 = 0.48$ ,  $F_{1,22} = 19.1$ ,  $P = 0.0003$ ; Fig. 3a). We also found that the C:N ratio of organo-mineral limed soils was negatively associated with pMC ( $R^2 = 0.64$ ,  $F_{1,22} = 37.5$ ,  $P < 0.0001$ ; Fig. 3b) suggesting a faster incorporation of C into the organo-mineral fraction of limed soils. In support of this idea (that more C has been processed and



1  
2  
3 incorporated into organo-mineral soils) we found that limed organo-mineral soils had  
4  
5 lower C:N ratios than those of unlimed soils ( $R^2 = 0.40$ ,  $F_{1,22} = 14.2$ ,  $P = 0.001$ ).  
6  
7

### 8 9 10 *GWP associated with liming and fertilization treatments*

11  
12 We found that limed plots all exhibited a net GWP which was lower than that of unlimed  
13  
14 plots (Table 2). The negative GWP of limed plots means higher mitigation potential of  
15  
16 greenhouse gases by permanent grasslands that receive regular liming applications. The  
17  
18 lower GWP of limed grasslands was mainly determined by their high soil  $C_{org}$   
19  
20 sequestration ability (Table 2) which offset the emissions of  $CO_2$  from the production,  
21  
22 transport and application of lime and fertilizers. Across the unlimed plots the PKNaMg  
23  
24 and PKNaMg +  $(NH_4)_2SO_4$  treatments showed a mitigation potential which was,  
25  
26 however, lower than the mitigation potential of the co respective fertilization treatments  
27  
28 under lime applications (Table 2). Finally, unlimed plots receiving no fertilization (Nil, in  
29  
30 Table 2) or receiving PKNaMg +  $NaNO_3$  showed a higher GWP which was mainly due  
31  
32 to loss of soil  $CO_2$  (positive values in Table 2) during the 21 years period.  
33  
34  
35  
36  
37  
38  
39  
40

### 41 **Discussion**

42  
43 Overall our results indicate that regular lime applications to permanent grassland not only  
44  
45 increased soil pH over time (Fig. 1a), but also increased and maintained the C sink  
46  
47 capacity of the soil to a greater extent than in unlimed soils. Soils that had never received  
48  
49 lime, except for a small amount in the 1880s, show no significant change in  $C_{org}$   
50  
51 sequestration between 1876 and 2005 (see Fig. 1a), which is consistent with a long-term  
52  
53 study at Park Grass that did not find any significant change in the  $C_{org}$  content of unlimed  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 soils between 1959 and 2002 (Hopkins *et al.*, 2009). The positive liming-effect on total  
4 soil  $C_{\text{org}}$  content was observed as early as 1932 (Richardson, 1938) and 1959 (Thurston *et*  
5 *al.*, 1976) in the same Park Grass plots, however it is the long-term changes in total soil  
6  $C_{\text{org}}$  content revealed in our study that shows the magnitude of the liming effect. In the  
7  
8 work reported here, the  $C_{\text{org}}$  sequestration measured in the 0-23 cm layer at different time  
9  
10 intervals since 1876 was 2 to 20 times greater in limed than in unlimed soils. Also total  
11  
12  $C_{\text{org}}$  content of limed soils, averaged for 1991, 1998, 2002 and 2005, was  $16.2 \pm 2.2 \text{ t ha}^{-1}$   
13  
14 greater than in unlimed soils. It is not clear what caused the low  $C_{\text{org}}$  content of limed  
15  
16 soils measured in 1984 (Fig. 1a), although this could be partly related to the temporary  
17  
18 absence of previously regular lime applications in two experimental plots since 1972.  
19  
20  
21  
22  
23  
24  
25  
26

27 The positive liming effect on net changes in soil  $C_{\text{org}}$  sequestration was observed  
28  
29 across different fertilization treatments, and the magnitude of such effect increased at  
30  
31 increasing soil pH differences between limed and unlimed plots under the same  
32  
33 fertilization treatment. For example, the unlimed grassland fertilized with  $(\text{NH}_4)_2\text{SO}_4$  (soil  
34  
35 pH = 3.6) has developed a thick 'mat' of undecomposed dead plant material which  
36  
37 represents a large organic C pool (i.e.  $\sim 24 \text{ t C ha}^{-1}$  as measured in 1959). However, even  
38  
39 after adding the  $C_{\text{org}}$  content of this 'mat' to the  $C_{\text{org}}$  content of the underlying mineral  
40  
41 soil (averaged for 1991, 1998, 2002 and 2005), the overall  $C_{\text{org}}$  content ( $85 \pm 1.3 \text{ t C ha}^{-1}$ )  
42  
43 was still significantly lower than the  $C_{\text{org}}$  content of limed soils fertilized with  $(\text{NH}_4)_2\text{SO}_4$   
44  
45 ( $103 \pm 5.3 \text{ t C ha}^{-1}$ ; soil pH = 7.2). The question remains, however, about what  
46  
47 mechanisms underlie such  $C_{\text{org}}$  accrual in the limed soils of the Park Grass experiment.  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

