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### ► **To cite this version:**

Iris Lochon, Pascal Carrère, Jean-Claude Yvin, Diane Houdusse-Lemenager, Juliette Bloor. Impacts of low-level liming on soil respiration and forage production in a fertilized upland grassland in Central France. *Science of the Total Environment*, 2019, 697, <10.1016/j.scitotenv.2019.134098>. <hal-02501454>

**HAL Id: hal-02501454**

**<https://hal.science/hal-02501454v1>**

Submitted on 4 Feb 2025

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HAL Authorization

**\*\*POSTPRINT\*\***

**Article type:** Full paper

**Title: Impacts of low-level liming on soil respiration and forage production in a fertilized upland grassland in Central France**

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## Graphical abstract



## Highlights

- Impacts of liming on soil respiration were studied in a two-year field trial.
- Liming increased soil pH but did not increase soil CO<sub>2</sub> emissions.
- Temperature was the main driver of soil respiration in both control and limed plots.
- Liming increased forage quality but did not affect forage production.
- Low-level liming may contribute to sustainable grassland management.

## **Abstract**

Liming is a common agricultural practice for improving acidic soils, but the addition of liming materials may also promote soil carbon dioxide (CO<sub>2</sub>) emissions, with adverse effects for climate regulation. In grasslands, current understanding of liming impacts on greenhouse gas emissions is limited by a lack of field data on liming and soil respiration. Here we used a two-year field trial and *in situ* chamber measurements to evaluate the effects of repeated, low-level liming on soil CO<sub>2</sub> emissions from an acidic managed grassland with high soil organic matter content. Soil pH, temperature and moisture were measured during the experiment, as well as microbial and plant biomass, in order to assess possible liming-induced changes to drivers of grassland carbon cycling. Soil CO<sub>2</sub> emissions showed significant variation during the two-year study, driven primarily by fluctuations in soil temperature. Soil respiration rates were unaffected by liming treatment, despite significant lime-induced increases in soil pH. Liming was associated with a decrease in biomass produced per gram nitrogen, as well as a decrease in forage C:N in the second year and transient decreases in microbial C:N, but neither plant nor microbial biomass showed significant responses to liming addition. Collectively, our results suggest that positive effects of low-level liming on plants and soil are not offset by increases in soil CO<sub>2</sub> emissions *in situ*, highlighting the potential for sustainable liming practices in fertilized grasslands.

**Keywords:** acid soils, Ca-amendment, CO<sub>2</sub> emissions, soil pH, soil microbial biomass, plant biomass

## 1. Introduction

Grasslands account for nearly 68% of global agricultural area (FAO 2013), playing an important role in the biosphere–atmosphere exchange of greenhouse gases and carbon dioxide (CO<sub>2</sub>) emissions from the agricultural sector (Soussana et al. 2007). In these grassland systems, soil respiration arising from plant roots and soil organisms is one of the main fluxes of terrestrial CO<sub>2</sub> to the atmosphere. Soil respiration is influenced by a number of abiotic and biotic factors such as soil moisture, temperature, pH, assimilate supply and microbial activities (Ryan & Law 2005; Bahn et al. 2008; Luo et al. 2016). Changes in biotic and abiotic soil conditions induced by management practices (e.g. mowing, grazing, fertilization) have the potential to modify soil respiration rates and affect the contribution of grasslands to atmospheric CO<sub>2</sub> emissions (Soussana & Lemaire 2014; Rumpel et al. 2015; Kunhikrishnan et al. 2016). Consequently, clear understanding of the effects of management practices on carbon cycling is required to identify efficient ways of reducing CO<sub>2</sub> emissions from grassland ecosystems (Fornara et al. 2011).

In grassland areas, nitrogen (N) fertilization and liming are two agricultural practices commonly used to increase plant productivity (Haynes & Naidu 1998). Given that mineral N inputs tend to promote soil acidification (Bolan & Hedley 2003), the application of carbonates and calcium-rich liming materials can be used to counteract acidification processes and improve the soil pH status in fertilized grasslands (Goulding, 2016; Kunhikrishnan et al. 2016). Liming leads to more optimal conditions for plant growth, alleviates aluminum toxicity associated with acid soils and enhances nutrient bioavailability (Haynes & Naidu 1998). Beyond the increase in pH, liming may improve the aggregation of soil particles and the circulation of water in soils (During et al. 1984; Haynes & Naidu 1998; Chan & Heenan 1999; Bolan et al. 2003). Liming may also promote nitrous oxide (N<sub>2</sub>O) reductase and the transformation of N<sub>2</sub>O to inert N<sub>2</sub>, providing a possible abatement strategy for greenhouse gas emissions (Garcia-Marco et al. 2016). However, the diverse

impacts of liming on plant and soil processes may also generate negative effects on ecosystem services. Liming-induced changes in soil pH and soil structure are expected to stimulate microbial activities generally, with implications for soil respiration and CO<sub>2</sub> emissions (Paradelo et al. 2015; Lochon et al. 2018). Liming also generates CO<sub>2</sub> emissions via the dissolution of liming materials *in situ* (IPCC 2006). Quantifying the magnitude of lime-induced changes of soil CO<sub>2</sub> emissions is an important step towards predicting the net outcome of liming on the grassland carbon balance and climate regulation services.

Previous studies have suggested mixed responses of soil CO<sub>2</sub> emissions to liming in grassland ecosystems (Johnson et al. 2005; Galbally et al. 2010; Egan et al. 2017; Lochon et al. 2018). However, this knowledge of liming impacts on CO<sub>2</sub> emissions in grasslands is largely based on laboratory incubations, and *in situ* soil respiration data from the field is extremely rare (Holland et al. 2018). There are a number of reasons why field measurements of soil respiration are essential for progressing our understanding of liming impacts. Firstly, measurement of soil respiration in disturbed soil samples under laboratory conditions may confound the interpretation and extrapolation of results (Herbst et al. 2016). Secondly, studies suggest that CO<sub>2</sub> emissions derived from the liming material alone may be much lower under field compared to laboratory conditions (Biasi et al 2008; Cho et al. 2019). Finally, recent work has shown that grassland management practices can influence the response of soil respiration to abiotic factors (Xue & Tang 2018; Moinet et al. 2019). It therefore seems reasonable to suppose that lime-induced changes in plant and soil properties could have indirect effects on the sensitivity of soil CO<sub>2</sub> emissions to drivers such as soil temperature or moisture content. Characterizing the temporal dynamics of soil respiration under field conditions offers the opportunity to both examine overall response patterns to liming, and determine whether liming addition modifies the sensitivity of soil CO<sub>2</sub> emissions to environmental drivers (Kunhikrishnan et al. 2016). To date, such effects have yet to be investigated.

The primary objective of the present study was to assess the impact of repeated, low-level liming on *in situ* soil respiration for fertilized, acidic soil. Our main hypotheses were that: (i) liming increases soil respiration via increases in pH, and (ii) liming modifies the sensitivity of CO<sub>2</sub> emissions to abiotic drivers (soil temperature, soil moisture). We tested these hypotheses by performing a field trial and measuring CO<sub>2</sub> emissions for two years from an Andosol under a managed grassland stand in Central France. We also examined the effects of liming on plant and microbial biomass, which play a key role for grassland carbon cycling.

