SOIL ORGANIC CARBON STOCK IN GRASSLANDS: EFFECTS OF INORGANIC FERTILIZERS, LIMING AND GRAZING IN DIFFERENT CLIMATE SETTINGS

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ABSTRACT

Grasslands store about 34% of the global terrestrial carbon (C) and are vital for the provision of various ecosystem services such as forage and climate regulation. About 89% of this grassland C is stored in the soil and is affected by management activities but the effects of these management activities on C storage under different climate settings are not known. In this study, we synthesized the effects of fertilizer (nitrogen and phosphorus) application, liming and grazing regime on the stock of SOC in global grasslands, under different site specific climatic settings using a meta-analysis of 341 datasets. We found an overall significant reduction (-8.5%) in the stock of SOC in global managed grasslands, mainly attributable to grazing (-15.0%), and only partially attenuated by fertilizer addition (+6.7%) and liming (+5.8%), indicating that management to improve biomass production does not contribute sufficient organic matter to replace that lost by direct removal by animals. Management activities had the greatest effect in the tropics (-22.4%) due primarily to heavy grazing, and the least effect in the temperate zone (-4.5%). The negative management effect reduced significantly with increasing mean annual temperature and mean annual precipitation in the temperate zone, suggesting that temperate grassland soils are potential C sinks in the face of climate change. For a sustainable management of grasslands that will provide adequate forage for livestock and mitigate climate change through C sequestration, we recommend that future tropical grassland management policies should focus on reducing the intensity of grazing. Also, to verify our findings for temperate grasslands and to better inform land management policy, future research should focus on the impacts of the projected climate change on net greenhouse gas exchange and potential climate feedbacks.

KEYWORDS: Grassland soils, soil improvement, land management, climate change, carbon sequestration, nitrogen amendment.

1. Introduction

Grasslands cover approximately 40% of the earth's surface (excluding Antarctica and Greenland), are distributed across all continents and over a wide range of geological and climatic conditions (Suttie *et al.,* 2005; White *et al.,* 2000). About 34% of the global terrestrial carbon (C) is stored in grasslands and a significant (89%) amount of the C sequestered by the grassland vegetation is stored in the soil (Ajtay *et al.,* 1979; White *et al.,* 2000), which is vital for the provision of ecosystem services and particularly for climate regulation (Buckingham *et al.,* 2013).

The distribution and productivity of grasslands is mainly limited by climate and inherent soil properties. Globally, 28% of grasslands are distributed in semi-arid areas, 19% in arid areas, 23% in humid areas and 20% in cold areas (White *et al.,* 2000). Climate exerts an overriding influence on the size of the grassland soil C store through its control on plant growth, and therefore rates of litter and plant exudate inputs to soil and the rates of C loss through decomposition, leaching and erosion, and these processes are particularly sensitive to precipitation and temperature patterns (Albaladejo *et al.,* 2013; Bellamy *et al*., 2005; Rees *et* *al*., 2005). Currently, climate is changing, with nearly 0.8ºC rise in global average temperature since the 19th Century and a greater warming as well as altered precipitation patterns expected throughout the 21st Century (IPCC, 2013; Jenkins *et al.,* 2008). Thus grasslands that naturally exist at the margins of their climatic and edaphic envelope, or whose continued existence depends on management activities may be particularly sensitive to climate change, with poorly understood consequences for soil C stocks and feedback to climate change.

Globally, grasslands are managed to increase biomass productivity in order to support livestock production, and are either being directly grazed, or cut for fodder, typically as hay or silage, or a combination of all three. Management activities are primarily used to change the status of soil properties thereby creating optimum conditions for plant growth. Soil characteristics that have been associated with rapid grassland establishment and increased productivity include relatively high sand and silt and low clay contents, and therefore moderate drainage, friable consistency, small aggregates, slightly acidic condition, and high nutrient levels (Epstein, 2012; Fay *et al.,* 2012; Gibbs, 1980). Nutrient levels and acid status can be improved by fertilisation and liming to raise the soil pH, and these are typically the most common management activities for improving or maintaining grassland productivity. As well as the intended increase in aboveground biomass, fertilisation and liming potentially lead to greater production of root exudates and litter, and often have unintended effects on soil properties such as microbial populations and their activities that influence decomposition processes (Alonso *et al.,* 2012; Hoffmann *et al.,* 2014; Soussana *et al.,* 2007). These management activities therefore have implications for soil C storage and sequestration.

Grazing regime itself may also influence net soil C storage. For example, soil C gain may result from over-compensatory plant growth (Tanentzap and Coomes, 2012) and increased inputs from enhanced root production (Frank *et al.,* 2002). Conversely, overgrazing could lead to soil C loss through reduced plant productivity and litter inputs (Conant and Paustian, 2002; Mestdagh *et al.,* 2006), or to exposure of bare soil and C loss via erosion (Evans, 1997). Thus a complex array of direct grazing effects and indirect grazing-related management effects on soil C storage may occur simultaneously. It is perhaps not surprising, therefore, that observed effects of liming, fertilizer application and grazing regime on soil C stock have been contradictory, and that increases, decreases and no change in soil C stock have been reported in different grassland ecosystems (Table S1) with specific climatic and soil conditions.

A number of global-scale reviews and meta-analyses have also reported inconsistent effects of grazing (Dlamini *et al.,* 2016; McShery and Ritchie, 2013; Pineiro *et al.,* 2010; Zhou *et al.,* 2016), fertilizer application (Geisseler *et al.,* 2016; Liu and Greaver, 2010; Lu *et al.,* 2011; Yue *et al.,* 2016), liming (Paradelo *et al.,* 2015) and grassland improvement (Conant *et al.,* 2001; 2017) on grassland soil C stock. For example, Zhou *et al.* (2016) reported a 10.28% grazing-induced reduction in soil C stock, whereas Pineiro *et al.* (2010) and McShery and Ritchie (2013) showed that grazing caused an increase, a decrease and no change in soil C stock with grazing effect size ranging from -0.33 to +0.38, depending on soil characteristics, climate and grazing intensity. Also, in separate analyses, N addition has been reported to cause a decrease (effect size = -0.0026; Lu *et al.,* 2011), no change (Liu *et al.,* 2010) and an increase (+19.75%; Yue *et al.,* 2016) in the C stock of grassland mineral soil layers. The differences in outcome could be attributed to a failure to account for context-specific differences in management, such as rates of fertilizer and lime application in different climatic zones (Dessureault-Rompré *et al.,* 2010; Iturri and Buschiazzo, 2016), or grazing regimes that vary depending on climatic influences on productivity (Oba *et al.,* 2000), or failure to consider the influence of soil type and characteristics (Mills *et al.,* 2005; Srinivasarao *et al.,* 2009).

The interactive effects of non-management factors (e.g. climate and soil) and fertilizer or lime application rates have not been synthesized for global grasslands. The few global studies (Dlamini *et al.,* 2016; McSherry and Ritchie, 2013; Zhou *et al.,* 2016) that considered interactive effects of grazing regime and non-management factors reported conflicting results. For example, McShery and Ritchie (2013) reported that grazing-induced changes in soil C stock were insensitive to either climate or soil texture, Dlamini *et al.* (2016) reported that significant soil C reduction due to over-grazing occurred only in cold (mean annual temperature, MAT $< 0^{o}C$) and dry (mean annual precipitation, MAP < 600 mm) climates, and in acidic (pH<5.0) and coarse-textured (< 32% clay) soils, whereas Zhou *et al.* (2016) found a significant reduction in soil C only in semi-humid and humid regions (MAP $\geq$ 400 mm). In order to inform appropriate management decisions in global grasslands and models that integrate climate and land management, there is need to resolve the conflicting results of previous studies. This may be better achieved if the effects of site-specific characteristics and grazing-related management activities within different climatic zones are considered.

