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

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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_2 sink, the long-term effects of liming on soil organic carbon (Corg) sequestration are largely unknown. Here, using data spanning 129 years of the Park Grass Experiment at Rothamsted (UK), we show net Corg 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 Corg 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 Corg content for similar plant productivity levels (i.e. hay yields); (2) higher 14C 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 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.

Increases in soil organic carbon sequestration can reduce the global warming

potential of long-term liming to permanent grassland

Running title: Liming effects on soil carbon sequestration

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Park Grass Experiment; 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_2 sink, the long-term effects of liming on soil organic carbon (C_{org}) sequestration are largely unknown. Here, using data spanning 129 years of the Park Grass Experiment at Rothamsted (UK), we show net C_{org} 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_{org} 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_{org} content for similar plant productivity levels (i.e. hay yields); (2) higher ^{14}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_{org} 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_2 emissions, but also increased CO_2 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^{-1} 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_2 from lime are currently calculated with the mass fraction of C (12%) in either limestone ($CaCO_3$) or dolomite ($MgCa(CO_3)_2$) 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_2 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_2 sink capacity of agricultural soils. First, lime dissolution could increase carbonic acid (HCO_3^-) concentrations in soil water solutions which could sequester 25-50% of lime C

3

in moderately acid soils (Hamilton *et al*., 2007). Second, long-term liming and

fertilization treatments may contribute to redistribute C_{org} from labile to more humified

soil pools which are resistant to decomposition (Manna *et al.,* 2007). This may occur

through positive calcium (Ca)-induced effects on soil aggregate stability (Grant *et al*.,

1992) and on the physical protection of soil C_{org} (Clough & Skjemstad, 2000), which

ultimately contribute to enhanced soil organic matter stabilization (Morris *et al.,* 2007).

Despite the evidence of positive lime-induced effects on the soil C_{org} pool, no

studies have yet quantified the effects of long-term liming on net changes in C_{org}

sequestration in humified soils. Such data is essential to estimate the net contribution of

liming to soil C dynamics and to quantify the global warming potential (GWP) of liming

in agro-ecosystems. In particular, it is important to know how different agricultural

practices contribute to net greenhouse gas emissions if we are to fully understand their

impact at the global scale (Robertson *et al.,* 2000; Smith *et al.,* 2008).

Here we examine long-term data collected from the Park Grass Continuous Hay

Experiment at Rothamsted (UK). Park Grass was established in 1856 (Lawes & Gilbert,

1859) and is the oldest ungrazed permanent grassland experiment the world. The data

was used to: (1) determine whether long-term liming has influenced net changes in soil

C_{org} sequestration across experimental plots which have received four different long-term

fertilization treatments (see Methods); (2) examine aboveground-belowground

interactions that could influence long-term lime-induced effects on soil C_{org}

sequestration; (3) estimate the global warming potential (GWP) of agricultural liming on

the basis of its impact on changes in soil C_{org} sequestration and CO_2 fluxes associated

with liming and fertilization treatments on the Park Grass Experiment.

Methods

Experimental design and data

The Park Grass Experiment is located at Rothamsted, Harpenden, Hertfordshire, in the

south-east of England ($51^0 48$'N and $0^0 22$'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 $tCaCO_3 ha^{-1}$ on the plots chosen for this study. A test of liming

started on half-plots in 1903 (1920 on plot 14) with 4.0 $tCaCO_3 \ ha^{-1}$ 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

5

unlimed plots respectively (Table 3 in Supporting Information 1). The unlimed sub-plot

given $(NH_4)_2SO_4$ (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^{-3} following a detailed protocol (Sohi *et al.,* 2001; see

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^{-3} which represents discrete free organic particles located between

6

stable soil aggregates; (b) an intra-aggregate organo-mineral fraction (IA-SOM) also at

density < 1.80 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 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

°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 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°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°C. Soil 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 CO_2 concentrations were analysed using a

Perkin Elmer Autosystem XL Gas Chromatograph fitted with a flame ionisation detector

and methaniser. Results were calibrated against a certified gas standard comprising 541

ppm CO_2 (BOC, UK). CO_2 fluxes rates per gram dry weight of soil were calculated using

a standard approach (Holland *et al.,* 1999) after applying linear regression to the CO_2

concentrations versus time data for each replicate.