## **2. Material and methods**

### *2.1. Study site*

The field trial was carried out at an upland permanent grassland site in the French Massif Central region (Laqueuille, Auvergne; 45°38'N, 2°44'E, 1050m a.s.l.). The site has an average annual temperature of 7.8 °C, 89 days of frost and an annual rainfall of 1094 mm (calculated over the period 1996-2015). The soil at this site is a silty andosol (21.1% clay, 60.2% silt and 18.7% sand) with a high organic matter content (22%) and a pH<sub>H2O</sub> of 5.2. The plant community is dominated by grasses (*Dactylis glomerata*, *Agrostis capillaris*, *Lolium perenne*, *Anthoxanthum odoratum*, *Poa pratensis*). Other species include forbs (*Veronica arvensis*, *Taraxacum officinale*) and legumes (*Trifolium repens*). Prior to the start of this experiment in 2014, the site had been subject to two cuts per year and one low-intensity cattle grazing event at the end of the plant growing season (autumn) for over ten years. Up until 2014, the site was fertilized with cattle manure in the autumn and mineral fertilizer in the spring (NPK, average inputs 63-20-29 kg ha<sup>-1</sup>), representing an estimated total input of 125 kg N per hectare, per year.

### *2.2. Experimental design and treatments*

Replicated plots (4 m x 2 m) were set up at the field site in October 2014 and assigned at random to either a control or limed treatment (total of eight plots in a blocked design with four blocks). Limed plots received a surface application of Calcimer T400 (TimacAgro, France) at a rate of 1200 kg ha<sup>-1</sup> in October 2015 and 2016; this calcium-rich soil improver (CaO = 40%, MgO = 1%) of marine origin has a neutralizing value of 41. The liming rates chosen here reflect regional recommendations for soil pH management in permanent grasslands with intermediate levels of management intensity (moderate fertilizer input, cutting rates and/or stocking rates), and correspond to a regular “maintenance liming” management practice (COMIFER 2009). All plots, both control and limed, received 100 kg N ha<sup>-1</sup> per year in the form of ammonium nitrate and in two splits (end of April, mid-June). Plots were mown twice each year (start of June, late September) to a height of 5 cm.

Two PVC collars (height 4 cm, Ø 10 cm) were positioned in small gaps in the vegetation in each plot and inserted 2.5 cm into the soil. These collars were left in place throughout the study period for respiration measurements (see below), and kept free of any encroaching vegetation. Collars were positioned at least 3 m apart within plots, and at least 30 cm from the plot edges to avoid possible boundary effects.

### *2.3. CO<sub>2</sub> flux measurements*

Measurement of CO<sub>2</sub> fluxes from soil were initiated six months after the liming application in 2015, and continued over a two-year period (2016 and 2017). Measurements were carried out during the plant growing season of each year (March to October; total of 28 measurement campaigns during the two-year study). No measurements were carried out during the winter due to the regular occurrence of snow at this upland site.

Flux measurements were made *in situ* using a Glen Spectra soil respiration chamber and a

portable infrared gas analyzer (IRGA; Li-6400, Li-Cor Biosciences, Lincoln, Nebraska, USA). The soil chamber was placed onto the collars in the experimental plots, and mean flux rates were calculated from duplicate measurements made on each collar. Flux measurements were made between 11:00 and 13:30h at each measurement date, and the order of measurements was randomized across blocks. At the same time, surface (5 cm depth) soil temperature and moisture was recorded for soil adjacent to the collars using a Licor soil temperature probe connected to the gas analyzer and an SM200 probe coupled to a HH2 moisture meter (Delta-T Devices, Cambridge, England). Daily air temperature and rainfall data were available from an automatic weather station which is part of a national weather monitoring network (hourly measurement intervals, INRA CLIMATIK network), and located within 500 m of the experimental site (Table 1).

#### *2.4. Measurement of belowground variables: soil pH, roots and microbial biomass*

Two intact soil cores were taken in each experimental plot four times each year: mid-April (beginning of the plant growing season), early June (immediately prior to the first cut of vegetation), mid-September (prior to the second vegetation cut) and early November. Soil cores were used to determine soil pH at all dates, but root and microbial biomass were only recorded for dates during the plant growing season (April, June, and September). The two soil cores ( $\varnothing$  2.1 cm, 0–10 cm depth) per plot were pooled at each sampling date, and sieved (2 mm mesh) to separate roots from soil. Soil  $\text{pH}_{\text{H}_2\text{O}}$  was determined for a 10 g sub-sample of freshly-sieved soil (soil:water ratio of 1:2) following Robertson et al. (1999). Root samples were washed, dried at 60°C for 48 h and weighed to determine dry mass. Microbial biomass was measured on 5 g subsamples using the chloroform fumigation-extraction method (Brookes et al. 1985) and extraction with 0.5M  $\text{K}_2\text{SO}_4$  solution. Microbial C was calculated as the difference in total C extracted in fumigated and unfumigated soils, with 0.35 as the adjustment factor (Sparling et al. 1990). Microbial N was

calculated as the difference in total N extracted in fumigated and unfumigated soils, with 0.54 as the adjustment factor (Brookes et al. 1985). An additional 5 g of soil samples were oven-dried (105°C, 24h) to determine gravimetric soil moisture content.

At the end of field trial (November 2017), additional soil cores (Ø 8cm, 0-10 cm deep) were taken within each soil respiration collar. Roots were carefully separated from the soil, washed, oven-dried (48h at 60°C) and weighed to determine dry mass.

### *2.5. Measurement of forage production and quality*

Aboveground plant biomass (> 5 cm) was collected from two 50 cm x 50 cm quadrats per plot at each cut (start of June, late September). A subsample of 150–250 g freshly harvested material per plot was sorted into plant functional groups (grasses, legumes, forbs), and all samples were then oven dried (60 °C, 48 h) before weighing to determine dry mass. After weighing, biomass samples were pooled per plot, homogenized using a cutting mill (1 mm mesh size, Retsch, WRb90), and a 3 g subsample of each mixture was then finely ground (Brinkmann ball grinder, Retsch, MM200). Total C and N content in aboveground biomass samples were determined for 5 mg of finely-ground material (Brinkmann ball grinder, Retsch, MM200) using an elemental combustion analyzer (Flash EA 1112 CNS analyzer, ThermoFinnigan, Milan, Italy). Annual forage production was calculated as the total of the two biomass cuts per year, and forage C:N was based on the total C and total N in the two cuts per year. Total plant N was also used to assess the ratio of forage biomass to N content (g dry mass g N<sup>-1</sup>); biomass:N ratios can be used as an indicator of nitrogen use efficiency in perennial grasses, and values typically decrease with increasing bioavailability of N (Vazquez de Aldana & Berendse 1997; Roscher et al. 2008).

### *2.6. Statistical analysis*

Means were taken for each variable measured in duplicate per experimental plot and per measurement date to avoid pseudoreplication. Mean seasonal CO<sub>2</sub> fluxes per plot were obtained by averaging the daily flux values by season (spring: March, April, May; summer: June, July, August; autumn: September, October) for each year. Impacts of liming on flux data, soil variables and forage production were analyzed using mixed model repeated measures analysis of variance (ANOVA), with block as a random factor. The influence of abiotic factors - temperature and soil moisture - on CO<sub>2</sub> fluxes was tested using covariance analysis (ANCOVA). Root biomass within the soil respiration collars was analyzed using one-way ANOVA (Treatment as fixed factor).

All the analyses were carried out using R software "packages" nlme and ggplot2 (R Core Team 2013) and assumptions of the statistical models were verified.