### *Increased C<sub>org</sub> content of the organo-mineral soil fraction*

We found that the increased C<sub>org</sub> content of limed soils was mainly due to increases in C<sub>org</sub> in the organo-mineral soil fraction (Fig. 1b), which is considered to be a stable-resistant soil fraction (Sollins *et al.*, 1996; Sohi *et al.*, 2001). Increased soil C<sub>org</sub> sequestration occurred even though air temperatures at Rothamsted also increased (by > 1.0 °C between 1876 and 2005; Fig. 1b); increases in temperature are known to enhance soil C losses due to enhanced decomposition and soil respiration (Zelles *et al.*, 1987; Badalucco *et al.*, 1992; Biasi *et al.*, 2008; Dorrepaal *et al.*, 2009), and were also detected in this study. Although increased aboveground productivity would be expected to play an important role in enhancing soil C<sub>org</sub> accrual in limed grasslands through greater C inputs to soil, we did not find evidence to support this in our study. Instead, we found that the organo-mineral fraction of limed soils had ~137% more C<sub>org</sub> than the same fraction of unlimed soils despite having similar hay yields across years. We also found that rates of soil C<sub>org</sub> accrual since 1984 increased significantly ( $P < 0.04$ ) with the proportion of legume biomass in both unlimed and limed plots fertilized with PKNaMg (but not N; see Thurston *et al.*, 1976; Crawley *et al.*, 2005), which suggests that legumes might play a role in soil C accumulation, as previously suggested in both grassland and cropping systems (Drinkwater *et al.*, 1998; Fornara & Tilman, 2008; De Deyn *et al.*, 2009). Accumulation of C<sub>org</sub> in limed grasslands could also depend on increased root C inputs to these soils, which is consistent with results of other studies at Park Grass (Jenkinson *et al.*, 2004) which showed that total root mass measured to 23 cm depth in limed soils receiving (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> was > 2 times greater than that of unlimed soils receiving NaNO<sub>3</sub> (9.1 vs 3.3 t ha<sup>-1</sup> respectively). However, unlimed unfertilized soils also showed high root

1  
2  
3 mass ( $9.7 \text{ t ha}^{-1}$ ; Jensen *et al.*, 1997), suggesting other processes (e.g. root C turnover  
4 rates, root exudation etc.) might play a significant role in  $C_{\text{org}}$  sequestration in these  
5  
6  
7  
8 grassland soils.  
9

### 10 11 12 *Changes in soil microbial community and soil respiration rates*

13  
14  
15 Liming had strong effects on the microbial community composition, resulting in lower  
16  
17  $G^+ : G^-$  ratios in limed compared to unlimed plots. Low  $G^+ : G^-$  ratios were in turn  
18  
19 associated with a higher temperature sensitivity of soil respiration, with greater soil  
20  
21 respiration rates and with higher microbial metabolic quotients. This suggests less  
22  
23 efficient microbial utilization of C in limed compared to unlimed soils, whereby microbes  
24  
25 respire more C per unit of degradable C (see Wardle & Ghani, 1995). These patterns  
26  
27 show similarities to soils that have received inputs of easily degradable C or lime  
28  
29 (Badalucco *et al.*, 1992; Thirukkumaran *et al.*, 2000), and support the notion that liming  
30  
31 increases both the abundance of  $G^-$  vs.  $G^+$  bacteria (Frostegård *et al.*, 1993; Pawlett *et al.*,  
32  
33 2000) and soil microbial activity (Zelles *et al.*, 1987; Badalucco *et al.*, 1992), resulting in  
34  
35 higher C losses through soil respiration. If greater soil biological activity is responsible  
36  
37 for higher soil respiration rates in our study, albeit measured using soils from a single  
38  
39 sampling, it is possible that increased biological activity will also contribute to enhanced  
40  
41 incorporation of plant C into more humified soil pools. Indeed, low  $G^+ : G^-$  ratios in limed  
42  
43 plots were associated with greater soil  $C_{\text{org}}$  of the organo mineral fraction. A potential  
44  
45 mechanism responsible for this may involve the role of  $G^-$  bacteria which are often root-  
46  
47 associated, are largely responsible for the turnover and processing of labile plant material  
48  
49 (Kramer & Gleixner, 2006), and can increase their utilization of root exudates in limed  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 grasslands (Rangel-Castro *et al.*, 2005). Also there is evidence that C-based compounds  
4  
5 from the mass of Gram – bacteria after cell death can significantly contribute to SOM  
6  
7 formation and stabilization (Kindler *et al.*, 2009).  
8  
9

