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Interactions between climate warming and land management regulate greenhouse gas fluxes in a temperate grassland ecosystem

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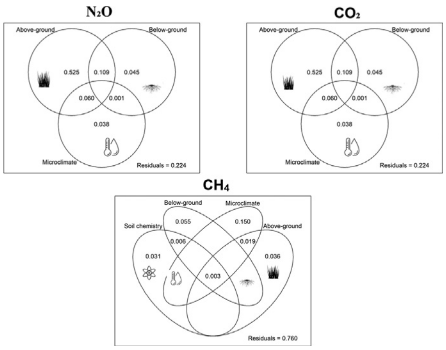
HIGHLIGHTS

- Warming and grassland management affect GHG.
- N-addition with cutting increased N_2O fluxes and reduced CO_2 fluxes.
- Warming and N-fertiliser increased CO_2 fluxes and reduced N_2O fluxes.
- CO_2 fluxes explained by above-ground, microclimate and below-ground metrics.
- N_2O fluxes explained by above-ground, microclimate and soil chemistry metrics.

GRAPHICAL ABSTRACT



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ABSTRACT

Greenhouse gas (GHG) fluxes from grasslands are affected by climate warming and agricultural management practices including nitrogen (N) fertiliser application and grazing. However, the interactive effects of these factors are poorly resolved in field studies. We used a factorial in situ experiment - combining warming, N-fertiliser and above-ground cutting treatments - to explore their individual and interactive effects on plant-soil properties and GHG fluxes in a temperate UK grassland over two years. Our results showed no interactive treatment effects on plant productivity despite individual effects of N-fertiliser and warming on above- and below-ground biomass. There were, however, interactive treatment effects on GHG fluxes that varied across the two years. In year 1, warming and N-fertiliser increased CO_2 and reduced N_2O fluxes. N-fertilised also interacted with above-ground biomass (AGB) removal increasing N_2O fluxes in year one and reducing CO_2 fluxes in year two. The grassland was consistently a sink of CH_4; N-fertilised increased the sink by 45% (year 1), AGB removal and warming reduced CH_4 consumption by 44% and 43%, respectively (year 2). The majority of the variance in CO_2 fluxes was explained by above-ground metrics (grassland productivity and leaf dry matter content), with microclimate (air and soil temperature and soil moisture) and below-ground (root N content) metrics also significant. Soil chemistry (soil mineral N and net mineralisation rate), below-ground (specific root length) and microclimate (soil moisture) metrics explained 49% and 24% of the variance in N_2O and CH_4 fluxes, respectively. Overall, our work demonstrates the importance of interactions between climate and management as determinants of short-term grassland GHG fluxes. These results show that reduced cutting combined with lower inorganic N-fertilisers would constrain grassland C and N cycling and GHG fluxes in warmer climatic conditions. This has implications for strategic grassland management decisions to mitigate GHG fluxes in a warming world.

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1. Introduction

According to the latest report by the Intergovernmental Panel on Climate Change (IPCC, 2021), the average global surface temperature is likely to rise by 1.5 to 4.4 °C by 2100. Consequently, it is expected that increases in soil temperature and reduced soil moisture (Brzostek et al., 2012) will affect the length of the growing season (Post et al., 2009) and thus plant productivity. These changes are likely to affect carbon (C), and nitrogen (N) cycling and greenhouse gases (GHGs) fluxes: carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4) from terrestrial ecosystems.

These changes are particularly important for the sustainability of agricultural ecosystems such as grasslands that underpin global food security and soil C sequestration (De Deyn et al., 2008; Erb et al., 2016; Garnett, 2009). In order to meet the growing demand for food, sustainable grassland management practices are needed to increase yields while reducing environmental impacts (Taube et al., 2014). Intensification through increases in N-fertiliser addition and the frequency of cutting are known to affect plant-soil nutrient cycling with feedbacks to productivity and GHG fluxes (Garnett, 2009). Global changes including climate warming are likely to interact with these grassland management practices with uncertain outcomes. Improved understanding of the mechanistic responses of grassland plant-soil systems to these interactions and the potential for synergistic or antagonistic effects is therefore critical.