### **3. Results**

#### *3.1. Soil pH, moisture and temperature*

Liming had a significant positive effect on soil pH in the 0-10 cm soil layer, with an average increase of 0.34 pH units across all dates ( $F_{1,6} = 23.7, P < 0.01$ , Fig. 1). However, the magnitude of increase in pH induced by liming varied with sampling date (Liming x Date interaction,  $F_{7,42} = 2.90, P < 0.05$ , Fig. 1). Liming-induced increases in pH were higher after the second lime application (October 2016), with an average increase of +0.47 pH units between limed and unlimed plots from November 2016 onwards compared to an average increase of +0.23 pH units from April to September 2016 (Fig. 1).

The soil moisture in the upper soil layer (0-5 cm) was  $37.9 \pm 1.02$  % on average at the sampling dates, whereas the average soil temperature was  $14.6 \pm 0.27$  °C during the study. Soil moisture and soil temperature varied depending on the date of flux measurement (data not shown), but did not show a significant difference between 2016 and 2017. Neither variable was affected by

liming treatment ( $P > 0.05$ ).

### 3.2. *CO<sub>2</sub> fluxes*

Daily soil CO<sub>2</sub> fluxes ranged from 1.34 to 9.77 g C-CO<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup> across treatments during the experimental period (Fig. 2). Mean values of soil respiration showed significant effects of measurement date, but liming had no effect on daily CO<sub>2</sub> emissions, either alone or in interaction with measurement dates (Table 2A, Fig. 2).

Soil temperature had a significant positive effect on daily soil respiration during the whole experimental period, irrespective of liming treatment (Fig. 3A). In contrast, soil moisture was unrelated to mean daily CO<sub>2</sub> emissions during the study ( $P > 0.05$ , Fig. 3B). Liming did not change the relationship between daily CO<sub>2</sub> emissions and abiotic factors across flux measurement dates ( $P > 0.05$ ).

Seasonal averages of CO<sub>2</sub> emissions varied depending on season and year (Year x season interaction, Table 2B, Fig. 4). Although CO<sub>2</sub> emissions were consistently higher in summer, average CO<sub>2</sub> emitted in spring was higher than that emitted in autumn in 2016. Overall, CO<sub>2</sub> emissions were significantly higher in 2016 than in 2017 (average of 5.72 g C-CO<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup> versus 3.49 g C-CO<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup> for 2016 and 2017 respectively, Table 2B). Liming did not have a significant effect on average seasonal CO<sub>2</sub> emissions, but there was a tendency for higher soil respiration in autumn in the limed compared to the control plots (Fig. 4).

### 3.3. *Biomass of microbes and plants*

Liming had no significant effect on the microbial biomass C in soil cores taken during the study, although there was a tendency for lower microbial C in limed plots in June and September each year (Date x Treatment interaction significant at the 10% level, Table 3, Fig. 5A). In contrast,

liming was associated with a significant decrease in the microbial C:N ratio in April of each year (Date x Treatment interaction, Table 3, Fig. 5B). Liming-induced decreases in microbial C:N persisted for a longer period in 2017 compared to 2016, since liming effects were still apparent in June 2017 (Year x Treatment interaction significant at the 10% level, Table 3). No liming effects were apparent on microbial C:N in September of each year (Fig. 5B).

Root biomass in soil cores taken from plots during the experimental period showed significant annual and seasonal variations, with highest values in April and June 2016 (Table 3, Fig. 5C). Liming had no significant effects on root biomass in experimental plots due to high variation within treatments (Table 3). Moreover, root biomass collected at the end of the study in soil respiration collars did not show any significant differences between control and liming treatments ( $P > 0.05$ ).

Liming had no overall effect on aboveground biomass production or total plant N content during the study, but caused a significant decrease in plant C:N and the biomass:N ratio in harvested plant material (Table 4). The decrease in plant C:N was driven by a strong negative response to liming in the second year of the study (Table 4). Liming did not modify the abundance of functional groups (legumes, grasses, forbs) in the plant community, and legume abundance was generally lower in 2016 compared to 2017 across all plots (Table 4).

#### **4. Discussion**

The current challenge for agriculture is to develop sustainable management practices that maintain soil quality and food production, reduce greenhouse gas emissions and increase soil C storage (Lal et al. 2011). Characterizing soil respiration in managed grasslands is critical for predicting soil C fluxes and carbon source-sink relationships under soil management changes, and has practical implications for agricultural managers (Xue & Tang 2018). This study presents a

detailed examination of liming impacts on *in situ* soil CO<sub>2</sub> emissions, and provides valuable insights into the effects of low-level, repeated lime addition on overall grassland function.

#### 4.1. Responses of soil respiration to liming

Rates of soil CO<sub>2</sub> emissions recorded in the present study in control plots were within the range of soil respiration ( $R_s$ ) reported elsewhere for upland temperate grasslands, confirming the high levels of soil CO<sub>2</sub> efflux from this ecosystem (Bahn et al. 2008). Based on results from previous studies in agricultural soils and cropping systems (Bezdicsek et al. 2003; Kemmit et al. 2006), we hypothesized that  $R_s$  would be increased by liming via an increase in soil pH, and hence soil biological activities. This first hypothesis was not supported by our results, despite a significant increase in soil pH in the limed plots during the study. Liming-induced increases in soil pH were stronger in 2017 following the second application of lime, but liming had no significant effect on soil CO<sub>2</sub> emissions at the daily and seasonal timescale in either the first or second year of study. These results suggest that low-level liming does not increase soil CO<sub>2</sub> emissions in our fertilized grassland system in the medium term. Divergence between our results and those from cropping systems may partly arise from differences in lime application as well as quantity of lime material applied. Surface application of lime is less effective at incorporating lime into the soil layer than tillage (or harrowing), and consequently improvement of pH and soil properties at depth may be relatively limited in untilled systems (Rheinheimer et al. 2018). Absence of liming effects on soil CO<sub>2</sub> emissions in the present study could also partly reflect the cool soil temperatures at our upland study site, since previous incubation studies have suggested that soil respiration responses to liming may be limited at soil temperatures of less than 20°C (Fornara et al. 2011).

As far as we are aware, only one other grassland study to date has reported any measurements of *in situ*  $R_s$  in response to liming (Egan et al. 2017). Egan and coworkers found

mixed results of liming on soil CO<sub>2</sub> emissions measured on three dates in a long-term liming trial; liming had no effects on R<sub>s</sub> on two out of three dates and a negative effect on R<sub>s</sub> on the third date. In a more detailed study of grassland gas fluxes, Galbally et al. (2010) documented non-significant effects of liming on night-time ecosystem respiration (i.e. plant and soil respiration combined). However, neither our study nor those of Egan et al. (2017) and Galbally et al. (2010) recorded CO<sub>2</sub> emissions immediately after addition of lime, and therefore did not capture possible short-term increases in CO<sub>2</sub> emissions due to chemical dissolution of carbonate in the liming product (Kunhikrishnan et al. 2016). Indeed, short-term incubation experiments on grassland soil have shown positive liming effects on soil CO<sub>2</sub> emissions following the addition of lime (Lochon et al. 2018). Given that sieved soil and favorable incubation conditions (temperature and soil moisture) provide an indication of potential emissions alone (Herbst et al. 2016), future field studies of grassland greenhouse gas emissions and liming should include flux measurements immediately after lime application to determine ‘effective’ soil CO<sub>2</sub> emissions at this time point.