10 The idea that more plant C is being processed in limed soils is supported by  
11  
12 radiocarbon analyses, which showed higher  $^{14}\text{C}$  incorporation into organo-mineral  
13  
14 fractions of limed than unlimed soils after atomic bomb-testing in the 1950s. As a result  
15  
16 of their higher  $\text{C}_{\text{org}}$  turnover, the C:N ratio of organo-mineral limed soils was also lower  
17  
18 than that of unlimed soils and was negatively associated with pMC (see Fig. 3b). Finally,  
19  
20 despite limed soils receiving, on average,  $2.8 \pm 0.3 \text{ t C ha}^{-1}$  (from  $\text{CaCO}_3$ ) since 1990, we  
21  
22 estimated that only 2.7% of this C input remained by 2005. Therefore, although this C  
23  
24 input to limed soils contributed to the increase in inorganic C content, the bulk of the C  
25  
26 accrual in limed soils was via organic C inputs.  
27  
28  
29  
30  
31  
32  
33

#### 34 *Global warming potential of long-term liming to permanent grassland*

35  
36 The increased soil  $\text{C}_{\text{org}}$  sequestration in limed soils was a key cause of their lower GWP  
37  
38 when compared with the GWP of unlimed soils. This means the net contribution of  
39  
40 agricultural liming to global warming could be lower than previous estimates suggest  
41  
42 (IPCC 1997, 2006). Regular liming applications might prove to be a potential mitigation  
43  
44 strategy to global warming if the magnitude of soil  $\text{C}_{\text{org}}$  accrual we observed in our study  
45  
46 is confirmed across a variety of grassland systems (see Soussana *et al.*, 2007). But  
47  
48 controls on the use of N fertilizers are also likely to be necessary (Robertson *et al.*, 2000;  
49  
50 Smith *et al.*, 2008). Our results indicate that further reductions in the GWP of permanent  
51  
52 grassland systems may be achieved by combining the use of lime and PKNaMg fertilizers  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 (but not N, see Table 2) to increase the abundance of legumes (Crawley *et al.*, 2005),  
4  
5 which may provide the N necessary to sustain productivity and improve soil C  
6  
7  
8 sequestration.  
9

## 10 11 12 13 **Conclusions**

14  
15 Overall, our results show that regular lime applications to long-term grassland enhanced  
16  
17 soil organic C sequestration, primarily by increasing the C<sub>org</sub> content of a resistant soil  
18  
19 organo-mineral pool. Thus, the net contribution of agricultural liming to global warming  
20  
21 could be lower than previously estimated (IPCC 1997, 2006) at least for permanent  
22  
23 grasslands. More importantly our study highlights two aspects that, if proved general,  
24  
25 could affect current predictions on long-term soil C changes in managed grasslands. First,  
26  
27 our results show that C sequestration in limed soils was enhanced by elevated soil C  
28  
29 turnover rates, and that this was associated with increases in soil pH and changes in soil  
30  
31 biological activity. Second, large variations in the organo-mineral C<sub>org</sub> content of limed  
32  
33 soils over time (see Fig. 1b) raise questions about the long-term stability of these organo-  
34  
35 mineral pools. Further studies are required in both of these areas if we are to improve our  
36  
37 mechanistic understanding of the key processes controlling C dynamics in grassland  
38  
39 soils.  
40  
41  
42  
43  
44  
45  
46  
47

48 **Acknowledgments:** This research was supported by a Marie Curie Outgoing Fellowship  
49  
50 issued to DAF within the Work Programme 2004, “Structuring the European Research  
51  
52 Area” (2002-2006), and based at Lancaster University. We thank Helen Quirk and  
53  
54 Gerlinde De Deyn who provided support during the laboratory work at Lancaster  
55  
56  
57  
58  
59  
60

1  
2  
3 University, and David Tilman for helpful comments on early drafts of the manuscript. We  
4  
5 also thank NERC QUEST Thematic Programme for financial support to NPM. Finally,  
6  
7  
8 Rothamsted Research is an institute of the Biotechnology and Biological Sciences  
9  
10 Research Council and receives support from the Lawes Agricultural Trust.  
11  
12  
13

