Assessing the impacts of lime application to pastures on soils, biodiversity, and forage quality

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Introduction

The application of lime to arable land is a long-established agricultural practice to ameliorate acidic soils that has been carried out since at least Roman times to improve arable crop and grassland productivity (Goulding 2016, Holland, Bennett et al. 2018). Soil acidification occurs through natural processes via carbon, nitrogen and sulphur cycling, plant growth, acidic precipitation, and application of nitrogen fertilisers (see Holland, Bennett et al. (2018) for a review). The addition of lime reduces acidity through various changes in the physical and chemical properties of soils, which in turn influences the bio-availability of soil nutrients, impacts on the soil fauna, as well as influencing plant species composition and the quality of plants as a forage for livestock (Goulding 2016, Holland, Bennett et al. 2018). In the UK the addition of lime to agricultural land was strongly encouraged by the UK government through the provision of subsidies to farmers from 1937 – 1977/78 in order to increase food production but, since subsidies ceased, lime application has steadily declined leading to the gradual acidification of soils (Holland, Bennett et al. 2018).

Agricultural intensification has been associated with severe biodiversity decline through habitat loss, degradation and homogenisation, soil degradation, mechanisation and simplification of crop-rotations (Stoate, Boatman et al. 2001, Stoate, Baldi et al. 2009). Despite intense efforts via agri-environment schemes to halt and reverse farmland biodiversity loss these schemes have met with limited success and agricultural practices continue to pose a significant threat to biodiversity (Kleijn and Sutherland 2003, Kleijn, Baquero et al. 2006, Stoate, Baldi et al. 2009, IPBES 2019). Though, the widespread decline in farmland biodiversity affects all taxa the declines in farmland birds have been particularly acute and well documented (Inger, Gregory et al. 2015, Heldbjerg, Sunde et al. 2018, Tarjuelo, Margalida et al. 2020). In the UK Farmland wading birds have undergone particularly severe declines (Brown, Wilson et al. 2015, Eaton, Aebischer et al. 2015, Harris, Massimino et al. 2020, Woodward, Aebischer et al. 2020) with the Eurasian curlew classed as one of the most serious conservation issues in the UK (Brown, Wilson et al. 2015). The reasons for these declines include mechanisation of farming, loss of rough grassland and habitat heterogeneity, predation and a decline in the availability of food resources such as earthworms, that are a key food item during the pre-laying and fledging periods of wading birds (Galbraith 1989, Baines 1990, Beintema, Thissen et al. 1991, Benton, Bryant et al. 2002, Benton, Vickery et al. 2003, Sheldon 2003, Newton 2004, McCallum, Wilson et al. 2016, McMahon, Doyle et al. 2020). Earthworms are known to be sensitive to soil pH and previous work has shown that earthworm abundance is associated with addition of lime (Potthoff, Asche et al. 2008, McCallum, Wilson et al. 2016). Due to the anticipated biodiversity and agricultural benefits, the addition of lime has been suggested as a conservation measure to aid farmland wading birds (McCallum, Wilson et al. 2016).

In the face of severe biodiversity loss (Secretariat of the Convention on Biological Diversity 2014, WWF 2020) it is recognised that the conservation of biodiversity needs to be integrated with wider land use objectives to deliver multiple ecosystem benefits (Wilson, Chen et al. 2015, IPBES 2019, Leclère, Obersteiner et al. 2020). Identifying farming practices that improve agricultural productivity, improve biodiversity and mitigate climate change is essential if society is to provide food security for a growing population and tackle biodiversity loss together with deleterious changes to our climate. The application of lime to pastoral land which is associated with improved agricultural productivity and biodiversity benefits may be one such practice, but further investigation is required to determine whether liming can benefit both biodiversity and agricultural production without the potential to contribute to greenhouse gas emissions. In this study we investigate how the application of lime to pasture influences soil properties, plant growth, diversity, vegetative quality, worm abundance and biomass across two fields at three farms. We anticipate that lime will raise the soil pH, thereby increasing the bio-availability of soil nutrients leading to an increase in plant biomass production and a shift towards more palatable plant species for livestock. We predict that less acidic soil conditions will lead to higher numbers of earthworms, more active microbial communities, and a greater breakdown of soil organic matter.

Materials and Methods

Study sites and experimental design

The study was carried out at three farms, Hartwood (HW, 55.810248, -3.848397, 195 m a.m.s.l), Auchnerran (AN, 57.116147, -2.963729, 220 m a.m.s.l) and Glensaugh (GS, 56.893270, -2.540307, altitude between 120 and 450 m a.m.s.l) Scotland, UK. At each farm two nearby fields (denoted A and B) with similar characteristics in terms of management, vegetation composition and terrain were selected. At all farms, the fields comprised of semi-improved grassland, which were being used to graze sheep (Auchnerran) or sheep and cattle (Glensaugh and Hartwood). Prior to the experiment fields had an initial pH of between 4.55 and 5.78 (Table 1). Each of the fields were divided in half and magnesium-based lime was applied to one half of each field in autumn 2017 at a rate intended to raise the pH to 7 (Table 1). The individual farm managers advised on the application rates based on their own experience of the sites and following recommendations from standard soil pH analyses and lime addition recommendations based on soil type and agricultural use (Sinclair, Crookes et al. 2014). Following the application of lime, the management of the fields continued as normal. Sheep and cattle were removed as required for routine farming purposes and the fields were free of livestock over winter when ground conditions were too wet (Hartwood). Control of thistles and rushes by herbicide application and toping continued, as did grass rolling (Auchnerran).

Soil measurements

In November and December 2016 (before liming) soils samples were collected from 8 randomly assigned points in each field (this equated to between 3 and 5 sample points per half field). At each point samples were collected from two depths, 0-5 cm and 5-15 cm (where soil depth allowed) using a 2.5 cm diameter soil corer. Soils were analyzed for pH using the calcium chloride ($CaCl_2$) method (McLean 1982, Sumner 1994, DEFRA 2003) and calcium (Ca) and magnesium (Mg) using acetic acid extractions (Anon 1985). Soil organic matter (SOM) was determined by calculating the loss on ignition at 450 °C (Gardner 1965, Pansu and Gautheyrou 2006). All analyses were carried out on each depth separately. Samples were collected from the same locations in subsequent years (2017 -2019, after liming though not from HW in 2019 due to a change in farm ownership) and the same analyses were conducted. Soil samples collected from 2017-2019 were also analysed for Nitrate (NO_3^-) and ammonium (NH_4^+) concentrations using 1M Potassium chloride (KCL) extractions.

Earthworms

Commencing in April 2017, and annually thereafter, earthworms were collected from between 5 and 11 random sample locations in each half field. Using a spade, soil was removed from a hole 25 cm x 25 cm and, where soil depth allowed, 25 cm deep. If the soil was shallower than 25 cm a note of the depth was recorded. All soil from the hole was placed on a plastic sheet and hand searched for worms in the field. Once removed, the worms were placed in pots, covered with a porous lid to allow air flow and later sorted on return to the lab. Worms were sorted, based on size, colouration and macro-features, into three broad functional groups; Endogeic, Epigeic, or Anecic as it is not possible to identify juvenile worms to species level. Worms from each group, in each sample location were counted, stored in 70% ethanol, before air drying at 30 °C and weighing. Due to small numbers of Epigeic and Anecic worms, counts of all worms were combined for reporting and statistical analyses.

Vegetation community composition

Commencing in July 2017 (before liming) and repeated each July in 2018 and 2019 (after liming) the plant species composition was recorded in 0.5 m x 0.5 m quadrats at 8 predefined locations in each half field. Quadrat locations were assigned randomly within each half field at the start of the experiment and the corners of each quadrat were marked with metal pegs to allow them to be relocated in subsequent years. In each quadrat all plant species (higher plants and bryophytes) were recorded, and the percentage cover of each species estimated.

Microresp

In spring 2018 and 2019 soil samples were collected within each half field using a 2.5 cm diameter corer. To ensure samples were representative of the half field a "W" route was walked across each half field and approximately 12 soil cores were collected along the way. Samples were divided into the first 0-5 cm and 5-15 cm depths and each depth bulked together for each half field. The samples were analysed using the MicroResp technique (Campbell, Chapman et al. 2003) to give an indication of the microbial activity in the soil. A sub-sample was taken of each bulked sample to determine the corresponding soil pH.

Biomass and forage quality

To measure the biomass production and changes in plant fibre, carbon (C) and nitrogen (N) content three grazing exclosure cages (80 cm x 80 cm x 60 cm) were placed at randomly assigned locations in each half field in March 2017 (before liming) at the beginning of the plant growing season. Adjacent to each cage, and in an area of vegetation that resembled that under the cage, all vegetation was harvested from a 20 cm x 20 cm quadrat. This provided a reference biomass value. In July/August 2017 (at the end of the plant growing season) a biomass sample was collected from a 20 cm x 20 cm quadrat placed in the centre of the exclosure cage and another reference sample was taken from an area outside of the cage. The cages were moved to new randomly assigned locations and this was repeated in 2018 and 2019. All samples were oven dried at 80 °C for 72 hours and weighed to determine the biomass produced. Samples were subsequently milled and analysed for forage quality using Near Infrared Reflectance Spectroscopy (NIRS) (Pérez Fernández 2014) which provided a measure of fibre content (neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid insoluble lignin (AIL)), carbon and nitrogen. NDF provides a measure of total fibre (cellulose, hemi cellulose and lignin) and gives an indication of potential voluntary intake by livestock i.e., livestock are likely to consume less forage with a high percentage NDF compared to forage with a lower NDF as it provides more bulk to their diet. Whereas ADF provides a measure of cellulose and lignin, the least digestible components of the plant, this relates to the energy which is likely to be obtained from the forage. Vegetation with a high percentage of ADF has low digestibility and will generally provide less energy to the animal than vegetation with a lower ADF. Finally AIL is a measure of lignin which is an undigestible component of the plant cell wall (Ball, Collins et al. 2001, Anon 2008, Rasby and Martin 2021)

Data analysis

Soil pH, Mg, Ca, NO_3^-, NH_4^+, SOM, biomass production, off-take, forage quality (NDF, ADF, AIL, C and N), and vegetation diversity indices (Shannon diversity and Simpson diversity) were all analysed using linear mixed-effects models (R package 'lme4', version 1.1.23, (Bates, Mächler et al. 2015)) with a fixed model of Treatment, Year and Treatment-Year interaction terms, and a random model of sampling location nested within plot (half-field/treatment), nested within block (field), nested within site (farm). NO_3^- and NH_4^+ were both log transformed before analysis to take account of their non-linear distribution. Plant species richness consisted of count data and was therefore analysed using a generalised linear mixed-effects model with a poisson distribution (R package 'lme4', version 1.1.23, (Bates, Mächler et al. 2015)). In each model Year was treated as a factor to allow for any year to year variation due to the short nature of the study. Separate analyses for 0-5 cm and 5-15 cm soil depths were carried-out for each of the soil responses. To obtain pairwise contrasts and estimated effect sizes for the effect of lime on soil pH, Mg, Ca, NO_3^-, NH_4^+ SOM, biomass production, offtake, NDF, ADF, AIL, forage C and forage N and the vegetation diversity indices, predicted means were computed and significant contrasts accessed using 'emmeans' package (version 1.5.0, (Russell 2020)). A Principle Response Curve analysis were carried out, in the 'vegan' package (version 2.5.6, (Oksanen, Blanchet et al. 2019)) to further investigate the plant species composition. Analyses were carried out in R (version 3.3.3, (R Core Team 2020)). The statistical significance ($p < 0.05$) of fixed model terms was assessed by backwards stepwise deletion of non-significant terms from the full model.

The earth worm data were analysed using generalised linear mixed-effect models using the 'glmer' function in the R package 'lme4' (version 1.1.23, (Bates, Mächler et al. 2015)). The worm count data where analysed using a Poisson model with a log link function, whereas the worm dry mass data were first loge +1 transformed and analysed using a normal gaussian error distribution with an identity link function. To accommodate varying hole depth, thereby sampling effort, for the earthworm samples loge transformed hole depth was used as an offset for the analyses of earthworm count and biomass data. The small sample size of study sites and the highly nested structure of random terms made model fitting difficult and initial analyses yielded convergence warnings. We therefore used the alternative "bobyqa" optimiser (via the nADQ = 0 argument) and for the worm dry mass analysis we had to specify model start values to ensure model convergence and stable results.