Our aim in this study is to investigate how grassland SOC stock responds to management activities in different climatic zones, and the influence of soil properties, in a single meta-analysis. Specifically, we determine the effect size (relative size of change in SOC stock) attributable to grazing-related management (liming and fertilizer addition) and grazing regime in different climatic settings, using a global meta-analysis approach. We focus on soil C stock rather than greenhouse gas inventory because understanding the fate of C stock is important not just for climate change mitigation but the provision of other ecosystem services such as maintaining soil quality, which is of immediate concern to farmers that manage the grasslands for livestock production. The result of this study will not only help to detect the overall pattern of response of SOC stock to major grassland management activities but also identify grasslands that are most likely to serve as either a C sink or a C source in the face of climate change. This will better inform policy decisions on future grassland management for sustainable provision of ecosystem services. We hypothesize that 1) the response of SOC stock to management activities will be significantly influenced by site-specific climatic setting and soil characteristics, and 2) fertilizer application, liming and grazing will result in an overall reduction in SOC stock.

2. Methodology

2.1 Data selection and extraction

All the data used for this study were extracted from peer-reviewed journal articles published before January 2017. A search for the articles was conducted in Web of Science between June and December 2016, using all combinations of the following groups of search terms: 1) management, liming, lime addition, fertilizer, nitrogen addition, nitrogen fertilizer application or grazing, 2) soil carbon, soil carbon stock, soil carbon storage or carbon sequestration, 3) grassland, pasture or meadow.

Our searches produced 2881 journal articles which we screened following a number of criteria: 1) they were grassland field studies in which SOC data (concentration in % or g/kg, stock in g/m^2 or Mg/ha, or both) were recorded in response to either liming, fertilizer application or grazing regime, 2) SOC data were recorded for both the managed field and a well-defined control field, and measurements were made at the same temporal and spatial scales, 3) only one of the target management practices such as grazing regime or nitrogen fertilizer varied while other management activities were absent or remained constant, 4) the depth of soil samples used for SOC determination were clearly specified, 5) the mean, sample sizes, measures of variability such as standard deviation, standard error or coefficient of variation can be extracted from the study, 6) experimental and control plots were established within the same ecosystem and had similar environmental characteristics at the beginning of the study, 7) management activities such as grazing intensity were clearly described quantitatively and/or qualitatively, and 8) experimental duration was clearly specified and was at least one entire growing season in order to avoid the effect of short term noise. In cases where two or more studies reported the same data from the same experiment, we chose one of the studies and excluded others, except if they provided supporting environmental information about the site. In order not to violate the key assumption of meta-analysis that studies must be independent, we chose data for the last year of sampling in studies where sampling was conducted annually from the same site. We excluded studies where either multiple nutrient fertilizers (e.g. NPK fertilizers) or organic manure (e.g. livestock slurry or industrial effluent) were applied. This was done to enable us to detect the exact effects of single nutrient fertilizers and prevent the confounding effects of high C and multiple nutrient contents of organic manures. We considered different management levels (e.g. different N levels or forms, or livestock stocking densities) sharing the same control plot as independent observations.

After a thorough screening, we selected 136 articles which yielded 341 pairs of independent studies (Tables S1 and S2), distributed among management activities as follows: 232 (grazing regime), 89 (fertilizer application) and 20 (liming). The selected studies, especially those on grazing, were distributed in most continents (Figure 1). Data was only available for N and phosphorus (P) fertilizers. We extracted data directly from tables or texts in the selected articles, or indirectly from figures using WebPlot Digitizer (http://arohatgi.info/WebPlotDigitizer/app/).

In addition to SOC, management, and duration data, we extracted data for the following characteristics when available: longitude, latitude, elevation, MAT, MAP, SOC at the beginning of the experiments (initial SOC), aboveground biomass (AGB), belowground biomass (BGB), clay content of the soil and soil bulk density. When MAT or MAP or either were not reported, we used ArcMap to extract the data from WorldClim-global climate database (http://worldclim.org/) with a spatial resolution of 30 arc seconds. Where only standard errors were reported, we converted them to standard deviations using the sample size.

Thirty–one studies did not report any measures of variability and we calculated their standard deviations following a method used by Geisseler *et al.* (2016). This involved calculating the average coefficient of variation (CV) across each management activity for all the datasets for which standard deviations were reported and using these average CVs to calculate the missing standard deviations. This was done separately for the control and the experimental datasets.

The equivalent soil mass method is recommended for comparing SOC stock changes in managed ecosystems (Lee *et al.,* 2009), in order to overcome the effect of compaction. This approach was not used here because not all the studies that we selected reported their SOC data on such basis. However, we considered extent of sampling depth as a moderator in our meta-analysis and grouped our data into three depth categories: 0 – 19cm, 0 – 40cm and 0 – 100cm. In order to compare SOC stock across studies we converted reported SOC concentrations (%) to SOC stock (Mg/ha) using reported bulk density and sampling depth values as follows:

$$SOC\ stock\ (Mgha^{-1}) = SOC\ concentration\ (\%) \times Bulk\ density\ (gcm^{-3}) \times depth(cm) \quad (1)$$

Thirty–one studies reported SOC only as concentrations in %, and did not report their corresponding bulk density values. To overcome this problem and maximise the number of studies available for meta-analysis, we estimated bulk density based on the relationship between SOC (%) and bulk density in all other studies (Figure S1). The best function with the highest coefficient of determination was exponential:

$$Bulk\ density\ (gcm^{-3}) = 1.3961e^{SOC\ concentration\ (\%)}\ (R^2 = 0.6246, p < 0.05) \quad (2)$$

Bulk densities calculated with equation (2) were subsequently used to convert SOC concentrations to stock using equation (1). This approach has previously been used to calculate missing bulk density values (e.g. Hopkins *et al.,* 2009; Xiong *et al.,* 2016).