## 14 15 **References**

- 16  
17 Badalucco L, Grego S, Dell'Orco S, Nannipieri, P (1992) Effect of liming on some  
18  
19 chemical, biochemical, and microbiological properties of acid soils under spruce  
20  
21 (*Picea abies* L.). *Biology and Fertility of Soils*, **14**, 76-83.  
22  
23  
24 Biasi C, Lind SE, Pekkarinen NM *et al.* (2008) Direct experimental evidence for the  
25  
26 contribution of lime to CO<sub>2</sub> release from managed peat soil. *Soil Biology*  
27  
28 *Biochemistry*, **40**, 2660-2669.  
29  
30  
31 Brady NC, Weill RR (2002) *The nature and properties of soils*. Prentice Hall, 13<sup>th</sup>  
32  
33 edition, Upper Saddle River, New Jersey 07458, USA.  
34  
35  
36 Clough A, Skjemstad JO (2000) Physical and chemical protection of soil organic carbon  
37  
38 in three agricultural soils with different contents of calcium carbonate. *Australian*  
39  
40 *Journal of Soil Research*, **38**, 1005–1016.  
41  
42  
43 Crawley MJ, Johnston AE, Silvertown J *et al.* (2005) Determinants of species richness in  
44  
45 the Park Grass Experiment. *American Naturalist*, **165**, 179-192.  
46  
47  
48 De Deyn GB, Quirk H , Yi Z, Oakley S, Ostle NJ, Bardgett RD (2009) Vegetation  
49  
50 composition promotes carbon and nitrogen storage in model grassland  
51  
52 communities of contrasting soil fertility. *Journal of Ecology*, **97**, 864-875.  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 Dorrepaal E, Sylvia T, van Logtestijn RSP, Swart E, van de Weg MJ, Callaghan TV,  
4  
5 Aerts R (2009) Carbon respiration from subsurface peat accelerated by climate  
6  
7 warming in the subarctic. *Nature*, **460**, 616-619.  
8  
9  
10 Drinkwater LE, Wagoner P, Sarrantonio M (1998) Legume-based cropping systems have  
11  
12 reduced carbon and nitrogen losses. *Nature*, **396**, 262-265.  
13  
14  
15 Edmeades DC, Ridley AM (2003) *Using lime to ameliorate topsoil and subsoil acidity*.  
16  
17 In: *Handbook of soil acidity*, (eds Rengel Z), pp. 297-336, Marcel Dekker, Inc.,  
18  
19 New York, Basel.  
20  
21  
22 Fornara DA, Tilman D (2008) Plant functional composition influences rates of soil  
23  
24 carbon and nitrogen accumulation. *Journal of Ecology*, **96**, 314-322.  
25  
26  
27 Frostegård Å, Bååth, E., Tunlid A (1993) Shifts in the structure of soil microbial  
28  
29 communities in limed forests as revealed by phospholipid fatty acid analysis. *Soil*  
30  
31 *Biology and Biochemistry*, **25**, 723-730  
32  
33  
34 Grant CD, Dexter AR, Oades JM (1992) Residual effects of additions of calcium  
35  
36 compounds on soil structure and strength. *Soil and Tillage Research*, **22**, 283-297.  
37  
38  
39 Hamilton SK, Kurzman AL, Arango C, Jin L, Robertson GP (2007) Evidence for carbon  
40  
41 sequestration by agricultural liming. *Global Biogeochemical Cycles*, **21**, 1-12.  
42  
43  
44 Holland EA, Robertson GP, Greenberg G, Groffman PM, Boone RD, Gosz JR (1999)  
45  
46 Soil CO<sub>2</sub>, N<sub>2</sub>O and CH<sub>4</sub> exchange. In: *Standard soil methods for long-term*  
47  
48 *ecological research*. (eds Robertson GP, Coleman DC, Bledsoe CS, Sollins P),  
49  
50 pp. 185–201. Oxford University Press, Oxford.  
51  
52  
53 Hopkins DW, Waite IS, McNicol JW, Poulton PR, Macdonald, AJ, O'Donnell AG (2009)  
54  
55 Soil organic carbon contents in long-term experimental grassland plots in the UK  
56  
57  
58  
59  
60



(Palace Leas and Park Grass) have not changed consistently in recent decades.

*Global Change Biology*, **15**, 1739-1754.

IPCC (1997) *Revised 1996 IPCC Guidelines for National Greenhouse Gas Inventories*

(eds Houghton JT, Meira Filho LG, Lim B, Tranton K, Mamaty I, Bonduki Y,

Griggs DJ, Callander BA). Intergovernmental Panel on Climate Change,

Meteorological Office, Bracknell, United Kingdom.

IPCC (2006) Agriculture, Forestry and Other Land Use. In: *2006 IPCC Guidelines for*

*National Greenhouse Gas Inventories*. (eds Egglestone S, Buendia L, Miwa K,

Ngara T, Tanabe K) Institute for Global Environmental Strategies, Hayama,

Japan.

Jenkinson DS, Poulton PR, Johnston AE, Powlson DS (2004) Turnover of nitrogen-15-

labeled fertilizer in old grassland. *Soil Science Society America Journal*, **68**, 865-

875.

Jensen LS, Mueller T, Nielsen NE, Hansen S, Crocker GJ, Grace PR, Klir J (1997)

Simulating trends in soil organic carbon in long-term experiments using the soil-

plant-atmosphere model DAISY. *Geoderma*, **81**, 5-28.

Kindler R, Miltner A, Thullner M, Richnow H-H, Kästner M (2009) Fate of bacterial

biomass derived fatty acids in soil and their contribution to soil organic matter.

*Organic Geochemistry*, **40**, 29-37.