Results

Soil measurements

In 2016, prior to liming, the soil pH was similar across control plots and those intended for liming the following year (Fig. 1a). This pattern was true at both 0-5 cm and 5-15 cm depths. Following the lime applications in 2017 pH in the 0-5 cm soil depth was consistently and significantly higher than in the control plots in all years (Table 2, Fig. 1a, treatment; $F_{1,5}=24.24$, $p<0.01$, year; $F_{3,124}=34.79$, $p<0.001$; treatment:year; $F_{3,125}=19.19$, $p<0.001$). The response at the 5-15 cm depth was slower and the pH was only found to be significantly higher in limed plots from 2018 onwards (Table 2; Fig. 1b, treatment; $F_{1,5}$=2.98, p=0.14, year; $F_{3,123}$=11.49, p=<0.001, treatment:year; $F_{3,125}$=7.92, p=<0.001).

Magnesium (Mg), a constituent element of the lime applied to the fields, was found at similar levels across plots at both depths prior to the lime application (Figs. 2a & 2b). In the first 0-5 cm, Mg concentrations were higher in the limed plots than the control plots in both 2017 and 2019 (Table 2, treatment; $F_{1,8}$=15.15, p<0.01, year $F_{3,158}$=15.25 p<0.001, treatment:year; $F_{3,160}$=9.68, p<0.001) and remained higher. However, despite following this trend, in 2018 the difference between Mg levels in the control and limed plots was not found to be statistically significant (Fig. 2a, Table 2). At the 5-15 cm depth no significant differences were seen between treatments over the first 2 years. However, by 2019 Mg was significantly higher at 5-15 cm in the limed plots (Table 2, Figure 2b, treatment; $F_{1,44}$=0.80, p=0.38, year; $F_{3,124}$=4.47, p<0.01, treatment:year $F_{3,126}$=4.95, p=<0.01).

Calcium (Ca), another major constituent of the lime applied to the fields, showed a similar pattern to Mg (Figs. 2, 3). Prior to lime application, no significant differences were seen between plots (Table 2). By 2017 Ca levels were significantly higher in the first 0-5 cm of soil on the limed plots compared to the control plots and remained higher, albeit non-significant (Table 2, treatment; $F_{1,5}$=8.84, p=0.03, year; $F_{3,123}$=13.30, p<0.001, treatment:year; $F_{3,125}$=7.86, p<0.001). At the 5-15 cm depth no significant differences in Ca concentration were seen in any year of the experiment, although there was an indication that Ca level were starting to increase in 2019 (Table 2, Fig. 3b, treatment; $F_{1,43}$=0.14, p=0.70, year; $F_{3,123}$=2.67, p=0.05, treatment:year $F_{3,125}$=1.67, p=0.18).

Nitrate (NO_3^-) and ammonium (NH_4^+) showed no significant differences between limed and unlimed plots at either depth (Fig 4 & 5, Table 2, NO_3^- 0-5 cm, treatment; $F_{1,118}$=1.55, p=0.22, year; $F_{2,119}$=0.79, p=0.46, treatment:year; $F_{2,117}$=0.81, p=0.45, NH_4^+ 0-5 cm, treatment; $F_{1,120}$=0.40, p=0.53, year; $F_{2,121}$=1.87, p=0.16, treatment:year; $F_{2,120}$=0.73,p=0.48). However, NO_3^- was higher in the first 0-5 cm of soil on the limed plots compared to the controls (Fig 5). There were no obvious trends at 5-15cm (Fig 4 & 5, Table 2, NO_3^- 5-15 cm treatment; $F_{1,5}$=1.69, p=0.25, year; $F_{2,115}$=4.20, p=0.17, treatment:year; $F_{2,117}$=2.05, p=0.13, NH_4^+ 5-15 cm, treatment; $F_{1,117}$=6.29, p=0.01, year; $F_{2,117}$=19.04, p<0.001, treatment:year; $F_{2,117}$=0.09, p=0.91).

Loss on ignition at 450 ˚C which provides an indication of soil organic matter (SOM) showed little change over time as a result of the liming treatment (Table 2, Fig. 6, 0-5 cm treatment; $F_{1,5}$=0.03, p=0.87, year; $F_{3,125}$=0.07, p=0.97, treatment:year; $F_{3,127}$=2.74, p=0.05, 5-15cm treatment; $F_{1,5}$=0.42, p=0.54, year; $F_{3,124}$=8.09, p<0.001, treatment:year; $F_{3,126}$=1.27, p=0.29).

Total multi- substrate induced respiration (MSIR) which provides an indication of soil microbial activity was higher in limed plots at 0-5 cm depth than in the control plots (Table 2, Fig. 13 0-5 cm treatment; $F_{1,15}$=11.89, p=<0.01, year; $F_{1,15}$=18.26, p,0.001, treatment:year; $F_{1,15}$=0.84, p=0.37). However, no difference in MSIR was seen between limed and unlimed plots at 5-15 cm depth (Table 2, Fig 13, treatment; $F_{1,15}$=0.05, p=0.83, year; $F_{1,15}$=13.36, p<0.01, treatment:year; $F_{1,15}$=0.08, p=0.79).

Worm counts and dry mass

Standardised worm counts and dry mass estimates both show appreciable variability within and between sites and between years (Fig. 7). Worm abundance was significantly influenced by a treatment-year interaction (ChiSq = 37.97, p < 0.001) with the effect of treatment increasing with time. However, lime addition nor year alone had strong effects on estimated worm numbers (reporting from the full model: treatment; z = -1.96, p= 0.05 year(2018); z = -1.76, p > 0.08, year(2019) z = -11.40, p < 0.001). There was no strong treatment-year interaction on standardised worm dry mass (Chisq = 5.696, p = 0.058) and the effect of treatment was also not significant (Chisq = 0.3161, p = 0.57395). Year, however, had a highly significant effect on worm dry mass (Chisq = 86.1845, p < 0.001). Overall, liming appears to marginally increase worn abundance and dry mass, but these effects are less than the effects of year and appear to be largely driven by changes at one site (Hartwood) and are year dependant.

Vegetation: Species diversity and community composition

There were no statistically significant differences between limed and non-limed plots for any of the vegetation diversity indices (species richness, Simpson diversity or Shannon diversity) (Figs. 8a-c, Table 2 Simpson diversity, treatment; $F_{1,5}$=0.13, p=0.73, year; $F_{2,188}$=0.09, p=0.91, treatment:year, $F_{2,188}$=1.54, p=0.22, Shannon diversity, treatment; $F_{1,5}$=0.09, p=0.78, year; $F_{2,188}$=0.21, p=0.81, treatment:year; $F_{2,188}$=1.95, p=0.14). Similarly, we found no evidence of significant changes in the plant community composition over the course of the study (Fig. 9).

Vegetation: Biomass, Off-take, and forage quality

In the first year after lime application there was no significant difference between the biomass production in limed fields compared to un-limed ones. However, by 2019 there was a non-significant trend towards higher biomass production in limed fields, though the difference was not statistically different (Table 2, Fig. 10, treatment; $F_{1,82}$, p=0.11, year; $F_{2,83}$=19.17, p=<0.001, treatment:year; $F_{2,82}$=1.09, p=0.34). Similarly, while there was no significant difference in offtake in 2017 and 2018, off-take was significantly greater by 2019 (Table 2, Fig.11, treatment; $F_{1,6}$=4.22, p=0.09, year; $F_{2,58}$=17.45, p<0.001, treatment:year; $F_{2,60}$=1.29, p=0.28).

Neither the levels of ADF or NDF within cages differed significantly between treatments at the start of the experiment, and though the levels of each increased on limed fields over the course of the study neither showed a significant difference by the end of the experiment (Fig. 12, Table 2, ADF, treatment; $F_{1,5}$=0.03, p=0.88, year; $F_{2,43}$=3.85, p=0.03, treatment:year; $F_{2,45}$=4.56, p= 0.016, NDF, treatment; $F_{1,6}$=0.04, p=0.86, year; $F_{2,54}$=14.14, p=<0.001, treatment:year; $F_{2,54}$=2.46, p=0.09). AIL values did not differ between treatment fields at the start of the experiment. AIL values peaked in both control and treatment fields in 2018 with limed fields showing significantly greater levels of AIL. There was no clear trend in the levels of AIL over the three years of the study (Table 2, Fig. 12 treatment; $F_{1,29}$=1.02, p=0.32, year; $F_{2,57}$=13.7, p<0.001, treatment:year $F_{2,60}$=1.74, p=0.18). The levels of forage C, forage N and the corresponding C:N ratio did not differ significantly between treatments at the start of the study (Fig. 12, Table 2) and the level of forage C remained relatively stable over the course of the study, while forage N in limed fields declined over the course of the study. However, neither these differences, nor the final C:N ratio were statistically significant (Fig. 12, Table 2, C, treatment; $F_{1,6}$=0.32, p=0.59, year; $F_{2,77}$=4.13, p=0.02, treatment:year; $F_{2,79}$=0.52, p=0.60, N, treatment; $F_{1,5}$=0.17, p=0.70, year; $F_{2,76}$=1.57, treatment:year; $F_{2,78}$=2.21, p=0.12, C:N, treatment; $F_{1,5}$=0.02, p=0.90, year; $F_{2,77}$=2.40, p=0.10, treatment:year; $F_{2,78}$=2.21, p=0.12).

Discussion

Liming has been used for centuries to improve soil quality and arable and livestock productivity, and previous studies have demonstrated that the addition of lime is associated with improved soil health, agricultural productivity, and biodiversity benefits (Goulding 2016, Holland, Bennett et al. 2018). However, here we found evidence for only limited effects of liming on soil pH and microbial activity.

The application of lime led to an increase in the soil pH across all three sites. This was particularly apparent in the surface soil (0-5 cm) where the increase in pH was seen within 3 months of lime application and continued to rise for the duration of the study. The application of lime had less of an effect at greater soil depths (5-15 cm), only raising pH one year after lime application and to a lesser magnitude than that seen in the soil surface. These patterns have also been shown in other studies where lime surface dressings tended to only affect the pH at the top of the soil profile in the short term while affects deeper down only become apparent after 12 months or more (Li, Conyers et al. 2019, Nunes, Denardin et al. 2019, Azam and Gazey 2021).

Raising the soil pH increases the bioavailability of soil nutrients including, nitrogen (N) phosphorous (P) and potassium (K) (Haynes 1982, Lyngstad 1992, Neale, Shah et al. 1997, Bolan, Adriano et al. 2003). In addition to the increased bioavailability of existing soil minerals, the application of lime also increases the total amount of Ca and Mg in the soil system. In this study we applied magnesium-based lime to the pastures as Mg is often a limiting factor in plant growth and livestock nutrition (Mayland, Greene et al. 1990, Riggs, Syers et al. 1995). The increase in Mg and Ca concentrations in the first 0-5 cm of soil mirrored the change in the soil pH. The fact that Ca concentrations increased as well as Mg is likely due to Ca being a constituent of Mg based lime and often used as a carrier to aid application. Both Mg and Ca showed reduced levels in 2018 which was a particularly dry year. This could be attributed to the lime breaking down more slowly in the dry soil

Nitrate (NO_3^-) and ammonium (NH_4^+) can both aid plant growth so understanding how lime application effects these concentrations in the soil is important for understanding the impact of lime on plant productivity (Lack and Evans 2001). In our study the concentrations of NO_3^- and NH_4^+ in the soil showed no significant differences between limed and unlimed plots. Below the soil surface (5-15 cm) NO_3^- concentrations were very variable. Kemmitt, Wright et al. (2006) also found concentrations of NO_3^- and NH_4^+ to be unaffected by lime addition. However, it has been speculated that pH induced changes to the community of bacteria and fungi could lead to increased mineralisation of organic nitrogen (Stevens and Laughlin 1996). Therefore, in some situations soils of a higher pH may contain higher levels of NH_4^+ which in turn are converted to NO_3^- by nitrifying bacteria. In our study these differences were not detectable.