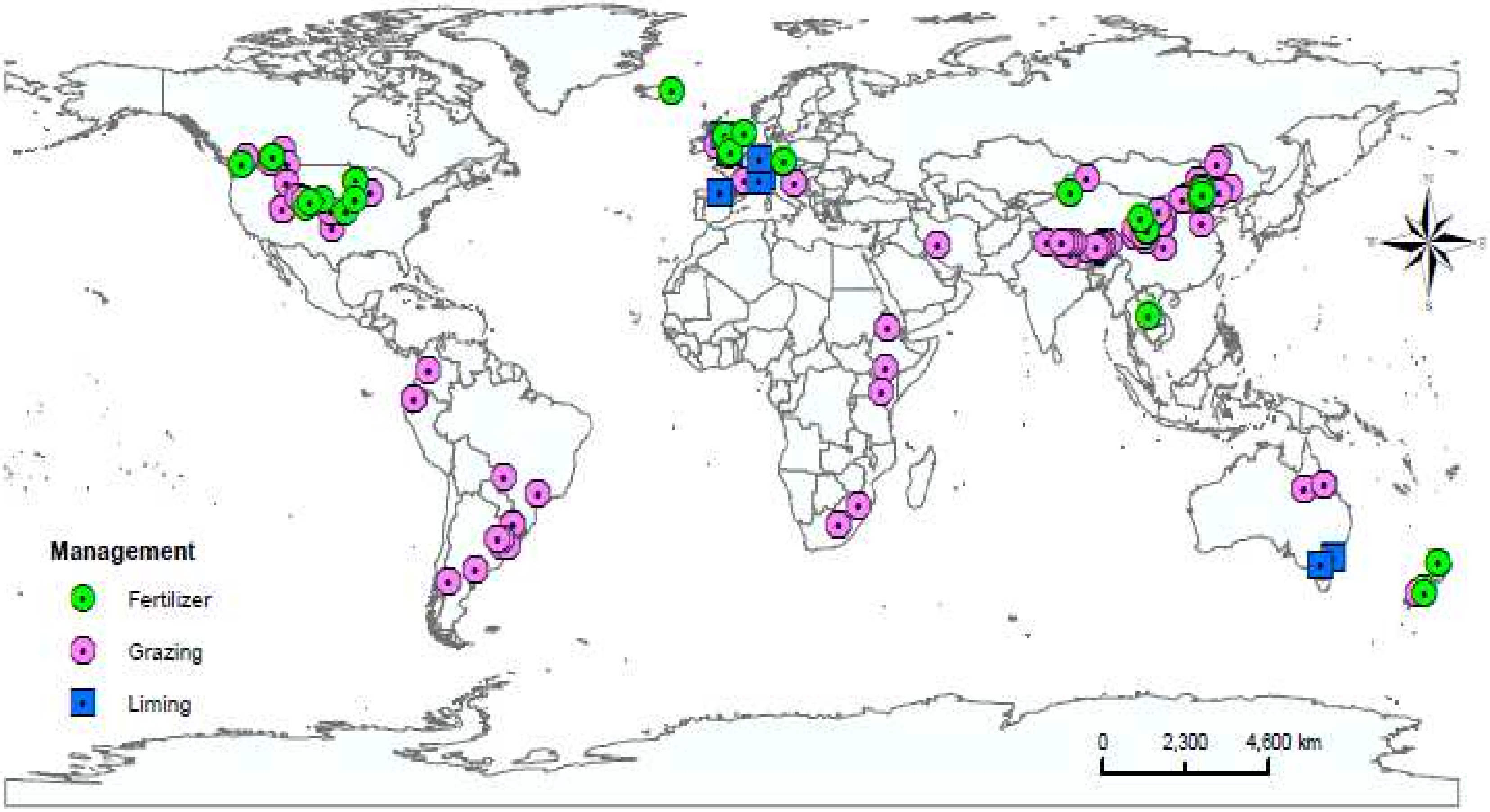

Figure 1: Global distribution of datasets used for meta-analysis.

The management activities (fertilizer application, liming and grazing) were divided into different categories so as to understand the variations within each management activity. We adopted the grazing intensity classification (i.e. light grazing, moderate grazing and heavy grazing) as used by the authors. This qualitative description was chosen because: 1) there was no consistency in the quantitative description of grazing given by the various authors, with different animals (e.g. sheep and cattle) and different units of measurement (e.g. sheep equivalent and percentage forage utilization) reported, and 2) we recognise that the carrying capacity of grasslands will vary depending on their climatic and geomorphic setting. In a few instances in humid areas where no clear qualitative description was given by the authors, we grouped the reported stocking densities into grazing intensities as follows: light grazing, < 5 sheep/ha; moderate grazing, 5 – 10 sheep/ha; heavy grazing, > 10 sheep/ha. We considered this classification appropriate for humid areas unlike the arid and semi-arid areas where the use of 2 sheep/ha could be considered as heavy grazing because of lower plant productivity. In some of the studies, grasslands were grazed by either cattle or yak. In such cases, we used 5 sheep units for 1 cattle and 3 sheep units for 1 yak based on equivalence values suggested by Li *et al.* (2011) and Xie and Wittig (2004). Similarly, where grazing intensity was reported only as percentage forage utilization (FU), we adopted the following classification based on the reports of Evans *et al*. (2012) and Krzic *et al.* (2014): light grazing (< 40% FU), moderate grazing (40 – 65% FU) and heavy grazing (> 65% FU).

The fertilizer studies were first grouped into N fertilizer type (ammonium chloride, ammonium nitrate, ammonium sulphate, calcium nitrate, potassium nitrate and urea) and P fertilizer type (calcium phosphate, potassium phosphate and sodium phosphate). As with our classification of grazing intensity, we adopted qualitative descriptions of N fertilizer intensity (low N, moderate N, and high N) as used by the authors of individual studies. Where this was not indicated by the author, we grouped the intensity of N fertilizers into three N rates: low N, < 50 kg N/ha; moderate N, 50 – 150 kg N/ha; high N, > 150 kg N/ha. The lowest class boundary (50kg N/ha) was chosen to give an appropriate classification that could account for the wide range of N fertilizers (from less than 10 kg N/ha to over 600 kg N/ha) used globally. We also used the qualitative description of P fertilizer intensity given by the authors, and where this was not available, we grouped P fertilizer rates as follows: low P, < 50 kg P/ha; moderate P, 50 – 100 kg P/ha and high P, > 100 kg P/ha. Calcium lime was used in all the liming experiments and could not be further categorised based on lime form, however, liming intensity was categorised into three rates: low lime, < 3 t/ha lime; moderate lime, 3 – 5 t/ha lime; and high lime, > 5 t/ha lime. This is based on the range of lime rates recommended for application to grasslands (e.g. DEFRA, 2011; Edmeades *et al.,* 1985).

We also categorised other factors which we thought could influence the effect of management activities on SOC. Based on the latitudes where experiments were conducted, three climatic zones were identified: Tropics (0 – 23.5° N and S), Subtropics (24 – 40° N and S), and Temperate (41 – 66° N and S). MAT in °C was divided into five categories: -5.0 to -0.1, 0.0 – 5.0, 5.1 – 10.0, 10.1 – 20.0 and 20.1 – 30. MAP was grouped into three categories: dry (< 600 mm), intermediate (600 – 1000 mm) and wet (> 1000 mm) based on previously identified global climate regimes (Dai and Wang, 2017). The duration of management activities was grouped into three: short term (< 10 years), medium term (10 – 30 years) and long term (> 30 years). The clay content was used to group soils into three textural classes: sand (< 20% clay), loam (20 – 30% clay) and clay (> 30% clay) which have been shown to be suitable for modelling large scale soil processes (Bormann, 2007).

2.2 Data analysis

Descriptive statistics such as minimum, maximum, mean and standard deviations were first computed for all the variables we considered, using SPSS Statistics (version 22). Subsequently, we conducted a meta-analysis based on the response-ratio approach described by Hedges *et al.* (1999) using the mixed-effect model of MetaWin software (Rosenberg *et al.,* 2000). In brief, the effect size of management activities (liming, fertilizer addition and grazing regime) on SOC stock was estimated using the natural logarithm of the response ratio (R), which is the ratio of the mean SOC stock in managed plots to mean SOC stock in control plots. i.e.

$$Effect\ size = \ln R = \ln\left(\frac{SOC\ in\ managed\ plot}{SOC\ in\ control\ plot}\right) \quad (3)$$

As some of the management practices have relatively small sample sizes, the 95% confidence intervals of average effect sizes were generated through 4999 bootstrap iterations in order to overcome any violation of normality assumptions. The management effect size was considered significant (at 5% probability level) if the 95% confidence intervals did not overlap zero. A negative effect size means that management resulted in a reduction in SOC stock whereas a positive effect size implies a management-induced increase in SOC stock.