The application of mineral N-fertiliser as a means to raise yields is standard practice in many temperate grassland ecosystems (Kidd et al., 2017; Lee et al., 2010). However, there are environmental impacts as N additions can augment soil N_2O fluxes by stimulating microbial nitrification and denitrification processes (Ussiri and Lal, 2012). Many factors influence these N_2O-formation processes. Temperature regulates microbial N_2O formation and N-mineralisation rates (Cantarel et al., 2012), while changes in soil water-filled pore space (WFPS) affect oxygen availability to microbes involved in nutrient cycling and N_2O production. It also affects the gas diffusion rates with implications for N_2O consumption in the soil. Climate warming can change both soil temperature and soil water content with potential to affect soil N availability. Increases in temperature can accelerate N mineralisation with synergistic interactive effects on plant available N (Rustad et al., 2001) and N_2O production. The application of N-fertiliser may also alter C cycling by increasing soil microbial CO_2 production (Melillo et al., 2011) or decreasing it (due to increased biomass production) to affect soil C stocks (van Groenigen et al., 2006). However, studies have found large variations in the effects of N addition; with increases or decreases in CO_2 fluxes (depending on N-fertiliser forms and application rates) (Zhu et al., 2016) or no effects (Ambus and Robertson, 2006). The combined effect of N and warming can increase respiration rates (Zhao et al., 2017) due to raised metabolic activity (Graham et al., 2014). Fluxes of CH_4 can also be affected by increases in N availability, decreasing CH_4 uptake by soils (Zhang et al., 2017). Warming effects on CH_4 are rarely studied: decreased CH_4 uptake in semi-arid rangelands was reported (Dijkstra et al., 2011) and also observed early in the growing season in a multifactor grassland experiment (Blankinship et al., 2010). Although few studies have examined the influence of individual effects of warming and N addition (Graham et al., 2014; Jiang et al., 2010), their interactive effects on grassland GHG fluxes remain uncertain.

Removal of above-ground biomass through grazing or mowing (e.g. haycut) is an intrinsic part of temperate grassland management which significantly affects plant-soil properties and GHG fluxes as a result of changes to plant biomass and mineral inputs from animal excreta (Fetzel et al., 2017; Petz et al., 2014). Cutting or harvesting the sward has been shown to accelerate plant regrowth and enhance root exudation (Leriche et al., 2001) with impacts on N cycling (Gusewell et al., 2005). This, in turn, can affect soil organic matter dynamics to liberate mineral N with feedbacks to plant productivity (Hamilton and Frank, 2001; Yoshitake et al., 2015), and N_2O fluxes. The grass cover percentage is also known to affect N_2O fluxes (Chirinda et al., 2019). Cutting can also affect C cycling and photosynthetic capacity, decreasing CO_2 fluxes and affecting plant C allocation (Bahn et al., 2008) by decreasing root biomass. Furthermore, the interactive effect of cutting and N addition may increase N_2O fluxes, due greater soil N availability, and increase CO_2 fluxes due to greater plant photosynthate production and root exudation triggering soil microbial activity (Bahn et al., 2006; Guitian and Bardgett, 2000). The effect of cutting on CH_4 uptake is mostly dependent on changes in evaporation and transpiration rates which alters soil water content (Wang et al., 2015). Lower soil moisture is likely to increase microbial methanotroph activities, stimulating CH_4 uptake (Dijkstra et al., 2012). However, it is possible that cutting and N addition interactions may increase mineral N availability in the soil, decreasing CH_4 uptake (Täumer et al., 2021). The interactive effect of warming and cutting in grassland soils depends on frequency (Zhou et al., 2007), affecting soil properties (Bahn et al., 2006), and the microclimate (Luo et al., 2001). Both warming and cutting may also affect nutrient mineralisation directly affecting GHG fluxes.

Most studies have investigated single factors despite the likelihood that multiple drivers operate concurrently. Considering the important role of managed grasslands in producing food and sequestering C under a changing climate it is important to determine whether the combined effects of multiple factors counteract or strengthen one another as regulators of plant-soil C and N cycling and ecosystem GHG fluxes.

The aim of this study was to investigate and quantify how climate warming and temperate grassland management, specifically N addition and above-ground biomass (AGB) removal, interact to affect plant-soil properties and ecosystem GHG fluxes. It was hypothesised that: H1) N addition and warming interact synergistically to increase plant productivity, N_2O and CO_2 fluxes; H2) above-ground biomass (AGB) removal and N addition interact antagonistically to decrease plant productivity and decrease N_2O and CO_2 fluxes due to C-limitation; and H3) AGB removal and warming interact antagonistically to reduce root productivity and diminish overall GHG fluxes, lowering CO_2 and N_2O fluxes. To test these hypotheses a two year field experiment was conducted with a full factorial design including interactions between warming, N addition and AGB removal. The interactive effects of these treatments on plant-soil C and N cycling were determined through the measurement of above- and below-ground plant productivity, soil properties and GHG fluxes over two years.

