

1 **Does liming grasslands increase biomass productivity without causing detrimental**  
2 **impacts on net greenhouse gas emissions?**

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19 **Keywords:** grassland, lime, N<sub>2</sub>O, CO<sub>2</sub>, CH<sub>4</sub>, SOC, net greenhouse gas emissions.

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55 **Abstract**

56 Soil acidification has negative impacts on grass biomass production and the potential of  
57 grasslands to mitigate greenhouse gas (GHG) emissions. Through a global review of research  
58 on liming of grasslands, the objective of this paper was to assess the impacts of liming on soil  
59 pH, grass biomass production and total net GHG exchange (nitrous oxide (N<sub>2</sub>O), methane  
60 (CH<sub>4</sub>) and net carbon dioxide (CO<sub>2</sub>)). We collected 57 studies carried out at 88 sites and  
61 covering different countries and climatic zones. All of the studies examined showed that liming  
62 either reduced or had no effects on the emissions of two potent greenhouse gases (N<sub>2</sub>O and  
63 CH<sub>4</sub>). Though liming of grasslands can increase net CO<sub>2</sub> emissions, the impact on total net  
64 GHG emission is minimal due to the higher global warming potential, over a 100-year period,  
65 of N<sub>2</sub>O and CH<sub>4</sub> compared to that of CO<sub>2</sub>. Liming grassland delivers many potential  
66 advantages, which justify its wider adoption. It significantly ameliorates soil acidity, increases  
67 grass productivity, reduces fertiliser requirement and increases species richness. To realise the  
68 maximum benefit of liming grassland, we suggest that acidic soils should be moderately limed  
69 within the context of specific climates, soils and management.

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71 **Keywords:** grassland, lime, N<sub>2</sub>O, CO<sub>2</sub>, CH<sub>4</sub>, SOC, net greenhouse gas emissions.

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## 73 **1. Introduction**

74 Soil acidification (i.e. low soil pH) is a natural process that reduces grass productivity by  
75 reducing soil base status and nutrient availability, and increasing the solubility of metals such  
76 as aluminium (Al), iron (Fe) and manganese (Mn) that can be toxic to grass (Holland et al.,  
77 2018; Horan et al., 2018). It has been accelerated by higher nitrogen (N) and sulphur (S)  
78 deposition related to human activities (Kunhikrishnan et al., 2016). Soil acidification influences  
79 both top- and sub-soils, and decreased productivity is readily apparent where grass biomass is  
80 harvested (Goulding and Annis, 1998). Studies have shown that soil acidification negatively  
81 impacts the potential of grasslands to mitigate greenhouse gas emissions (GHG; N<sub>2</sub>O, CH<sub>4</sub> and  
82 CO<sub>2</sub>) from soils (Goulding, 2016). Soil pH is generally controlled by land use, geology and  
83 climate (Fabian et al., 2014), however, the main drivers of acidification are *via* precipitation  
84 (wet deposition) and deposition of acid gases and particles (e.g. nitric acid, ammonia and  
85 sulphur dioxide; dry deposition), especially in heavily industrialised regions such as western  
86 Europe, USA and Australia (Bouwman et al., 2002). For an alpine grassland, Wang et al. (2020)  
87 found that acidification changed plant composition, root morphology and litter  
88 decomposability and temporarily increased soil C stock. However, in the long-run acidification  
89 negatively impacts nutrient cycling and consequently, reduces grass productivity. The Park  
90 Grass experiment at Rothamsted in the United Kingdom, began in 1856, has shown that regular  
91 application of N fertiliser as ammonium sulphate progressively made the soil more acidic  
92 (Silvertown et al., 2006). However, regular application of lime is a common practice used to  
93 neutralise and control soil acidification. Previous studies on agriculture (croplands and  
94 grasslands) and forests reported that liming decreases Al toxicity, increases soil pH, increases  
95 soil phosphorus (P) and magnesium (Mg) availability, and improves soil physical condition  
96 (Tunney et al., 2010; Holland et al., 2018). Liming enhances soil nitrification (Meiwes, 1995)  
97 and thereby, increases soil nitrate concentration and N availability for grass uptake (Fuentes et  
98 al., 2006; Holland et al., 2018). It improves soil structure, mitigates soil degradation through  
99 its buffering capacity (Keiblinger and Kral, 2018), increases earthworm activities and makes  
100 grass more palatable to animals (DAERA, 2021). Additionally, the use of high Mg lime for  
101 soil treatment increases both soil pH and Mg contents in the soils and thereby, reduces the risks  
102 of livestock hypomagnesaemia (i.e. abnormally low magnesium levels in their blood) (Bide et  
103 al., 2021).

104 Research on liming found that application of lime optimised plant growth, by adjusting  
105 soil pH, and mitigating N<sub>2</sub>O emissions, but the impact on soil organic carbon (SOC) was  
106 inconsistent in the literature (e.g. Goulding, 2016). Liming of grasslands can enhance SOM

107 mineralisation and emission of CO<sub>2</sub> (Holland et al., 2018) but also increase SOM and create a  
108 net C sink due to high biomass production (Fornara et al., 2011). Barcelos et al. (2021) found  
109 the effects of lime on C cycling through microbial biomass, especially in the subsoil, were  
110 minimal. Eze et al. (2018) noted that the increase in global SOC due to liming (+5.8%) and  
111 fertilisation (+6.7%) was not large enough to replace that lost by heavy grazing (-15%),  
112 especially in the tropics. Though Johnson et al. (2005) reported that liming grasslands with or  
113 without N fertiliser decreased soil respiration by 37% and 70% respectively, the authors argued  
114 that these reductions in the CO<sub>2</sub> emissions by liming were either due to low concentrations of  
115 labile SOC, which decreased over time or by adaptation of the microbial community to acidic  
116 soil and low pH (Keller et al., 2005). In contrast, Hinsinger et al. (2003) reported that liming  
117 could increase CO<sub>2</sub> emissions by stimulating rhizosphere priming. Liming could change the  
118 structure of microbial community to one with a lower C use efficiency and higher respiration  
119 (Keiblinger et al., 2010). In acidic soils, high availability of Al<sup>3+</sup> ions inhibits CH<sub>4</sub> oxidiser  
120 activity, whereas the addition of lime increases their activity thereby, reducing the total GHG  
121 emissions (Hilger et al., 2000; Kunhikrishnan et al., 2016). In a global review of agricultural  
122 lands, Holland et al. (2018) found that liming affected soil C storage variably due to differences  
123 in soil type, land use, climate and management. Furthermore, Rangel-Castro et al. (2005) found  
124 that the microbial communities in limed soils were more complex and active in utilising  
125 recently released C compounds than those of un-limed soils. However, regular application of  
126 lime to grasslands indirectly increased biomass production by increasing soil available  
127 nutrients (Holland et al., 2018), improved grass quality and hence livestock production.

128 Unlike cropland, where there is a general awareness of the importance of liming for  
129 crop production, liming of grassland is often neglected, especially when the overall profit of  
130 grassland is low. Due to scarcity of field data, it is still unknown how lime exactly influences  
131 grass productivity and nutrient use efficiency at different initial soil pH, number of grass  
132 species and agro-climatic zones. A meta-analysis where enough data are available, and a review  
133 of available studies where data are insufficient for a full analysis, could help to assess the  
134 present evidence on the importance of liming for grassland and fill this gap in knowledge. This  
135 meta-analysis and review aims to use the available literature globally to assess the impacts of  
136 liming grasslands on soil pH, biomass production and net GHG emissions. We first review the  
137 effects of liming on the soil pH and biomass production. We then review and assess the impacts  
138 of liming on GHG and net CO<sub>2</sub> emissions. Finally, we assess the impacts of liming on the total  
139 net GHG emissions and give some suggestions for future research to support the sustainability  
140 of grasslands. The specific hypotheses we critically evaluated were as follow: (a) liming

141 grasslands ameliorates soil acidity; (b) liming grasslands increases grass productivity and  
142 species richness and; (c) liming grasslands increases SOC and has no effects on total net GHG  
143 emissions.

144

## 145 **2. Materials and methods**

### 146 *2.1 Data collection*

147 This review is part of a more extensive reviewing process for European permanent grasslands  
148 under a project called “Developing Sustainable Permanent Grassland Systems and Policies”  
149 (SUPER-G) (Schils et al., 2022). To review peer-reviewed publications on the impacts of  
150 liming on soil pH, grassland dry matter production and total net GHG emissions (i.e. nitrous  
151 oxide (N<sub>2</sub>O), methane (CH<sub>4</sub>) and net CO<sub>2</sub> emissions), we carried out a comprehensive search  
152 on the Web of Science database (accessed between September 2020 and November 2021). Due  
153 to the scarcity of published data on European grasslands alone, we collected all papers  
154 published globally between 1980 and 2021. We used the keywords: grassland, lime, N<sub>2</sub>O, CO<sub>2</sub>,  
155 CH<sub>4</sub>, SOC and net greenhouse gas emissions. To comprehensively cover all available papers,  
156 we examined all references cited in the papers collected. Our search covered all types of  
157 managed grasslands, as shown in Tables 1 and 3. We excluded laboratory, greenhouse, pot and  
158 modelling papers and only included studies that were carried out in the field and had a control  
159 treatment. We defined the control treatment as a grassland on which no lime was applied. The  
160 total number of papers from the Web of Science search was 12,470 but the majority were  
161 excluded, in most cases, either because there was no control treatment or because the study did  
162 not meet the other criteria described above. Only 57 papers with studies carried out at 88 sites  
163 covering different countries and climatic zones were found suitable for this review (Table 1).  
164 From these papers, we found 33 papers on soil pH and grass production and 24 papers on SOC  
165 or GHG emissions. This shows that data on the impacts of liming grasslands on GHG emissions  
166 are very scarce in the literature. Therefore, our quantitative analysis was confined to data on  
167 soil pH and grass biomass production, while the papers on SOC (15 studies) and GHG  
168 emissions (N<sub>2</sub>O (4 studies); CH<sub>4</sub> (2 studies) and CO<sub>2</sub> (5 studies)) were only reviewed and  
169 summarised. Most of the studies were short-term liming experiments of 2-4 years, though a  
170 few of them were long-term studies (>10 years). Where the original papers reported data from  
171 multiple years, we used the average of all years. All types of lime materials were converted to  
172 calcium carbonate equivalent (CCE) which is the neutralizing value of a liming material  
173 compared to pure calcium carbonate (Moore et al., 1987). Annual grass biomass production  
174 was measured in tonnes of dry matter ha<sup>-1</sup>. The GHG emissions (N<sub>2</sub>O, CH<sub>4</sub> and CO<sub>2</sub>) data were

175 reported from studies that measured the gas flux from grasslands and used a static / automated  
176 chamber gas measurement method or Eddy Covariance (EC). SOC (0 to 50 cm) was measured  
177 by direct sampling and laboratory analysis. We considered net CO<sub>2</sub> emissions as the balance  
178 between SOC stored in the soil and CO<sub>2</sub> emitted to the atmosphere and total net GHG emissions  
179 as the sum of net CO<sub>2</sub> emissions and N<sub>2</sub>O and CH<sub>4</sub> emissions.