The mean effect sizes of management categories were also calculated using the approach described in the preceding paragraph. The total heterogeneity (Q_T) in each type of management practice was calculated and partitioned into within group heterogeneity (Q_W) and between group heterogeneity (Q_B). A significant Q_B (at 5% probability level) meant that management categories within that management type differed in their effects, and the exact effect of any management category was considered significantly different from that of another category when their 95% confidence intervals did not overlap. The percentage effect size of management activities was calculated from the equation:

$$Percentage\ effect\ size = (\exp(\ln R) - 1) \times 100 \qquad (4)$$

The effect size of management activities was further categorised according to duration of management, extent of sampling depth, clay content, climatic zone, MAT and MAP, using the categorical meta-analytic model of MetaWin software. Also, the continuous model (a weighted least square regression) of the Meta-Win software was used to analyse the linear relationships between the management effect sizes and elevation, MAT, MAP, initial SOC, clay content of the soils and duration of management. The linear model is represented as:

$$Effect\ size\ = a + b(Independent\ variable) + \mathcal{E} \qquad (6)$$

Where a = intercept, b = slope of the model, Ɛ = error term. The value and significance of the slope (at 5% probability level) was used to assess the influence of the independent variables on the effect of management activities on SOC stock. A negative slope indicates a greater management-induced reduction in SOC stock whereas a positive slope indicates that the negative effect of management on SOC is decreasing. Finally, we ensured that there was no publication bias (i.e. the tendency for only statistically significant results to be published by journals) by running a fail-safe test in MetaWin software.

3. Results

The datasets used in this study (Table 1) covered a wide range of climatic and elevation gradients ranging from latitudes 44°S to 65°N, longitudes 121°W to 175°E and altitudes 14 to 4800 m above sea level, with MAT that ranges from -4.8 to 26.8°C and MAP of 120 to 2000 mm. Most of the experiments were conducted in permanent grasslands with few in sown grasslands or rotated pasture (Table S1). Fertilizers were applied at the rates of 10 to 376 kg P/ha and 10 to 640 kg N/ha whereas 0.4 to 25.0 t/ha of lime was applied. The duration of management activities was from 0.5 to 146 years. The belowground biomass was generally higher than aboveground biomass, and both belowground biomass and aboveground biomass were slightly higher in unmanaged sites (2074 g/m^2 and 357 g/m^2) than in managed sites (2034 g/m^2 and 348 g/m^2) respectively. The grassland soils varied in texture from sandy (1.37% clay) to clayey (60% clay) and the average stock of OC within the top 2.5 to 100 cm of the soils was 40 ± 32 Mg/ha in managed grasslands and 43 ± 35 Mg/ha in unmanaged grasslands.

3.1 Effects of management activities on SOC stock

Our meta-analysis showed that management types and their intensity (Table 2 and Figure 2) and management duration (Table 3) affected SOC stock in different ways. Liming, fertilizer application and grazing resulted in an overall significant reduction (-8.5%) in SOC stock. The three management activities differed significantly ($p < 0.05$) in their separate effects on SOC stock. Grazing significantly reduced SOC stock by -15%, liming resulted in a non-significant increase (+5.8%) whereas fertilizer application significantly increased SOC stock by +6.7%. Significant variability was observed between the categories of each of these management practices. There was a progressive decline in SOC stock as the intensity of grazing increased from light (-6.9%) and moderate grazing (-13.2%) to heavy grazing (-27.1%), and the reduction in SOC stock was statistically significant at all the three levels of grazing intensity.

There was a very small and insignificant (+0.3%) effect of P fertilizer addition on soil C stock but N fertilizers significantly increased (+8.1%) SOC stock (Table 2). Considering the forms of N fertilizers applied, ammonium nitrate, ammonium sulphate and calcium nitrate increased SOC stock by +12.6%, +13.1% and +26.9% respectively, while there were no significant effects of ammonium chloride, urea, and potassium nitrate (Table 2). Low N rates resulted in a non-significant increase (+0.3%) in SOC stock whereas moderate N and high N rates significantly increased SOC by +5.2% and +13.3% respectively. The response of soil C stock to increasing lime intensity followed a completely different pattern: there were non-significant increase in SOC stock at both low (+6.8%) and high (+2.8%) lime rates, whereas moderate lime rate led to a significant increase (+14.1%) in soil C stock (Table 2).

There were no significant relationships ($p > 0.05$) between the duration of liming, fertilizer addition and grazing regime, and their individual effects on SOC stock (Table 3). Generally, an increase in the duration of liming and fertilizer addition was associated with a greater decline in SOC stock; on the other hand, an increase in the duration of grazing leads to an increase in SOC stock. However, the overall effect of these three management activities on SOC stock was statistically significant in the short (< 10 years; -5.3%) and medium (10 – 30 years; -14.3%) term but was not significant in the long term (> 30 years; -4.5%).

3.2 Influence of climate and other site-specific characteristics on the response of SOC stock to grassland management

The effects of liming, fertilizer addition and grazing on SOC stock were statistically significant irrespective of elevation, clay content, the extent of soil depth sampled and the SOC contents at the start of management (Table 4). However, the modifying effect of clay on management activity was only significant in fertilized grasslands, with increasing clay content resulting in a greater reduction in SOC stock ($b = -0.0008$, $p = 0.042$) (Table 4).

The overall management-induced reductions in SOC stock were significant across all climatic zones in the order: tropics > subtropics > temperate, and across all MAT classes with effect size increasing with increasing temperature class above 0ºC (Table 5). There were statistically significant relationships between MAP, MAT and the effects of management practices on SOC stock. The smallest effect of management on SOC stock was found when MAT was in the range of 0 – 5.0ºC (-4.9%). Management effects on SOC stock were greater at MAT below 0ºC (-8.4%) and above 5.0ºC (-17.2%). The management effect was only significant when MAP was below 600 mm (-11.7%; Table 5).

On further analysis, it was only in the temperate zone that the relationships between MAT, MAP and the effects of management activities on SOC stock were statistically significant. Within the temperate zone, the overall negative effect of management practices decreased with increasing MAT ($b = 0.0108$, $p = 0.00054$, $n = 195$) and MAP ($b = 0.0002$, $p = 0.0000$, $n = 195$) (Table 5). When management activities were considered individually, the effects of grazing and N fertilizer application exhibited a positive relationship with MAT and MAP, whereas the negative effect of liming decreased with increasing MAT and increased with increasing MAP (Table 6; Figure 3). When the relationships between management and temperate zone climatic parameters were explored by management intensity, the MAP-grazing relationship was only significant for heavy grazing ($b = 0.0007$, $p = 0.00063$), the MAP-N fertiliser relationship was only significant for low N fertilisation ($b = 0.0004$, $p = 0.01167$), the MAT-N fertiliser relationship was only significant for moderate N fertiliser ($b = 0.0082$, $p = 0.04342$), and MAP-lime ($b = -0.0001$, $p = 0.02246$) and MAT-lime ($b = 0.0198$, $p = 0.00885$) relationships were only significant for low lime categories (Table 6).

Table 1: Characteristics of datasets used for meta-analysis. n = number of datasets.