#### *4.2. Temporal variation in soil respiration and linkages with soil temperature*

As with other studies of R<sub>s</sub> in temperate upland grasslands (Bahn et al. 2008; Grand et al. 2016), we found that soil CO<sub>2</sub> emissions showed considerable temporal variation, and that soil temperature was a stronger predictor of soil CO<sub>2</sub> emissions than soil moisture on our 28 dates of flux measurements. Soil temperature is a key factor driving soil CO<sub>2</sub> emissions due to the temperature dependence of the biochemical processes and enzyme activities associated with R<sub>s</sub>, as well as temperature effects on substrate availability in soil (Davidson & Janssens 2006). However, the higher soil CO<sub>2</sub> emissions observed overall in 2016 compared to 2017 were not driven by temperature differences, since we found no significant difference in soil temperature for flux measurement dates in 2016 and 2017. Instead, the higher values of R<sub>s</sub> in 2016 probably reflect the

higher root biomass recorded in experimental plots in 2016, which may have increased labile carbon availability via root exudates. The temporal resolution of flux measurement dates appears to have been insufficient to capture the higher spring and summer air temperatures of 2017, and it is possible that soil temperatures on some flux measurement dates may have been buffered by recent rainfall events or soil moisture levels. Although the manual chamber technique used in the present study is ideally-suited to spatially-extensive measurements, automated systems with a high temporal frequency of measurements would provide more robust assessments of soil respiration responses to fluctuating environmental conditions (Savage & Davidson 2003).

Contrary to our expectations and second hypothesis, liming did not change the relationship between  $R_s$  and soil temperature (or moisture). This differs from previous work on grazing and grassland restoration practices which found that management modified the sensitivity of soil  $CO_2$  emissions to abiotic drivers (Xue et al. 2018; Moinet et al. 2019), and likely reflects the absence of liming effects on plant or microbial biomass observed in the present study. Plant biomass can modulate  $R_s$  responses to soil temperature via changes in assimilate supply (Bahn et al. 2008), as well as causing direct changes to soil temperature via altered aboveground microclimate or soil moisture conditions. Our results imply that the temperature dependency of soil respiration is not affected by addition of lime when the levels of liming and the degree of soil pH change are relatively low. However, this response pattern needs to be confirmed in other grasslands since effects of liming on grassland productivity (and potential knock-on effects on  $R_s$ ) may be mediated by soil type (Mijangos et al. 2010), and/or botanical composition (Poozesh et al. 2010).

#### *4.3. Effects of liming on plant and microbial biomass*

In the present work, liming had no effect on either plant or microbial biomass; whilst our results for microbial biomass are in agreement with previous grassland studies (Fornara et al. 2011;

Lochon et al. 2018), the absence of plant biomass responses to liming was more unexpected (Galbally et al. 2010; Mijangos et al. 2010; but see Hejzman et al. 2010). Dicotyledonous species have been shown to respond more strongly to liming than grasses in long-term liming trials (Poozesh et al. 2010), and the absence of biomass effects in our study may partly reflect the dominance of grasses in all plots (>80% of harvested biomass) throughout the experimental period. Liming did not alter the relative abundance of plant functional groups in our study, probably due to the shorter study length and smaller contrast in pH between control and limed plots compared with previous trials.

Despite the absence of microbial biomass responses, we found that liming decreased microbial C:N at the start of the plant growing season each year. Shifts in microbial C:N may reflect changes in the microbial community, with decreases in C:N linked to a greater dominance of bacteria relative to fungi in the soil (de Vries et al. 2016). Such a response pattern is consistent with reports that bacteria are promoted by increases in pH (Rousk et al. 2009). Shifts in microbial C:N may also occur due to plasticity in biomass composition and/or nutrient use efficiency which allows the microbial community to deal with stoichiometric imbalances in resources (Mooshammer et al. 2014). In parallel, liming decreased plant biomass:N ratios (and plant C:N in aboveground biomass in the second year of study), suggesting enhanced nutrient bioavailability in limed plots. Decreases in plant biomass:N values can be interpreted as a decrease in N use efficiency (NUE), and are consistent with grassland plant responses to increased N availability (e.g. Vazquez de Aldana & Berendse 1997; Xi et al. 2014). Decreased plant C:N is an indicator of improved forage quality, and also supports the idea of increased N availability in limed plots. It is perhaps notable that the decrease in plant C:N occurred in the second year when both liming-induced increases in pH were highest and decreases in microbial C:N persisted for a longer period. Although plant C:N values

may be influenced by shifts in botanical composition and/or plant phenological stage as well as by changes in soil N availability, this did not appear to be the case here.

Changes in both microbial and plant stoichiometry have implications for biogeochemical cycling and plant soil feedbacks (Mooshammer et al. 2014; Heyburn et al. 2017). More direct measurements of process rates and microbial community composition, via isotopic labelling and molecular techniques respectively, would allow clearer understanding of the mechanisms of grassland responses to liming. Nevertheless, our results agree with the idea that liming impacts are expressed at different time-scales for different ecosystem properties, with initial effects on nutrient cycling that ‘ripple’ and promote changes in plant production in a subsequent time-step (Holland et al. 2018).

## **5. Conclusion**

Collectively, our results suggest that repeated, low-level liming can improve soil fertility and hence forage quality in moderately-fertilized grasslands without increasing CO<sub>2</sub> emissions. These positive impacts of low lime inputs are of particular importance because amounts of lime applied by farmers are often below what is necessary for maintaining recommended soil pH values due to either economic (Goulding 2016) or environmental (Mijangos et al. 2010) considerations. We propose that repeated, low-level liming can contribute to sustainable management practices for fertilized, permanent grasslands. Liming-induced increases in nutrient bioavailability may even allow reductions in mineral N inputs (and their associated risks of N losses) in the longer-term. Additional work is needed to determine whether the findings obtained here for moderately-fertilized grasslands can be generalized under different land use intensities. Indeed, a growing number of studies suggest that N fertilization and liming may interact on grassland function (Poozesh et al. 2010; Lochon et al. 2018). Further work should also consider both soil CO<sub>2</sub>

emissions and other greenhouse gas fluxes in order to better quantify the global warming balance of liming in managed grasslands.

### **Acknowledgements**

The authors thank Alexandre Salcedo for help with data collection and monitoring the experiment, and the staff of INRA-Herbipôle for help with site maintenance. Thanks also to Laurence Andanson and Sandrine Revaillet for assistance with chemical analyses. This study was supported by a CIFRE doctoral fellowship to IL, in partnership with CMI.