180 We used the global climate zones criteria proposed by Smith et al. (2008) to divide our  
181 dataset. The climatic zones were distinguished based on temperature and moisture regimes  
182 (cool, warm, dry and moist zones). The cool zone covers the temperate (oceanic, sub-  
183 continental and continental) and boreal (oceanic, sub-continental and continental) areas, whilst  
184 the warm zone covers the tropics (lowland and highland) and subtropics (summer rainfall,  
185 winter rainfall, and low rainfall). The dry zone includes the areas where the annual precipitation  
186 is  $\leq 500$  mm, whilst the moist zone includes areas where the annual precipitation is  $> 500$  mm.  
187 The four climate categories were moist cool (MC), moist warm (MW), dry cool (DC) and dry  
188 warm (DW). However, all datasets on liming grasslands were found under MC and MW  
189 climate zones only. The two other climatic zones have no available research / observations. We  
190 also divided the data into monoculture and multi-species grasses. Multispecies grass contains  
191 two or more species (not including perennial rye grass or white clover) (FAS, 2021). For the  
192 different studies, different methods were used to measure soil pH, for example using a pH  
193 probe or meter in deionised water or 0.01 M CaCl<sub>2</sub> in 1:1 and 1:2 or 1:5 v:v soil:solution ratios.  
194 We made no adjustment for analytical methods used for soil pH, and where a range of values  
195 was reported, we took the arithmetic mean. The mean annual air temperature (MAAT, in °C)  
196 and mean annual precipitation (MAP, in mm) values for each study were collected from the  
197 original published papers.

198

## 199 *2.2 Data analyses*

200 We explored, analysed, and visualised the data with R version 4.1.0 (R Core Team, 2021). The  
201 distributions of soil pH and grass dry matter production measurements were characterised using  
202 the “fitdistrplus” package version 1.1-5 (Delignette-Muller & Dutang, 2015). To investigate  
203 differences between the control and limed treatments on soil pH and grass dry matter  
204 production (t ha<sup>-1</sup>) under the two climatic zones (MC and MW) and different number of grass  
205 species, we used the “glmer” method with random effect (different studies) and Gamma (link  
206 “log”) or gaussian (link “log”) distribution (“lme4” package version 1.1-27) (Bates et al.,  
207 2015), while p-values were calculated in order to confirm the significance of the relationships  
208 using the “lmerTest” package version 3.1-3 (Kuznetsova et al., 2017). In addition to linear

209 mixed-effects modelling, we also used the response ratio (RR) (Hedges et al., 1999) of liming  
210 on the grass dry matter production between treatment and control, to confirm our results. We  
211 calculated the RR and performed analyses with it according to Li et al. (2019) using the  
212 “metafor” package version 3.0-2 (Viechtbauer, 2010). Based on recommendations (Wiebe et  
213 al., 2006; Kambach et al. 2020), we used multiple imputations of missing variance measures  
214 to overcome the problem of incompletely reported primary studies (standard deviations were  
215 missing in 28.6% of studies). The data imputation was made by “mice” package version 3.13.0  
216 (van Buuren and Groothuis-Oudshoorn, 2011). We have not applied the RR method on soil pH  
217 due to unavailability of standard deviation values in the original papers.

218 Linear regression models were used to show relationships between the changes in soil  
219 pH and clay, silt and sand contents in the soil. In addition, linear regression models were also  
220 used to show relationships between the changes in grass dry matter production and soil pH due  
221 to liming, calcium carbonate equivalent lime, MAAT and MAP. For the relationship between  
222 calcium carbonate equivalent and applied N fertiliser and dry matter production in different  
223 climate zones and different grassland types, we created interpolated contour plots using the  
224 package “akima” version 0.6-2 (Akima et al., 2016). A contour plot is a graphical technique  
225 for representing a three-dimensional surface by plotting constant z slices on a two-dimensional  
226 format. That is, given a value for z, lines are drawn for connecting the (x, y) coordinates where  
227 that z value occurs. We performed linear regressions to show relationships between the calcium  
228 carbonate equivalent and applied N fertiliser variables against grass dry matter production. For  
229 exploring the fits of different models, inspection of residuals patterns for the entire model and  
230 posterior predictive simulation were used as diagnostic tools (Gelman and Hill, 2006; Bates et  
231 al., 2015; Harrison et al., 2018).

232

### 233 **3. Results and Discussion**

#### 234 *3.1. Impacts of liming on soil pH*

235 In this global systematic analysis and review, we quantitatively analysed the data collected on  
236 the impacts of liming grasslands on soil pH and grass dry matter production (Table 1). A paired  
237 test with random effects showed that liming significantly increased the initial soil pH values  
238 ( $p < 0.001$ ;  $n = 85$ ) (Table 2). Similar impacts of liming on soil pH were reported in previous  
239 studies (e.g. Corbett et al., 2021; Zurovec et al., 2021). The optimum soil pH for the growth  
240 and development of grasslands is variable due to the tolerance of some grass-species to high  
241 soil acidity (Anderson et al., 2013). These grass species are less sensitive to soil acidity because  
242 they resist Al toxicity (Poozesh et al., 2010). For example, the grass species Browntop

243 (*Agrostis tenuis* Sibth) and Chewings fescue (*Festuca rubra* L. subsp. *commutata* Gaud) are  
244 not affected by  $Al^{3+}$  up to 30 and 12  $\mu M$ , respectively. However, Yorkshire fog (*Holcus*  
245 *lanatus* L.), Veld grass (*Ehrharta calycina* Smith) and Paspalum (*Paspalum dilatatum* Poir)  
246 are tolerant to Al with a 50% reduction in attainable yield, under optimal management and N  
247 input, due to  $Al^{3+}$  of 13, 10 and 7  $\mu M$ , respectively (Edmeades et al., 1991). Although liming  
248 is an important management to improve biodiversity, it could negatively influence species with  
249 different pH optima in the species pool (De Graaf et al., 1998). These significant increases in  
250 soil pH due to liming were also found when the data were segregated by climatic zones; MC  
251 ( $p < 0.001$ ;  $n = 55$ ) and MW ( $p < 0.001$ ;  $n = 30$ ) or by the number of grass species in the field:  
252 monoculture ( $p < 0.001$ ;  $n = 48$ ) and multi-species grasses ( $p < 0.001$ ;  $n = 37$ ) (Table 2). A previous  
253 synthesis on agricultural systems by Goulding (2016) also found significant increases in soil  
254 pH under variable numbers of grass species. Changes in soil pH due to liming have  
255 significantly positive correlations with amounts of clay ( $t = 3.69$ ,  $p < 0.01$ ,  $R^2 = 0.39$ ,  $n = 23$ )  
256 and silt ( $t = 2.27$ ,  $p < 0.05$ ,  $R^2 = 0.24$ ,  $n = 18$ ) contents in soils but no correlation with the amounts  
257 of sand was observed ( $p > 0.05$ ) (Fig.1). Corbett et al. (2021) and He et al. (2021) reported  
258 significant correlations between soil pH and clay contents in soils. Thus, the initial soil pH and  
259 clay and silt soil particles should all be considered when deciding on the amounts of lime  
260 applied to soils. These soil parameters are related to the cation exchange capacity and base  
261 saturation percentage, which are in effect what determine the lime requirement.

262

### 263 3.2. Impacts of liming on grassland dry matter production

264 On one hand, a paired test with random effects for all available data showed that liming had  
265 statistically significant positive effects on the grass dry matter production compared to the  
266 control treatments ( $p < 0.001$ ;  $n = 63$ ) (Table 2). Soil acidity leads to low base status and high  
267 aluminium (Al) saturation (Horan et al., 2018) and therefore, reduces grass dry matter  
268 production (Mijangos et al., 2010) and abundance of desirable species (Olsson et al., 2009).  
269 Unlike N fertiliser, which aims to increase grass production by adding mineral N, liming aims  
270 to do so by optimising nutrient availability and plant growth conditions. Thus, correcting soil  
271 pH through liming provides the right environment for grassland to reach its growth potential.  
272 This reduces the need for animal supplementary feeding and improves the efficiency and  
273 sustainability of grazing livestock production. Significant increases in grass dry matter  
274 production due to liming were also found under the two climatic zones; MC ( $p < 0.01$ ;  $n = 37$ )  
275 and MW ( $p < 0.001$ ;  $n = 26$ ) and under the different numbers of grass species: monoculture  
276 ( $p < 0.001$ ;  $n = 34$ ) and multi-species grasses ( $p < 0.01$ ;  $n = 29$ ) (Table 2).



277 On the other hand, as illustrated in Fig. 2a, a response ratio analysis ( $\pm$  95% confidence  
278 intervals) showed that in the MC climate, the increase in grass dry matter production of 20.8%  
279 due to liming was significantly higher and the increase of 14.6% in the MW climate was higher  
280 but not significantly different from the control. Here, although temperature can increase grass  
281 productivity, it could also increase plant decomposition and microbial response to other  
282 perturbations (e.g. liming) (Ågren and Hyvonen, 2003; Wennman and Katterer, 2006; Jabro et  
283 al., 2008). The increase in grass dry matter production due to liming for both monoculture  
284 (17.4%) and multi-species (17.7%) grass were both significantly higher compared to the  
285 control ( $p < 0.05$ ) (Fig. 2b). The response ratio analysis showed that significantly higher biomass  
286 production of 34.4% could be achieved by liming grasslands grown on medium soil, and of  
287 42.1% by applying an annual N fertiliser ranging from 100 to 200 kg N ha<sup>-1</sup>. However, although  
288 the increases in grass dry matter production due to liming for other soil types / applied amounts  
289 of N fertiliser were higher, no significant differences were observed (Fig. 2c and d,  
290 respectively).