The Q_{10} values were then calculated from an exponential model fitted to the CO_2

flux data for the four incubation temperatures. The Q_{10} is the proportional increase in

respiration as the temperature increases by $10^{\circ}C$ where the $Q_{10} = (R2/R1)^{10/(T2-T1)}$ with R1

and R2 being the respiration rates at two given temperatures (T1, T2) separated by $10^{\circ}C$.

Microbial analyses

Phospholipid fatty acid (PLFA) analysis was used to gain insight into the effects of

fertilization and liming treatments on microbial community composition. Four fresh soil

samples were collected in June 2008 from each subplot 0-10 cm soil depth, stored in a

cooler box for 16 hours, and freeze-dried. Microbial lipids were extracted from 2 g of

each freeze-dried soil with a solvent system that included methanol, chloroform, and a

phosphate buffer. Total extracted lipids collected in the organic phase were fractionated

into neutral, glyco-, and polar lipids with chloroform, acetone, and methanol using silicic

acid chromatography. We used i15:0, a15:0, i16:0, i17:0 and a17:0 PFLAs as indicative

of Gram + bacteria whereas 16:1ω7, 16:1ω9, 16:1ω5 and 18:1ω7 PFLAs were indicative

of Gram – bacteria (Frostegård *et al*., 1993; Pawlett *et al*., 2000; Kramer & Gleixner,

2006).

^{14}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_2 was transferred into a glass tube

cooled with liquid nitrogen containing the iron catalyst necessary for reduction. The CO_2

was reduced to graphite at 600°C with an excess of hydrogen gas. The resulting graphite

was analyzed by ^{14}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 ^{14}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_2, CH_4 and N_2O-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_2, over a specific time span, here 21 years (see also

Robertson *et al.,* 2000). We estimated the net CO_2 fluxes taking into account: (1) net soil

CO_2 changes between 1984 and 2005; (2) liming contribution to GWP through increased

soil CO_2 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_2O and

CH_4 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^2) 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_{org} sequestration might occur across years

depending on different liming and fertilization treatments rather than to compare the total

soil C_{org} content of different experimental plots. To determine potential liming and

fertilization effects on soil C_{org} 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_{org} 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_{org} (r). To do this, we used the C_{org} content of each sub-plot in 1984 and in 2005, with r

being:

$r = Log_e[(C_{org\ 1984}/C_{org\ 2005}]/t$, where t, time, is 21 years. This gives, for each sub-plot, the

rate of change in the C_{org} content of the organo-mineral fraction with units of yr^{-1}.

Results

Soil C_{org} sequestration

We found that the C_{org} 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_{org}

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_{org} content of limed soils, averaged for four

recent years (i.e., 1991, 1998, 2002 and 2005), was 16.2 ± 2.2 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_{org} sequestration between 1876 and 2005 (P =

0.34, Fig. 1a). We found the increased C_{org} content of limed soils was mainly due to

increases in C_{org} in the organo-mineral soil fraction ($F_{1,5}$ = 29.9, P = 0.002; Fig. 1b).

Liming however did not affect the C_{org} content of more labile soil fractions (Table 1;

Table 4 in Supporting Information 1). Increased soil C_{org} sequestration in the limed plots

occurred while air temperatures at Rothamsted also increased > 1.0 °C between 1876 and

2005 (Fig. 1b).

Soil C_{org} and aboveground plant productivity

We tested whether soil C_{org} 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 ± 0.4 t ha^{-1}) than unlimed plots (4.9 ± 0.3 t ha^{-1}), and was

significantly lower (P < 0.0001) in unfertilized (3.2 ± 0.1 t ha^{-1}) than fertilized plots (6.3

± 0.2 t ha^{-1}; see Fig. 4d in Supporting Information 1). However, we could not detect any

significant relationship between plant aboveground productivity and total soil C_{org} in our

plots ($F_{1,22}$ = 0.21, P = 0.65), or between plant productivity and net changes in soil C_{org}

between 1984 and 2005 ($F_{1,7}$ = 2.08, P = 0.2). Instead, we found that for similar hay

yields across years, the organo-mineral fraction of limed soils had ~137% more C_{org} than

the same fraction of unlimed soils (see Fig. 5 in Supporting Information 1). We also

found that in both unlimed and limed plots fertilized with PKNaMg (but not N) the rates

of soil C_{org} accrual since 1984 increased significantly (P < 0.04) with the proportion of

legume biomass measured in a previous study (Crawley *et al.,* 2005).