2. Material and methods

2.1. Study site

The experimental site was located at Lancaster University, Lancaster, UK (54° 1′50” N, 2.7° 46′30”W, 94.1 m a.s.l.) adjacent to Hazelrigg Weather Station. This site is a 61 ha area of permanent unfertilised grassland intermittently grazed by sheep and used as a hay meadow. The site was not grazed by sheep for one year prior to the treatments being imposed or during the experimental years. The site is under maritime temperate climatic conditions, with mean annual temperature of 13 °C and mean annual precipitation of 1049 mm between 1981 and 2010. The soil is semi-permeable, seasonally wet, acidic, loamy and clayey according to the National Soil Resources Institute (NSRI), UK soil classification survey (Farewell et al., 2011). Initial analyses of the properties of the upper 10 cm of the soil profile were: total N content 0.3%, total C content 3.5%, C/N ratio of 12, available P of 24 mg kg^{-1}, K of 121 mg kg^{-1}, pH of 5.3 and bulk density of 1.06 g cm^{-3}.

2.2. Experimental design

The field experiment used a full-factorial design to test the interactive effects of warming, N addition and AGB removal totalling eight treatment combinations with five replicates across five blocks. The treatments consisted of: soil control, warming, N addition, AGB removal and their interactions N + warming, AGB removal + N, AGB removal + warming, and AGB removal + N + warming. Each block is comprised of 25 plots (9 m^2) in a 5 × 5 grid and 1 m between plots. For this study, four plots per block were randomly selected and split to give eight nested treatments (Fig. S1).

The warming treatment was accomplished using open-top passive conical chambers with an upper opening of 0.66 m, base diameter of 1.12 m and a height of 0.40 m - based on the International Tundra Experiment design (ITEX) (Marion et al., 1997). The transparent material was 2 mm thick polycarbonate sheet (Polycarbonate Shop, Broughton Astley, UK) which allows 92% of the photosynthetically active radiation. The ITEX warming chambers were installed in the field one month prior to the beginning of measurements in April 2015.

N addition was applied in May (Spring) as ammonium nitrate (NH_4NO_3) at a rate of 100 kg N ha^{-1} y^{-1} (consistent with typical grassland management recommendations for hay meadows in the UK). For each N addition plot, the fertiliser was dissolved in 5 L deionised water and applied using a watering can over both plants and soil. NoN plots received an equivalent amount of deionised water only. N was applied in solution to allow an even spread of the fertiliser to the small experimental plots.

AGB removal was achieved by cutting 2 cm above-ground level and removing the plant biomass when it reached 5 cm height (i.e. by continuous cutting during the growing season over six cuts - May until October in 2015 and 2016). The repeated cuttings was to test the effect of plant removal (either by harvesting and/or grazing) on C and N cycling. There is no deposition of dung/urine or effect of animal trampling in the soil, so the treatment does not simulate grazing completely.

2.3. Greenhouse gas fluxes

A closed static chamber method was used to measure GHG concentration (CO_2, N_2O and CH_4) (Ward et al., 2009). A 30 cm diameter, 20 cm high gas sampling base ring was fitted in place to 5 cm soil depth one month prior to the measurements. For each flux measurement, the opaque chamber was attached to the base ring and 20 mL of chamber air was sampled via a septum using a syringe and needle. Samples were taken after 0, 15, 30 and 45 min with 10 mL of the chamber air transferred into a pre-evacuated 3 mL exetainer vial (Labco, Lampeter, UK). Samples were analysed using a PerkinElmer AutoSystem XL Gas Chromatograph (GC) (PerkinElmer, Waltham, MA, USA) with a Flame Ionisation Detector fitted with a methaniser and Electron Capture Detector operating at 360 °C. The GC was fitted with a stainless steel Porapak Q 50–80 mesh column (length 2 m, outer diameter 3.17 mm) maintained at 60 °C. All results were calibrated against certified gas standards (Air Products, Waltham on Thames, UK) (Case et al., 2012). Gas fluxes were calculated by fitting linear regressions through sampling time points and corrected for temperature and barometric pressure following the Clapeyron ideal gas law (Holland et al., 1999): $pV = nRT$, which relates absolute pressure p to absolute temperature T, with volume V of the chamber and the amount n (in moles) of gas, and R being the molar gas constant. N_2O and CH_4 fluxes were converted into CO_2-equivalente (CO_2eq) according to their global warming potentials (GWP100) of 25 and 298, respectively (Forster et al., 2007).