Soil organic matter (SOM) content is important for retaining soil nutrients, reducing acidification, and regulating water flow through soils (Lal 2009). We saw no change in the amount of SOM during the 2 years of monitoring following lime application. In a review by (Haynes and Naidu 1998) they suggest that lime application could either lead to a reduction in SOM if microbial communities are more active and break down organic matter more quickly or conversely SOM could increase due to greater plant growth and the subsequent addition of plant foliage and root material to the soil.

We found no significant differences in the abundance or biomass of worms between limed and unlimed areas. However, the raw data did suggest that worms were more abundant at one of the farms (HW- Hartwood) compared to the other two. This could be attributed to the soils in these fields being deeper and moister. At Hartwood we also saw a slight trend towards more worms where lime had been applied relative to the unlimed areas. These results are inconsistent with other studies that have demonstrated a more convincing effect of lime application on earthworm abundance and biomass (Bishop (2003) and McCallum, Wilson et al. (2016)). In an earthworm census of annually limed fields (6 t $CaCO_3$ha-^{1} over 3 years) and unlimed fields at Sourhope, UK Bishop (2003) found total worm abundance to be significantly higher in limed soils compared to unlimed soil. However, the liming effect differed between worm species with not all species increasing as a result of liming. In particular, *Octolasion cyaneum* showed no significant difference between limed or unlimed soil. In our study many of the worms collected were juveniles and identification to species was not possible, but many of the mature specimens collected across all sites resembled *Octolasion cyaneum.* As this species appears to be unaffected by lime application this could explain why we did not detect any change in overall worm abundance and biomass. In contrast, Bishop (2003) found more *Allolobophora chlorotica* in limed soil, albeit a non-significant increase, likewise McCallum, Wilson et al. (2016) found this species to be associated with higher pH values (most abundant at pH 5.2 and not found below pH 4.5). We only recorded *A. chlorotica* at Hartwood and this may, at least partially, explain the trend towards greater worm abundance at Hartwood in limed areas. Furthermore, the worm samples collected by McCallum, Wilson et al. (2016) came from soils with pH values ranging from pH 3.5 to 6, with almost 20% of their samples derived from soils below pH 4.5 which contained very few worms. Our pH values were generally much higher, even in unlimed plots. Therefore, our study sites did not include the more acidic soils where McCallum, Wilson et al. (2016) only found very low numbers of worms. It is also worth recognising that we collected worms from much larger soil samples (25 cm x 25 cm x 25 cm) which was more comparable with Bishop (2003) (42 cm long x 30 cm wide x 25 cm deep), while (McCallum, Wilson et al. 2016) used much smaller soil cores (10cm depth x 10.5cm diameter), notably shallower. However, as most worms were found near to the surface this may not have had a large influence. McCallum, Wilson et al. (2016) showed that SOM actually had a stronger effect on worm numbers than soil pH, with higher levels of SOM (indicating peaty soils) associated with fewer earthworms. We did not look at the relationship between SOM and worm abundance, though in our study SOM content was similar between limed and control fields at each farm.

We saw no significant change in plant biomass production following lime application over the course of this study, although biomass showed a non-significant increase two years after lime application. These results are consistent with the findings of Cregan, Hirth et al. (1989) and Lochon, Carrere et al. (2019) who also found no change in plant biomass production. Though, other studies have shown an increase in biomass production after lime application, either in the short-term (3-5 years) (Stevens and Laughlin 1996) or in the long-term (18 years – where lime & basic slag $CaSiO_3$ were applied) (Davies 1987a). The lack of response in biomass production following liming in our study could be attributed to mineralized nitrogen being a limiting factor in plant growth or the plant species present in the swards being poor responders to an increase in the pH obtained in this study.

We looked at the NDF (Neutral detergent fibre), ADF (Acid detergent fibre), and AIL (Acid insoluble lignin) together with forage C and forage N as measures of vegetation quality for grazing livestock. Few studies have investigated these parameters in relation to liming. However, Yu, Fraser et al. (2011) found NDF to be lower where lime had been applied. Likewise, we anticipated that liming would reduce NDF together with ADF and AIL due to a shift in the sward composition towards more palatable species with a lower lignin content. However, two years after liming we did not detect any change in plant species richness or Shannon diversity and there were no significant changes in the sward composition, a finding consistent with Crawley, Johnston et al. (2005) who also found no change in response to lime addition. Therefore, it is not surprising that we didn't find any change in ADF, NDF or AIL contents as the plants had not responded to the raised pH

That we did not identify any change in plant species richness and community composition is likely influenced by the wide pH tolerance of the existing suite of species as well as a limited source of alternative species from neighbouring areas. Where there are few species present which can exploit the raised pH conditions to a greater extent than any of the other species present the existing community is likely to persist. Alternatively, poor soil fertility could be a limiting factor. For example, Crawley, Johnston et al. (2005) only found a change in plant species richness when ammonium sulphate was applied together with the lime.

Soil micro-organisms are important for many soil functions, in particular nutrient cycling. Microbial activity can also determine whether soils act as carbon sinks or carbon sources (Kunhikrishnan, Thangarajan et al. 2016). Here we found a significant increase in multi substrate-induced respiration (MSIR) in both years following lime application, indicating that liming has made the soil conditions more favourable for soil microbial activity. This finding is consistent with the results of Neale, Shah et al. (1997) who have shown that soil basal respiration increases with the addition of lime, yet both our results are contrary to the findings of Johnson, Leake et al. (2005) who found soil basal respiration to reduce with lime addition. Where soil microbial activity increases as a result of liming Kemmitt, Wright et al. (2006) suggest that this can be the result of three processes. First, an increase in plant production as a result of liming which enables more plant material to enter the soil, however, this is not supported by our findings, second, higher pH enables nitrifying bacteria to be more active, though we didn't see any significant evidence of increase nitrification and finally it decreases Aluminium (Al) in the soil which enables greater bioavailability of substrate. The fact that we have found a significant increase in microbial respiration in soils subject to liming but few other effects in terms of soil processes and plant production may be due to high variability in these measures. If this increased MSIR persists, then it could lead to a reduction in SOM and subsequently a reduction in soil carbon storage.

Monitoring the effect of lime application on soil properties, plant growth, diversity, vegetive quality, worm abundance and biomass across two fields at three farms has demonstrated surprisingly little impact of the addition of lime to these pastures.

The reasons we found only limited and restricted effects likely reflect both the short-term nature of our experiment, small sample size of farms, and limited replication. The effects of lime on soil, vegetation, and earth worms are likely to be long-term and take longer than the three years monitoring which we were able to undertake. The effects of lime and changing pH on earthworms are complex and differ between functional groups and species, and interpretation of results is further complicated by difficulties in identifying the large number of juvenile worms to species level (Bishop 2003). Studies that have shown that earthworms benefit from liming have been studies that assessed the effects of long-term liming (McCallum, Wilson et al. 2016). In addition, our study did not include examples of the highly acidic soils included in McCallum, Wilson et al. (2016) study on the effects of lime addition on earthworms meaning that earthworms may have already been comparatively abundant in the less acidic soils found at our study sites.

To produce sufficient food for a growing human population while tackling climate change and biodiversity loss, carbon neutral, biodiversity positive and productive agricultural practises are required. There is a renewed interest in the practise of applying lime to pastures as a way of improving soil health and enhancing the biodiversity of land under extensive agricultural production. Here we describe the results of a large-scale experiment that studied the effects of adding lime, at recommended rates, to pastures on soil pH, soil nutrients, soil processes, earthworms, and vegetation. Our results suggest that the effects of lime are not clear cut and that results likely require several years to become apparent. Further work is needed to better understand the relationship between liming and earthworms. Increased microbial respiration could be an early sign that soil organic matter is reduced under liming as the balance between inputs from vegetation and microbial respiration has shifted.

Acknowledgements

We would like to thank Donald Barrie, John Rattray and the Game and Wildlife Conservation Trust for the use of their fields, their willingness to arrange the application of lime, and for overseeing the stocking and routine management of the fields for the duration of the study. We would also like to thank the Analytical team at the James Hutton Institute who carried out the analysis on the soil samples, Jean Robertson and Resa Haghi who provided expertise in the NIRS analysis and Clare Cameron who led on the MicroResp analysis. We also appreciated comments from Ruth Mitchell that helped to improve this report. This work was funded by the Scottish Government's Rural and Environment Science and Analytical Services' Strategic Research Programme.

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Tables

Table 1. Baseline pH and lime application rates for each field.

Farm	Field	Initial pH*	Lime application tonnes/ha	Date of application
GS	A	4.99	3	02/08/2017
	B	4.55	3	02/08/2017
AN	A	4.87	5	07/09/2017
	B	4.94	5	07/09/2017
HW	A	5.78	2.3	02/09/2017
	B	4.75	9	02/09/2017

*mean value taken from the first 5 cm of soil across each field, pH derived using the CaCl method.

Table 2. Statistical differences between limed and control plots in each year of the experiment. Contrasts assessed using the 'emmeans' package (version 1.5.0, (Russell 2020)

Contrast	Year	Estimate	SE	df	t.ratio	p.value	lower.CL	upper.CL
Soil pH 0-5 cm								
control/limed	2016	0.05	0.12	11.37	0.40	0.70	-0.21	0.30
control/limed	2017	-0.44	0.12	11.37	-3.81	**<0.001**	-0.70	-0.19
control/limed	2018	-0.69	0.12	11.37	-5.96	**<0.001**	-0.95	-0.44
control/limed	2019	-0.78	0.13	17.80	-5.82	**<0.001**	-1.06	-0.49
Soil pH 5-15 cm								
control/limed	2016	0.12	0.09	17.31	1.34	0.20	-0.07	0.30
control/limed	2017	-0.01	0.09	17.31	-0.08	0.94	-0.19	0.18
control/limed	2018	-0.33	0.09	17.31	-3.81	**<0.01**	-0.52	-0.15
control/limed	2019	-0.22	0.10	27.95	-2.09	**0.05**	-0.43	0.00
Soil Mg 0-5 cm								
control/limed	2016	5.59	75.82	16.36	0.07	0.94	-154.85	166.03
control/limed	2017	-343.26	75.82	16.36	-4.53	**<0.001**	-503.70	-182.82
control/limed	2018	104.02	75.82	16.36	-1.37	0.19	-264.46	56.42
control/limed	2019	432.17	90.11	26.34	-4.80	**<0.001**	-617.28	-247.06
Soil Mg 5-15 cm								
control/limed	2016	12.70	20.14	12.98	0.63	0.54	-30.82	56.22
control/limed	2017	-3.51	20.14	12.98	-0.17	0.86	-47.04	40.01
control/limed	2018	6.81	20.14	12.98	0.34	0.74	-36.72	50.33
control/limed	2019	-72.39	23.44	20.89	-3.09	**<0.01**	-121.15	-23.62

Contrast	Year	Estimate	SE	df	t.ratio	p.value	lower.CL	upper.CL
Soil Ca 0-5 cm								
control/limed	2016	69.94	329.61	16.74	0.21	0.83	-626.32	766.21
control/limed	2017	-1476.71	329.61	16.74	-4.48	**<0.001**	-2172.97	-780.45
control/limed	2018	-204.72	329.61	16.74	-0.62	0.54	-900.98	491.55
control/limed	2019	-1275.56	392.52	26.94	-3.25	**<0.001**	-2081.03	-470.08
Soil Ca 5-15 cm								
control/limed	2016	135.55	141.08	11.65	0.96	0.36	-172.85	443.96
control/limed	2017	154.30	141.08	11.65	1.09	0.30	-154.10	462.71
control/limed	2018	57.09	141.08	11.65	0.40	0.69	-251.32	365.49
control/limed	2019	-175.31	162.22	18.49	-1.08	0.29	-515.47	164.86
Soil NO_3 (Log) 0-5 cm								
control/limed	2017	1.05	0.23	30.33	0.24	0.81	0.68	1.64
control/limed	2018	0.73	0.16	30.33	-1.48	0.15	0.47	1.13
control/limed	2019	0.79	0.22	31.07	-0.86	0.40	0.45	1.38
Soil NO_3^- (Log) 5-15 cm								
control/limed	2017	1.44	0.22	25.32	2.39	0.02	1.05	1.98
control/limed	2018	0.95	0.15	25.32	-0.32	0.75	0.69	1.31
control/limed	2019	1.08	0.21	29.49	0.40	0.69	0.73	1.61
Soil NH_4^+ (Log) 0-5 cm								
control/limed	2017	0.80	0.19	30.42	-0.95	0.35	0.50	1.29
control/limed	2018	0.80	0.19	30.42	-0.96	0.34	0.50	1.28
control/limed	2019	1.19	0.35	30.44	0.58	0.56	0.65	2.17