Variable	n	Minimum	Maximum	Mean	Standard deviation
SOC of managed sites (Mg/ha)	341	0.93	204.12	40.14	32.30
SOC of control sites (Mg/ha)	341	1.80	200.81	43.39	35.35
Aboveground biomass of managed sites (g/m^2)	131	7.20	7998.00	348.43	988.55
Aboveground biomass of control sites (g/m^2)	131	7.40	6225.00	356.75	686.30
Belowground biomass of managed sites (g/m^2)	50	27.70	32487.00	2033.77	4650.11
Belowground biomass of control sites (g/m^2)	50	82.50	26188.00	2074.02	4111.07
Initial SOC of sites (%)	35	0.19	17.40	4.46	4.42
Clay content of study sites (%)	98	1.37	60.00	19.57	13.60
Elevation (m)	341	14.00	4800.00	1619	1324
Latitude (^{o})	341	-44.00	65.04	31.13	26.34
Longitude (^{o})	341	-121.75	175.75	45.36	91.90
Mean annual temperature (^{o}C)	341	-4.80	26.80	5.60	6.60
Mean annual precipitation (mm)	341	120.00	2000.00	594.00	377.00
Duration of management (years)	341	0.50	146.00	18.97	22.25
Soil depth (cm)	341	2.50	100.00	14.70	12.10
Calcium lime (t/ha)	20	0.40	25.00	7.44	8.32
Nitrogen fertilizer rate (kg N/ha)	71	10.00	640.00	137.41	129.30
Phosphorus fertilizer rate (kg N/ha)	18	10.00	376.00	83.08	88.64

Table 2: Effect of management (mgt) activities on SOC stock. * = significant, ns = not significant at 5% probability level, n = number of data.

Management type	Management category	Effect on SOC stock (%)	Management intensity	Effect on SOC stock (%)
All mgt type (n = 341)		-8.5^{*}		
Grazing (n = 232)		-15.0^{*}	Light grazing (n = 100)	-6.9^{*}
			Moderate grazing (n = 67)	-13.2^{*}
			Heavy grazing (n = 65)	-27.1^{*}
Liming (n = 20)		$+5.8^{ns}$	Low lime (n = 8)	$+6.8^{ns}$
			Moderate lime (n = 5)	$+14.1^{*}$
Fertilization (n =89)		$+6.7^{*}$	High lime (n = 7)	$+2.8^{ns}$
	All nitrogen fertilizer (n = 71)	$+8.1^{*}$		
	Ammonium nitrate (n = 28)	$+12.6^{*}$	Low nitrogen (n = 19)	$+0.3^{ns}$
	Ammonium chloride (n = 3)	-5.4^{ns}	Moderate nitrogen (n = 29)	$+5.2^{*}$
	Ammonium sulphate (n = 9)	$+13.1^{*}$	High nitrogen (n = 23)	$+13.3^{*}$
	Urea (n = 24)	$+3.6^{ns}$		
	Potassium nitrate (n = 4)	-1.0^{ns}		
	Calcium nitrate (n = 3)	$+26.9^{*}$		
	All phosphorus fertilizer (n = 18)	$+0.3^{ns}$	Low phosphorus (n = 8)	-2.1^{ns}
	Calcium phosphate (n = 10)	-5.0^{ns}	Moderate phosphorus (n = 6)	$+5.6^{ns}$
	Potassium phosphate (n = 5)	$+7.5^{*}$	High phosphorus (n = 4)	$+1.7^{ns}$
	Sodium phosphate (n = 3)	-7.8^{ns}		

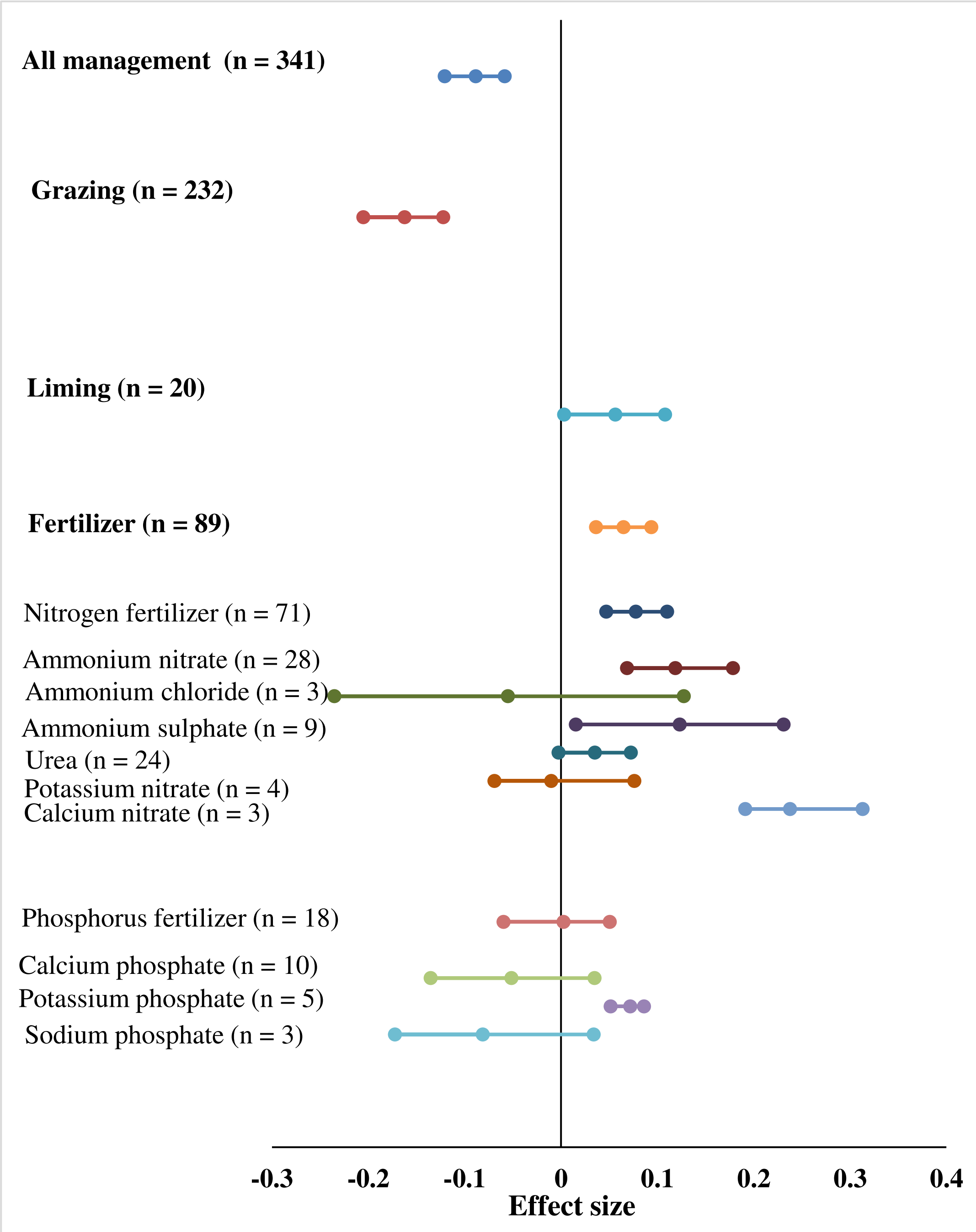

Figure 2: Effect sizes of fertilizer application, liming and grazing on SOC stock (bars represent mean plus and minus 95% confidence intervals)

Table 3: Influence of management duration on the response of SOC stock to fertilizer, liming and grazing. * = significant, ns = not significant at 5% probability level, n = number of datasets, a = intercept, b = slope, p-value^S = p-value for the regression slope.