291 Contour plots (Fig. 3) show the relationship between the amounts of lime, applied N  
292 fertiliser and dry matter production for different climate zones (i.e. MW and MC) or different  
293 numbers of grass species. Here, the amounts of lime and applied N fertiliser explain 42.3% of  
294 overall dry matter variations ( $n = 45$ ,  $p < 0.001$ ); dry matter correlated significantly with both  
295 calcium carbonate equivalent ( $t = -2.2$ ;  $p < 0.05$ ) and applied N fertiliser ( $t = 3.9$ ;  $p < 0.001$ ).  
296 Clear differences in vegetation and the number of species due to liming can be seen in the Park  
297 Grass Experiment in the UK (Fig. 4). In this experiment, combinations between N fertiliser and  
298 ground chalk lime resulted in a higher species richness compared to un-limed treatments. Many  
299 studies e.g. Jarvis (1984) and Poozesh et al. (2010) reported that liming also increased the total  
300 number of grass species, the proportion of di-cotyledons and the nodulation of white clover  
301 and thereby, increased N through symbiotic fixation. In contrast, Pavlu et al. (2021) reported  
302 no difference in species richness due to previously applied liming. Positive correlation between  
303 changes in soil pH and changes in grass dry matter production due to liming was found ( $t =$   
304 1.62,  $p = 0.134$ ,  $R^2 = 0.21$ ,  $n = 12$ ) (Fig. 5a). However, although this correlation was not  
305 statistically significant, due to limited data and high variability, it shows a clear trend between  
306 them. In a 60-year experiment investigating impacts of N deposition on wild plant  
307 communities, Berendse et al. (2021) noted a faster recovery of species richness and plant  
308 diversity in limed plots than in the un-limed ones. Awad et al. (1976) and Poozesh et al. (2007)  
309 found inverse relationships between grass growth and Al concentration in soils. As shown in  
310 Fig. 5b, the changes in dry matter production due to liming was significantly negatively

311 correlated with the applied amount of lime in the form of calcium carbonate equivalent ( $t =$   
312  $-2.71$ ,  $p < 0.01$ ,  $R^2 = 0.11$ ,  $n = 62$ ). Thus, to get the maximum benefit of liming grassland, acid  
313 soils should be regularly limed but at a low rate depending on soil type and initial soil pH.  
314 Grassland productivity is also reduced if soil acidity is combined with a low soil phosphorus  
315 concentration (P) (Tanaka et al., 1984). The maximum recommended lime rate for grasslands  
316 in England and Wales is  $7.5 \text{ t ha}^{-1}$  for each application (AHDB, 2021). Excess liming can  
317 decrease grass productivity due to reduced nutrient availability (e.g. phosphorus and minor  
318 nutrients) in alkaline conditions (Higgins et al., 2012). It can also result in a lower grass root  
319 mass, higher root decomposition and higher N mineralisation (Heyburn et al., 2017). Moreover,  
320 the application of lime in silvo-pastoral systems can increase grass biomass production but,  
321 although not significant, it slows down tree growth due to competition between the grasses and  
322 trees (Mosquera-Losada et al., 2011). Li et al. (2019), found grasses and legumes in grazing  
323 systems have a highly variable response to liming because of variations in species richness and  
324 the number of grazing livestock, which make nutrient cycling processes very complex (Hooper  
325 et al., 2005). Liming improves feed quality and reduces the amount of N fertiliser required by  
326 the grass (Higgins et al., 2012; Mkhonza et al., 2020). In contrast, few studies reported that  
327 liming increased soil pH but had no significant gains (Toxopeus, 1989; Viadé et al., 2011), or  
328 even had negative effects (Cregan et al., 1989; Carran, 1991; Ryant et al., 2016), on grass  
329 biomass productivity. However, Ryant et al. (2016) noted that this low productivity due to  
330 liming was due to the suppression of some grass species that adapted to acidic soils. Biomass  
331 production under liming treatments was positively correlated with MAP ( $t=2.3$ ,  $r^2=0.08$ ,  
332  $p < 0.05$ ,  $n=63$ ) but the correlation with MAAT was not significant, as illustrated in Fig. 5c and  
333 d. Here, a wet climate plays an important role in enhancing soil acidity due to leaching and  
334 acid rain (Slessarev et al., 2016).

335

### 336 *3.3. Impacts of liming on greenhouse gas emissions*

#### 337 *3.3.1 Impacts on N<sub>2</sub>O and CH<sub>4</sub> emissions*

338 Data on the impacts of liming grasslands on N<sub>2</sub>O and CH<sub>4</sub> emissions were scarce. Therefore,  
339 we analysed / summarized the collected studies qualitatively (Table 3). This represents a  
340 significant gap in knowledge which needs to be filled in order to better understand the benefits  
341 and impacts of liming practices. Available studies show that liming either decreased or had no  
342 significant effect on N<sub>2</sub>O emissions. According to Bakken et al. (2012) and Liu et al. (2014),  
343 under acidic soil the N<sub>2</sub>O reductase functioning in denitrifiers is weak. However, increasing  
344 soil pH by liming can improve the capacity of denitrifiers to reduce N<sub>2</sub>O to N<sub>2</sub> and thereby,

345 reduce N<sub>2</sub>O emissions. Likewise, Jha et al. (2020) found that liming increases *nosZ* gene  
346 abundance in grazed grassland soils causing lower N<sub>2</sub>O emissions and more complete bacterial  
347 denitrification. Wang et al. (2021) and Zurovec et al. (2021) found a decrease in both soil N<sub>2</sub>O  
348 and yield-scaled N<sub>2</sub>O emissions in limed grasslands compared to the un-limed grasslands and  
349 a negative linear relationship between soil pH and cumulative N<sub>2</sub>O emissions. Moreover,  
350 Williams et al. (2021) reported that liming of fertilised grasslands was most effective in  
351 lowering the yield-scaled N<sub>2</sub>O emissions compared to ploughing and reseeded of the  
352 grassland. In Norway, Byers et al. (2021) found that liming reduced N<sub>2</sub>O emissions from the  
353 plots with grass only but not from grass-clover or pure clover treatments. They argued that the  
354 increased decomposition and nitrification of the N-rich clover biomass in winter led to higher  
355 soil NO<sub>3</sub><sup>-</sup> and low O<sub>2</sub> and consequently, higher N<sub>2</sub>O emissions from denitrification. However,  
356 Galbally et al. (2010) reported that liming had no significant impact on the overall average N<sub>2</sub>O  
357 emissions (i.e.  $0.96 \pm 0.07$  mg N m<sup>-2</sup> d<sup>-1</sup> for acid plots compared to  $0.88 \pm 0.04$  mg N m<sup>-2</sup> d<sup>-1</sup>  
358 for limed plots) but decreased the yield-scaled N<sub>2</sub>O emissions due to the significant increase in  
359 grass yield. The reduction in the amount of N fertiliser requirement (Higgins et al., 2012;  
360 Mkhonza et al., 2020) due to higher grass biomass production (Zurovec et al., 2021) and higher  
361 soil nitrate (i.e. higher soil nitrification) (Clough et al. 2004) under liming, significantly  
362 mitigate N<sub>2</sub>O emissions from grasslands. Further, Cuhel et al. (2010) noted that soil pH is one  
363 of the main factors that determine end products of denitrification. They found N<sub>2</sub>O/ (N<sub>2</sub>O + N<sub>2</sub>)  
364 ratio increased with decreasing soil pH due to changes in the total denitrification activity but  
365 had no change in N<sub>2</sub>O production.

366 Available studies showed that liming either decreased or had no effect on CH<sub>4</sub>  
367 emissions from grassland soils. Although CH<sub>4</sub> emission from grasslands soils is less important  
368 compared to that release from ruminant livestock, anaerobic storage of manure (Corre, 2002)  
369 or N<sub>2</sub>O emissions from soils, it still needs to be mitigated because it contributes to the climate  
370 change problem (Garnett et al., 2017). The CH<sub>4</sub> is produced in soils by methanogenic archaea  
371 (Watanabe et al., 2007) and consumed as C and energy sources by methanotrophic  
372 microorganisms (Smith et al., 2003). Usually, well-drained grassland soils consume CH<sub>4</sub>,  
373 however there are important interactions with N fertilisation and soil pH that influence CH<sub>4</sub>  
374 consumption (Bodelier and Laanbroek, 2004). Specifically, in some cases, ammonium-N  
375 fertilisers reduce the soil oxidising capacity (Hütsch et al., 1994) by enhancing competing  
376 nitrifier communities that oxidise (i.e. consume) CH<sub>4</sub> at a slower rate than methanotrophs, or  
377 by increasing the threshold CH<sub>4</sub> concentration at which the methanotrophic activity starts  
378 (Mosier et al., 1998). The long-term Park Grass experiment (Hütsch et al., 1994; Stiehl-Braun

379 et al., 2011) showed that the interaction of soil pH with N fertilisation was important. Here,  
380 liming for more than 100 years did not restore the CH<sub>4</sub> oxidising capacity of the soil that had  
381 received NH<sub>4</sub>-N fertiliser, whereas it did in soils that received NO<sub>3</sub>-N fertiliser (Hütsch et al.,  
382 1994; Silvertown et al., 2006). The authors argued that NH<sub>4</sub>-N fertilisation had caused a shift  
383 in microbial population or had resulted in a very persistent NH<sub>4</sub><sup>+</sup> inhibition of CH<sub>4</sub> oxidation.  
384 Ammonium sulphate, which has an acidifying effect, seemed to cause an increase in CH<sub>4</sub>  
385 emissions at low soil pH when no lime was applied. In contrast, manure apparently had a  
386 buffering effect on CH<sub>4</sub> consumption, as CH<sub>4</sub> consumption was relatively stable at a varying  
387 soil pH (Stiehl-Braun et al., 2011). Soil pH strongly influences CH<sub>4</sub> consumption through  
388 several pathways, which are still not fully understood (Stiehl-Braun et al., 2011). Although soil  
389 acidity directly affects methanotrophs, availability of NH<sub>4</sub><sup>+</sup> as ammonia and toxic effects of  
390 Al<sup>3+</sup> ions at low soil pH could be possible explanations (Hütsch et al., 1994; Powlson et al.,  
391 1997; Stiehl-Braun et al., 2011). Moreover, the accumulation of NO<sub>2</sub><sup>-</sup> and NH<sub>2</sub>-OH compounds  
392 at low pH, are also toxic to methanotrophs (Kunhikrishnan et al., 2016). Powlson et al. (1997),  
393 observed that low soil pH (below 5.1) significantly decreased the CH<sub>4</sub> consumption capacity  
394 of the soil. In contrast to the Park Grass experiment, a natural grassland experiment in Puerto  
395 Rico where lime was incorporated into the soil by tillage showed that liming did not completely  
396 restore CH<sub>4</sub> uptake. Here, the soil microflora were adapted to the acidic environment (Mosier  
397 et al., 1998). Incorporation of lime in soils has greater impacts on net CH<sub>4</sub> emission than surface  
398 application, as the soil layers that most contribute to CH<sub>4</sub> oxidation are the deeper ones that are  
399 scarcely influenced by a surface liming practice (Hütsch, 2001).