### **References**

- Bahn, M., Rodeghiero, M., Anderson-Dunn, M., Dore, S., Gimeno, C., Drosler, M., Williams, M., Ammann, C., Berninger, F., Flechard, C., Jones, S., Balzarolo, M., Kumar, S., Newesely, C., Priwitzer, T., Raschi, A., Siegwolf, R., Susiluoto, S., Tenhunen, J., Wohlfahrt, G., Cernusca, A. 2008. Soil respiration in European grasslands in relation to climate and assimilate supply. *Ecosystems* 11, 1352-1367.
- Bezdicsek, D.F., Beaver, T., Granatstein, D. 2003. Subsoil ridge tillage and lime effects on soil microbial activity, soil pH, erosion, and wheat and pea yield in the Pacific Northwest, USA. *Soil Till. Res.* 74, 55-63.
- Biasi, C., Lind, S.E., Pekkarinen, N.M., Huttunen, J.T., Shurpali, N.J., Hyvönen, N.P., Repo, M.E., Martikainen, P.J. 2008. Direct experimental evidence for the contribution of lime to CO<sub>2</sub> release from managed peat soil. *Soil Biol. Biochem.* 40, 2660–2669.
- Bolan, N.S., Adriano, D.C., Curtin, D. 2003. Soil acidification and liming interactions with nutrient and heavy metal transformation and bioavailability. *Adv. Agron.* 78, 215-272.
- Bolan, N.S., Hedley, M.J. 2003. Role of carbon, nitrogen, and sulfur cycles in soil acidification. In

- Rengek, Z. (ed.), Handbook of soil acidity, pp. 29–56. Marcel Dekker, New York.
- Brookes, P.C., Landman, A., Pruden, G., & Jenkinson, D.S. 1985. Chloroform Fumigation and the Release of Soil Nitrogen: A Rapid Direct Extraction Method to Measure Microbial Biomass Nitrogen in Soil. *Soil Biol. Biochem.* 17, 837–842.
- Chan, K.Y., Heenan, D.P. 1999. Lime-induced loss of soil organic carbon and effect on aggregate stability. *Soil Sci. Soc. Am. J.* 63, 1841-1844.
- Cho, S.R., Jeong, S.T., Kim, G.Y., Lee, J.G., Kim, P.J., Kim, G.W. 2019. Evaluation of the carbon dioxide (CO<sub>2</sub>) emission factor from lime applied in temperate upland soil. *Geoderma* 337, 742-748.
- COMIFER. 2009. Le Chaulage des bases pour le raisonner. Groupe Chaulage COMIFER. ISBN : 978-235253-0442.
- Davidson, E.A., Janssens, I.A. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* 440, 165-173.
- de Vries, F.T., Brown, C., Stevens, C.J. 2016. Grassland species root response to drought: consequences for soil carbon and nitrogen availability. *Plant Soil* 409, 297–312.
- During, C., Jackson, B.L.J., Dyson, C.B. 1984. Lime effects on hill country: 2. Relation of soil moisture, pH, and aluminium values with pasture yield. *New Zeal. J. Agr. Res.* 27, 531–535.
- Egan, G., Crawley, M.J., Fornara, D.A. 2017. Effects of long-term grassland management on the carbon and nitrogen pools of different soil aggregate fractions. *Sci. Total Environ.* 613-614, 810–819.
- FAO. 2013. FAOSTAT Food and Agriculture Organization of the United Nations, Rome. Inputs\_Land Data, <http://faostat3.fao.org/home/E> (last access March 4, 2016)
- Fornara, D.A., Steinbess, S., McNamara, N.P., Gleixner, G., Oakley, S., Poulton, P.R., Macdonald, A.J., Bardgett, R.D. 2011. Increases in soil organic carbon sequestration can reduce the global

- warming potential of long-term liming to permanent grassland. *Glob. Chang. Biol.* 17, 1925–1934.
- Galbally, I.E., Meyer, M.C.P., Wang, Y-P., Smith, C.J., Weeks, I.A. 2010. Nitrous oxide emissions from a legume pasture and the influences of liming and urine addition. *Agr. Ecosyst. Environ.* 136, 262-272.
- Garcia-Marco, S., Abalos, D., Espejo, R., Vallejo, A., Mariscal-Sancho, I. 2016. No tillage and liming reduce greenhouse gas emissions from poorly-drained agricultural soils in Mediterranean regions. *Sci. Total Environ.* 566-567, 512-520.
- Goulding, K.W.T., 2016. Soil acidification and the importance of liming agricultural soils with particular reference to the United Kingdom. *Soil Use Manage.* 32, 390–399.
- Grand, S., Rubin, A., Verrecchia, E.P., Vittoz, P. 2016. Variation in soil respiration across soil and vegetation types in an Alpine Valley. *PLoS One* 11, e0163968.
- Haynes, R.J., Naidu, R. 1998. Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions: A review. *Nutr. Cycl. Agroecosystems* 51, 123–137.
- Hejman, M., Schellberg, J., Pavlu, V. 2010. Long-term effects of cutting frequency and liming on soil chemical properties, biomass production and plant species composition of *Lolium-Cynosuretum* grassland after the cessation of fertilizer application. *Appl. Veg. Sci.* 13, 257-269.
- Herbst, M., Tappe, W., Kummer, S., Vereecken, H. 2016. The impact of sieving on heterotrophic respiration response to water content in loamy and sandy topsoils. *Geoderma* 272, 73-82.
- Heyburn, J., McKenzie, P., Crawley, M.J., Fornara, D.A. 2017. Effects of grassland management on plant C:N:P stoichiometry: implications for soil element cycling and storage. *Ecosphere* 8, e01963

- Holland, J.E., Bennett, A.E., Newton, A.C., White, P.J., McKenzie, B.M., George, T.S., Pakeman, R.J., Bailey, J.S., Fornara, D.A., Hayes, R.C. 2018. Liming impacts on soils, crops and biodiversity in the UK: a review. *Sci. Total Environ.* 610-611, 316-332.
- Johnson, D., Leake, J., Read, D. 2005. Liming and nitrogen fertilization affects phosphatase activities, microbial biomass and mycorrhizal colonisation in upland grassland. *Plant Soil* 271, 157-164.
- Kemmitt, S., Wright, D., Goulding, K., Jones, D. 2006. pH regulation of carbon and nitrogen dynamics in two agricultural soils. *Soil Biol. Biochem.* 38, 898–911.
- Kunhikrishnan, A., Thangarajan, R., Bolan, N.S., Xu, Y., Mandal, S., Gleeson, D.B., Seshadri, B., Zaman, M., Barton, L., Tang, C., Luo, J., Dalal, R., Ding, W., Kirkham, M.B., Naidu, R. 2016. Functional Relationships of Soil Acidification, Liming, and Greenhouse Gas Flux. *Adv. Agron.* 139,1–71.
- Lal, R., Delgado, J. A., Groffman, P.M., Millar, N., Dell, C., Rotz, A. 2011. Management to mitigate and adapt to climate change. *J. Soil Water Conserv.* 66, 276–285.
- Lochon, I., Carrère, P., Revaillet, S., Bloor, J.M.G. 2018. Interactive effects of liming and nitrogen management on carbon mineralization in grassland soils. *Appl. Soil Ecol.* 130, 143-148.
- Luo, Q., Gong, J., Zhai, Z., Pan, Y., Liu, M., Xu, S., Wang, Y., Yang, L., Baoyin, T. 2016. The responses of soil respiration to nitrogen addition in a temperate grassland in northern China. *Sci. Total Environ.* 569-570, 1466-1477.
- Mijangos, I., Albizu, I., Epelde, L., Amezaga, I., Mendarte, S., Garbisu, C. 2010. Effects of liming on soil properties and plant performance on temperate mountainous grasslands. *J. Environ. Manage.* 91, 2066-2074.
- Moinet, G.Y.K., Midwood, A.J., Hunt, J.E., Rumpel, C., Millard, P., Chabbi, A. 2019. Grassland management influences the response of soil respiration to drought. *Agronomy* 9,124.