Kramer C, Gleixner G (2006) Variable use of plant- and soil-derived carbon by

microorganisms in agricultural soils. *Soil Biology and Biochemistry*, **38**, 3267-

3278.

- 1  
2  
3 Lal, R (2004) Soil carbon sequestration impacts on global climate change and food  
4 security. *Science*, **304**, 1623-1626.  
5  
6  
7  
8 Lawes JB, Gilbert JH (1859) *Report of experiments with different manures on permanent*  
9  
10 *meadow land*. Clowes, London.  
11  
12  
13 Lawes JB, Gilbert JH (1900) Agricultural, botanical and chemical results of experiments  
14  
15 on mixed herbage of permanent grassland, conducted for many years in  
16  
17 succession on the same land. *Philosophical Transactions of the Royal Society*  
18  
19 *Series B – Biological Sciences*, **192**, 139–210.  
20  
21  
22 Manna MC, Swarup A, Wanjari RH, Mishra B, Shahi, DK (2007) Long-term  
23  
24 fertilization, manure and liming effects on soil organic matter and crop yields.  
25  
26 *Soil and Tillage Research*, **94**, 397-409.  
27  
28  
29 Morris SJ, Bohm S, Haile-Mariam S, Paul EA (2007) Evaluation of carbon accrual in  
30  
31 afforested agricultural soils. *Global Change Biology*, **13**, 1145-1156.  
32  
33  
34 Norton LD, Chang XC (1998) Liming to improve chemical and physical properties of  
35  
36 soil. In: *Handbook of Soil Conditioners: Substances that enhance the physical*  
37  
38 *properties of soil* (eds Wallace A, Terry RE), pp. 309-332. Marcel Dekker, New  
39  
40 York.  
41  
42  
43 Pawlett M, Hopkins DW, Moffett BF, Harris JA (2009) The effect of earthworms and  
44  
45 liming on soil microbial communities. *Biology Fertility Soils*, **45**, 361-369.  
46  
47  
48 Rangel-Castro JI, Killham K, Ostle N *et al.* (2005) Stable isotope probing analysis of the  
49  
50 influence of liming on root exudate utilization by soil microorganisms.  
51  
52 *Environmental Microbiology*, **7**, 828–838.  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 Richardson HL (1938) The nitrogen cycle in grassland soils with special reference to the  
4  
5 Rothamsted Park Grass Experiment. *Journal of Agricultural Science*, **1**, 73-121.  
6  
7  
8 Robertson GP, Paul EA, Harwood RR (2000) Greenhouse gases in intensive agriculture:  
9  
10 Contributions of individual gases to the radiative forcing of the atmosphere.  
11  
12 *Science*, **289**, 1922–1925.  
13  
14  
15 Six J, Ogle SM, Breidt FJ, Conant RT, Mosier AR, Paustian K (2004). The potential to  
16  
17 mitigate global warming with no-tillage management is only realized when  
18  
19 practiced in the long term. *Global Change Biology*, **10**, 155-160.  
20  
21  
22 Sohi SP, Mahieu N, Arah JRM, Powlson DS, Madari B, Gaunt JL (2001) A procedure to  
23  
24 isolating soil organic matter fractions suitable for modeling. *Soil Science Society*  
25  
26 *America Journal*, **65**, 1121-1128.  
27  
28  
29 Sollins P, Homann P, Caldwell, BA (1996) Stabilization and destabilization of soil  
30  
31 organic matter: mechanisms and controls. *Geoderma*, **74**, 65-105.  
32  
33  
34 Soussana JF, Allard V, Pilegaard K *et al.* (2007) Full accounting of the greenhouse gas  
35  
36 (CO<sub>2</sub>, N<sub>2</sub>O, CH<sub>4</sub>) budget of nine European grassland sites. *Agriculture Ecosystems*  
37  
38 *and Environment*, 121, 121–134.  
39  
40  
41 Smith P, Martino D, Cai Z *et al.* (2008) Greenhouse gas mitigation in agriculture.  
42  
43 *Philosophical Transactions of the Royal Society B*, **363**, 789–813.  
44  
45  
46 Thirukkumaran CM, Parkinson D (2000) Microbial respiration, biomass, metabolic  
47  
48 quotient and litter decomposition in a lodgepole pine forest floor amended with  
49  
50 nitrogen and phosphorous fertilizers. *Soil Biology and Biochemistry*, **32**, 59-66.  
51  
52  
53 Thurston JM, Williams, ED, Johnston AE (1976) Modern developments in an experiment  
54  
55 on permanent grassland started in 1856: Effects of fertilisers and lime on  
56  
57  
58  
59  
60

1  
2  
3 botanical composition and crop and soil analyses. *Annales Agronomiques*, **27**,  
4 1043-1082.  
5  
6

7  
8 Tilman D, Fargione J, Wolff B *et al.* (2001) Forecasting agriculturally driven global  
9 environment change. *Science*, **292**, 281–284.  
10  
11