400

### 401 3.3.2 Net CO<sub>2</sub> emissions

402 The balance between SOC stored in the soil and CO<sub>2</sub> emitted to the atmosphere (net CO<sub>2</sub>  
403 emission) under liming was assessed. Although most studies collected and reviewed (Table 3)  
404 have shown lower SOC associated with liming, few reported contrasting results with either  
405 small increases (Fornara et al., 2011; Sochorová et al., 2016) or similar effects to un-limed  
406 treatments (Aye et al., 2016; Egan et al., 2018). Liming of grasslands can enhance SOM  
407 mineralisation and emission of CO<sub>2</sub> (Holland et al., 2018) but can also increase SOM and create  
408 a net C sink due to high biomass and root production (Fornara et al., 2011). The combination  
409 between liming and other sward management, such as frequent cutting and heavy grazing,  
410 reduced SOC (Forster et al., 2021). Moreover, Barcelos et al. (2021) found the effects of lime  
411 on C cycling through microbial biomass, especially in the subsoil, were minimal. The effect of  
412 liming grasslands on net CO<sub>2</sub> emission is the result of several processes that take place

413 simultaneously. Firstly, there can be greater OM inputs due to increased biomass production.  
414 Secondly, the lime application can lead to increased OM mineralisation due to favourable soil  
415 pH, since soil biological activities that promote OM mineralisation and accelerate OM turnover  
416 rates are stimulated (Marcelo et al., 2012). If these higher microbial activities remain constant  
417 over time, they can result in higher CO<sub>2</sub> emissions and lower SOC stocks (Paradelo et al., 2015;  
418 Lochon et al., 2018). Thirdly, liming is a source of inorganic C and thereby, it enhances CO<sub>2</sub>  
419 efflux (Raza et al., 2021). Fourthly, high Ca<sup>2+</sup> concentrations and ionic strength following lime  
420 application can also improve the aggregation of clay minerals and the formation of stable  
421 aggregates, thereby protecting SOC (Haynes and Naidu, 1998). According to Foereid et al.  
422 (2006), after a short-term isotope study, the control plots stored more C in the soil than the  
423 limed treatment. They found that although the limed treatment had a greater primary  
424 productivity, the throughput was slower in the control treatment. Therefore, the control  
425 treatment could accumulate more C in the soil in the longer-term (Foereid et al., 2006).  
426 Generally, liming grasslands resulted in higher net CO<sub>2</sub> emissions because of increased CO<sub>2</sub>  
427 emissions and decreased SOC. To reduce this net CO<sub>2</sub> emission, Snyder et al. (2009) suggested  
428 applying lime in the form of an oxide (e.g. quicklime or slaked lime) rather than as carbonate  
429 materials.

430 Liming has a direct chemical effect on inorganic C transformations, and an indirect  
431 biological effect on organic C transformations through diverse C flux pathways in the  
432 rhizosphere and mycorrhizosphere (Hinsinger et al., 2003; Ahmad et al., 2014). The results of  
433 these effects and the mechanisms behind them depend on the ecosystem, including many  
434 factors such as weather, soil type, soil OM content and liming practice (Soussana et al., 2014).  
435 In a long-term study by Kemmitt et al. (2006), enhanced CO<sub>2</sub> emissions continued for 16 years  
436 after the last liming event, as a consequence of direct pH effects on the functioning of the  
437 microbial community (e.g. nitrifiers), and on (decreasing) toxic Al concentrations, which in  
438 turn, indirectly increased substrate availability and biomass production. The long-term legacy  
439 effect of a single lime application in a subalpine ecosystem was confirmed by Schaffner et al.  
440 (2012). The authors found that the Ca<sup>2+</sup> pool in soil was still significantly higher in limed than  
441 in the control treatments, but the soil C concentration was not affected. However, in a 30-  
442 month-experiment by Lochon et al. (2019), no longer-term effects of liming were found  
443 probably due to the relatively low amount applied. Moreover, decreased net CO<sub>2</sub> emissions  
444 were observed on peatlands after six years of annual liming and N and P fertilisation. In this  
445 case, liming increased available and total N and P at the surface peat, increased soil pH and  
446 shifted the dominant plant community but no impact on microbial C cycling was observed

447 (Keller et al., 2005). Other than the amount, type and quality of lime applied, the liming method  
448 may have an impact on the effectiveness of liming in increasing CO<sub>2</sub> emissions (Marcelo et al.,  
449 2012). In some studies, where the quantitative effect of lime on potentially mineralisable C was  
450 observed, the amount of respired C was proportional to liming rate (Kemmitt et al., 2006;  
451 Marcelo et al., 2012). However, liming increases grass productivity and SOC decomposition  
452 in organic and low productive soils (Alison et al., 2019). Furthermore, in an experiment in a  
453 silvo-pastoral system, Mosquera-Losada et al. (2011) showed that lime application in  
454 combination with sewage sludge fertilisation (200 kg total N ha<sup>-1</sup>) reduced soil OM due to the  
455 increased mineralisation rate, which can reduce the soil capacity to store C. Further, Wang et  
456 al. (2016) noted that the total SOC (0-10 cm depth) decreased or remained constant after long-  
457 term liming, depending on the lime application rates, though the decrease in SOC occurred  
458 mainly in the labile C pools.

459

### 460 3.3.3 Total net GHG emissions

461 Liming grasslands showed neutral or even favourable effects on N<sub>2</sub>O and CH<sub>4</sub> emissions.  
462 However, the main disadvantage of liming is the risk of high net CO<sub>2</sub> emissions, especially  
463 when over-applied (Aye et al., 2016). As the global warming potential of CO<sub>2</sub> is low compared  
464 to N<sub>2</sub>O and CH<sub>4</sub> (the GWPs of N<sub>2</sub>O and CH<sub>4</sub> are 273 and 27.2 to 29.8 times that of CO<sub>2</sub>,  
465 respectively, over a 100-year period; IPCC, 2021), increased CO<sub>2</sub> efflux from liming of  
466 grassland could still have limited impact on total net GHG emissions. Here, the increase in net  
467 CO<sub>2</sub> emissions due to liming will be compensated by the saving in GHG emissions due to the  
468 reduction in N<sub>2</sub>O and CH<sub>4</sub> emissions. In a meta-analysis, Wang et al. (2021) reported that  
469 liming global agricultural acid soils would have neutral impacts on total net GHG emissions  
470 with a significant increase in crop productivity. Thus, liming could help to fulfil the  
471 environmental targets proposed in the EU Green Deal and the Farm to Fork and Biodiversity  
472 strategies (EC, 2021). A new emerging technology to raise soil pH and potentially sequester  
473 CO<sub>2</sub> is the application of Mg or Ca silicates (mineral carbonation of e.g. wollastonite or olivine  
474 or other mafic rock powders) (O'Connor et al., 2001; ten Berge et al., 2012). However, to the  
475 best of our knowledge, no study has yet been carried out on grassland. Future research should  
476 focus on the development of methods to increase SOC with liming. This could be achieved by,  
477 for example, breeding of grass species with deeper or more extensive root systems  
478 e.g. *Festulolium* (ryegrass x fescue hybrid). These types of grass have a greater resource use  
479 efficiency (e.g. water), high biomass productivity, high contribution to SOC (Humphreys et al.,  
480 2003; Kell, 2011) and induce lower enteric CH<sub>4</sub> emissions when fed to ruminants if

481 supplemented with feed diets (Celis-Alvarez et al., 2021). Moreover, practising of extensive  
482 grazing can also help to maintain soil C stocks due to regular organic matter input by livestock  
483 (Abdalla et al., 2018; Forster et al., 2021). However, for less profitable farms, acid-tolerant  
484 grass species can be grown. Long-term field experiments on grasslands should be conducted  
485 to investigate further the potential antagonistic or synergistic effects of lime on total net GHG  
486 emissions.

487

#### 488 **4. Conclusions**

489 In this global systematic review and analysis, we found that liming grasslands significantly  
490 raised soil pH and enhanced grass biomass production in acidic soils. Liming either decreased  
491 or had no effects on N<sub>2</sub>O and CH<sub>4</sub> emissions. There is a trade-off between the impacts of liming  
492 on grass biomass production and the soil net CO<sub>2</sub> emissions. However, as the global warming  
493 potential of CO<sub>2</sub> is low compared to N<sub>2</sub>O and CH<sub>4</sub>, the impacts on total net GHG emissions  
494 will be minimal. In conclusion, liming grassland increases the net CO<sub>2</sub> emissions, but it makes  
495 sense to lime acidic grasslands to increase nutrient use efficiency within livestock grazing  
496 systems. However, the application rate should be optimised according to soil type, climate and  
497 management.