Contrast	Year	Estimate	SE	df	t.ratio	p.value	lower.CL	upper.CL
Soil NH_4^+ (Log) 5-15 cm								
control/limed	2017	1.19	0.16	30.25	1.26	0.22	0.90	1.58
control/limed	2018	1.29	0.18	30.25	1.83	0.08	0.97	1.71
control/limed	2017	1.19	0.16	30.25	1.26	0.22	0.90	1.58
Loss on Ignition 0-5 cm								
control/limed	2016	-1.16	0.82	30.80	-1.41	0.17	-2.84	0.52
control/limed	2017	0.35	0.82	30.80	-0.43	0.67	-2.03	1.33
control/limed	2018	1.83	0.82	30.80	2.22	**0.03**	0.15	3.51
control/limed	2019	0.04	1.02	42.32	0.04	0.97	-2.03	2.10
Loss on Ignition 5-15 cm								
control/limed	2016	-0.27	0.66	16.62	-0.40	0.69	-1.66	1.13
control/limed	2017	0.41	0.66	16.62	0.62	0.55	-0.99	1.80
control/limed	2018	1.12	0.66	16.62	1.70	0.11	-0.27	2.52
control/limed	2019	0.00	0.79	26.86	0.00	1.00	-1.61	1.61
Total multi-substrate respirations (MSIR) 0-5 cm								
control/limed	2018	-33.60	10.88	13.33	-3.09	**<0.01**	-57.04	-10.15
control/limed	2019	-19.46	10.88	13.33	-1.79	0.10	-42.90	3.99
Total multi-substrate respirations (MSIR) 5-15 cm								
control/limed	2018	0.93	2.62	13.33	0.35	0.73	-4.73	6.58
control/limed	2019	-0.10	2.62	13.33	-0.04	0.97	-5.75	5.56

Contrast	Year	Estimate	SE	df	t.ratio	p.value	lower.CL	upper.CL
Plant Species Richness								
control/limed	2017	1.00	0.08	N/A	-0.01*	0.99	0.85	1.17
control/limed	2018	1.01	0.08	Inf	0.08*	0.93	0.85	1.19
control/limed	2019	0.99	0.08	Inf	-0.07*	0.95	0.85	1.17
Shannon diversity (Loge)								
control/limed	2017	-0.06	0.08	6.96	-0.77	0.47	-0.25	0.13
control/limed	2018	0.04	0.08	6.96	0.49	0.64	-0.15	0.23
control/limed	2019	-0.04	0.08	6.96	-0.53	0.61	-0.23	0.15
Simpson diversity								
control/limed	2017	-0.03	0.03	7.49	-1.03	0.33	-0.10	0.04
control/limed	2018	0.01	0.03	7.49	0.25	0.81	-0.06	0.08
control/limed	2019	-0.01	0.03	7.49	-0.20	0.85	-0.08	0.06
Biomass production (g)								
control/limed	2017	-3.79	6.66	28.54	-0.57	0.57	-17.43	9.85
control/limed	2018	-0.57	6.41	26.67	-0.09	0.93	-13.74	12.59
control/limed	2019	-15.44	8.37	28.38	-1.85	0.08	-32.56	1.69
Offtake (g)								
control/limed	2017	-6.03	5.92	27.69	-1.02	0.32	-18.16	6.10
control/limed	2018	-0.98	5.70	25.83	-0.17	0.86	-12.69	10.73
control/limed	2019	-15.57	7.43	28.12	-2.10	**0.05**	-30.78	-0.36

Contrast	Year	Estimate	SE	df	t.ratio	p.value	lower.CL	upper.CL
ADF (Inside)								
control/limed	2017	-0.44	0.80	28.36	-0.55	0.58	-2.08	1.19
control/limed	2018	-0.57	0.76	26.34	-0.75	0.46	-2.13	0.99
control/limed	2019	-0.64	0.99	28.03	-0.65	0.52	-2.67	1.39
NDF (Inside)								
control/limed	2017	-1.37	0.78	28.35	-1.76	0.09	-2.97	0.23
control/limed	2018	-0.69	0.74	26.33	-0.93	0.36	-2.22	0.84
control/limed	2019	-1.68	0.97	28.03	-1.74	0.09	-3.67	0.30
AIL (Inside)								
control/limed	2017	1.05	0.65	16.25	1.60	0.13	-0.34	2.43
control/limed	2018	1.58	0.62	14.59	2.54	**0.02**	0.25	2.92
control/limed	2019	0.60	0.79	21.76	0.76	0.45	-1.03	2.23
Forage C (Inside)								
control/limed	2017	-0.05	0.11	16.79	-0.43	0.68	-0.28	0.19
control/limed	2018	0.01	0.11	15.17	0.09	0.93	-0.22	0.23
control/limed	2019	0.13	0.13	22.27	0.96	0.35	-0.15	0.41
Forage N (Inside)								
control/limed	2017	0.00	0.09	22.26	-0.04	0.97	-0.18	0.17
control/limed	2018	0.03	0.08	20.31	0.33	0.75	-0.14	0.20
control/limed	2019	0.17	0.10	25.63	1.57	0.13	-0.05	0.38

Contrast	Year	Estimate	SE	df	t.ratio	p.value	lower.CL	upper.CL
Forage C:N (Inside)								
control/limed	2017	0.16	1.57	18.29	0.10	0.92	-3.14	3.46
control/limed	2018	-0.12	1.50	16.50	-0.08	0.93	-3.29	3.04
control/limed	2019	-2.13	1.91	23.32	-1.12	0.28	-6.08	1.82

*z.ratio

Figures

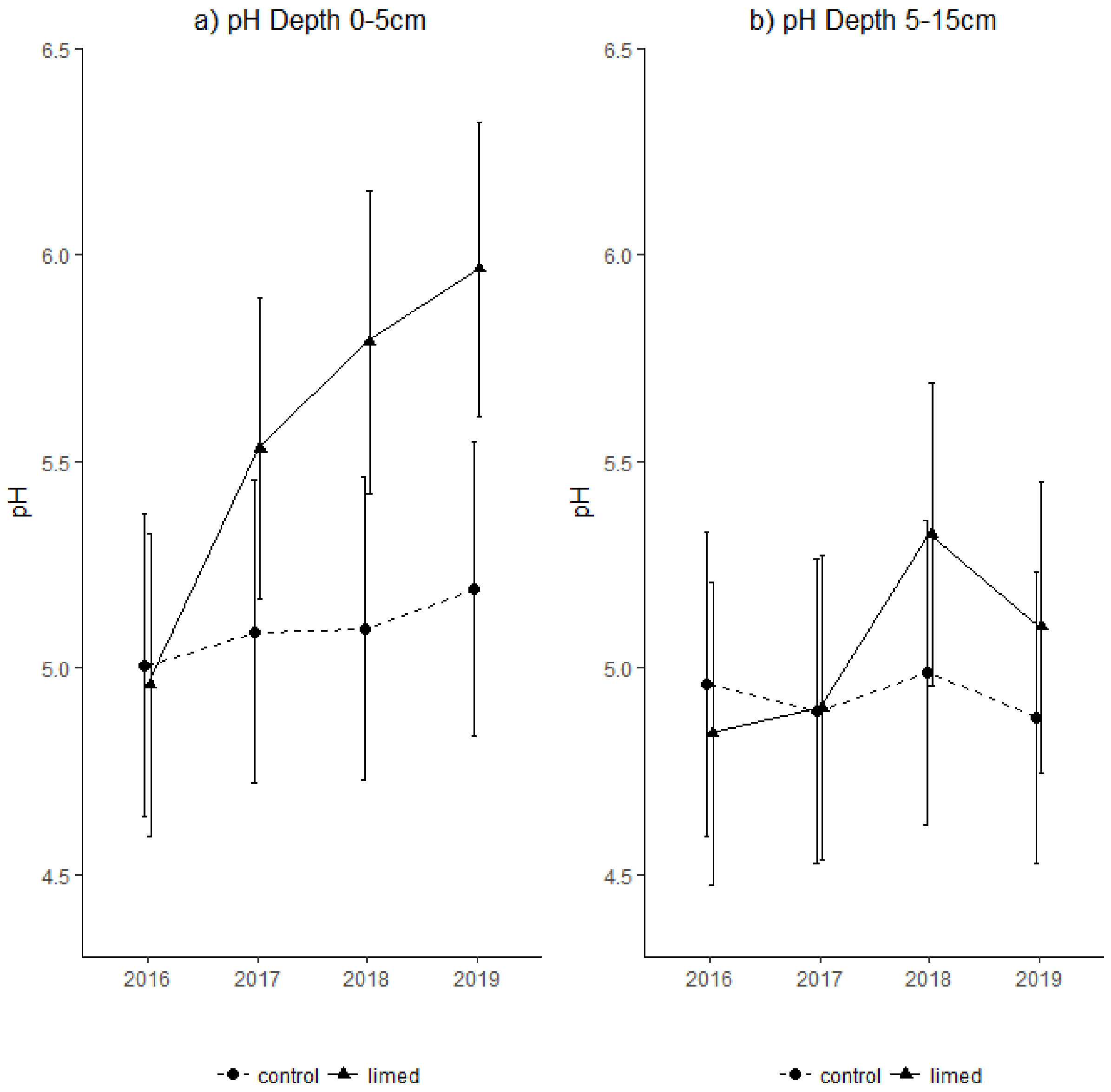


Figure 1. Estimated means and CI from linear mixed-effects models for a) soil pH 0-5cm depth and b) soil pH 5-15cm depth