To identify potential belowground mechanisms responsible for increased C_{org} in

limed organo-mineral soils, we performed a linear regression which included soil pH as

predictor variable. This analysis (R^2 = 0.6, $F_{1,40}$ = 19.3, P < 0.0001) shows that the C_{org} of

the organo-mineral soil pool strongly increased with soil pH (Fig. 2a). We did not find

any significant fertilization treatment effect on total soil C_{org} of the organo-mineral pool

(ANOVA, $F_{3,40}$ = 0.77, P > 0.51). Also we could not find any significant fertilization

effect on soil C_{org} of the organo-mineral pool across years (MANOVA, $F_{3,4}$ = 0.12, P =

0.94). However, we found that rates of soil C_{org} change in the organo-mineral pool

between 1984 and 2005 were 5.4 times greater in limed than unlimed plots under the

$(NH_4)_2SO_4$ treatment (see blue bars in the small bars plot framed in Fig. 2a), and 9.2

times greater in unfertilized limed soils (red bars).

Changes in soil microbial community composition and soil respiration rates

We tested whether potential liming and fertilization effects on soil C_{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_{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}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

13

incorporated into organo-mineral soils) we found that limed organo-mineral soils had

lower C:N ratios that those of unlimed soils ($R^2 = 0.40$, $F_{1,22} = 14.2$, $P = 0.001$).

GWP associated with liming and fertilization treatments

We found that limed plots all exhibited a net GWP which was lower than that of unlimed

plots (Table 2). The negative GWP of limed plots means higher mitigation potential of

greenhouse gases by permanent grasslands that receive regular liming applications. The

lower GWP of limed grasslands was mainly determined by their high soil C_{org}

sequestration ability (Table 2) which offset the emissions of CO_2 from the production,

transport and application of lime and fertilizers. Across the unlimed plots the PKNaMg

and PKNaMg + $(NH_4)_2SO_4$ treatments showed a mitigation potential which was,

however, lower than the mitigation potential of the co respective fertilization treatments

under lime applications (Table 2). Finally, unlimed plots receiving no fertilization (Nil, in

Table 2) or receiving PKNaMg + $NaNO_3$ showed a higher GWP which was mainly due

to loss of soil CO_2 (positive values in Table 2) during the 21 years period.

Discussion

Overall our results indicate that regular lime applications to permanent grassland not only

increased soil pH over time (Fig. 1a), but also increased and maintained the C sink

capacity of the soil to a greater extent than in unlimed soils. Soils that had never received

lime, except for a small amount in the 1880s, show no significant change in C_{org}

sequestration between 1876 and 2005 (see Fig. 1a), which is consistent with a long-term

study at Park Grass that did not find any significant change in the C_{org} content of unlimed

soils between 1959 and 2002 (Hopkins *et al.,* 2009). The positive liming-effect on total

soil C_{org} content was observed as early as 1932 (Richardson, 1938) and 1959 (Thurston *et*

al., 1976) in the same Park Grass plots, however it is the long-term changes in total soil

C_{org} content revealed in our study that shows the magnitude of the liming effect. In the

work reported here, the C_{org} 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. Also total

C_{org} content of limed soils, averaged for 1991, 1998, 2002 and 2005, was 16.2 ± 2.2 t ha^{-1}

greater than in unlimed soils. It is not clear what caused the low C_{org} content of limed

soils measured in 1984 (Fig. 1a), although this could be partly related to the temporary

absence of previously regular lime applications in two experimental plots since 1972.

The positive liming effect on net changes in soil C_{org} sequestration was observed

across different fertilization treatments, and the magnitude of such effect increased at

increasing soil pH differences between limed and unlimed plots under the same

fertilization treatment. For example, the unlimed grassland fertilized with $(NH_4)_2SO_4$ (soil

pH = 3.6) has developed a thick 'mat' of undecomposed dead plant material which

represents a large organic C pool (i.e. ~ 24 t C ha^{-1} as measured in 1959). However, even

after adding the C_{org} content of this 'mat' to the C_{org} content of the underlying mineral

soil (averaged for 1991, 1998, 2002 and 2005), the overall C_{org} content (85 ± 1.3 t C ha^{-1})

was still significantly lower than the C_{org} content of limed soils fertilized with $(NH_4)_2SO_4$

(103 ± 5.3 t C ha^{-1}; soil pH = 7.2). The question remains, however, about what

mechanisms underlie such C_{org} accrual in the limed soils of the Park Grass experiment.