498

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504

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Table 1. Published studies on the impacts of liming on grass dry matter production

| Coordinates<br>(Country)    | Grass type                  | MAAT*<br>(°C) | MAP**<br>(mm) | Climate<br>zone | Soil texture | Average<br>initial pH | Type/ rate of lime (t<br>ha <sup>-1</sup> ) | Calcium<br>carbonate<br>equivalent<br>(t ha <sup>-1</sup> ) | Duration<br>(year) | Lime<br>effects/ Δ<br>dry matter<br>(t ha <sup>-1</sup> ) | Ref. |
|-----------------------------|-----------------------------|---------------|---------------|-----------------|--------------|-----------------------|---|---|--------------------|---|------|
| 50.21°N, 06.85° E<br>(DE)   | Grassland                   | 6.9           | 811           | MC              | N/A          | 5.3                   | Calcium oxide (1)                           | 1.8   | 7                  | Increased   | 1    |
| 43.12° N, 2.85° W<br>(SP)   | Multi- species<br>grassland | 10.1          | 2000          | MC              | Clay loam    | 4.6                   | Calcium carbonate<br>(2.4)                  | 2.4   | 0.5                | 1.07  | 2    |
|                             |                             |               |               | MC              | Sandy loam   | 4.2                   | Calcium carbonate<br>(4.7)                  | 4.7   | 0.5                | -0.43   | 2    |
| 39.60° S, 176.58° E<br>(NZ) | Ryegrass/clover             | 13.2          | 838           | MW              | Silt loam    | 5.4                   | Limestone (0.5)                             | (0.5)   | 3                  | 0.23  | 3    |
|                             |                             |               |               |                 |              | 5.4                   | Limestone (1)                               | (1)   | 3                  | 0.83  | 3    |
|                             |                             |               |               |                 |              | 5.4                   | Limestone (2)                               | (2)   | 3                  | 0.69  | 3    |
|                             |                             |               |               |                 |              | 5.4                   | Limestone (4)                               | (4)   | 3                  | 0.63  | 3    |
|                             |                             |               |               |                 |              | 5.4                   | Limestone (8)                               | (8)   | 3                  | 0.84  | 3    |
| 43.23° N, 7.35° W<br>(SP)   | Silvopastoral <sup>▲</sup>  | 11.5          | 1083          | MC              | Sandy soil   | 5.2                   | Calcium carbonate<br>(2.5)                  | 3.4   | 6                  | 0.24  | 4    |
|                             |                             |               |               |                 |              | 5.2                   | Calcium carbonate<br>(2.5)                  | 3.4   | 6                  | 0.23  | 4    |
|                             |                             |               |               |                 |              | 5.2                   | Calcium carbonate<br>(2.5)                  | 3.4   | 6                  | 0.15  | 4    |
| 49.50° N, 15.96° E<br>(CZ)  | Permanent grass             | 5.8           | 758           | MC              | Loamy soil   | 4.4                   | Dolomite limestone<br>(1.8)                 | 3.4   | 2                  | Decreased   | 5    |
| 27.48° N, 81.91° W<br>(US)  | Bahiagrass                  | 17.2          | 1117          | MW              | N/A          | 4.3                   | Dolomite limestone<br>(1)                   | 1.09  | 5                  | No<br>significant<br>effects                              | 6    |
| 38.33° S, 175.16° E<br>(NZ) | Multi-species<br>grassland  | 13.8          | 1502          | MW              | Loam         | 5.6                   | Ground lime (2.5)                           | 2.5   | 3                  | -0.49   | 7    |
|                             |                             |               |               |                 |              | 5.7                   |   | 2.5   | 3                  | 0.10  | 7    |
|                             |                             |               |               |                 |              | 6.2                   |   | 2.5   | 3                  | 0.38  | 7    |
| 43.50° N, 20.86° E<br>(RS)  | Natural grassland           | 9.1           | 1400          | MC              | N/A          | 4.1                   | Hydrated lime (1)                           | 1.36  | 2                  | 3.22  | 8    |
| 43.15° N, 07.48° W<br>(SP)  | Italian<br>ryegrass/clover  | 19            | 1019          | MC              | Sandy loam   | 4.2                   | Magnesium<br>limestone (3)                  | 3.27  | 3                  | No<br>significant<br>effects                              | 9    |

|                             |  |         |          |    |                           |           |  |                          |      |                        |    |
|-----------------------------|--|---------|----------|----|---------------------------|-----------|--|--------------------------|------|------------------------|----|
| 25.11° N, 103.48° E<br>(CN) | Ryegrass/white clover                  | 12      | 1008     | MW | Fine loamy soil           | 4.5-5.4   | Quick (or burnt) Limestone (0.5,1, 1.5)          | (0.99, 1.79, 2.69)       | 3    | No significant effects | 10 |
| 55.72° N, 21.45° E<br>(LT)  | Cocksfoot/reed canary grass            | 6-7     | 518      | MC | Loam                      | 4.25-4.85 | Calcium carbonate (6)                            | 6                        | 3    | Increased              | 11 |
| 43.9° N, 20.31° E<br>(RS)   | Red clover/oat grass                   | 12      | 680      | MC | N/A                       | 4.8       | Calcium carbonate (3)                            | 3                        | 3    | 0.38                   | 12 |
|                             | Red clover/oat grass                   | 12      | 680      | MC | N/A                       | 4.8       | Calcium carbonate (6)                            | 6                        | 3    | 1.25                   | 12 |
| 56.55° N, 23.73° E<br>(LV)  | Perennial grasses (red clover/timothy) | 5.6     | 670      | MC | Loam                      | 4.7-5.6   | Dolomite limestone (2.58, 5.7, 11.4)             | (2.81, 6.21, 12.43)      | 30   | 0.1                    | 13 |
| 15.58° S, 47.70° W<br>(BR)  | Green panic grass                      | 22      | 1230     | MW | N/A                       | 4.2       | Dolomite limestone (1.6)                         | 3.04                     | 0.75 | Increased              | 14 |
| 22.85° S, 48.38° W<br>(BR)  | Congo signal grass                     | 26.1    | 1358     | MW | Clayey                    | 4.2       | Lime stone (3.8)                                 | 3.8                      | 2    | 1.03                   | 15 |
| 36.12° N, 97.07° W<br>(US)  | Red clover                             | 17.6    | 615      | MW | Silt loam                 | 4.1-4.7   | Calcium carbonate (0.4, 0.7, 1.2, 2.0, 3.7)      | (0.4, 0.7, 1.2, 2, 3.7)  | 3    | Increased              | 16 |
| 93.25° N, 12.08° E<br>(NO)  | Grass leys                             | 0.7-6.3 | 728-1708 | MC | Multi-soils               | 5.2-5.54  | Dolomite limestone (2.5, 5); Stone meal (2.5, 5) | (2.5, 5)                 | 4    | Increased              | 17 |
| 42.09° N, 01.32° W<br>(FR)  | Cocksfoot grass                        | 12-13.4 | 751-1093 | MC | Loam                      | 3.9       | (Calcium carbonate + Calcium sulphate) (2.37)    | 2.37                     | 2    | 2.65                   | 18 |
| 42.33° N, 07.86° W<br>(SP)  | Multi-species grassland                | 13      | 1400     | MC | Loam                      | 5.04      | Limestone (1.8)                                  | 1.8                      | 1    | 5.39                   | 19 |
| 54.46° N, 06.08° W<br>(UK)  | Meadow/perennial ryegrass              | 8.6     | 1024     | MC | Clay loam/silty clay loam | 5.8       | Ground limestone (0.10, 0.15, 0.23, 0.30)        | (0.10; 0.15, 0.23, 0.30) | 3    | 4.0                    | 20 |
| 57.78° N, 06.49° W<br>(UK)  | Multi-species grassland                | 8.6     | 865      | MC | Multi-soil types          | 4.7-5.6   | Ground limestone (4,8)                           | (4, 8)                   | 4    | No significant effects | 21 |
| 37.73° S, 142.01° E<br>(NZ) | Multi-species pasture                  | 14.9    | 1500     | MW | Clay loam                 | 4.6-5.4   | Limestone (3)                                    | 3                        | 1    | 2.5                    | 22 |
|                             |  |         |          |    |                           |           |  | 3                        | 1    | 1.0                    | 22 |
|                             |  |         |          |    |                           |           |  | 3                        | 1    | 6.2                    | 22 |

|                             |  |      |             |    |                    |         |                                  |                      |   |                              |    |
|-----------------------------|--|------|-------------|----|--------------------|---------|----------------------------------|----------------------|---|------------------------------|----|
| 17.45° S, 52.60° W<br>(BR)  | Urochloa pasture   | 28.9 | 1447        | MW | Clay soil          | 4.7     | Dolomite limestone<br>(2)        | 2.18                 | 2 | No<br>significant<br>effects | 23 |
| 35.38° S, 147.5° E<br>(AU)  | Perennial pasture<br>( <i>Phalaris aquatica</i><br><i>L.</i> ) | 15.8 | 500-<br>800 | MW | Sandy/clay<br>loam | 4-4.2   | Limestone (3.3,<br>4.1)          | (3.3, 4.1)           | 5 |                              | 24 |
|                             | Annual pasture   | 15.8 | 500-<br>800 | MW | Sandy/clay<br>loam | 4-4.2   | Limestone (3.3,<br>4.1)          | (3.3, 4.1)           | 5 | 0.46                         | 24 |
| 35.90° S, 146.93° E<br>(AU) | Perennial/ legume<br>mixture                                   | 17.6 | 630         | MW | N/A                | 4.3     | Calcium carbonate<br>(N/A)       | N/A                  | 2 | Increased                    | 25 |
| 29.01° S, 29.86° E<br>(SA)  | Italian ryegrass   | 16   | 1166        | MW | Sandy clay<br>loam | 4.1     | Dolomite limestone<br>(4, 8, 12) | (7.6, 8.72,<br>13.1) | 2 | 0.19                         | 26 |
| 28.01° S, 50.42° W<br>(BR)  | Natural pasture  | 16.6 | 1441        | MW | Clay               | 3.9-4.3 | Limestone (7.2,<br>14.4)         | 7.2                  | 4 | 0.30                         | 27 |
|                             |  |      |             |    |                    |         |                                  | 14.4                 | 4 | 0.10                         | 27 |
| 39.60° S, 176.58° E<br>(NZ) | Ryegrass/clover<br>(grazed)                                    | 14.3 | 838         | MW | Silt loam          | 5.4     | Limestone (2)                    | 2                    | 6 | 0.54                         | 28 |
|                             | Ryegrass/clover<br>(grazed)                                    |      |             |    |                    |         | Limestone (7.5)                  | 7.5                  | 6 | 0.10                         | 28 |
|                             | Ryegrass/clover<br>(cut)                                       | 14.3 | 838         | MW | Silt loam          | 5.4     | Limestone (2)                    | 2                    | 6 | 0.44                         | 28 |
|                             | Ryegrass/clover<br>(cut)                                       |      |             |    |                    |         | Limestone (7.5)                  | 7.5                  | 6 | 0.03                         | 28 |
| 39.33° S, 174.28° E<br>(NZ) | Ryegrass/clover<br>(grazed)                                    | 12   | 2000        | MW | Sandy loam         | 5.3     | Limestone (0.5)                  | 0.5                  | 4 | 0.74                         | 29 |
| 55.59° N, 2.43° W<br>(UK)   | Upland grassland   | 8.4  | 716         | MC | Loam               | 4.9     | Calcium carbonate<br>(6)         | 6                    | 4 | 0.27                         | 30 |
|                             | Upland grassland   | 8.4  | 716         | MC | Loam               | 4.9     | Calcium carbonate<br>(6)         | 6                    | 4 | 1.23                         | 30 |
|                             | Upland grassland   | 8.4  | 716         | MC | Loam               | 4.9     | Calcium carbonate<br>(6)         | 6                    | 4 | 3.06                         | 30 |
|                             | Upland grassland   | 8.4  | 716         | MC | Loam               | 4.9     | Calcium carbonate<br>(6)         | 6                    | 4 | 3.01                         | 30 |
| 59.65° N, 10.75° W<br>(NO)  | Grassland  | 5.7  | 795         | MC | N/A                | 5.2     | Dolomite limestone<br>(23)       | 25.1                 | 4 | -0.03                        | 31 |
|                             | Timothy/Perennial<br>ryegrass/meadow                           | 5.7  | 795         | MC | N/A                | 5.2     | Dolomite limestone<br>(23)       | 23                   | 4 | -0.12                        | 31 |



|                         |                                 |      |     |    |            |     |                          |     |   |      |    |
|-------------------------|---------------------------------|------|-----|----|------------|-----|--------------------------|-----|---|------|----|
|                         | Clover                          | 5.7  | 795 | MC | N/A        | 5.2 | Dolomite limestone (23)  | 23  | 4 | 0.39 | 31 |
| 07.42° N, 38.66° E (ET) | Grass/ herbaceous/ legume/ forb | 20.5 | 825 | MW | Clay loam  | 5.9 | Dolomite limestone (7.5) | 7.5 | 5 | 2.34 | 32 |
|                         |                                 |      |     |    | Sandy loam | 5.9 | Dolomite limestone (7.5) | 7.5 | 5 | 2.50 | 32 |
| 51.97° N, 05.63° W (NL) | Multi- species grassland        |      |     | MC | Clay       | 4.9 | Dolomite limestone (0.7) | 0.7 | 2 | 0.94 | 33 |