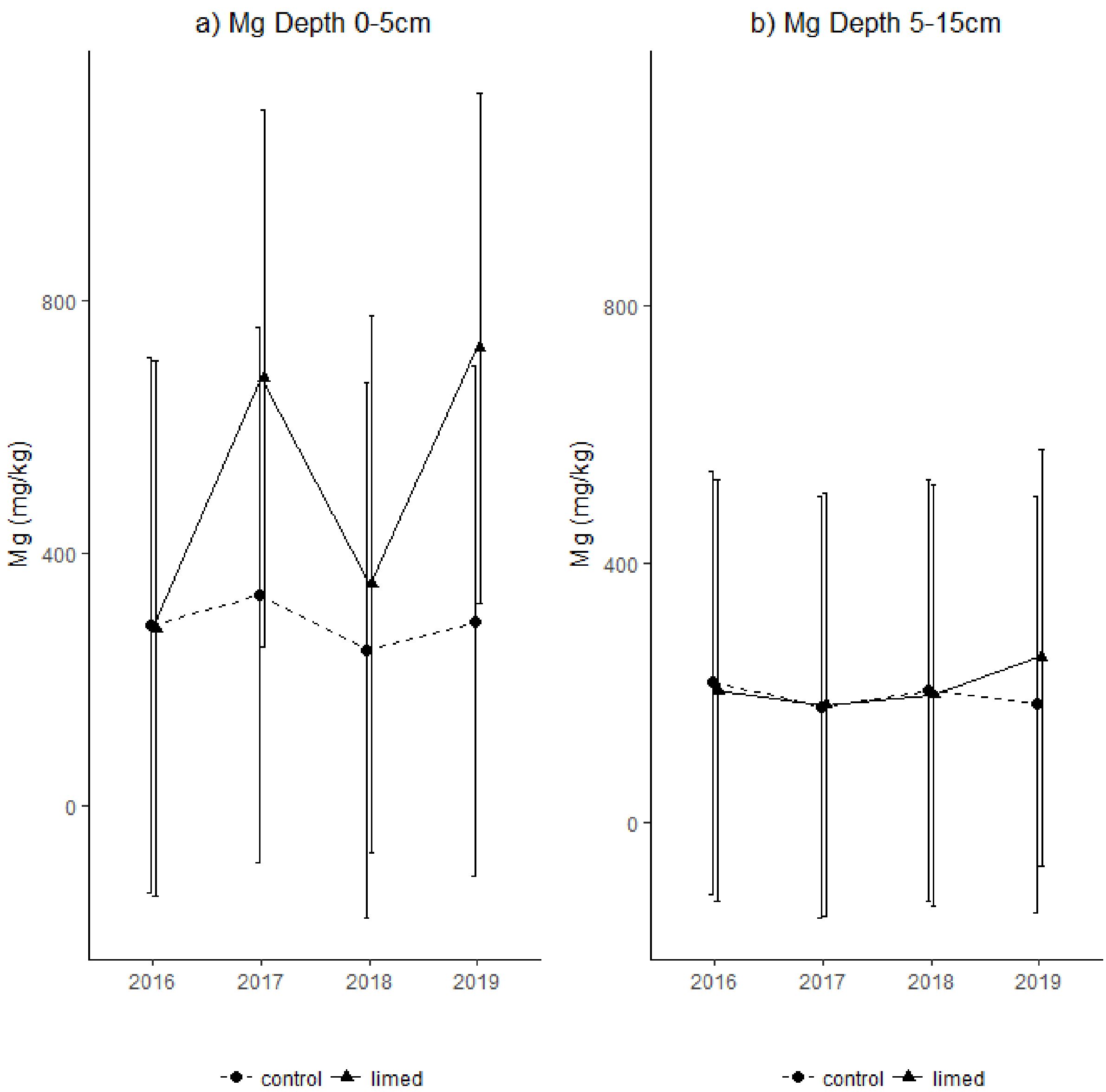


Figure 2. Estimated means and CI from linear mixed-effects models for a) soil Mg 0-5cm depth and b) soil Mg 5-15cm depth

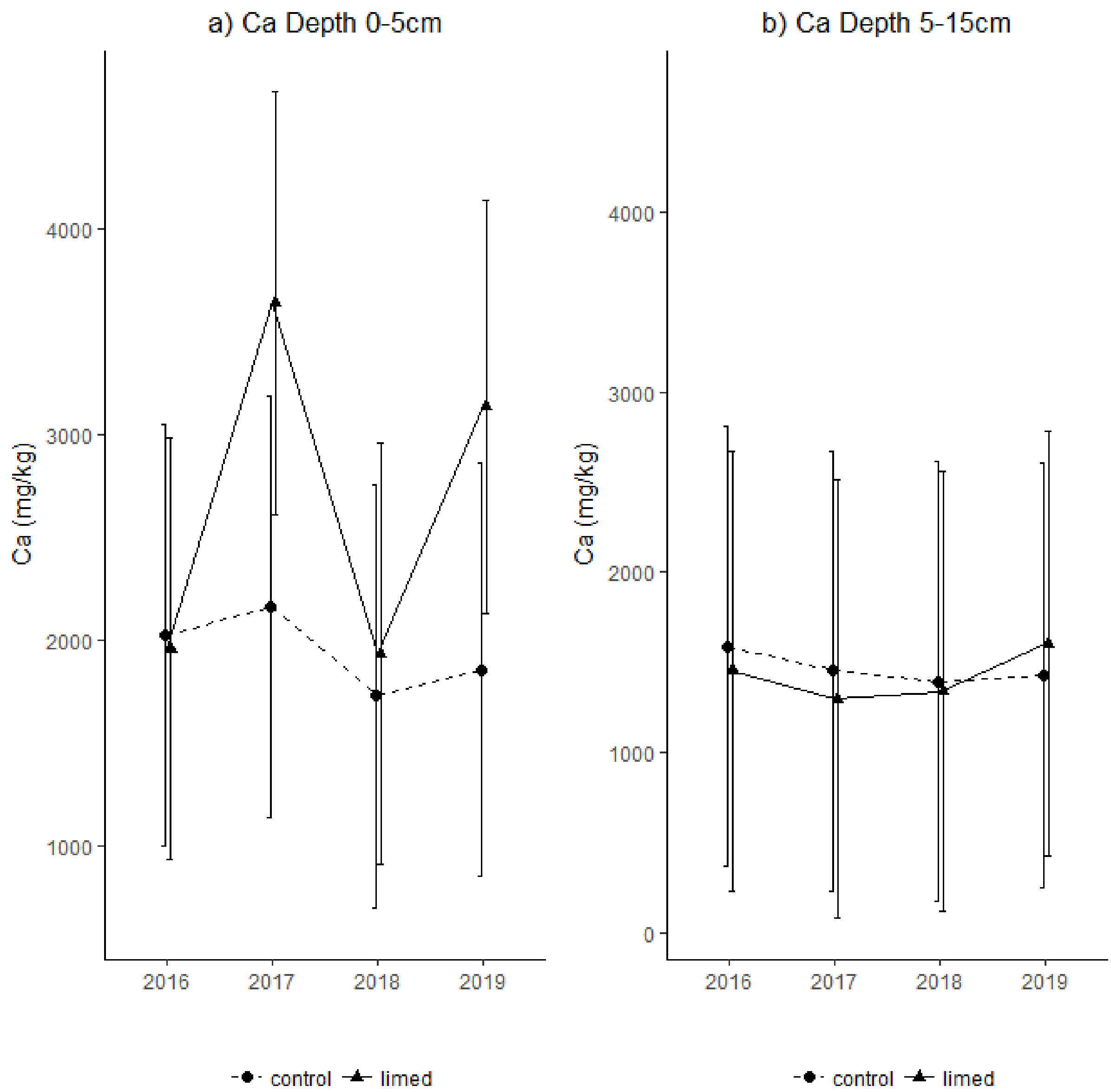


Figure 3. Estimated means and CI from linear mixed-effects models for a) soil Ca 0-5cm depth and b) soil Ca 5-15cm depth.

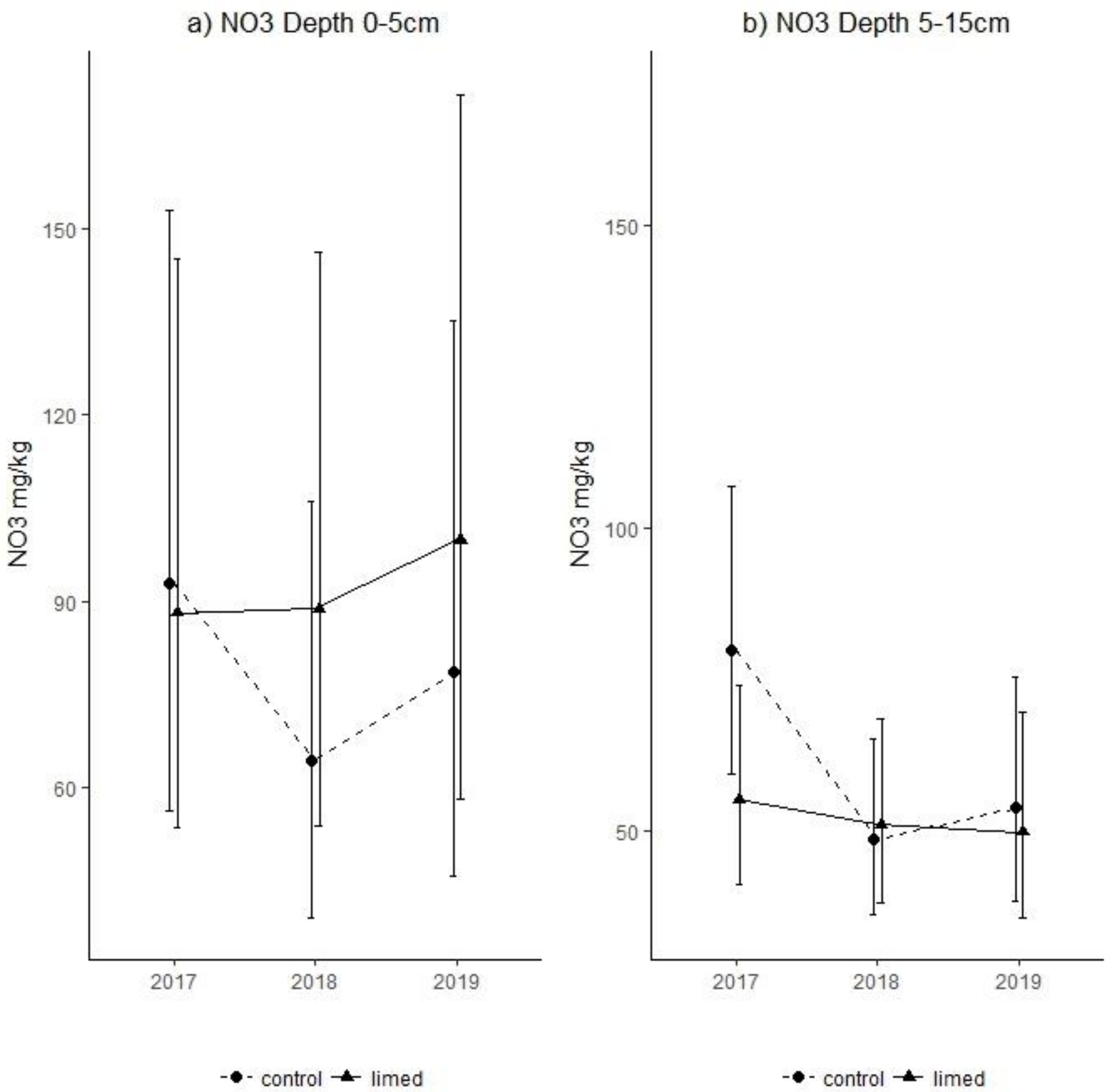


Figure 4 Estimated means and CI from linear mixed-effects models for a) soil NO_3^- 0-5cm depth and b) soil NO_3 5-15cm depth.