- Mooshammer, M., Wanek, W., Zechmeister-Boltenstern, S., Richter, A. 2014. Stoichiometric imbalances between terrestrial decomposer communities and their resources: mechanisms and implications of microbial adaptations to their resources. *Front. Microbiol.* 5, 1-10.
- Paradelo, R., Virto, I., Chenu, C. 2015. Net effect of liming on soil organic carbon stocks: A review. *Agr. Ecosyst. Environ.* 202, 98–107.
- Poozesh, V., Castillon, P., Cruz, P., Bertoni, G. 2010. Re-evaluation of the liming-fertilization interaction in grasslands on poor and acid soils. *Grass Forage Sci.* 65, 260-272.
- Rheinheimer dos Santos, D., Tiecher, T., Santanna, M.A., Brunetto, G., da Silva, L.S. 2018. Long-term effect of surface and incorporated liming in the conversion of natural grassland to no-till system for grain production in a highly acidic sandy-loam Ultisol from South Brazilian Campos. *Soil Till. Res.* 180, 222-231.
- Robertson, G.P., Coleman, D.C., Bledsoe, C.S., Sollins, P. 1999. *Standard Soil Methods for Ecological Research.* Oxford University Press.
- Roscher, C., Thein, S., Schmid, B., Scherer-Lorenzen, M. 2008. Complementary nitrogen use among potentially dominant species in a biodiversity experiment varies between two years. *J. Ecol.* 96, 477-488.
- Rousk, J., Brookes, P.C., Bååth, E. 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. *Appl. Environ. Microbiol.* 75, 1589–96.
- Rumpel, C., Crème, A., Ngo, P.T., Velásquez, G., Mora, M.L., Chabbi, A. 2015. The impact of grassland management on biogeochemical cycles involving carbon, nitrogen and phosphorus. *J. Soil Sci. Plant Nutr.* 15, 353–371.
- Ryan, M.G., Law, B.E. 2005. Interpreting, measuring, and modeling soil respiration. *Biogeochemistry.* 73, 3-27.

- Savage, K.E., Davidson, E.A. 2003. A comparison of manual and automated systems for soil CO<sub>2</sub> flux measurements: trade-offs between spatial and temporal resolution. *J. Exp. Bot.* 54, 891-899.
- Sparling, G.P., Feltham, C.W., Reynolds, J., West, A.W., Singleton, P. 1990. Estimation of soil microbial C by a fumigation–extraction method: use on soils of high organic matter content, and re-assessment of the K<sub>ec</sub>-factor. *Soil Biol. Biochem.* 22, 301–307.
- Soussana, J.F., Allard, V., Pilegaard, K., Ambus, P., Amman, C., Campbell, C., Ceschia, E., Clifton-Brown, J., Czobel, S., Domingues, R., Flechard, C., Fuhrer, J., Hensen, A., Horvath, L., Jones, M., Kasper, G., Martin, C., Nagy, Z., Neftel, A., Raschi, A., Baronti, S., Rees, R.M., Skiba, U., Stefani, P., Manca, G., Sutton, M., Tuba, Z., Valentini, R. 2007. Full accounting of the greenhouse gas (CO<sub>2</sub>, N<sub>2</sub>O, CH<sub>4</sub>) budget of nine European grassland sites. *Agr. Ecosyst. Environ.* 121, 121–134.
- Soussana, J.-F., Lemaire, G. 2014. Coupling carbon and nitrogen cycles for environmentally sustainable intensification of grasslands and crop-livestock systems. *Agr. Ecosyst. Environ.* 190, 9–17.
- Vázquez de Aldana, B.R.V., Berendse, F. 1997. Nitrogen-use efficiency in six perennial grasses from contrasting habitats. *Func. Ecol.* 11, 619-626.
- Xi, N., Carrère, P., Bloor, J.M.G. 2014. Nitrogen form and spatial pattern promote asynchrony in plant and soil responses to nitrogen inputs in a temperate grassland. *Soil Biol. Biochem.* 71, 40-47.
- Xue, H., Tang, H. 2018. Responses of soil respiration to soil management changes in an agropastoral ecotone in Inner Mongolia, China. *Ecol. Evol.* 8, 220-230.



**Table 1.** Rainfall and temperature data for the Laqueuille grassland site. Annual and seasonal values are presented for 2016 and 2017, as well as the long-term average (data from the INRA CLIMATIK network).

	Annual values	Seasonal values (during flux measurements)		
		Spring	Summer	Autumn
<b><i>Total rainfall (mm)</i></b>				
Long-term average (1996-2015)	1094 ± 41.2	274 ± 21.4	310 ± 16.0	191 ± 17.5
2016	1184	436	207	148
2017	935	279	297	118
<b><i>Average temperature (°C)</i></b>				
Long-term average (1996-2015)	7.81 ± 0.19	6.64 ± 0.25	14.8 ± 0.25	10.8 ± 0.37
2016	8.21 ± 0.34	5.33 ± 0.49	15.2 ± 0.45	11.6 ± 0.61
2017	8.27 ± 0.38	7.88 ± 0.53	16.2 ± 0.45	10.4 ± 0.48

**Table 2.** Effects of liming treatment on CO<sub>2</sub> emissions recorded over different timescales during the experiment: A) Interactions between liming and date of measurement on mean daily soil respiration in 2016 and 2017; B) Interactions between liming, season and year on seasonal averages of CO<sub>2</sub> emissions. Plot and Block are random factors for the ANOVA presented.

Fixed factors	df num	df den <sup>1</sup>	F value	P value <sup>§</sup>	
<b>A) Mean daily soil respiration</b>					
<b>2016</b>	Date	12	70	42.13	<b>&lt; 0.001</b>
	Treatment	1	6	0.01	0.917
	Date x Treatment	12	70	0.39	0.963
<b>2017</b>	Date	14	84	10.86	<b>&lt; 0.001</b>
	Treatment	1	6	1.56	0.259
	Date x Treatment	14	84	0.39	0.975
<b>B) Seasonal averages of CO<sub>2</sub> emissions</b>					
	Year	1	30	174.8	<b>&lt; 0.001</b>
	Season	2	30	113.7	<b>&lt; 0.001</b>
	Treatment	1	6	0.54	0.489
	Year x Season	2	30	8.86	<b>&lt; 0.001</b>
	Year x Treatment	1	30	0.29	0.592
	Season x Treatment	2	30	0.29	0.732
	Year x Season x Treatment	2	30	0.09	0.918

<sup>1</sup> estimate of the degree of freedom for the mixed model (package R "lmerTest")

<sup>§</sup>P values in bold are significant at <0.05.

**Table 3.** Effects of liming, year and date of measurement on microbial and root biomass recorded in the 0-10cm soil layer. P-values derived from analysis of variance are shown (P values in bold are significant at <0.05, P values in italics are significant at the 10% level.).

Effect	df num	df den <sup>1</sup>	Microbial biomass	Microbial C:N ratio	Root biomass
Year	1	30	0.207	< <b>0.001</b>	< <b>0.001</b>
Date	2	30	<b>0.024</b>	< <b>0.001</b>	<b>0.006</b>
Treatment	1	6	0.322	<i>0.067</i>	0.101
Year x Date	2	30	<i>0.095</i>	< <b>0.001</b>	0.185
Year x Treatment	1	30	0.817	<i>0.065</i>	0.897
Date x Treatment	2	30	<i>0.095</i>	<b>0.026</b>	0.280
Year x Date x Treatment	2	30	0.470	0.675	0.275

<sup>1</sup> estimate of the degree of freedom for the mixed model (package R "lmerTest")

**Table 4.** Effects of liming on total annual forage production, forage quality (C:N), aboveground biomass:N ratio and abundance of grasses and legumes in the forage during the study. P-values derived from analysis of variance are shown (ns = no significant difference).