\*MAAT= mean annual air temperature. \*\*MAP= mean annual precipitation. Ref. = reference. 1= Hejcman et al. (2010); 2=Mijangos et al. (2010); 3= Morton et al. (2005); 4= Mosquera-Losada et al. (2011); 5= Ryant et al. (2016); 6= Silveira et al. (2012); 7=Toxopeus (1989); 8=Zornic et al. (2019); 9= Viadé et al. (2011); 10= Junquan et al. (2007); 11= Šiaudinis et al. (2014); 12=Tomic et al. (2018); 13= Vigovskis et al. (2016); 14= Braga et al. (2013); 15=Castro and Crusciol (2013); 16= Caddel et al. (2004); 17=Fystro and Bakken (2005); 18=Poozesh et al. (2010); 19= Fernandez-Sanjurjo et al. (2010); 20=Higgins et al. (2012); 21=Adams (1984); 22= During et al. (1984); 23=Da Silva et al. (2018); 24=Li et al.(2006); 25= Hayes et al. (2008); 26= Manson (1995); 27= Prestes et al. (2016); 28= Morton et al. (1998); 29=Thomson (1982); 30=Rangel-Castro et al. (2004); 31=Byers et al. (2021); 32= Bedaso et al. (2021); 33=Berendse et al. (2021). NA= not available. ▲Three N fertilisation rates were applied (0, 200 and 400 kg N/ha).

AU= Australia; BR= Brazil; CH= Switzerland; CN= China; CZ= Czech Republic; DE= Germany; ET= Ethiopia; FR= France; LT= Lithuania; LV= Latvia; NO= Norway; NZ= New Zealand; RS= Serbia; SA= South Africa; SP= Spain; UK= United Kingdom and US= United States of America.

Table 2: Statistical analysis of the impacts of liming ( $\text{t ha}^{-1}$ ) on soil pH and grass dry matter production ( $\text{t ha}^{-1}$ ) under different climatic zones (MC = moist, cool; MW = moist, warm) and number of grass species. N is the number of observations.

|                  | Soil pH             | Control (Mean $\pm$ SD) | Limed (Mean $\pm$ SD) | N  | t-value | p-value |
|------------------|---------------------|-------------------------|-----------------------|----|---------|---------|
| Soil pH          | All data            | 4.93 $\pm$ 0.71         | 5.70 $\pm$ 0.84       | 85 | 16.36   | <0.001  |
|                  | MC                  | 4.87 $\pm$ 0.74         | 5.56 $\pm$ 0.96       | 55 | 10.94   | <0.001  |
|                  | MW                  | 5.04 $\pm$ 0.66         | 5.96 $\pm$ 0.49       | 30 | 14.94   | <0.001  |
|                  | Monoculture grass   | 4.87 $\pm$ 0.67         | 5.81 $\pm$ 0.77       | 48 | 13.69   | <0.001  |
|                  | Multi-species grass | 5.00 $\pm$ 0.77         | 5.55 $\pm$ 0.92       | 37 | 4.68    | <0.001  |
| Grass dry matter | All data            | 5.21 $\pm$ 2.64         | 6.18 $\pm$ 2.93       | 63 | 6.39    | <0.001  |
|                  | MC                  | 4.66 $\pm$ 2.12         | 5.70 $\pm$ 2.69       | 37 | 3.89    | <0.001  |
|                  | MW                  | 5.99 $\pm$ 3.13         | 6.86 $\pm$ 3.17       | 26 | 4.45    | <0.001  |
|                  | Monoculture grass   | 5.49 $\pm$ 2.29         | 6.37 $\pm$ 2.67       | 34 | 5.66    | <0.001  |
|                  | Multi-species grass | 4.88 $\pm$ 3.02         | 5.95 $\pm$ 3.24       | 29 | 4.31    | <0.001  |

Table 3. Published studies on the impacts of liming grasslands on greenhouse gas (GHG) emissions and soil organic carbon (SOC)

| Coordinates<br>(Country)  | Grass type   | Average<br>N<br>fertiliser<br>(kg ha <sup>-1</sup> ) | MAAT<br>*<br>(°C) | MAP<br>**<br>(mm) | Soil<br>texture       | Initial<br>pH | Type/rate of<br>lime (t ha y <sup>-1</sup> ) | Calcium<br>carbonate<br>equivalent<br>(t ha <sup>-1</sup> )*** | GHG /<br>SOC<br>measured | Duration<br>(year) | Impact on<br>GHG<br>emissions | Mechanism(s)<br>of response        | Ref. |
|---------------------------|--|--|-------------------|-------------------|-----------------------|---------------|--|--|--------------------------|--------------------|-------------------------------|------------------------------------|------|
| 48.87°N, 14.22°E<br>(CZ)  | <i>Lolium perenne</i><br>and <i>Phleum<br/>pratense</i>  | N/A  | 7                 | 650               | N/A                   | 6.8           | N/A  | N/A  | N <sub>2</sub> O         | 0.8                | No significant<br>effects     | Increased<br>pH                    | 1    |
| 35.38°S, 147.50°E<br>(AU) | Rye grass/<br>clover   | N/A  | 16.2              | 570               | N/A                   | <6            | N/A  | N/A  | N <sub>2</sub> O         | 1                  | No significant<br>effects     | Increased<br>pH                    | 2    |
|                           |  |  |                   |                   |                       |               |  |  | N <sub>2</sub> O         | 1                  | No significant<br>effects     | Increased<br>pH                    | 2    |
|                           |  |  |                   |                   |                       |               |  |  | N <sub>2</sub> O         | 1                  | No significant<br>effects     | Increased<br>pH                    | 2    |
| 52.28°N, 147.50°W<br>(AU) | Perennial<br>Ryegrass  | 300  | 10.4              | 1037              | Loam                  | 5             | Ground<br>limestone<br>(1.5)                 | 1.5  | N <sub>2</sub> O         | 1                  | Decrease                      | Increased<br>pH                    | 3    |
|                           |  |  |                   |                   |                       |               |  | 1.5  | N <sub>2</sub> O         | 1                  | Decrease                      | Increased<br>pH                    | 3    |
|                           |  |  |                   |                   |                       |               |  | 1.5  | N <sub>2</sub> O         | 1                  | Decrease                      | Increased<br>pH                    | 3    |
| 59.65°N, 0.75°E<br>(NO)   | Perennial<br>Ryegrass,<br>Meadow Grass<br>Red<br>clover/Perennial<br>Ryegrass,<br>Meadow Grass<br>Red clover | 140  | 5.7               | 795               | Clay<br>loam          | 8.2           | Dolomite<br>limestone<br>(23)                | 25.1   | N <sub>2</sub> O         | <1                 | Decrease                      | Increased<br>pH                    | 4    |
|                           |  |  |                   |                   |                       |               |  |  |                          |                    | No significant<br>effects     | Increased<br>pH                    | 4    |
|                           |  |  |                   |                   |                       |               |  |  |                          |                    | No significant<br>effects     | Increased<br>pH                    | 4    |
| 51.81°N, 0.36°W<br>(UK)   | Mixed grass<br>species.  | 65-240   | 9.1               | 700               | Silty<br>clay<br>loam | 5.7           | Ground/slaked<br>lime<br>(N/A)               | N/A  | CH <sub>4</sub>          | >80                | Decrease                      | Increased<br>pH<br>Increased<br>pH | 5    |

|                           |   |     |      |      |                 |      |                                   |             |                 |   |                                       |  |    |
|---------------------------|---|-----|------|------|-----------------|------|-----------------------------------|-------------|-----------------|---|---------------------------------------|--|----|
| 18.48°N, 67.04°W<br>(US)  | Guinea grass<br>( <i>Panicum maximum</i> )    | 75  | 24   | 1650 | clay            | 4.5  | Powder lime<br>(10)               | 10          | CH <sub>4</sub> | 1.8   | No significant effects                | Increased pH   | 6  |
| 47.01°N, 92.58°W<br>(US)  | Fen dominated by graminoids                   | N/A | 3.2  | 497  | Peaty soil      | 4.9  | Calcium carbonate<br>(N/A)        | N/A         | CO <sub>2</sub> | 6<br>(measurement 6 years after liming)                       | Decrease net CO <sub>2</sub> flux     | Reduced available nutrients                                      | 7  |
| 51.83°N, 0.42°W<br>(UK)   | Grassland<br>( <i>Festuca rubra L.</i> )      | N/A | 9.8  | 733  | Silty clay loam | 3.45 | Calcium carbonate<br>(15, 25, 53) | 15, 25, 53  | CO <sub>2</sub> | 37 (SR <sup>Δ</sup> measurements 16 year after latest liming) | Increased longer-term CO <sub>2</sub> | Increased pH; indirectly increased primary production substrate  | 8  |
| 51.98°N, 0.58°W<br>(UK)   | Grassland<br>( <i>Lolium multiflorum L.</i> ) | N/A | 9.6  | 642  | Sandy loam      | 3.7  | Calcium carbonate<br>(9, 25, 45)  | 9, 25, 45   | CO <sub>2</sub> | 37 (in 4 steps; SR measurements 16 years after latest liming) | Increased longer-term CO <sub>2</sub> | Increased pH; indirectly: increased primary production substrate | 8  |
| 45.63°N, 2.73°E<br>(FR)   | Upland grassland                              | 100 | 7.8  | 1094 | Silty clay loam | 5.2  | Calcimer T400 (1.2)               | 1.2         | CO <sub>2</sub> | 2.5 (twice, SR measurements 6 months after latest liming)     | No significant effects                | Increased soil pH.   | 9  |
| 37.72°S, 145.05°E<br>(AU) | Unimproved pasture <sup>▲</sup>               | 0   | 14.8 | 666  | Clay soil       | 4.8  | Limestone<br>(3, 12.5, 25)        | 3, 12.5, 25 | CO <sub>2</sub> | 5 and 34 (SR measurements at 5 and 34 years)                  | Increased basal CO <sub>2</sub>       | Increased pH; slow downward movement of alkalinity.              | 10 |