Increased C_{org} content of the organo-mineral soil fraction

We found that the increased C_{org} content of limed soils was mainly due to increases in

C_{org} 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_{org}

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_{org} 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_{org} than the same fraction of

unlimed soils despite having similar hay yields across years. We also found that rates of

soil C_{org} 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_{org} 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 $(NH_4)_2SO_4$ was > 2 times greater than that of unlimed soils receiving $NaNO_3$

(9.1 *vs* 3.3 t ha^{-1} respectively). However, unlimed unfertilized soils also showed high root

16

mass (9.7 t ha^{-1}; Jensen *et al.,* 1997), suggesting other processes (e.g. root C turnover

rates, root exudation etc.) might play a significant role in C_{org} sequestration in these

grassland soils.

Changes in soil microbial community and soil respiration rates

Liming had strong effects on the microbial community composition, resulting in lower

G+:G– ratios in limed compared to unlimed plots. Low G+:G– ratios were in turn

associated with a higher temperature sensitivity of soil respiration, with greater soil

respiration rates and with higher microbial metabolic quotients. This suggests less

efficient microbial utilization of C in limed compared to unlimed soils, whereby microbes

respire more C per unit of degradable C (see Wardle & Ghani, 1995). These patterns

show similarities to soils that have received inputs of easily degradable C or lime

(Badalucco *et al.,* 1992; Thirukkumaran *et al.,* 2000), and support the notion that liming

increases both the abundance of G– *vs*. G+ bacteria (Frostegård *et al.*, 1993; Pawlett *et al.*,

2000) and soil microbial activity (Zelles *et al.,* 1987; Badalucco *et al.,* 1992), resulting in

higher C losses through soil respiration. If greater soil biological activity is responsible

for higher soil respiration rates in our study, albeit measured using soils from a single

sampling, it is possible that increased biological activity will also contribute to enhanced

incorporation of plant C into more humified soil pools. Indeed, low G+:G– ratios in limed

plots were associated with greater soil C_{org} of the organo mineral fraction. A potential

mechanism responsible for this may involve the role of G– bacteria which are often root-

associated, are largely responsible for the turnover and processing of labile plant material

(Kramer & Gleixner, 2006), and can increase their utilization of root exudates in limed

17

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

The idea that more plant C is being processed in limed soils is supported by radiocarbon analyses, which showed higher ^{14}C incorporation into organo-mineral fractions of limed than unlimed soils after atomic bomb-testing in the 1950s. As a result of their higher C_{org} turnover, the C:N ratio of organo-mineral limed soils was also lower than that of unlimed soils and was negatively associated with pMC (see Fig. 3b). Finally, despite limed soils receiving, on average, 2.8 ± 0.3 t C ha^{-1} (from CaCO$_3$) since 1990, we estimated that only 2.7% of this C input remained by 2005. Therefore, although this C input to limed soils contributed to the increase in inorganic C content, the bulk of the C accrual in limed soils was via organic C inputs.

Global warming potential of long-term liming to permanent grassland

The increased soil C_{org} sequestration in limed soils was a key cause of their lower GWP when compared with the GWP of unlimed soils. This means the net contribution of agricultural liming to global warming could be lower than previous estimates suggest (IPCC 1997, 2006). Regular liming applications might prove to be a potential mitigation strategy to global warming if the magnitude of soil C_{org} accrual we observed in our study is confirmed across a variety of grassland systems (see Soussana *et al.,* 2007). But controls on the use of N fertilizers are also likely to be necessary (Robertson *et al*., 2000; Smith *et al.,* 2008). Our results indicate that further reductions in the GWP of permanent grassland systems may be achieved by combining the use of lime and PKNaMg fertilizers

(but not N, see Table 2) to increase the abundance of legumes (Crawley *et al.,* 2005),

which may provide the N necessary to sustain productivity and improve soil C

sequestration.