Plot microclimate conditions were recorded at each sampling; air and soil temperature (at 5 cm below and above soil surface, respectively) were recorded inside the chambers using a Tiny Tag temperature logger with integral stab probe (Gemini Data Loggers, UK) and soil moisture with a ML2x Theta Probe and Meter HH2 (Delta T Devices, UK). GHG gas samples were taken immediately after N application in May (approximately 9 a.m.), daily in week one, then twice a week in week two followed by every month until October for both experimental years. GHG fluxes were measured from the beginning of May 2015 until October 2016.

2.4. Soil sampling and analyses

Three soil cores (diameter = 1 cm, depth = 4.5 cm) were taken from each replicate treatment plot for the mineral N and total C and N analysis (as the experimental plot was small, soil sampling was done at 4.5 cm depth to avoid disturb the plants-soil system too much). Soil samples were taken on days 3, 32 and 72 after N application in 2015 and on days 6, 14 and 63 in 2016 (May, June and July 2015/2016). Soil gravimetric moisture content was determined after drying at 105 °C for 24 h. Water-filled pore space was calculated by the ratio of volumetric soil water content to total soil porosity. Mineral N (NH_4^+ + NO_3^-) was assessed with 1 M KCl in a 1:5 (soil weight: extractant volume) ratio extraction by analysis with a spectrophotometer (Auto Analyser 3 Digital colorimeter BRAN + LUEBBE). Net mineralisation (net NH_4^+ + NO_3^- production) and net nitrification (net NO_3^- production) rate were determined by incubating the soil at 25 °C for 14 days analysing the final mineral N content as described above, then calculating the daily mineral N production rate as the difference between final and initial N content, divided by the incubation period. Soil C and N were determined on dried, finely ground soil samples, using an elemental analyser (TruSpec® CN, St. Joseph, MI) with furnace temperature at 950 °C.

2.5. Plant and root sampling and analyses

For the AGB removal treatment (i.e. continuous cutting during the growing season), plant matter within the GHG measurement chamber was cut on days 8, 22, 42, 72, 120 and 156 (in 2015) and 6, 14, 35, 62, 109 and 155 (in 2016) after N application.

On day 72 and 63, in 2015 and 2016 respectively, all plots were harvested to simulate hay meadow management and plant tissue samples were dried at 70 °C, weighed, ground and analysed for total C and N content using an elemental analyser (TruSpec® CN, St. Joseph, MI) at furnace temperature 950 °C. Leaf traits were assessed by measuring plant height, leaf dry matter content (LDMC), leaf N content (LNC), leaf C content (LCC) and leaf C/N ratio according to Pérez-Harguindeguy et al. (2013).

On the day of the final harvest in each year, a soil core (diameter = 5 cm, depth = 10 cm) was taken from each plot to determine the below-ground biomass after washing all roots. Before drying the roots at 105 °C for 24 h to determine the biomass, roots were stored in the fridge with 10% ethanol solution to measure the following root traits: specific root length (SRL), root dry matter content (RDMC), root N content (RNC), root C content (RCC) and root C/N ratio according to Pérez-Harguindeguy et al. (2013). Root length and diameter were analysed using WinRhizo® root analysis software (Regent Instruments Inc., Sainte-Foy-Sillery-Cap-Rouge, QC, Canada) coupled to an Epson flatbed scanner. Root total C and N content were determined on dried root samples, ground and analysed using an elemental analyser (TruSpec® CN, St. Joseph, MI) with furnace temperature at 950 °C.

2.6. Statistical analyses

Linear mixed effects models (LME) were used to test the interactive effects of warming, N addition and AGB removal on microclimate (air temperature, soil temperature and soil moisture), plant productivity (above- and below-ground biomass and root/shoot ratio) and GHG fluxes (CO_2, N_2O and CH_4). Fixed effects were warming, N addition and AGB removal and their interactions. The random effect was split-plot nested within block to take account of the experimental split-plot design. For all LME models, data were checked for normality and equal variances using the residual plots method and log-transformed where necessary before analysis. Weight functions were used to account for unequal variances following Zuur et al. (2011). The significance of the fixed effects was determined by comparing models with and without the factor of interest using a likelihood ratio test (LRT). All statistical analyses were made using R programming language 3.4.3 (R Development Core Team, 2017) with additional packages *nlme* (Pinheiro et al., 2019) and *plyr* (Wickham, 2011).