|                          |                       |     |      |        |                 |         |  |             |     |     |                      |                        |   |    |
|--------------------------|-----------------------|-----|------|--------|-----------------|---------|--|-------------|-----|-----|----------------------|------------------------|---|----|
| 37.71° S, 145.04° E (AU) | Unimproved pasture    | N/A | 14.8 | 666    | Silty           | 4.8     | Limestone (3, 12.5, 25)                | 3, 12.5, 25 | SOC | 1   | after latest liming) | No significant effects | Increased plant biomass & thereby, offset faster mineralization | 11 |
| 55.47° N, 2.24° W (UK)   | Acid upland grassland | N/A | 7.5  | 995    | Sandy silt loam | 4.9     | Calcium carbonate (6)                  | 6           | SOC | 1   |                      | Decrease               | Increased soil pH   | 12 |
| 55.47° N, 2.24° W (UK)   | Acid upland grassland | N/A | 7.5  | 995    | Sandy silt loam | 4.9     | Calcium carbonate (6)                  | 6           | SOC | 1   |                      | No significant effects | Increased soil pH; influenced microbial composition.            | 13 |
| 51.41° N, 0.64° W (UK)   | Grassland             | 100 | 9.6  | 754    | Sandy           | 4.8     | Calcium carbonate (5 every 5 to 10 yr) | 5           | SOC | 1   |                      | No significant effects | Increased soil pH.  | 14 |
| 55.47° N, 2.24° W (UK)   | Acid upland grassland | N/A | 7.5  | 995    | Sandy silt loam | 3.7     | Calcium carbonate (6)                  | 6           | SOC | 0.5 |                      | Decrease               | Increased soil pH.  | 15 |
| 51.80° N, 0.37° W (UK)   | Permanent pasture     | N/A | 10.4 | 733    | Silty clay loam | 3.6-6.1 | Calcium carbonate (N/A)                | N/A         | SOC | 129 |                      | Decrease               | Increased soil pH   | 16 |
| 37.70° S, 142.11° E (AU) | Permanent pasture     | N/A | 13.4 | 684    | Clay loam       | 4.56    | Calcium carbonate (N/A)                | N/A         | SOC | 1   |                      | Decrease               | Increased soil pH   | 17 |
| 43.15°N, 7.33° W (SP)    | Silvopastoral system  | N/A | 12   | 1222.3 | Sandy loam      | 5.2     | Calcium carbonate (2.5)                | 2.5         | SOC | 1   |                      | Decrease               | Increased soil pH   | 18 |
| 37.70° S, 145.03° E (AU) | Unmanaged pasture     | N/A | 14.8 | 645    | N/A             | 4.3-5   | Calcium carbonate (12.5, 25)           | 12.5, 25    | SOC | 1   |                      | Decrease               | Increased soil pH   | 19 |

|                             |                                    |     |      |      |                       |             |                                      |            |     |       |                           |  |    |
|-----------------------------|------------------------------------|-----|------|------|-----------------------|-------------|--------------------------------------|------------|-----|-------|---------------------------|--|----|
| 50.22° S, 06.85° E<br>(DE)  | Permanent<br>grassland             | 100 | 6.9  | 811  | Pseudo<br>gley        | 5.3         | Calcium<br>hydroxide<br>(N/A)        | N/A        | SOC | 70    | Increase                  | Increased<br>soil pH.                        | 20 |
| 46.63° N, 07.85° E<br>(CH)  | Subalpine<br>grassland             | N/A | 14.8 | 1338 | N/A                   | 4.5         | Calcium<br>carbonate<br>(0.8)        | 0.8        | SOC | 4     | Decrease                  | Increased<br>microbial<br>activities.        | 21 |
| 31.25° S, 146.92° E<br>(AU) | Perennial<br>pasture               | N/A | 12   | 645  | N/A                   | N/A         | Limestone<br>(2.5)                   | 2.5        | SOC | 10-35 | Decrease                  | Increased<br>microbial<br>decompositi<br>on. | 22 |
| 51.83° N, 0.42° W<br>(UK)   | Grassland (red<br>fescue)          | N/A | 13.7 | 649  | Silty<br>clay<br>loam | 3.5-<br>7   | Calcium<br>carbonate<br>(15, 25, 53) | 15, 25, 53 | SOC | 21    | Decrease                  | Increased<br>microbial<br>decompositi<br>on. | 23 |
| 52.00° N, 0.42° W<br>(UK)   | Grassland<br>(Italian<br>ryegrass) | N/A | 10.3 | 696  | Sandy<br>loam         | 3.7-<br>6.1 | Calcium<br>carbonate<br>(9, 25, 45)  | 9, 25, 45  | SOC | 21    | Decrease                  | Increased<br>microbial<br>decompositi<br>on. | 23 |
| 552.29° N, 2.14° W<br>(UK)  | Upland<br>grassland                | N/A | 8    | 964  | Brown<br>soil         | 3.3         | Calcium<br>carbonate<br>(6)          | 6          | SOC | 6     | No significant<br>effects | Increased<br>soil pH                         | 24 |

\*MAAT= mean annual air temperature. \*\*MAP= mean annual precipitation. <sup>Δ</sup>Soil respiration. Ref. = reference. 1=Cuhel et al. (2010); 2=Galbally et al. (2010); 3=Zurovec et al. (2021); 4=Byers et al. (2021); 5= Stiehl-Braun et al. (2011); 6= Mosier et al. (1998); 7= Keller et al. (2005); 8= Kemmitt et al. (2006); 9= Lochon et al. (2019); 10= Aye et al. 2016; 11= Aye et al. (2016); 12=Rangel-Castro et al. (2004); 13=Rangel-Castro et al. (2005); 14=Egan et al. (2018); 15= Foereid et al. (2006); 16=Fornara et al. (2011); 17= Grover et al. (2017); 18=Mosquera-Losada et al. (2011); 19=Wang et al. (2016); 20= Sochorova et al. (2016); 21=Schaffner et al. (2012); 22=Orgill et al. (2015); 23=Kemmitt et al. (2006); 24= Grieve et al. (2005). NA = not available. <sup>▲</sup>grassland have never been ploughed, reseeded or heavily fertilised. AU= Australia; CH= Switzerland; CZ= Czech Republic; DE= Germany; FR= France; NO= Norway; SP= Spain; UK= United Kingdom and US= United States of America

## Figures

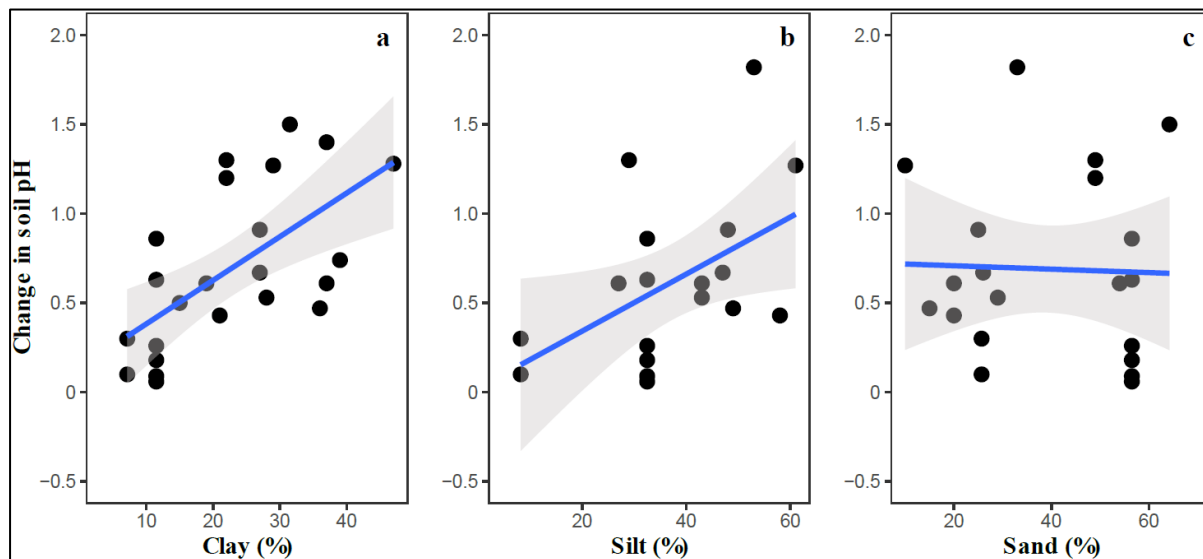


Fig. 1: Relationships between soil pH and clay (a) silt (b) and sand (c) contents. Clay was positively correlated with changes in pH ( $t = 3.69$ ,  $p < 0.01$ ,  $R^2 = 0.39$ ,  $n = 23$ ). Silt was positively correlated with changes in pH ( $t = 2.27$ ,  $p < 0.05$ ,  $R^2 = 0.24$ ,  $n = 18$ ). Sand was not significantly correlated with the changes in pH ( $t = -0.14$ ,  $p > 0.05$ ,  $R^2 = 0.001$ ,  $n = 20$ ).