12 Wardle DA, Ghani A (1995) A critique of the microbial metabolic quotient ( $q\text{CO}_2$ ) as a  
13 bioindicator of disturbance and ecosystem development. *Soil Biology and*  
14 *Biochemistry*, **27**, 1601-1610.  
15  
16  
17

18  
19 West TO, McBride AC (2005) The Contribution of agricultural lime to carbon dioxide  
20 emissions in the United States: dissolution, transport, and net emissions.  
21  
22 *Agriculture Ecosystem and Environment*, **108**, 145–154.  
23  
24  
25

26  
27 Zelles L, Stepper K, Zsolnay A (1987) The effect of lime on microbial activity in spruce  
28 (*Picea abies* L.) forests. *Biology and Fertility of Soils*, **9**, 78-82.  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**Table 1.** Results of density fractionation analyses performed on soil samples collected from the Park Grass plots. Total C and N are expressed both as percentage and as unit weight per kilogram of soil (mean  $\pm$  S.E.) and averaged for three recent years 1991, 1998 and 2005. FR-SOM = free-aggregate soil fraction; IA-SOM = intra-aggregate soil fraction. "Recovered" indicates the percentage of total C and N recuperated at the end of the density fractionation normalized to the initial mass of soil fractionated.

		C (%)	N (%)	C:N	C (g C kg <sup>-1</sup> soil)	N (g N kg <sup>-1</sup> soil)
Bulk soil	Limed	3.89 $\pm$ 0.1	0.31 $\pm$ 0.01	12.6	39.2 $\pm$ 1.19	3.16 $\pm$ 0.11
	Unlimed	3 $\pm$ 0.08	0.24 $\pm$ 0.008	12.5	30.1 $\pm$ 0.88	2.39 $\pm$ 0.08
FR-SOM	Limed	7.7 $\pm$ 0.84	0.42 $\pm$ 0.03	18.3	1.70 $\pm$ 0.28	0.09 $\pm$ 0.02
	Unlimed	7.5 $\pm$ 0.89	0.36 $\pm$ 0.01	20.8	1.73 $\pm$ 0.24	0.08 $\pm$ 0.01
IA-SOM	Limed	11.4 $\pm$ 0.66	0.47 $\pm$ 0.02	24.3	0.59 $\pm$ 0.007	0.02 $\pm$ 0.002
	Unlimed	12.1 $\pm$ 0.59	0.45 $\pm$ 0.03	26.8	0.61 $\pm$ 0.006	0.02 $\pm$ 0.002
Organo-mineral	Limed	3.37 $\pm$ 0.09	0.29 $\pm$ 0.007	11.6	31.2 $\pm$ 0.89	2.67 $\pm$ 0.06
	Unlimed	2.5 $\pm$ 0.06	0.20 $\pm$ 0.005	12.5	23.4 $\pm$ 0.62	1.91 $\pm$ 0.05
All fractions	Limed				33.5 $\pm$ 1.20	2.78 $\pm$ 0.02
	Unlimed				25.7 $\pm$ 1.02	2.01 $\pm$ 0.02
Recovered (%)	Limed				85.4 $\pm$ 5.2	87.9 $\pm$ 6.1
	Unlimed				85.5 $\pm$ 5.9	84.1 $\pm$ 5.5

**Table 2.** Relative GWPs for different fertilization and liming treatments based on measurements and estimates of multiple CO<sub>2</sub> sinks and sources (see Supplementary material\_2 for details). GWP was estimated for a 21-year period between 1984 and 2005. Negative values indicate a global warming mitigation potential. Units are CO<sub>2</sub> equivalents (g CO<sub>2</sub> m<sup>-2</sup> y<sup>-1</sup>).

Grassland management	CO <sub>2</sub> sinks and sources (g CO <sub>2</sub> m <sup>-2</sup> y <sup>-1</sup> )						
	Net soil CO <sub>2</sub> sequestration for 21 years	Lime	N <sub>2</sub> O-N	CH <sub>4</sub>	Fertilizer	Fuel	Net GWP
<b>Limed plots</b>							
Nil	-260	47.6	2.2	-2.2	0.0	1.8	-211
PKNaMg	-286	43.3	3.8	-2.2	10.4	2.1	-229
PKNaMg + (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	-551	101	3.3	-1.6	33.2	2.1	-413
PKNaMg + NaNO <sub>3</sub>	-231	21.9	7.0	-2.2	97.2	2.1	-105
<b>Unlimed plots</b>							
Nil	90.6	0.0	1.8	-1.2	0.0	1.8	93.0
PKNaMg	-84.1	0.0	3.1	-1.4	10.4	1.9	-70.0
PKNaMg + (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	-64.8	0.0	2.5	-0.1	33.2	1.9	-27.3
PKNaMg + NaNO <sub>3</sub>	23.3	0.0	5.1	-1.4	97.2	1.9	126