Warming, N addition and AGB removal treatments affected microclimate, soil C and N cycling and plant productivity metrics with consequences for GHG fluxes. To understand how changes in these properties influenced GHG fluxes we used multiple regressions to estimate the variance in GHG fluxes explained by these variables individually and in broad groups (above-ground, below-ground, soil chemistry and microclimate metrics). Firstly, data were checked for collinearity using the variance inflation factor (VIF) and scatterplots. Collinear variables were removed from the analysis. Model selection was applied using the remaining variables in both forward and backward selection searching for the lowest AIC (Akaike information criterion). The best-fitted model for each GHG was then checked for normality using a residual plot method. Metrics were included in broad groups (i.e. above-ground, below-ground, soil chemistry and microclimate) and the variation partition was conducted with the R package *vegan* (Oksanen et al., 2017) to determine which groups of variables explained CO_2, N_2O and CH_4 fluxes.

3. Results

3.1. Local climate

Climate data from the Hazelrigg weather station showed that during measurement periods, mean air and soil temperature were similar in both years (approx. 10 °C), but it was wetter in 2015 (total rainfall of 1332 mm and 1193 mm in 2015 and 2016, respectively). The growing season between May and October was drier in 2016 than in 2015 (Fig. S2).

3.2. Treatment effects on plot microclimate

Mean air temperature increased by 2.3 and 2.6 °C in 2015 and 2016 in the warming plots relative to the non-warmed plots ($P < 0.0001$; $P < 0.0001$, Fig. 1, Table S1). In both years, there was a significant interaction between AGB removal and warming ($P < 0.0001$; $P < 0.0003$, Table S1); warming increased soil temperature when AGB was removed. In both years, N addition significantly affected soil temperature independently of warming or AGB removal in both years ($P = 0.02$, $P = 0.002$, Table S1). There was a significant three-way interaction between warming, N addition and AGB removal on soil moisture in both years; the effect of warming on soil moisture varied whether the N addition or AGB removal treatments were also imposed (Table S1). Warming increased soil moisture when plots were N fertilised in both years ($P = 0.02$, $P = 0.0007$, Table S1).

3.3. Plant productivity

There were no interactive effects of the treatments on AGB or below-ground biomass, however, there were significant individual treatment effects (Table 1). AGB was unsurprisingly reduced in the AGB removal plots by 64% and 68% in both years ($P < 0.0001$; $P < 0.0001$, respectively) (Fig. 2, Table 1). This decrease in AGB coincided with a small increase in below-ground biomass in 2015 with no significant effect in 2016. AGB increased 46% and 60% in response to N addition in both years ($P < 0.0001$; $P < 0.0001$, respectively) (Fig. 2, Table 1), but below-ground biomass was unaffected. The effect of warming was less consistent across the two years. Warming increased AGB by 3.7% in 2015 ($P = 0.001$) (Fig. 2, Table 1) with no effect in 2016, while below-ground biomass decreased with warming biomass by 16% in 2016 ($P = 0.0009$; $P = 0.001$), but was unaffected in 2015 (Fig. 2, Table 1).

Root/shoot ratio was affected by an interactive effect of the treatments in 2015, but not in 2016; AGB removal antagonistic interacted with warming decreasing root/shoot ratio, and when AGB removal interacted with N addition it increased root/shoot ratio ($P = 0.03$, $P = 0.02$, Fig. 2, Table 1). Warming-only and N addition-only decreased root/shoot ratio ($P = 0.002$, $P < 0.0001$), while AGB removal-only increased it in 2016 ($P < 0.0001$, Fig. 2, Table 1).