Management duration (years)	Range (years)	Management effect on SOC (%)	Effect size		
			a	b	p-value^S
< 10 years (n = 164)		-5.3*			
10 – 30 years (n = 124)		-14.3*			
> 30 years (n = 53)		-4.5^{ns}			
Grazing duration (n = 232)	1 – 91		-0.1731	0.0006	0.37041
Liming duration (n = 20)	0.5 – 73		0.0877	-0.0007	0.57725
Fertilizer duration (n = 89)	3 – 146		0.0567	-0.0002	0.62739
All management duration (n = 341)	1 – 146		-0.0960	0.0004	0.48688

Table 4: Overall effect of fertilizer, liming and grazing on SOC stock under different site characteristics. * = significant, ns = not significant at 5% probability level, n = number of datasets, I = initial, a = intercept, b = slope, p-value^S = p-value for the regression slope.

Factor	Factor category	Range	Effect on SOC (%)	Effect size		
				a	b	p-value^S
Depth extent	0 – 19 cm (n = 248)		-6.6*			
	0 – 40 cm (n = 85)		-11.9*			
	0 – 100 cm (n = 8)		-24.0*			
Clay content	< 20% Clay (n = 57)		-11.9*			
	20 to 30% Clay (n = 27)		-11.8*			
	> 30% Clay (n = 14)		-16.9*			
	%Clay in grazed site (n = 70)	1.4 – 60		-0.2477	0.0025	0.22596
	%Clay in limed site (n = 5)	29 – 29		-0.0254	-0.0005	1.00000
	%Clay in fertilized site (n = 23)	4.3 – 23		0.1135	-0.0088*	0.04248
Elevation	Elevation (m) (n = 341)	14 – 4800		-0.1007	0.0000	0.38066
ISOC	ISOC of grazed site (%)(n = 18)	0.2 – 7.0		-0.1241	-0.0029	0.86654
	ISOC of limed site (%) (n = 4)	6.4 – 17.4		0.1584	-0.0087	0.62526
	ISOC of fertilized site (%) (n = 13)	0.2 – 8.6		0.0642	-0.0153	0.47418

Table 5: Overall effect of fertilizer, liming and grazing on SOC stock under different climate conditions. MA = mean annual, T = temperature, P = precipitation, Mgt = management, n = number of datasets, bold values are significant at 5% probability level, italicized value is the largest slope, a = intercept, b = slope, p-value^S = p-value for the slope.

Factor	Factor category	Range	Mgt effect on SOC stock (%)	Effect size		
				a	b	p-value^S
Climatic zone	Tropics (n = 24)		**-22.4**			
	Subtropics (n = 122)		**-12.5**			
	Temperate (n = 195)		**-4.5**			
MAT	-5.0 to -0.1°C (n = 55)		**-8.4**			
	0 to 5.0°C (n = 138)		**-4.9**			
	5.1 to 10.0°C (n = 84)		**-10.2**			
	10.1 to 20.0°C (n = 48)		**-12.8**			
	20.1 to 30.0°C (n = 16)		**-17.2**			
MAP	< 600 mm (n = 223)		**-11.7**			
	600 to 1000 mm (n = 66)		0.4			
	> 1000 mm (n = 52)		-5.2			
MAT	MAT (°C) (n = 341)	-4.8 – 26.8		-0.0581	**-0.0053**	0.00465
	MAT tropics (°C) (n = 24)	4 – 26.8		-0.4056	0.0074	0.40919
	MAT subtropics (°C) (n = 122)	-4.3 – 19.0		-0.0753	**-0.0092**	0.00008
	MAT temperate (°C) (n = 195)	-4.8 – 15.5		-0.0878	***0.0108***	0.00054
MAP	MAP (mm) (n = 341)	120 – 2000		-0.1387	**0.0001**	0.01079
	MAP tropics (mm) (n = 24)	520 – 1230		-0.5338	0.0004	0.07645
	MAP subtropics (mm) (n = 122)	120 – 1850		-0.1332	0.0000	0.91539
	MAP temperate (mm) (n = 195)	120 – 2000		-0.1362	**0.0002**	0.00000

Table 6: The relationship between climatic variables in the temperate zone and the effect sizes of management intensity on SOC stock. Bold values are significant at 5% probability level, n = number of datasets, a = intercept, b = slope, p-value^S = p-value for the slope.

Management effect size	Temperate MAT				Temperate MAP			
	n	a	b	p-value^S	n	a	b	p-value^S
Grazing	113	-0.1702	0.0057	0.32638	113	-0.2511	**0.0002**	0.00219
Light grazing	50	-0.0709	-0.0062	0.52051	50	-0.1291	0.0001	0.36422
Moderate grazing	39	-0.1591	0.0136	0.12257	39	-0.2135	0.0002	0.05223
Heavy grazing	24	-0.4712	0.0250	0.13653	24	-0.7038	**0.0007**	0.00063
Liming	14	-0.0709	**0.0235**	0.00196	14	0.2492	**-0.0001**	0.01021
Low lime	7	-0.0543	**0.0198**	0.00885	7	0.2273	**-0.0001**	0.02246
Moderate lime	4	-1.6644	0.1869	0.45149	4	0.2451	-0.0001	0.51269
High lime	3	-1.5918	0.1792	0.06106	3	0.2756	-0.0002	0.86640
Nitrogen fertilizer	56	0.0468	**0.0107**	0.00356	56	0.0170	**0.0001**	0.04711
Low nitrogen	10	0.0059	0.0130	0.15058	10	-0.1680	**0.0004**	0.01167
Moderate Nitrogen	23	0.0261	**0.0082**	0.04342	23	0.0112	0.0001	0.20137
High nitrogen	23	0.0847	0.0132	0.15513	23	0.0699	0.0001	0.36717