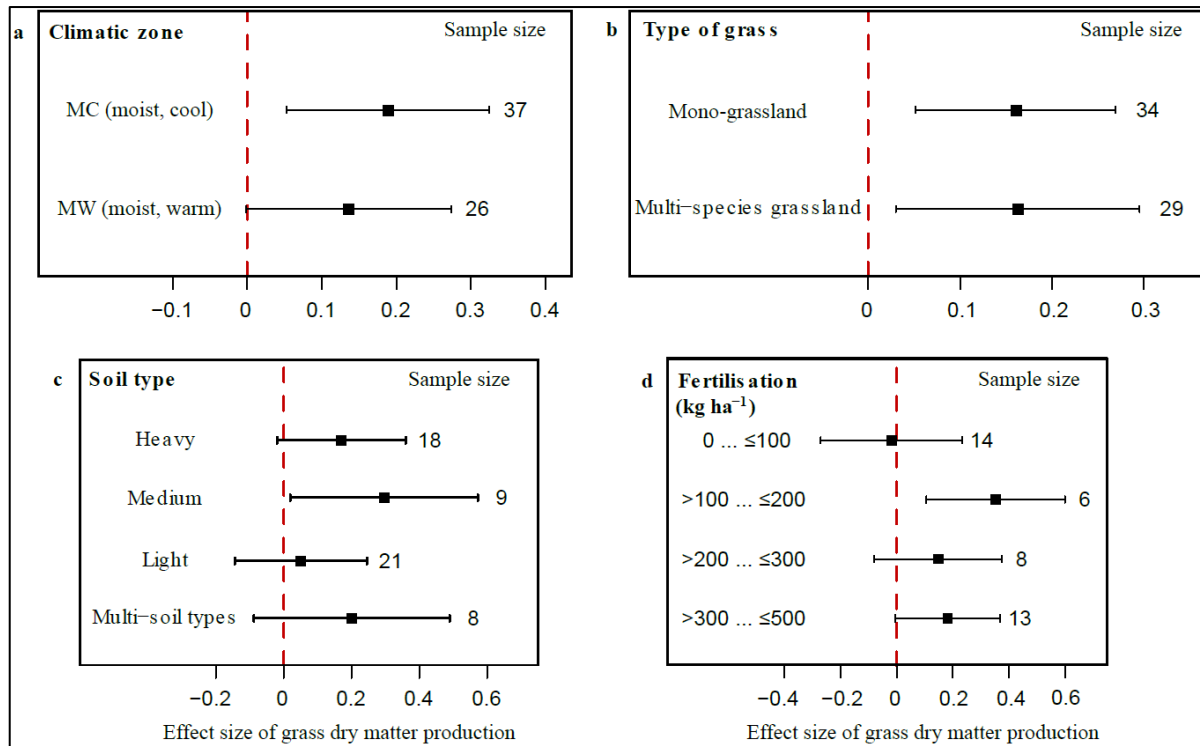


Fig. 2: Responses of grass dry matter production to liming in the different climatic zones (a), number of species (b), soil types (c) and amounts of fertilisation (d). Effect size stands for the response ratio between treatment and control. Bars represent the 95% confidence intervals. The number of observations of each variable is noted beside the bar. Response ratio  $\pm$  95% confidence intervals do not overlap 0 means  $p < 0.05$ .



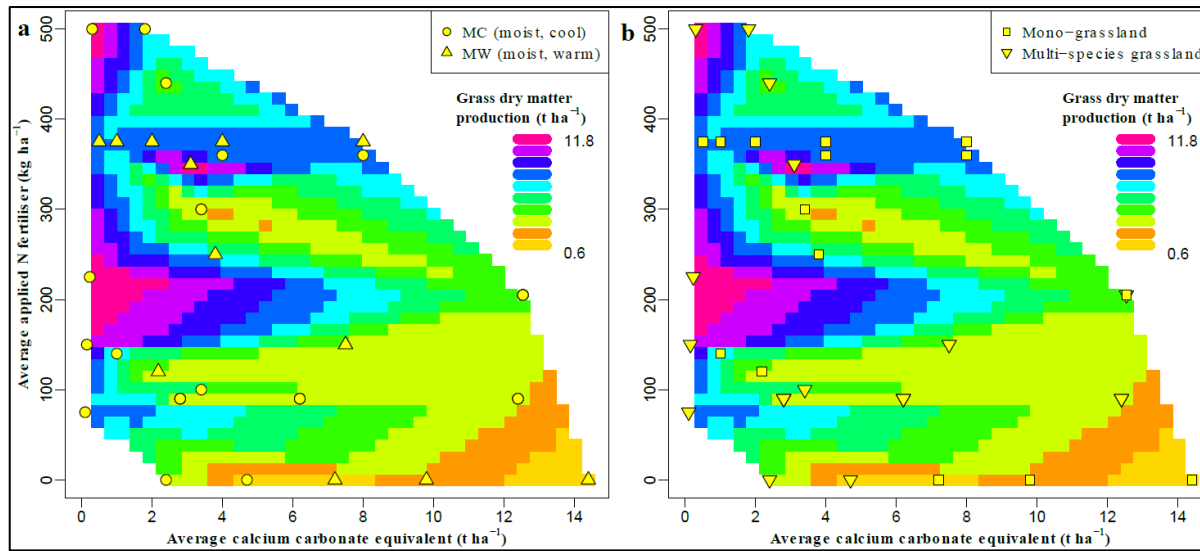


Fig. 3: Contour plots showing relationships between calcium carbonate equivalent, applied N fertiliser and grass dry matter production (a) in different climate zones and (b) different grassland types. Calcium carbonate equivalent and applied N fertiliser explain 42.3% of overall grass dry matter variations ( $n = 45$ ,  $p < 0.001$ ); the grass dry matter correlated significantly with calcium carbonate equivalent ( $t = -2.2$ ;  $p < 0.05$ ) and applied N fertiliser ( $t = 3.9$ ;  $p < 0.001$ ).



Fig. 4: Aerial picture of the Park Grass Experiment in 2005 showing plot boundaries due to differences in fertiliser treatments producing different vegetation (top left); differences in the type and number of plant species (top right and bottom right) due to the different N fertiliser and lime combinations. Plots with lime show more plant species. The bottom left picture shows sub-plots a, b, c and d. Ground chalk has been applied as necessary to maintain soil pH (0-23cm) for sub-plots a (pH 7), b (pH 6) and c (pH 5), respectively. Sub-plot d received no chalk.

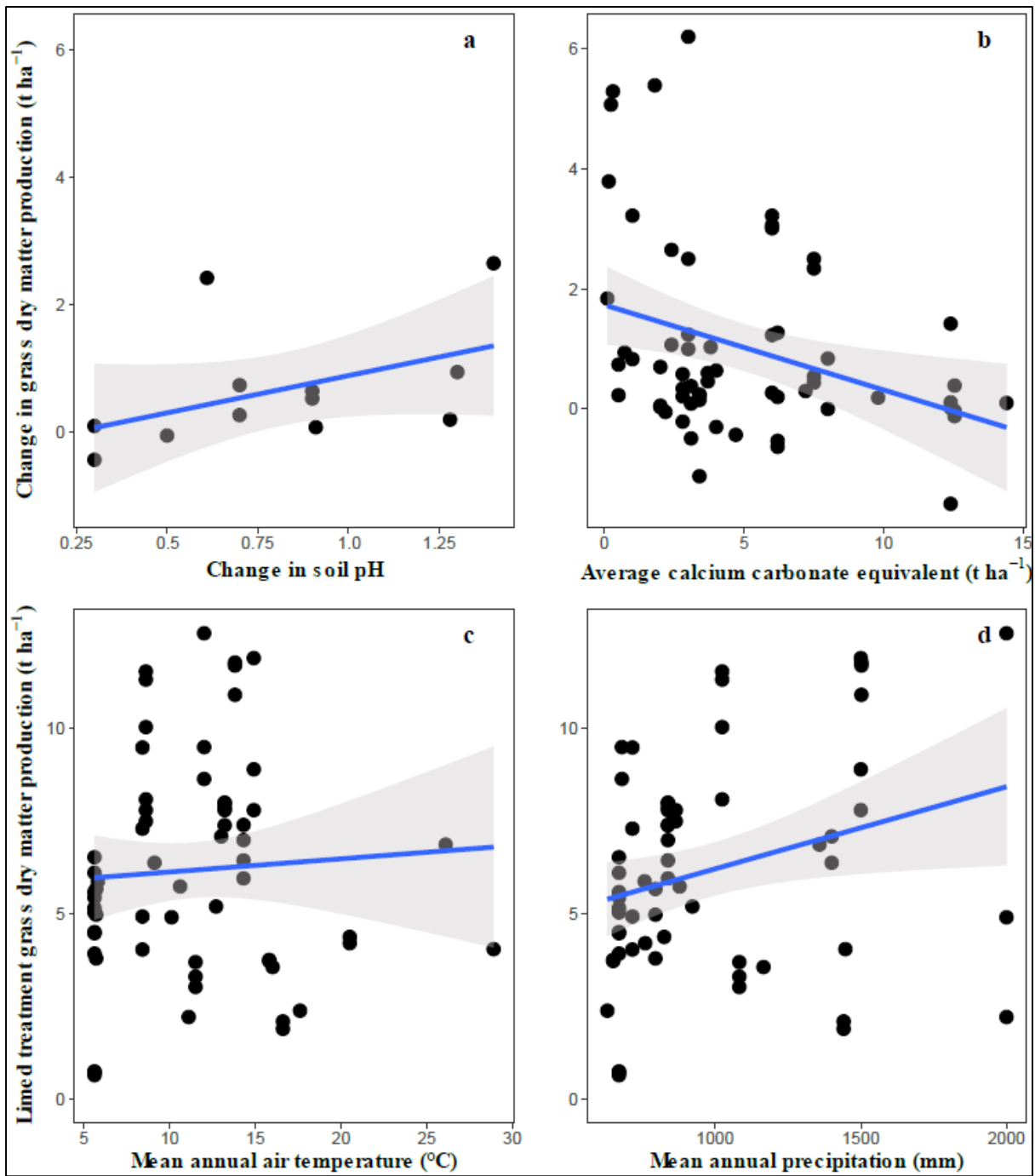


Fig. 5: Relationships between grass dry matter production and change in soil pH (a); amounts of lime in calcium carbonate equivalent (b); mean annual air temperature (c); and mean annual precipitation (d). Change in soil pH was positively correlated with change in grass dry matter production ( $t = 1.62$ ,  $p = 0.134$ ,  $R^2 = 0.21$ ,  $n = 12$ ). Calcium carbonate equivalent was negatively correlated with change in grass dry matter production ( $t = -2.71$ ,  $p < 0.01$ ,  $R^2 = 0.11$ ,  $n = 62$ ). Three outliers were removed in each case. Mean annual air temperature was not significantly correlated with the dry grass matter production ( $p > 0.05$ ,  $n = 63$ ). Mean annual precipitation was positively correlated with grass dry matter production ( $t = 2.3$ ,  $R^2 = 0.08$ ,  $p < 0.05$ ,  $n = 63$ ).