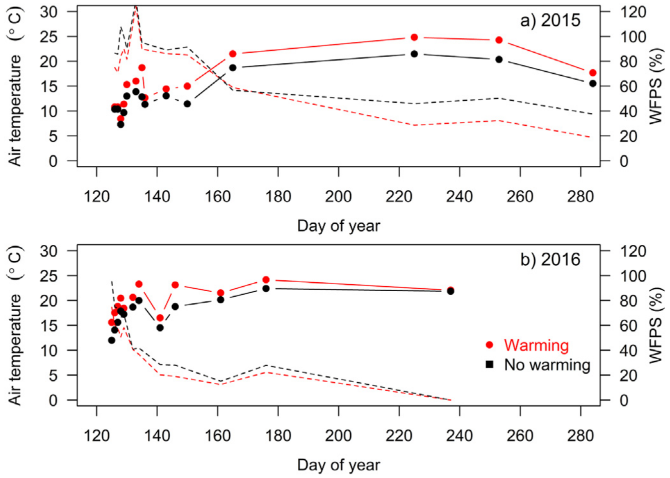

Fig. 1. Seasonal variation in the warmed and no-warmed plots. Mean air temperature (°C) represented by solid lines, and water-filled pore space (WFPS %) represented by dashed lines over a) 2015 and b) 2016. Statistical analysis reported in Table S1.

3.4. Greenhouse gas fluxes

Warming, N addition and AGB removal significantly affected CO_2 fluxes, however, different interactions were observed with N depending on the year. During the first year, there was a synergistic interaction between warming and N addition; the increase in CO_2 fluxes with warming was greater in the N fertilised plots in 2015, but not observed in 2016 ($P = 0.04$, Fig. 3, Table 2). In the second year, AGB removal interacted with N addition; the decrease of CO_2 fluxes with AGB removal was greater in N fertilised plots in 2016 ($P = 0.04$, Fig. 3, Table 2). CO_2 fluxes was reduced by 21% in AGB removal plots in 2015, while warming-only increased fluxes by 10% in 2016 ($P < 0.0001$, $P = 0.0006$, respectively) (Fig. 3, Table 2).

There were interactive effects of treatments on N_2O fluxes in the first year of the experiment. Warming decreased the effect of N addition, with an antagonist interaction reducing N_2O fluxes ($P = 0.04$, Fig. 4, Table 2). In addition, AGB removal synergistically interacted with N addition, with greater N_2O fluxes from the soil ($P = 0.02$, Fig. 4, Table 2). During the second year, only the main effects significantly affected N_2O fluxes; warming and N addition increased fluxes while AGB removal also increased them ($P = 0.01$, $P < 0.0001$, $P = 0.05$, Fig. 4, Table 2).

All grassland plots were consistent sinks of CH_4. No interactive effects were found for CH_4 fluxes. In the first year, N addition was the only treatment to significantly affect CH_4 fluxes, increasing the sink by 45% ($P = 0.01$, Fig. 5, Table 2). In year two, AGB removal-only and warming-only lower consumption of CH_4 by 44% and 43%, respectively ($P = 0.02$, $P = 0.04$, Fig. 5, Table 2).

3.5. Relationship between plant traits, climate and GHG fluxes

Using multiple regression, we investigated how plant productivity (above- and below-ground), leaf and root traits, soil chemistry and microclimate metrics explain changes in GHG fluxes. The best fit model describing CO_2 fluxes contained seven metrics (AGB, LDMC, root diameter, root N content, air temperature, soil temperature and soil moisture) explaining 77% of the variation (Table 3). Using variance partitioning, significant variables were divided into three groups (Above-ground: grassland productivity, LDMC; Below-ground: root diameter, root N content; and microclimate: air temperature, soil temperature and soil moisture). Variance of CO_2 fluxes can be mostly explained by above-ground metrics (52.5%, Fig. 6) with some shared variance with below-ground and microclimate metrics (10.9% and 6%, respectively, Fig. 6).

The best model for describing N_2O fluxes contained four metrics (soil mineral N, SRL, soil moisture and N mineralisation rate) and explained 49% of the variation (Table 3). Dividing variables into three groups (Soil chemistry: soil mineral N, mineralisation rate; Below-ground: SRL; Microclimate: soil moisture). Soil chemistry explained most of the variation (22%, Fig. 7) with shared variance explained by microclimate (8.9%, Fig. 7) and below-ground and microclimate metrics together (2.7%, Fig. 7).

Table 1

The effect of warming (warm), AGB removal and nitrogen addition (Nadd) on above-ground and below-ground biomass and root/shoot ratio over 2015 and 2016. Significance tests using likelihood ratio test (LRT) comparing models with or without parameter of interest where degree of freedom (d.f.) shows the difference in degrees of freedom between the models. Significant effect ($P < 0.05$) are shown in bold. Arrows represent the direction of the significance effects.