1  
2  
3 **Fig. 1. (a)** Liming effects on bulk soil  $C_{org}$  ( $t\ C\ ha^{-1}$ ) in the Park Grass Experiment.  
4  
5  
6 MANOVA was performed on  $C_{org}$  values for 1876, 1932, 1959, 1984, 1991, 1998, 2002  
7  
8 and 2005. Archived soils were also available for limed plots in 1971 and for unlimed  
9  
10 plots in 1976. The short vertical bars near the x axis indicate main lime applications (in  
11  
12 1976 lime was applied to only two plots). Solid lines indicate soil pH values for both  
13  
14 limed and unlimed plots. **(b)** Liming effects on  $C_{org}$  of the organo-mineral fraction for  
15  
16 different years (bar histograms; MANOVA was performed on  $C_{org}$  values for 1876, 1984,  
17  
18 1991, 1998 and 2005); also mean increase in annual temperatures (solid line) since 1876  
19  
20 at Rothamsted.  
21  
22  
23  
24  
25

26 **Fig. 2. (a)** The relationship between soil  $C_{org}$  of the organo-mineral fraction and soil pH  
27  
28 (empty circles = unlimed plots, filled circles = limed plots). Different colours indicate  
29  
30 different fertilization treatments. The regression analysis included soil  $C_{org}$  data for 1876,  
31  
32 1971, 1976, 1984, 1991, 1998 and 2005. The small bars plot framed in the left-upper  
33  
34 corner shows the rate of soil  $C_{org}$  change for 21 years [ $\text{Log}(\text{soil C } 2005/\text{soil C } 1984)]/21$   
35  
36 of the organo-mineral fraction (filled bars = limed plots; empty bars = unlimed plots  
37  
38 within each fertilization treatment). **(b)** The relationship between Gram+:Gram- bacteria  
39  
40 ratio on soil pH as both measured in 2008 (Estimate = - 0.19, t-ratio = -10.8,  $P < 0.0001$ ).  
41  
42  
43  
44  
45  
46

47 **Fig. 3. (a)** The relationship between the percent of modern C (pMC) in the organo-  
48  
49 mineral soil fraction and the  $C_{org}$  in this fraction (values are for 1984, 1998 and 2005),  
50  
51 and **(b)** on the C:N ratios of the same soil fraction. Filled circles = limed plots; empty  
52  
53 circles = unlimed plots. Triangles indicate 'pre-bomb' test  $^{14}C$  measurements on archived  
54  
55 soils from 1870, 1876 and 1906 (not included in the regression analysis).  
56  
57  
58  
59  
60