	Treatments*		ANOVA results (P values <sup>§</sup> )		
	Control	Limed	Treatment	Year	Tmt x Yr
<b>Forage yield (g m<sup>-2</sup>)</b>					
2016	571 ± 29.7	548 ± 47.0	0.467	<i>0.079</i>	0.818
2017	667 ± 60.2	624 ± 45.2			
<b>Forage C:N (g g<sup>-1</sup>)</b>					
2016	16.9 ± 0.66	16.6 ± 0.60	<b>0.033</b>	<i>0.089</i>	0.117
2017	18.3 ± 0.54	16.6 ± 0.50			
<b>Biomass:N ratio (g dry mass g<sup>-1</sup> N)</b>					
2016	36.1 ± 1.02	34.9 ± 1.28	<b>0.018</b>	0.186	0.174
2017	38.4 ± 1.26	34.9 ± 1.01			
<b>Abundance of grasses (%)</b>					
2016	81.3 ± 3.92	79.2 ± 0.90	0.222	<i>0.067</i>	0.534
2017	89.7 ± 3.19	83.7 ± 2.30			
<b>Abundance of legumes (%)</b>					
2016	7.4 ± 2.97	7.2 ± 3.05	0.443	<b>0.049</b>	0.401
2017	0.9 ± 0.44	4.4 ± 1.01			

\*Data are means ± standard errors, n=4.

<sup>§</sup>P values in bold are significant at <0.05. P values in italics are significant at the 10% level.

## Figure Legends

**Fig. 1.** Soil pH recorded under different liming treatments during the two-year experiment. Means and standard errors are presented ( $n = 4$ ).

**Fig. 2.** Soil respiration rate (Rs) during the growing season (April to October) in 2016 and 2017. Means and standard errors are presented ( $n=4$ ).

**Fig. 3.** Effects of (A) soil temperature and (B) soil moisture on mean daily soil respiration rate in limed and control plots during the experimental period. The solid line represents a significant linear regression ( $P < 0.05$ ).

**Fig. 4.** Effects of liming on seasonal soil CO<sub>2</sub> emissions in 2016 and 2017. Average values and standard errors are presented ( $n= 3-6$ ).

**Fig. 5.** Microbial biomass (A), microbial C/N ratio (B) and root biomass (C) in the 0-10cm soil layer under experimental liming treatments. Means and standard errors are presented ( $n = 4$ ).

Fig.1.

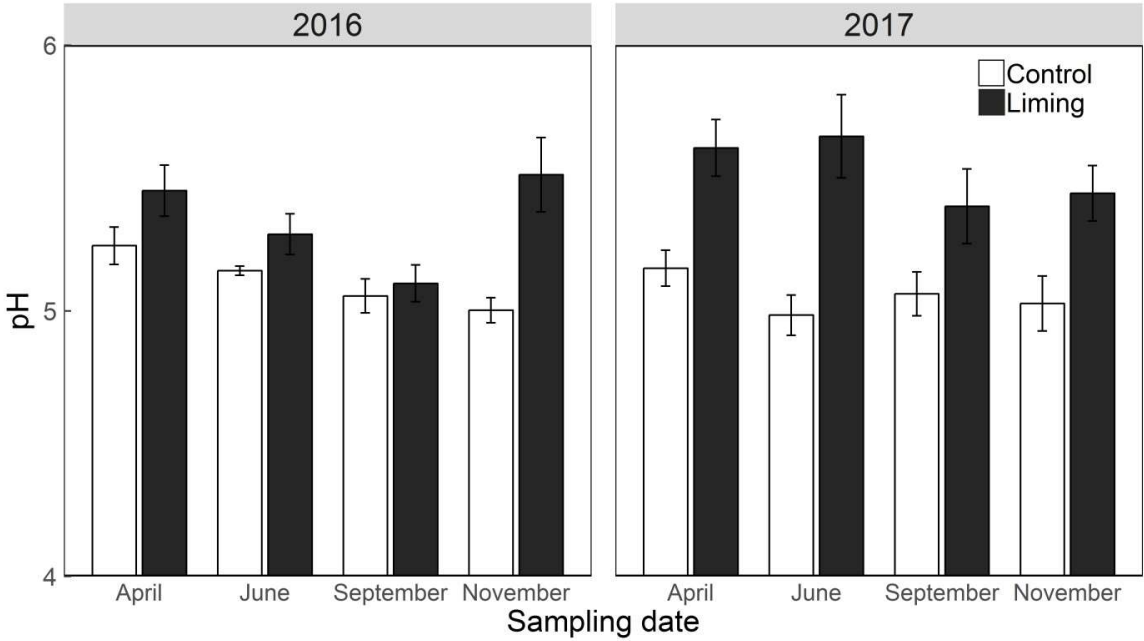
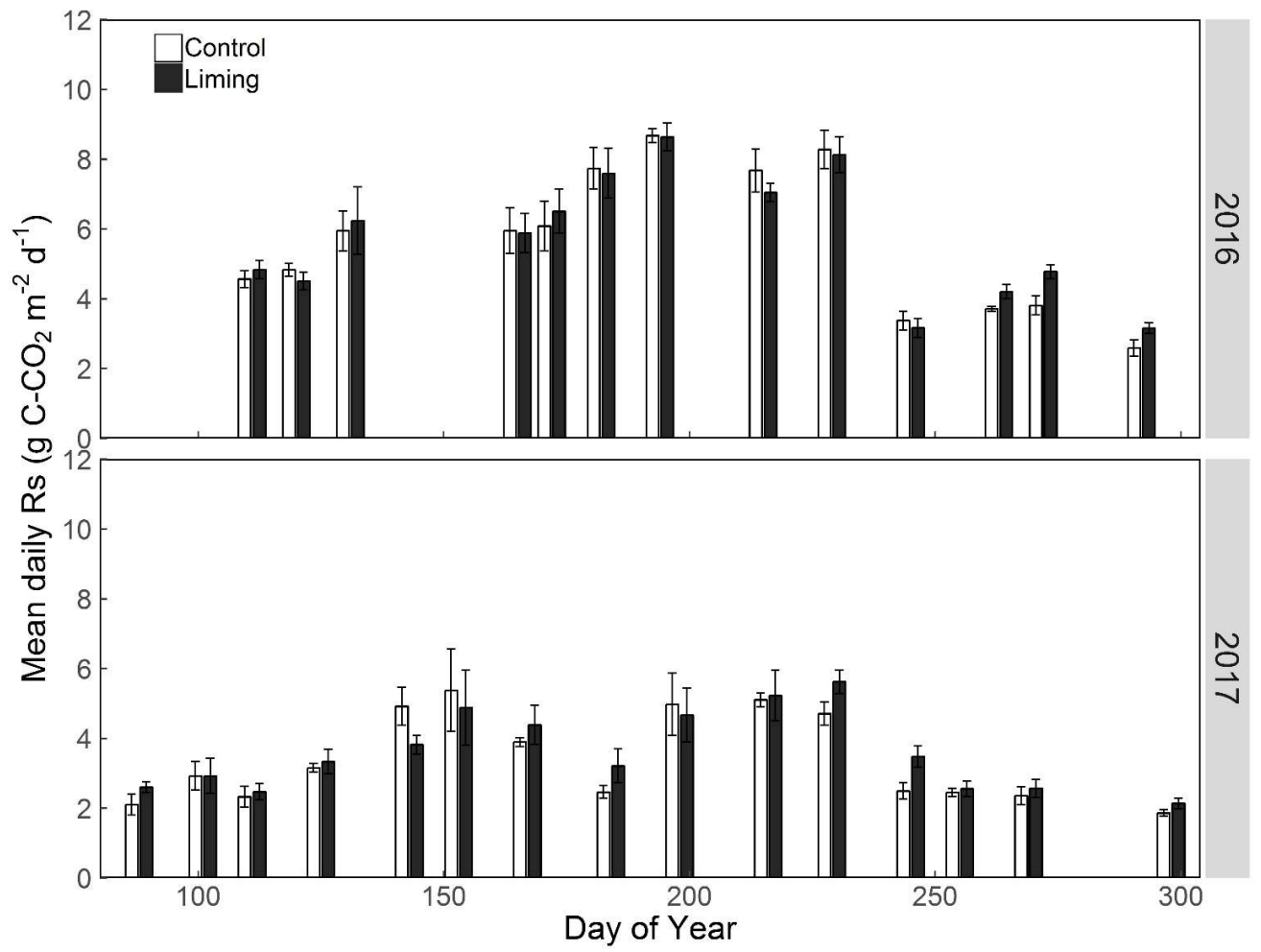