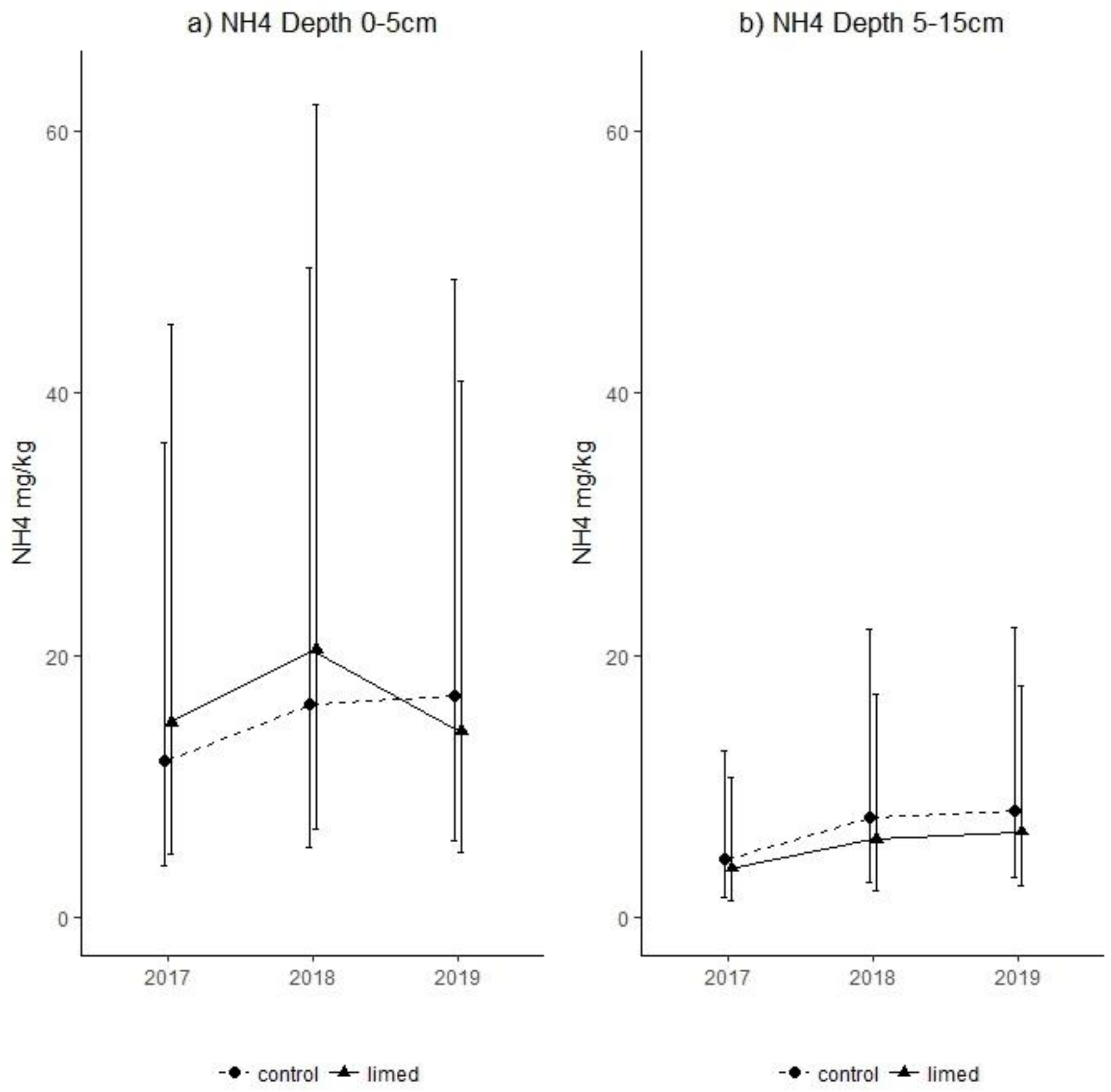


Figure 5 Estimated means and CI from linear mixed-effects models for a) soil NH_4^+ 0-5cm depth and b) soil NH_4^+ 5-15cm depth.

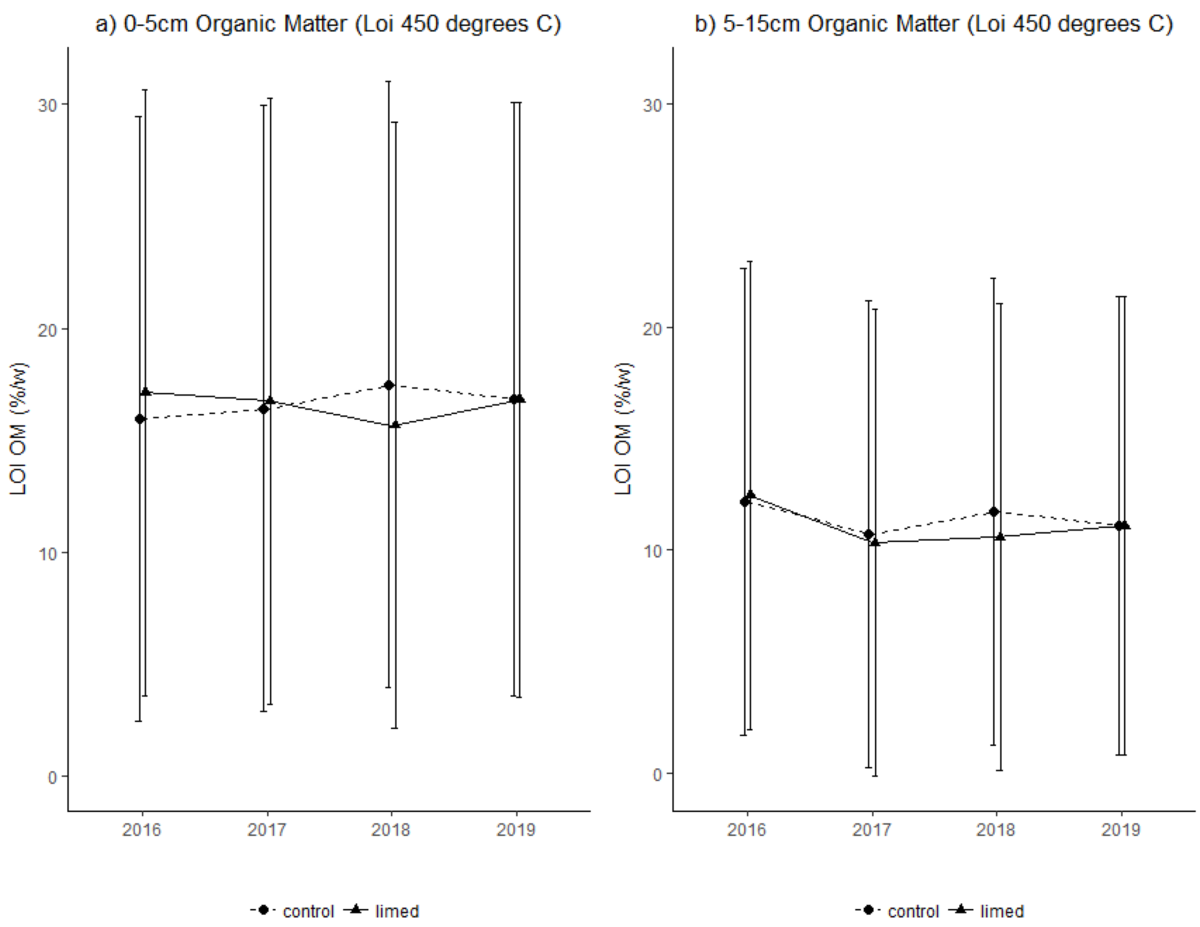
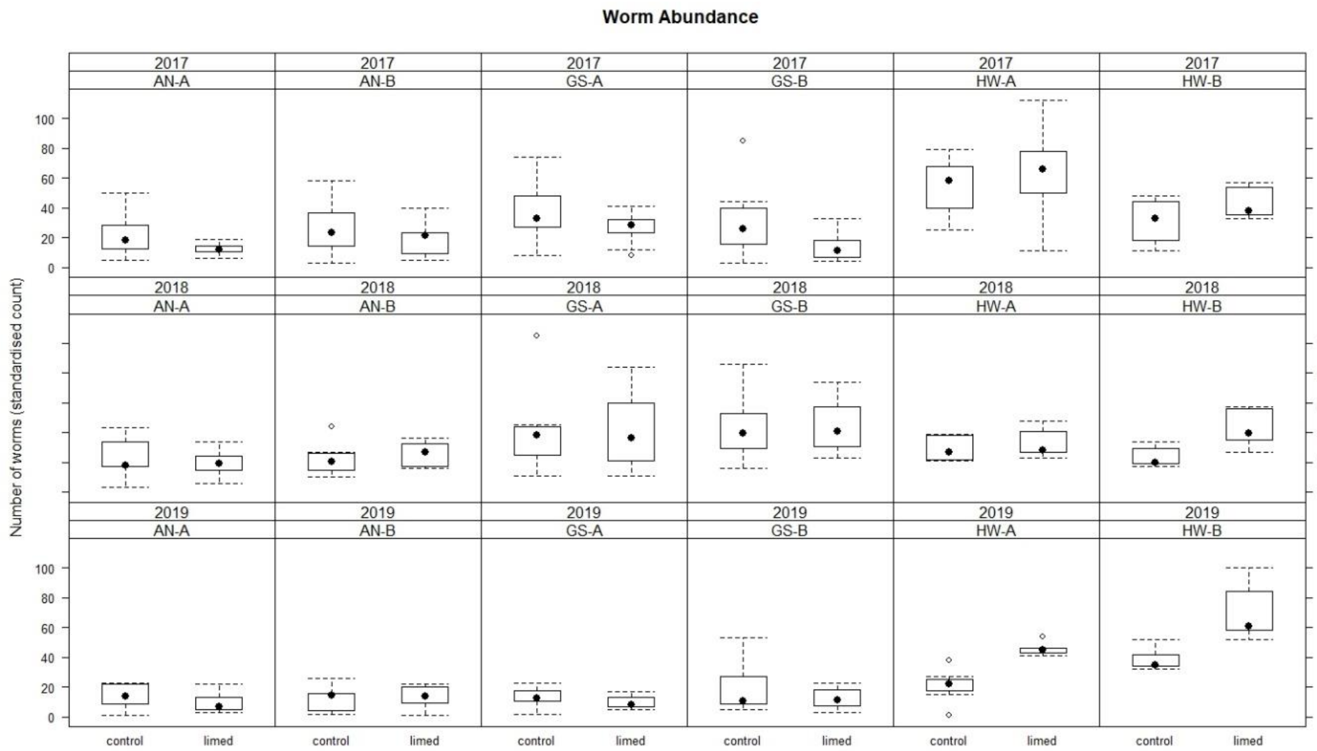


Figure 6. Estimated means and CI from linear mixed-effects models for loss on ignition for samples; a) 0-5cm depth, and b) 5-15cm depth.

a)



b)

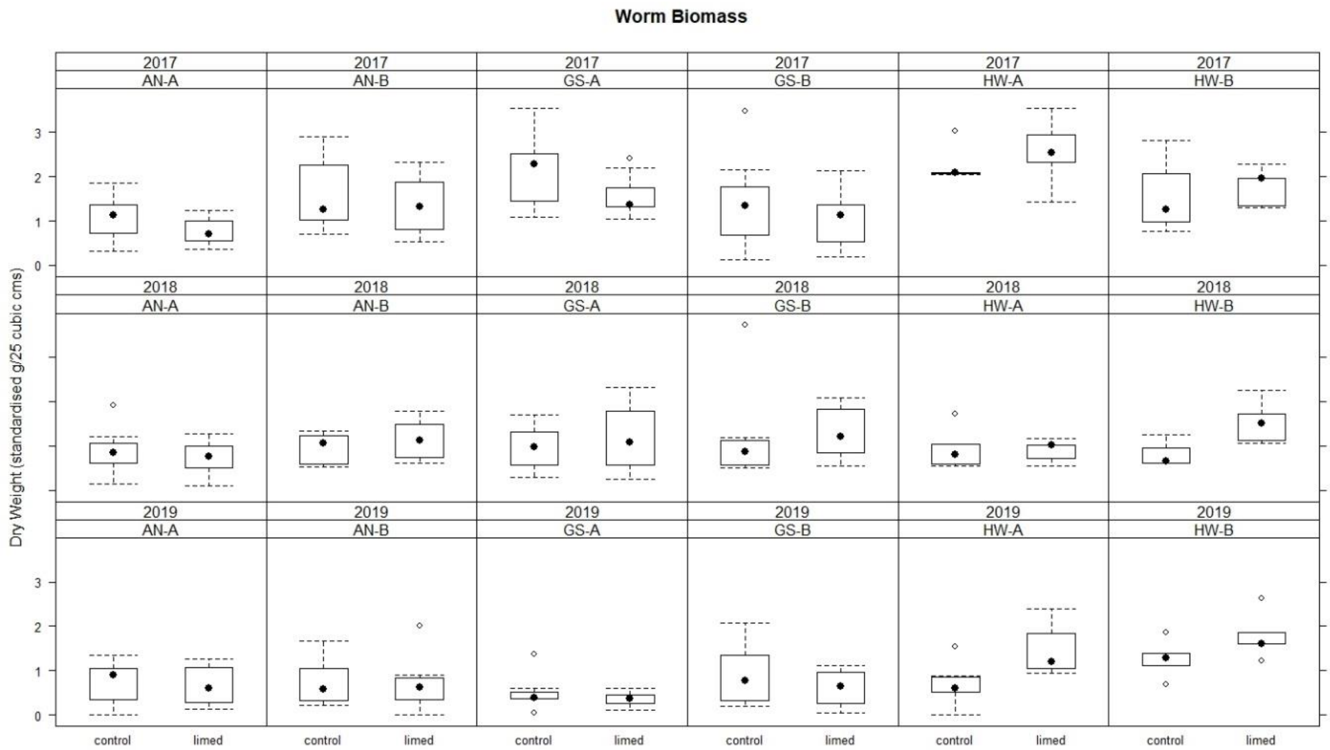


Figure 7. Box-plots of standardised; a) worm counts – worms per 25 cm^{-3}, and b) worm dry mass for each field and year (g 25 cm^{-3}). Box-plots show the median (black closed-circle), 25 and 75% quartiles (box), and 1st and 99th percentiles of each variable based on empirical data. Counts and dry mass are standardised to 25 cm^3 to allow comparison between sample pits of different depth (< 25 cm).