2015		Above-ground biomass g m^{-2}		Below-ground biomass g m^{-2}		Root/shoot ratio	
	d.f.	LRT	*P*	LRT	*P*	LRT	*P*
warm	1	**↑10.39**	**0.001**	2.88	0.09	0.48	0.49
Nadd	1	**↑22.06**	**<0.0001**	0.15	0.69	**11.89**	**0.001**
AGB removal	1	**↓66.63**	**<0.0001**	**↑11.09**	**0.001**	**67.59**	**<0.0001**
Warm *x* Nadd	1	1.20	0.27	0.30	0.58	1.88	0.17
AGB removal *x* Nadd	1	1.85	0.17	2.32	0.13	**5.34**	**0.02**
warm *x* AGB removal	1	0.04	0.84	2.16	0.14	**4.78**	**0.03**
warm *x* Nadd *x* AGB removal	1	0.76	0.38	3.34	0.07	0.17	0.67

2016		Above-ground biomass g m^{-2}		Below-ground biomass g m^{-2}		Root/shoot ratio	
	d.f.	LRT	*P*	LRT	*P*	LRT	*P*
warm	1	0.00	0.93	**↓10.76**	**0.001**	**↓9.18**	**0.002**
Nadd	1	**↑32.46**	**<0.0001**	0.12	0.73	**↓23.53**	**<0.0001**
AGB removal	1	**↓58.32**	**<0.0001**	1.98	0.16	**↑73.81**	**<0.0001**
Warm *x* Nadd	1	0.53	0.46	0.15	0.69	0.93	0.33
AGB removal *x* Nadd	1	3.76	0.06	0.03	0.87	2.91	0.09
warm *x* AGB removal	1	1.59	0.21	0.18	0.66	0.54	0.46
warm *x* Nadd *x* AGB removal	1	0.08	0.77	0.18	0.66	0.00	0.94