Conclusions

Overall, our results show that regular lime applications to long-term grassland enhanced

soil organic C sequestration, primarily by increasing the C_{org} content of a resistant soil

organo-mineral pool. Thus, the net contribution of agricultural liming to global warming

could be lower than previously estimated (IPCC 1997, 2006) at least for permanent

grasslands. More importantly our study highlights two aspects that, if proved general,

could affect current predictions on long-term soil C changes in managed grasslands. First,

our results show that C sequestration in limed soils was enhanced by elevated soil C

turnover rates, and that this was associated with increases in soil pH and changes in soil

biological activity. Second, large variations in the organo-mineral C_{org} content of limed

soils over time (see Fig. 1b) raise questions about the long-term stability of these organo-

mineral pools. Further studies are required in both of these areas if we are to improve our

mechanistic understanding of the key processes controlling C dynamics in grassland

soils.

19

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

26

Table 2. Relative GWPs for different fertilization and liming treatments based on measurements and estimates of multiple CO_2 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_2 equivalents (g CO_2 m^{-2} y^{-1}).

Grassland management	CO_2 sinks and sources (g CO_2 m^{-2} y^{-1})						
Limed plots	Net soil CO_2 sequestration for 21 years	Lime	N_2O-N	CH_4	Fertilizer	Fuel	Net GWP
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 + $(NH_4)_2SO_4$	-551	101	3.3	-1.6	33.2	2.1	-413
PKNaMg + $NaNO_3$	-231	21.9	7.0	-2.2	97.2	2.1	-105
Unlimed plots							
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 + $(NH_4)_2SO_4$	-64.8	0.0	2.5	-0.1	33.2	1.9	-27.3
PKNaMg + $NaNO_3$	23.3	0.0	5.1	-1.4	97.2	1.9	126

27

Fig. 1. (a) Liming effects on bulk soil C_{org} (t C ha^{-1}) in the Park Grass Experiment.

MANOVA was performed on C_{org} values for 1876, 1932, 1959, 1984, 1991, 1998, 2002

and 2005. Archived soils were also available for limed plots in 1971 and for unlimed

plots in 1976. The short vertical bars near the x axis indicate main lime applications (in

1976 lime was applied to only two plots). Solid lines indicate soil pH values for both

limed and unlimed plots. **(b)** Liming effects on C_{org} of the organo-mineral fraction for

different years (bar histograms; MANOVA was performed on C_{org} values for 1876, 1984,

1991, 1998 and 2005); also mean increase in annual temperatures (solid line) since 1876

at Rothamsted.

Fig. 2. (a) The relationship between soil C_{org} of the organo-mineral fraction and soil pH

(empty circles = unlimed plots, filled circles = limed plots). Different colours indicate

different fertilization treatments. The regression analysis included soil C_{org} data for 1876,

1971, 1976, 1984, 1991, 1998 and 2005. The small bars plot framed in the left-upper

corner shows the rate of soil C_{org} change for 21 years [Log (soil C 2005/soil C 1984)]/21

of the organo-mineral fraction (filled bars = limed plots; empty bars = unlimed plots

within each fertilization treatment). **(b)** The relationship between Gram+:Gram- bacteria

ratio on soil pH as both measured in 2008 (Estimate = - 0.19, t-ratio = -10.8, P < 0.0001).

Fig. 3. (a) The relationship between the percent of modern C (pMC) in the organo-

mineral soil fraction and the C_{org} in this fraction (values are for 1984, 1998 and 2005),

and **(b)** on the C:N ratios of the same soil fraction. Filled circles = limed plots; empty

circles = unlimed plots. Triangles indicate 'pre-bomb' test ^{14}C measurements on archived