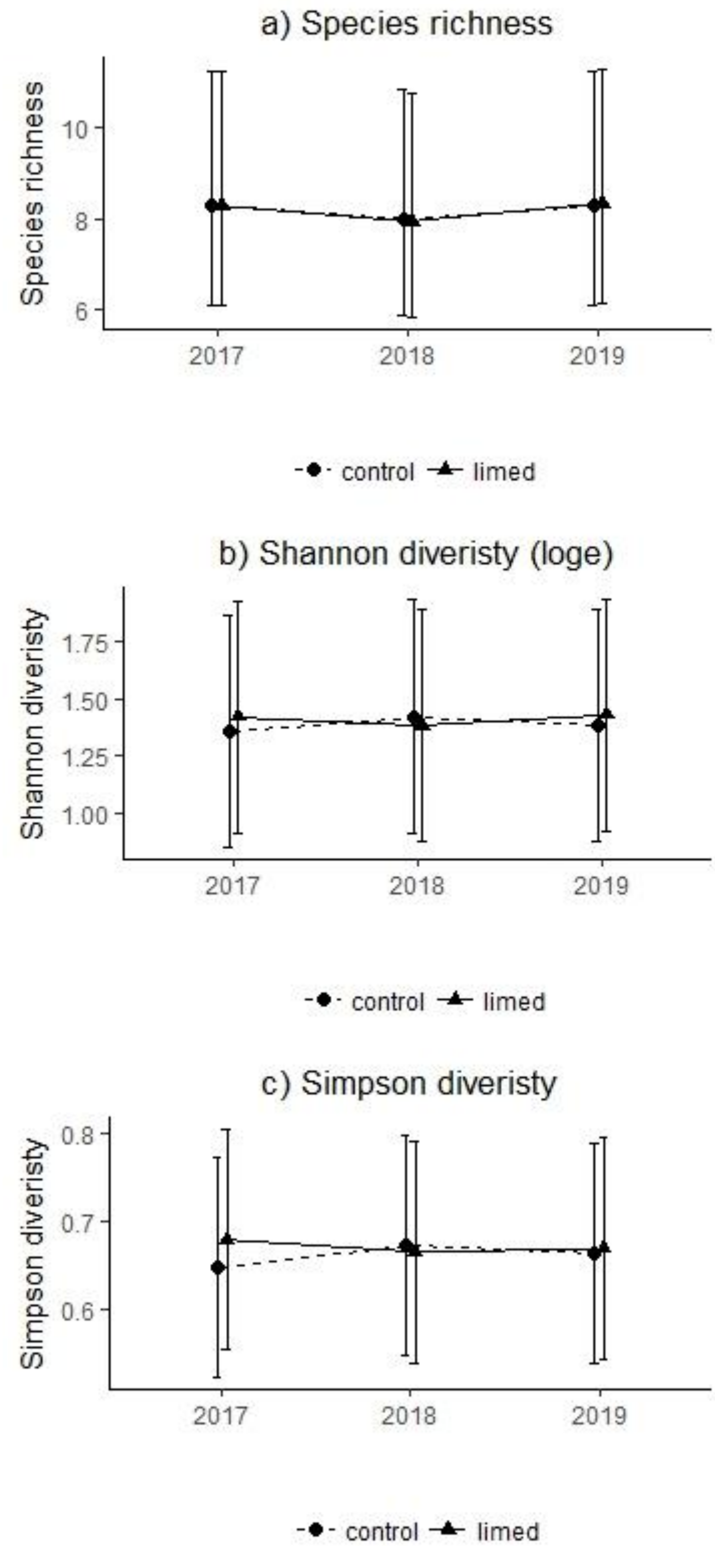


Figure 8. Estimated means and CI from the generalized linear mixed-effects model for a) Species richness and estimated means and CI from linear mixed-effects models for b) Shannon diversity calculated with Loge and c) Simpson diversity

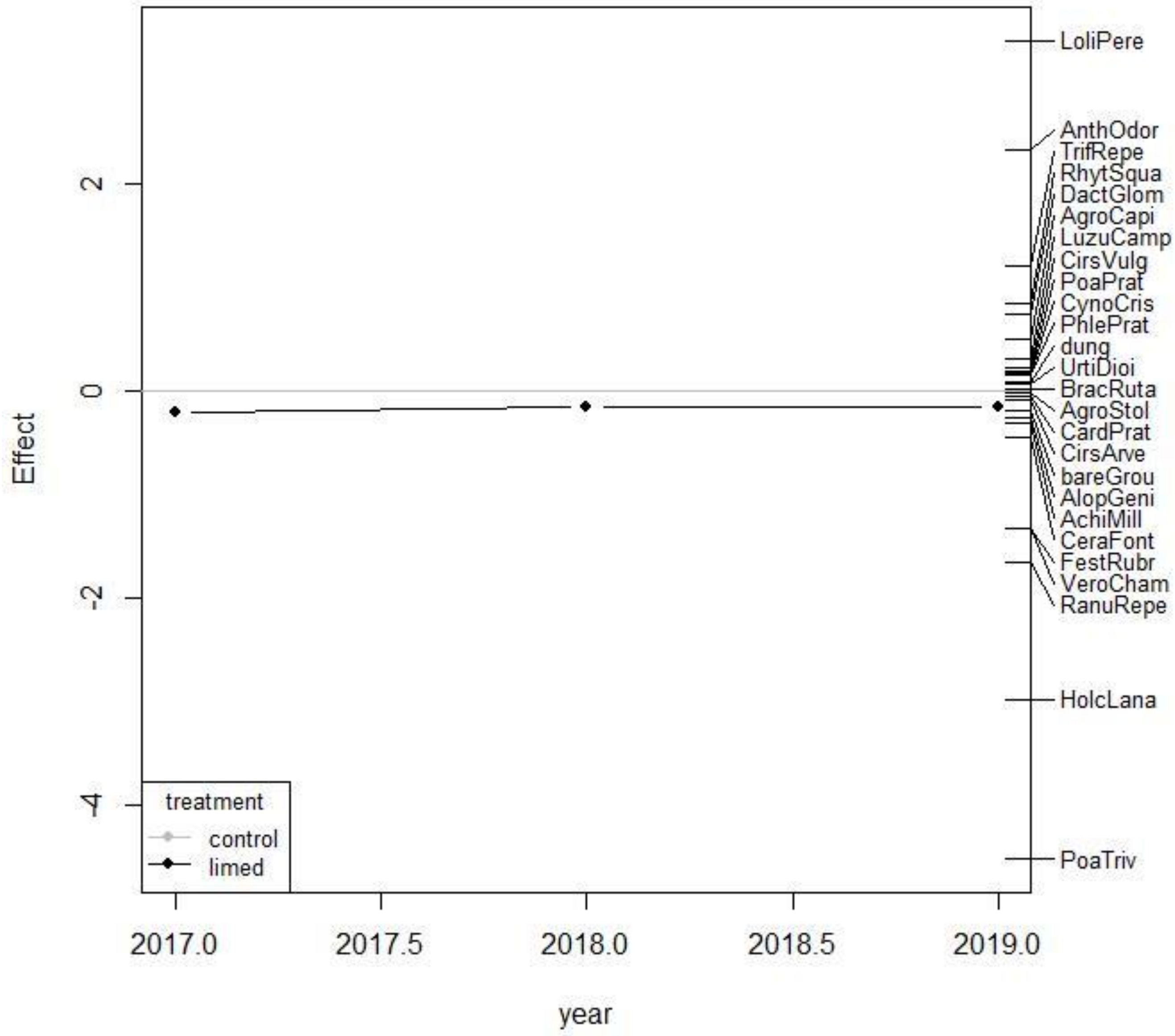


Figure 9: Principle response curve showing the effect of liming over 3 years (only those species with a sum of percentage covers over 30 are displayed for clarity).

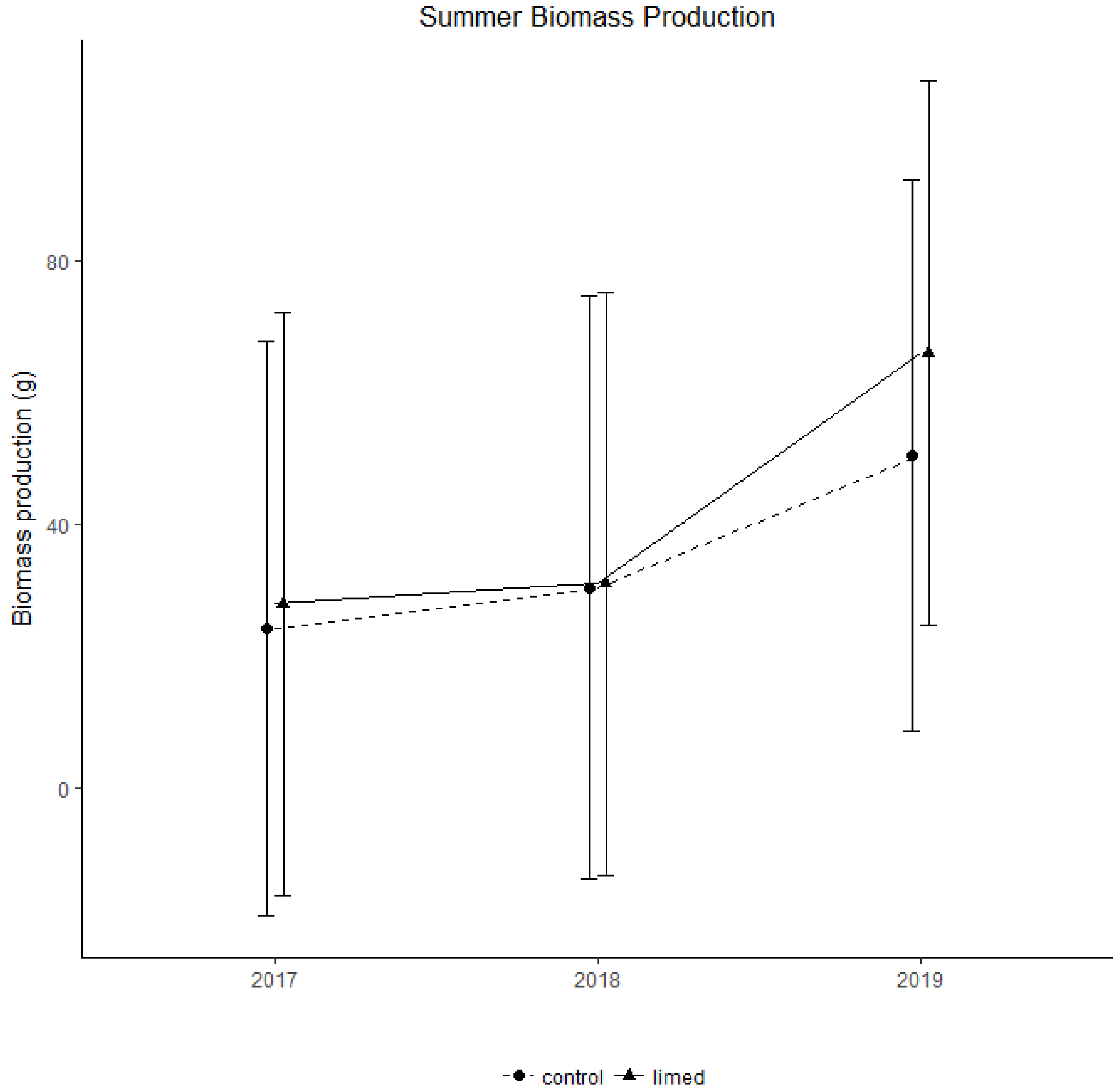


Figure 10. Estimated means and CI from linear mixed-effects models for summer biomass production (Mar-Jul/Aug)