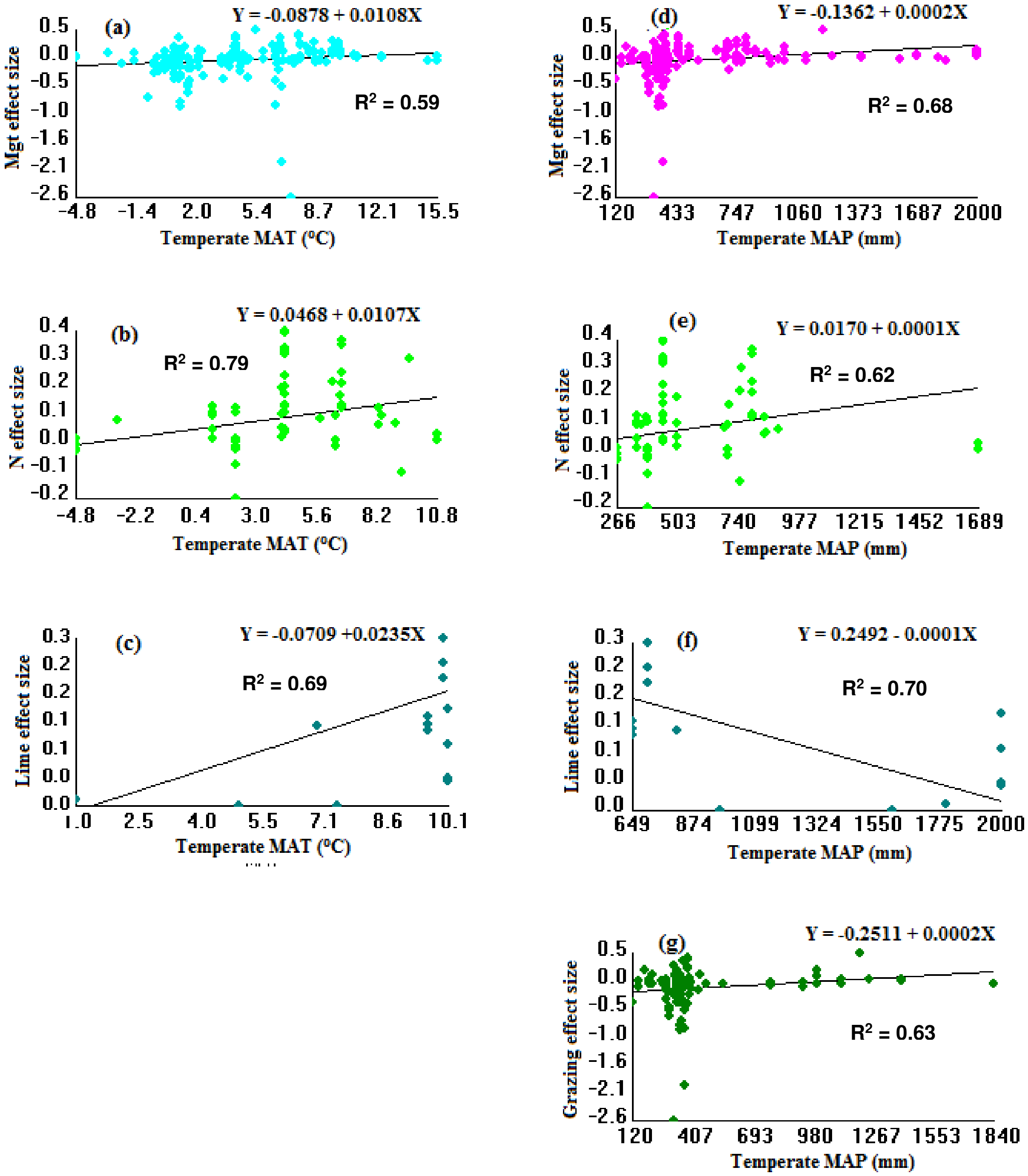

Figure 3: Statistically significant relationships between temperate MAT and the effect size of all management (a), nitrogen addition (b), and lime (c) on SOC stock; and between temperate MAP and the effect size of all management (d), nitrogen addition (e), lime (f) and grazing (g) on SOC stock. The interactive effect of grazing effect size and MAT was not significant and was therefore excluded in the figure.

4. Discussion

This study reveals an overall significant reduction (-8.5%) in the stock of SOC in global managed grasslands, mainly attributable to grazing (-15.0%), and only partially attenuated by fertilizer addition (+6.7%) and liming (+5.8%), indicating that management to improve biomass production does not contribute sufficient organic matter to replace that lost by direct removal by animals. Management activities had the greatest effect in the tropical zone (-22.4%) and in zones with low MAP (-11.7%), suggesting a sensitivity at extremes of the climate envelope. The large effect in the tropical zone likely reflects the drastic change in plant biomass inputs to soil when forests are converted to grasslands. In the temperate zone the overall size of the negative management effect was small (-4.5%), but was positively related to MAT and MAP, indicating that soil C stocks may be relatively robust to management under anticipated future regimes of climate warming. Our two hypotheses that grassland management practices will result in SOC decline and that this effect will be influenced by climatic settings were therefore confirmed.

4.1 The net effect of liming, fertilizer addition and grazing on the stock of C in grassland soils

This study showed that grazing had an overriding effect on grassland SOC stock. The significant reductions in SOC stock resulting from grazing (-15%) is larger but consistent with the results of previous published meta-analyses (e.g. -9%, Dlamini *et al.,* 2016; -10%, Zhou *et al.,* 2016). In our study, the negative effect of grazing doubles as the intensity of grazing increased from light to moderate grazing, and from moderate to heavy grazing. Heavy grazing therefore resulted in the most significant reduction in SOC stock (-27.1%) and this can primarily be attributed to excessive removal of vegetation and consequently limited litter returns to the soil. Hans *et al.* (2008) reported a 74% forage utilization under heavy grazing, with the amount of litter returns being only 45% of the values in lightly grazed sites. This happens because a significant proportion of grassland vegetation fed to livestock is subsequently lost from the ecosystem through animal respiration, methane expulsion and export of products such as milk (Soussana *et al.,* 2007). There is also the tendency for plant meristems to be removed under heavy grazing, leading to a reduction in plant growth capacity (Conant and Paustian, 2002; Mestdagh *et al.,* 2006). Of the plant C ingested by animals, only about 25-40% is returned to the grassland as excreta (Soussana *et al.,* 2007). Even this relatively small amount of C that is returned to the soil as animal excreta does not always imply an increase in soil C because the excreta contains readily utilizable substrates that stimulate soil microbial activities in grasslands (Clegg, 2006), leading to a greater decomposition of organic materials and soil C loss (Grayston *et al.,* 2004). This was demonstrated when livestock was excluded for 7 years from a grassland in northern England, which resulted in a 20% reduction in the activity of soil microorganisms (Medina-Roldan *et al.,* 2012). In addition, Ritz *et al.* (2004) found a higher microbial biomass due to increasing sheep urine patches and Williams *et al.* (2000) reported an increase in active bacteria number relative to fungi with the addition of synthetic sheep urine.

The removal of standing plants and litter also exposes the soil to erosive precipitation and wind thereby accelerating erosion and C loss (Steffens *et al.,* 2008; Tanentzap and Coomes, 2012; Xie and Wittig, 2004). For example, Han *et al.* (2008) found that only 33-36% of the ground was covered by vegetation after two years of heavy grazing. Heavy grazing may also cause soil C loss through trampling and poaching (Ma *et al.,* 2016). Trampling from grazing animals results in soil compaction, characterised by increased bulk density and reduced infiltration (Marshall *et al.,* 2014, 2009). This may increase runoff events and export particulate soil C to surface waters (Meyles *et al.,* 2006; Robroek *et al.,* 2010). It has also been reported that the rate of photosynthesis decreases significantly under simulated sheep trampling (Clay and Worrall, 2013). This reduces plant productivity and potential amount of C inputs to the soil.

The practice of improving grassland productivity by applying high rates of lime and N fertilizer is likely not to increase soil C storage under heavy grazing regimes. In this study, SOC stock declined from 14.1% at moderate liming (3 – 5 t/ha) to 2.8% at high liming rate (>5 t/ha). Wang *et al.* (2016) also found that increasing the rate of liming (e.g. by 12.5 t ha^{-1}) led to a decrease of about 14% in SOC at the surface (0-10 cm) soils of some pastures in Australia. At high lime rates, soil acidity-related constraints are removed (Orgill *et al.,* 2015) and this leads to an increase in microbial respiration, a faster decomposition of organic materials and an increase in the level of dissolved organic C (DOC) in soil solution, which is prone to leaching and erosion losses (Hornung *et al.,* 1986; Mijangos *et al.,* 2010; Staddon *et al.,* 2003). In addition, if root growth is stimulated by liming, there is an increase in the release of exudates from grass species at higher soil pH levels which acts as a primer to enhance SOM decomposition and C loss (Grayston *et al.,* 2004). Generally, the potential contribution of liming to global grassland SOC stock gain is limited because the practice is confined to acidic soils which are mostly found in sites that are heavily leached by precipitation (i.e. high MAP). This can be seen in this study because relative to all the management activities we considered, liming studies were very few and the bulk of the papers we used were from low MAP areas, reflecting the climatic zone in which grasslands naturally occur.