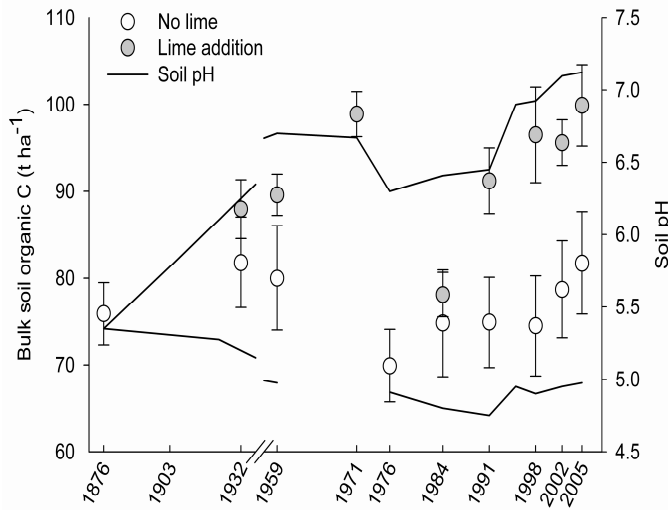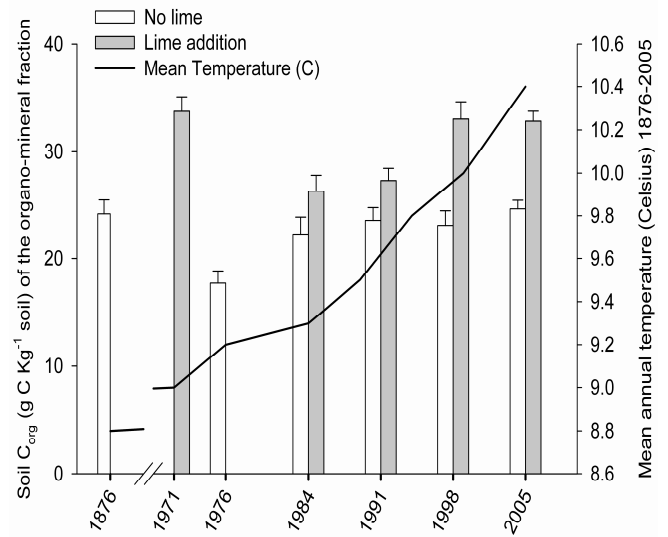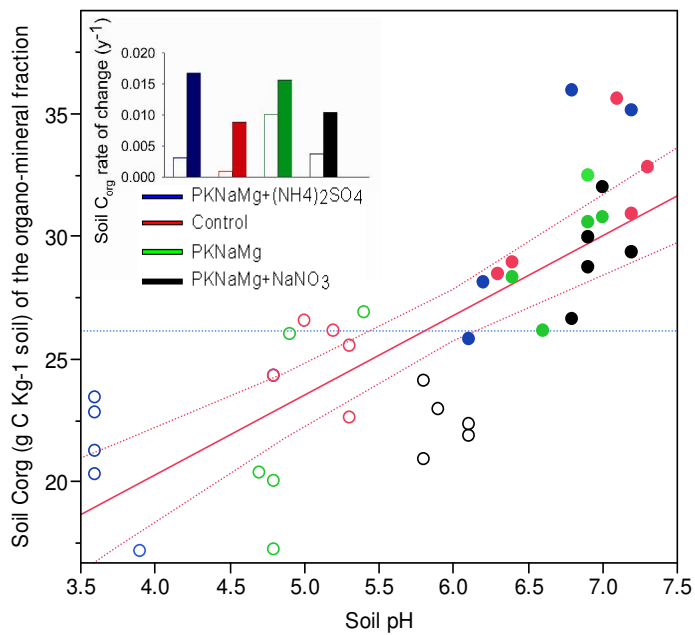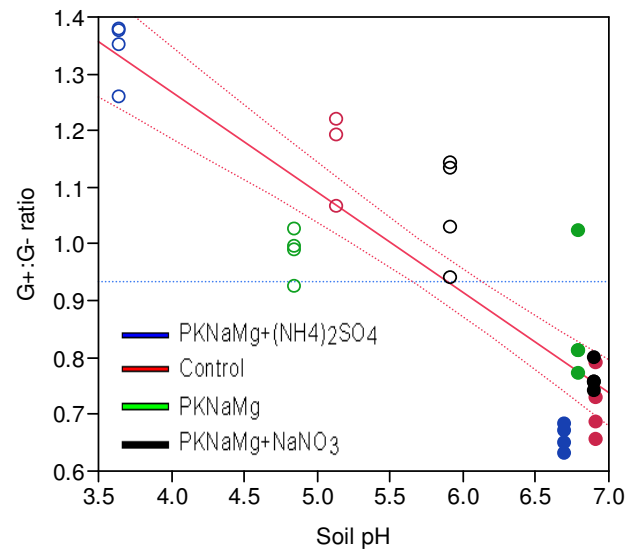soils from 1870, 1876 and 1906 (not included in the regression analysis).

Fig. 1a

Fig. 1b

Fig. 2a

Fig. 2b

Fig. 3a

Fig. 3b