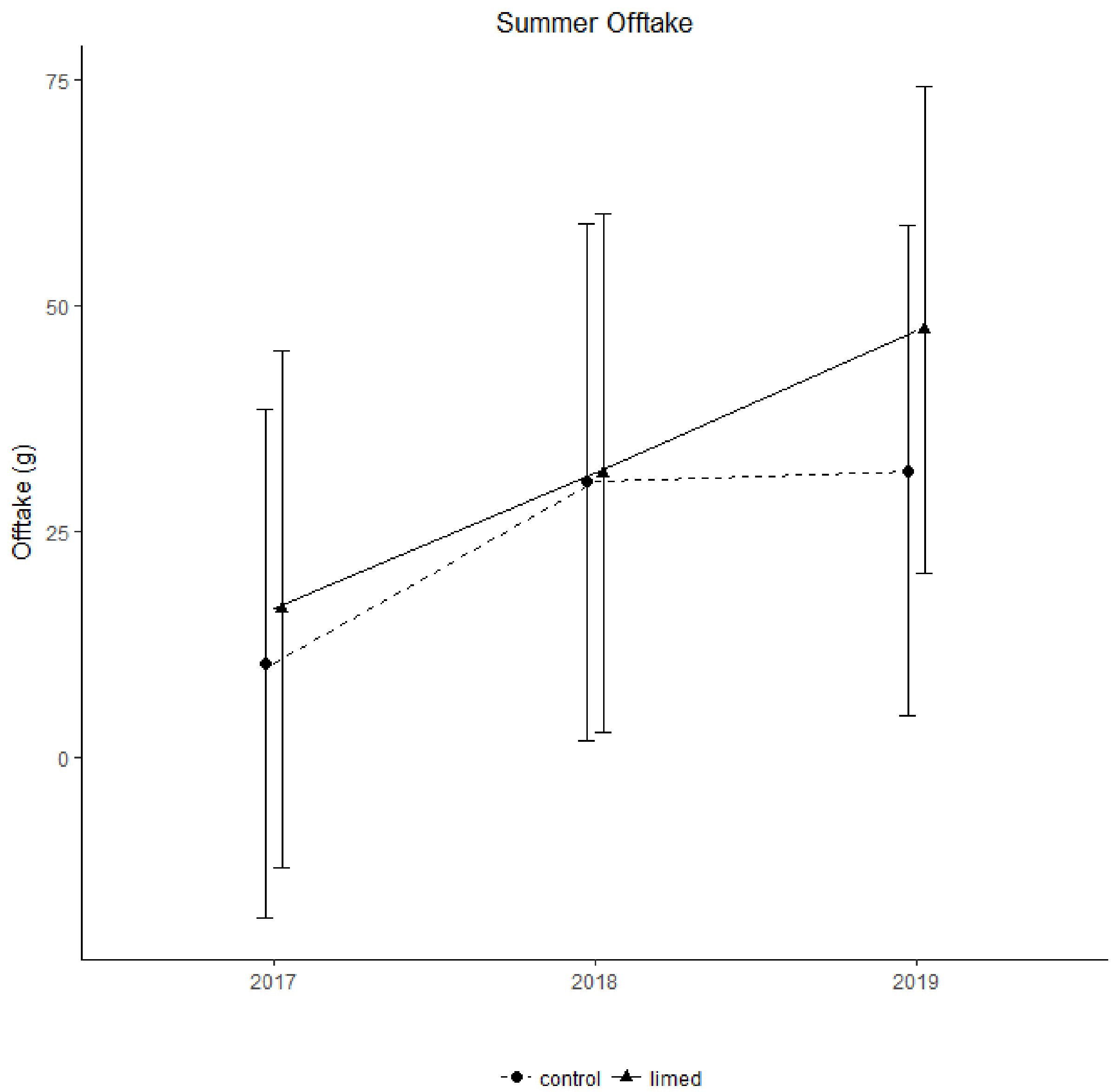


Figure 11. Estimated means and CI from linear mixed-effects models for summer vegetation off-take (Mar-Jul/Aug)

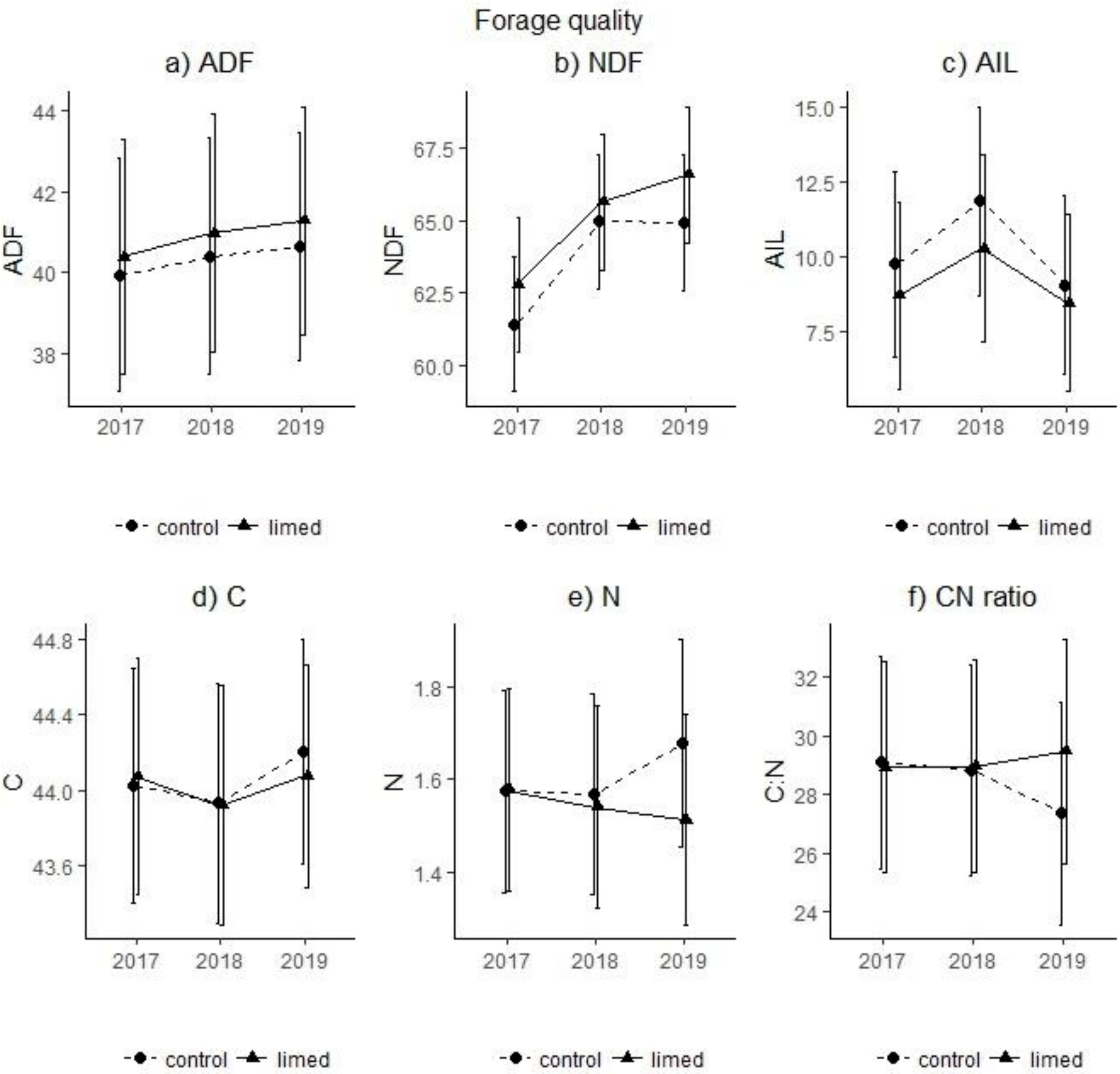


Figure 12. Estimated means and CI from linear mixed-effects models for a) ADF, b) NDF, c) AIL, d) carbon, e) nitrogen and f) C:N ratio

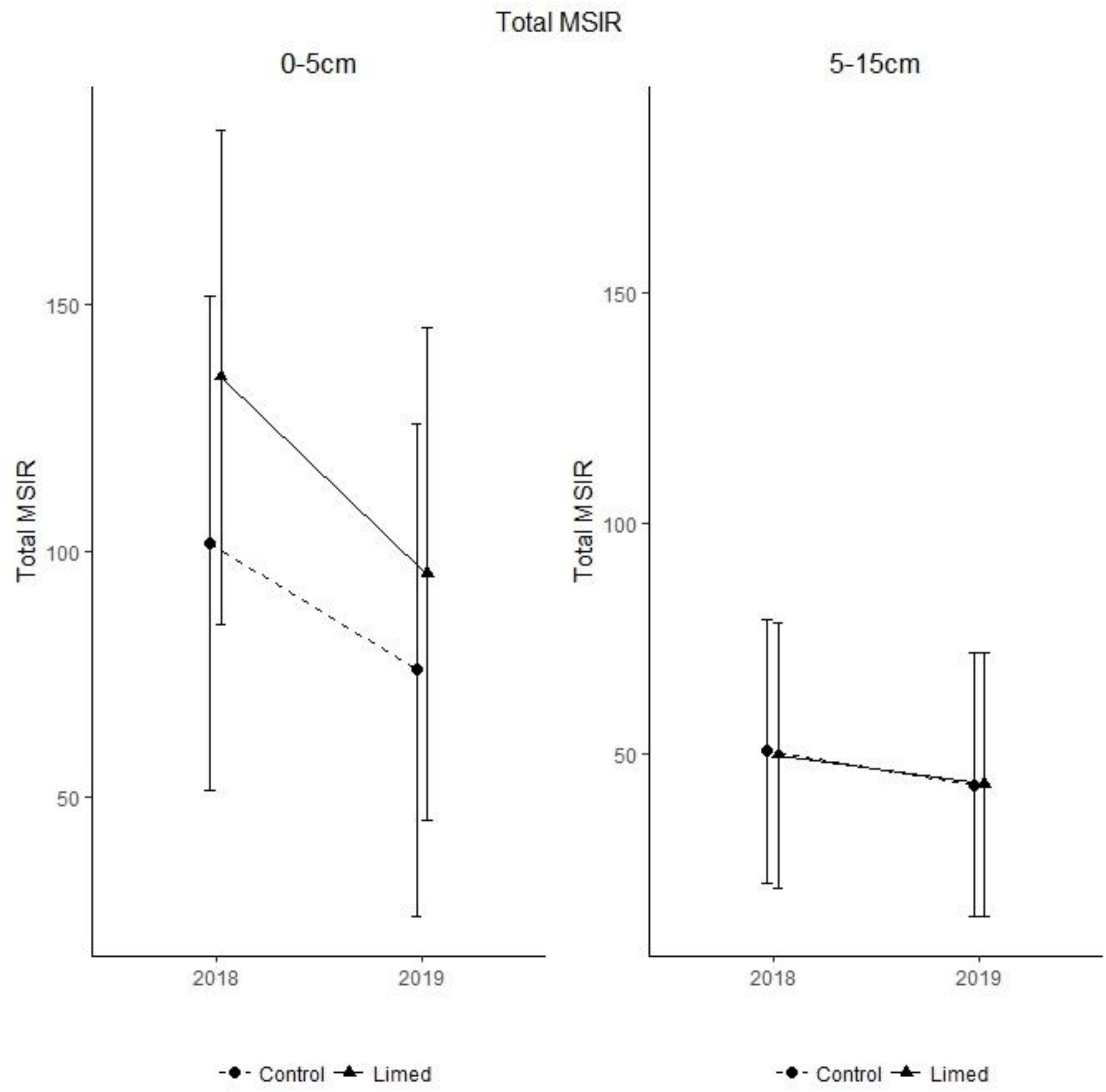


Figure 13. Estimated means and CI from linear mixed-effects models for total multi-substrate induced respiration (MSIR).