Fig.2.



**Fig.3.**

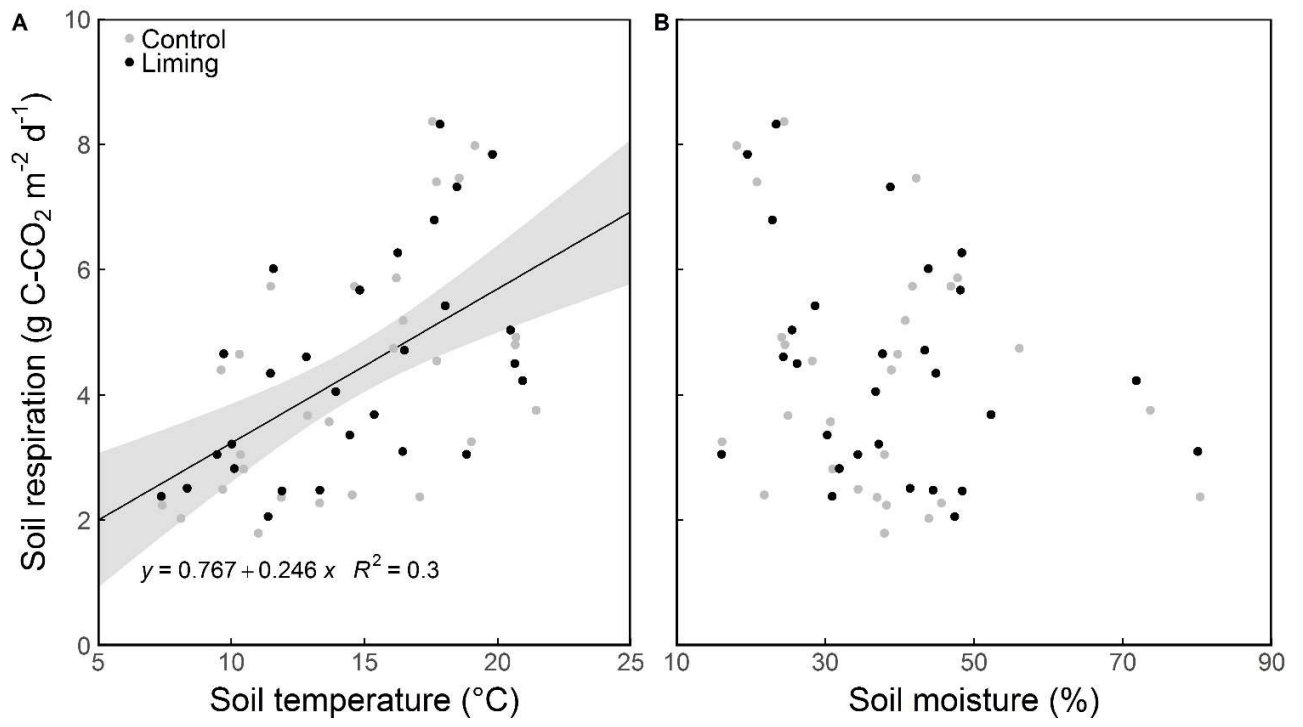


Fig. 4.

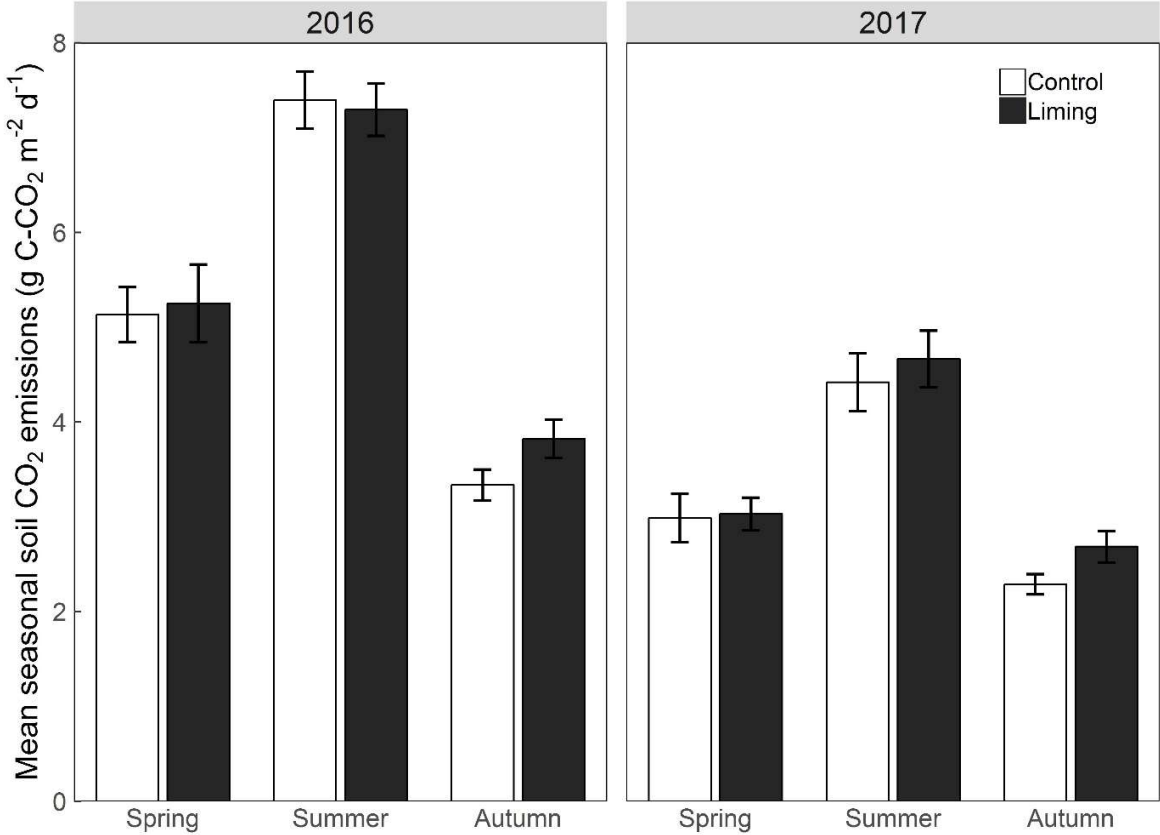


Fig. 5.