Fig. 1a

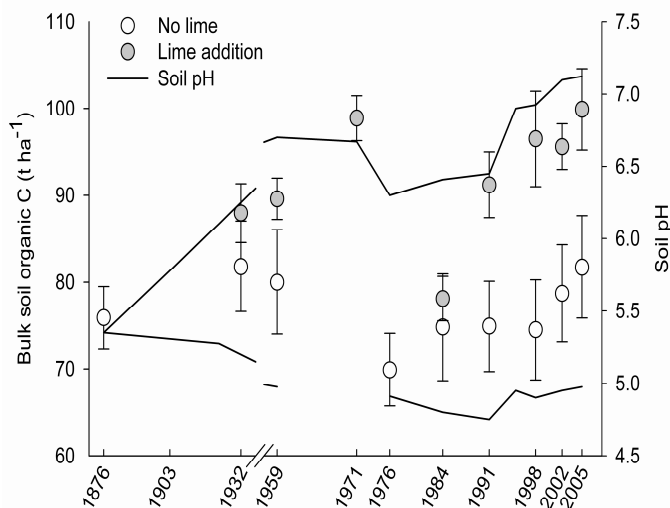


Fig. 1b

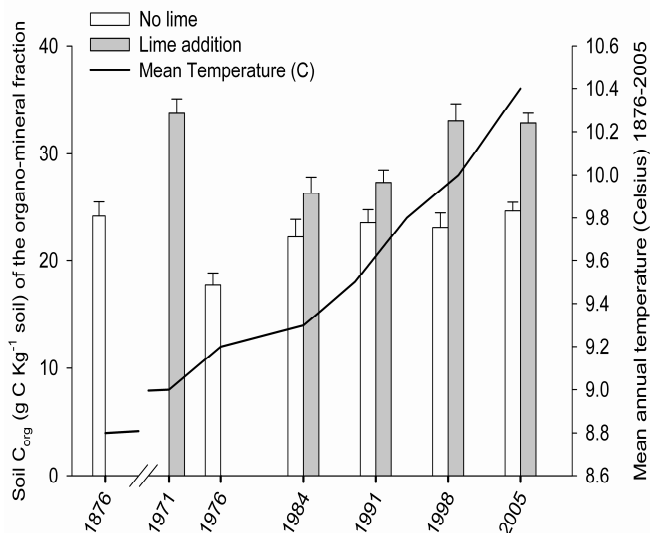


Fig. 2a

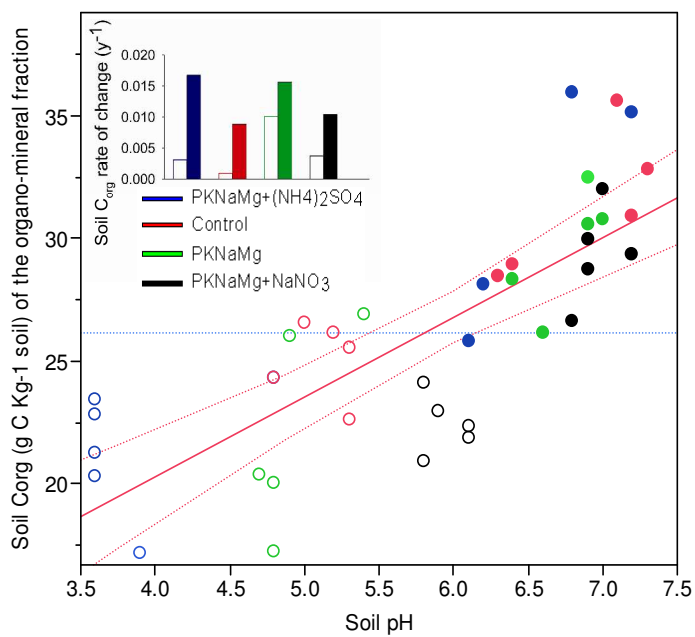


Fig. 2b

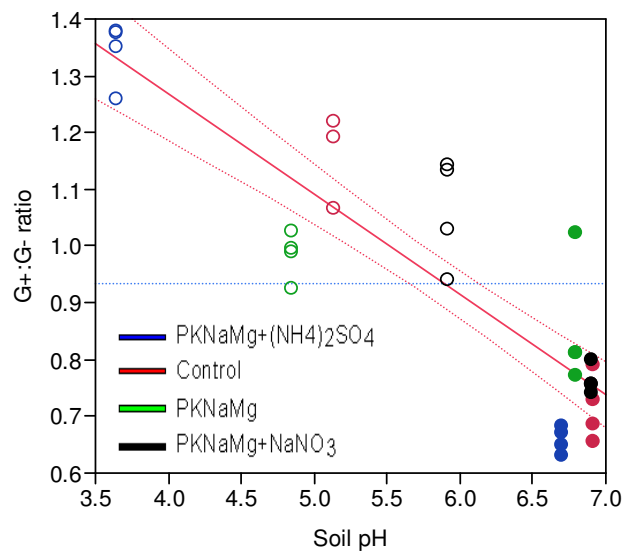




Fig. 3a

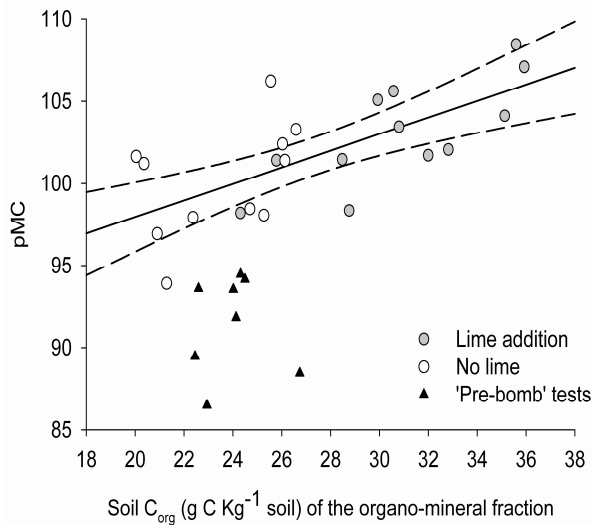
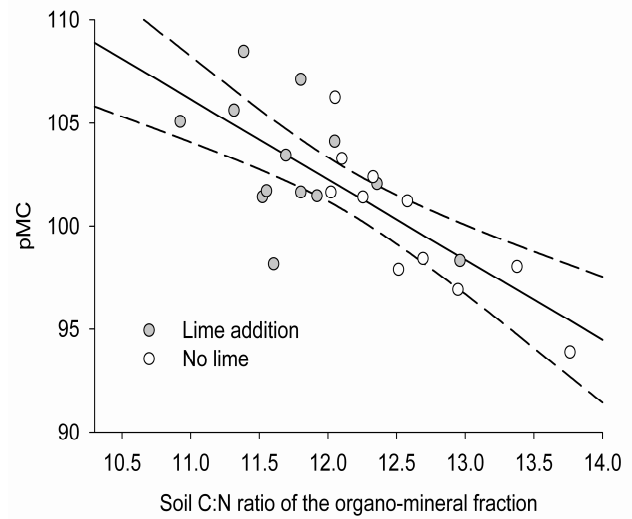


Fig. 3b



Review Only