At high rates of N fertilizer application, additional SOC stock gained by grassland soils for every unit of N fertilizer added has been shown to decline (i.e. a reduction in C gain efficiency at high rates of N fertilizer addition). Ammann *et al.* (2009) found that the application of 230 kg N ha^{-1} $year^{-1}$ for six years in a Swiss grassland caused only about 6% increase in soil C gain, compared to the 13% gain at high N rate (>150 kg N/ha) in our study. Fornara and Timan (2012) studied the effect of 27 years of N addition (ranging from 0 to 270 kg N/ha) on C sequestration in prairie grassland soils in Minnesota, USA, and found that SOC stock increased with increasing N rates but net C gain per unit of added N significantly decreased after 10 kg N/ha. Also, after six years of subjecting some grasslands in Northern China to six levels of N addition (ranging from 0 to 560 kg N/ha), He *et al*. (2013) reported an increase in SOC stock from 118 to 131 t/ha within the surface 0-100 cm of the soil, but there was a decreasing C gain efficiency as added N increased. These findings are comparable with our study because we found a greater decline in soil C stock as the duration of fertilization increased, however, this negative effect peaks in the medium term (10 – 30 years) and becomes insignificant afterwards. Overall, there is evidence that the positive effect of high N addition declines over time and may also increase the risk of emissions of other more potent greenhouse gases such as nitrous oxide (N_2O) into the atmosphere beyond background levels (Jarvis *et al*., 2001; Vuichard *et al*., 2007), thereby negating any C sequestration benefits. Our study focused only on the potential for long term C accrual in managed grassland soils rather than net greenhouse gas emissions, and there is a need for future studies to synthesize the net effect of management activities on the balance of greenhouse gases. This will provide a clearer picture of the full implication of grassland management to climate change.

This study indicates that intensive grazing-related grassland management activities (particularly liming and N fertilization) and heavy grazing are not a sustainable management regime. Management intensification depletes SOC stock potentially increasing the atmospheric CO_2 concentration and exacerbating the already climate warming trajectory. Future grassland management policy particularly in the tropics with the greatest management-induced decline in SOC stock, should therefore focus mainly on reducing the intensity of grazing. This can be best achieved by excluding grasslands from grazing (Xiong *et al.,* 2016). However, since there is need to balance the goal of soil C sequestration with the need for livestock production, grasslands should be maintained under moderate grazing regimes and governments should consider setting up environmental schemes as an incentive to encourage farmers to adopt less intensive management activities.

4.2 Influence of climate on the response of SOC stock to liming, fertilizer application and grazing

This study revealed that climate significantly influenced the overall effect of liming, fertilizer application and grazing on SOC stock. This is in line with reports in previous studies (e.g. Chimner and Welker, 2011; McSherry and Ritchie, 2013; Zhou *et al.,* 2016) which showed that climate exerts significant influence on the effects of management on grassland C cycling. In our study, the temperate zone had the smallest management-induced decline in SOC stock (-4.5%) and yet exhibited a greater interactive effect of climate compared to either the tropics or the sub-tropics. Negative effects of management declined significantly with increasing MAT and MAP which is a strong indication that increasing temperature and precipitation in temperate grasslands has the potential to reverse the overall management-induced decline in SOC stock of these areas and possibly increase C sequestration. The strong positive temperature-management interactive effects on SOC stock of temperate grasslands can be explained by temperature-induced increase in the length of growing season. Increasing temperature extends the length of the growing season in temperate environments (Hunt *et al.* 1991) thereby enhancing plant growth and C additions to the soil (Chang *et al.,* 2016). For example, relative to 1961-1990 average of 252 days, the length of growing season in England increased to 282 days in 2012 (DECC, 2013) in response to about 1.7^{o}C increase in MAT

(Jenkins *et al.* 2008). Xia *et al.* (2014) observed that increasing spring temperature at latitudes between 30 and 90 ^{o}N stimulates the onset of leaf unfolding which results in an earlier start of the growing season and enhances ecosystem productivity.

The positive response of SOC stock to increasing temperate and precipitation in the temperate zone may also result from greater nutrient availability and biomass production due to removal of restrictions on mineralization imposed by cold and dry conditions. In dry (MAP <600mm) and/or cold conditions (MAT<0^{o}C), characteristics of many temperate grasslands, there is lower biomass production which limits litter inputs to the soil leading to low C stock (De Deyn *et al.,* 2008; Garcia-Pausas *et al.,* 2007). As the climate becomes warmer and wetter, increased mineralisation of organic materials by soil microbes increases nutrients available for plants' uptake thereby increasing grassland biomass production (Davidson, 2015; Guo and Gifford, 2002; Xiong *et al.,* 2016) and litter returns to soil. High temperatures (e.g. > 20^{o}C) stimulates higher soil microbial activities such as respiration and organic matter (OM) decomposition, which results in the loss of soil C as CO_2 or methane (CH_4) into the atmosphere (Ward *et al.,* 2013) or as dissolved organic C (DOC) in soil solution. However, it is not likely that increased warming of the temperate zone in the 21st Century (IPCC, 2013) will stimulate higher C loss via soil microbial respiration compared to enhanced biomass production resulting from warmer and wetter climate and grassland improvement activities such as liming and N fertilization.

Thus, temperate grasslands will potentially serve as a C sink in the face of climate change due to increasing temperature. This will contribute significantly to global climate regulation because temperate grasslands are widely distributed in most continents (Dixon *et al.,* 2014) e.g. the Pampas of South America, the Plains and Prairies of North America, the Steppes of Eurasia, the Downs of Australia and New Zealand, and the Veldts of Africa. However, since the climate change trajectory in temperate zones is an all-season increase in temperature, an increase in winter precipitation and a decrease in summer precipitation (Jenkins *et al.,* 2008), there is still a possibility for the legacy effects of high rate of evapotranspiration in summer to negate effects of increased precipitation in winter, which may lead to drier conditions and enhance management-induced soil C loss.

Therefore, in order to ensure sustainable provision of various ecosystem services by temperate grasslands particularly forage for livestock and climate change mitigation via carbon sequestration, there is need to further study how projected changes in climatic conditions (e.g. warming, drought and wetter conditions) will influence SOC storage and fluxes. A number of manipulative experiments have already been conducted in temperate grasslands to study the effects of climate change on the ecosystem, and involved the use of regulated heating to simulate desired temperature increase, with either an addition or exclusion of water to simulate wet or drought condition. The results of these climate experiments in temperate grasslands were synthesised by White *et al.* (2012) but they found a mixed and complex results with no consistent pattern of grassland response. White *et al.* (2012) concluded that climate change effects on temperate grasslands remain poorly understood and this underscores the need for further research. As temperate grasslands are subjected to different management regimes, it is necessary to conduct more site specific experiments that consider the interactive effects of climate change and grassland management activities such as fertilizer application and liming under different grazing regimes. This will provide an improved understanding of mechanisms operating at each of the global regions of temperate grasslands, and help inform appropriate policy decisions.

5. Conclusion

There was an overall significant reduction (-8.5%) in the stock of SOC in global managed grasslands, due primarily to grazing (-15%), which was partly weakened by fertilizer addition (+6.7%) and liming (+5.8%). This indicated that grazing-related management activities to improve biomass production does not contribute sufficient soil C to replace the C loss via animal grazing. SOC loss was greatest in the tropics and mainly under heavy grazing, and we recommend that future grassland management policy should focus on reducing the intensity of grazing. Temperate grasslands had the least management-induced decline in SOC stock but it was positively related to MAT and MAP such that increasing MAT and MAP reduced the negative management effects. This was an indication that temperate grasslands are potential C sinks in the face of climate change. However, the understanding of the mechanisms of interactions between climate change and management activities in temperate grasslands is still poor. Therefore, in order to ensure a sustainable management of grasslands that will provide adequate forage for livestock and mitigate climate change through C sequestration, we recommend further studies looking at the interactive effects of projected climate change and management regimes on soil C stock.

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