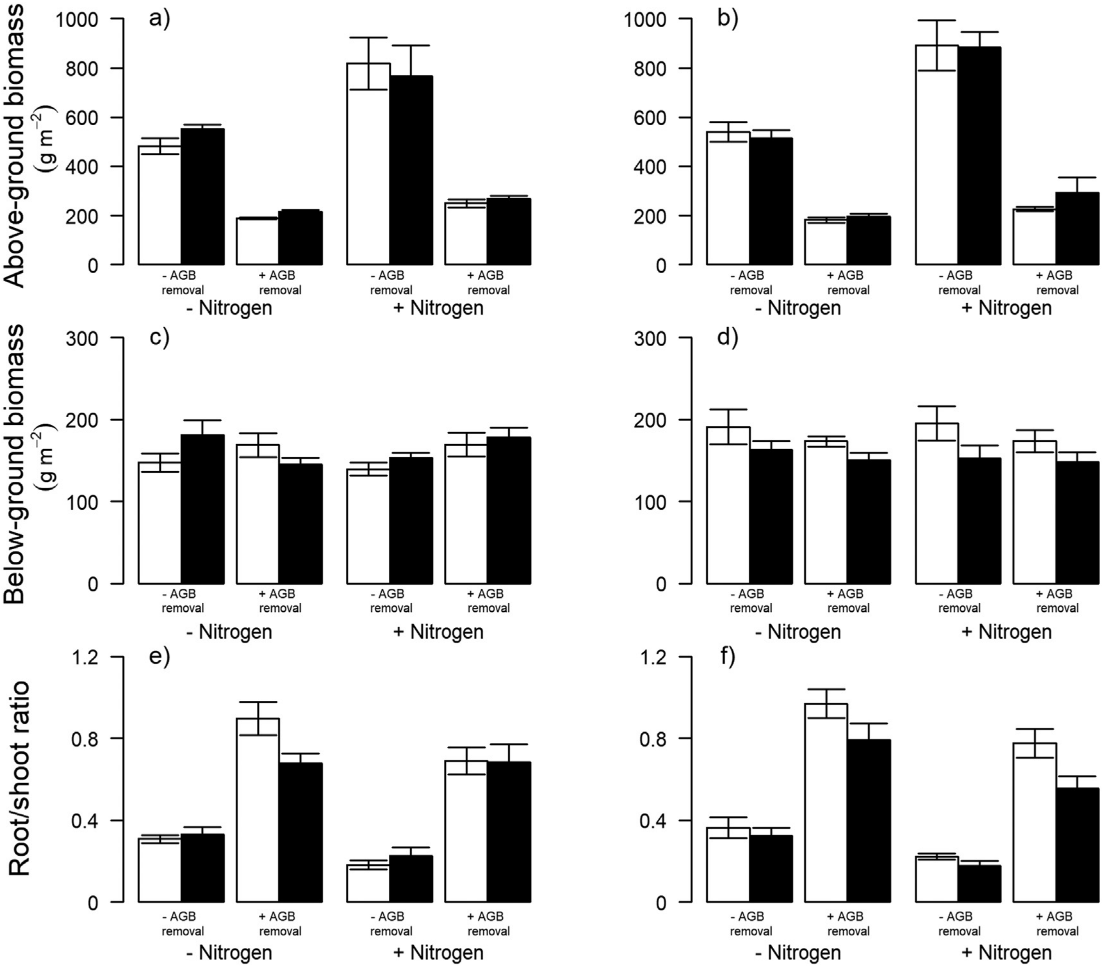

Fig. 2. Above- and below-ground biomass and root/shoot ratio in response to warming, AGB removal and nitrogen addition treatments over 2015 (a, c and e) and 2016 (b, d and f). White bars are for no-warmed and black bars are for warmed experimental field plots. Data are mean ± SE ($n = 5$). Statistical analysis reported in Table 1.

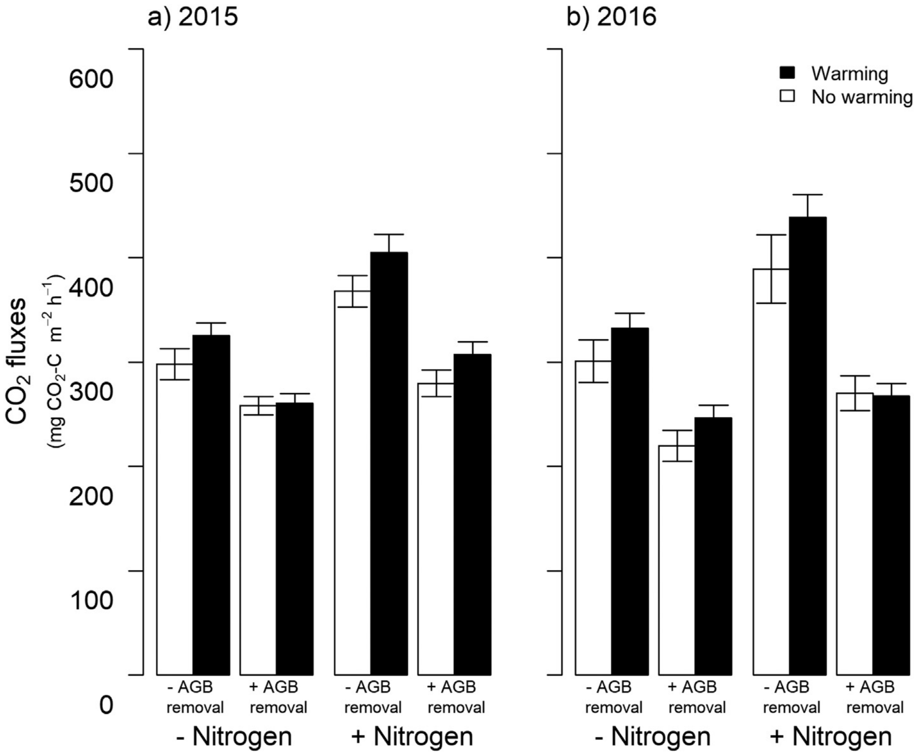

Fig. 3. CO_2 fluxes in response to warming, nitrogen addition and AGB removal treatments over (a) 2015 and (b) 2016. Data are means (mg CO_2-C m^{-2} h^{-1}) for all sampling dates ± SE (n = 14). Statistical analysis reported in Table 2.

The best model describing CH_4 fluxes contained six metrics (AGB, SRL, soil mineral N, RDMC, air temperature and soil moisture) explaining 24% of the variation found in CH_4 fluxes (Table 3). Using variation partition and dividing variables into four groups (Soil chemistry: soil mineral N; Below-ground: SRL, RDMC; Microclimate: soil moisture, air temperature; Above-ground: grassland productivity). Microclimate explained most of the variation in CH_4 fluxes (15%) with shared variance explained by above-ground (1.9%, Fig. 8), and by all of the other three metrics together (0.3%, Fig. 8).

4. Discussion

The aim of this study was to investigate how climate warming, N addition and AGB removal interact to affect plant-soil properties and GHG fluxes in a temperate UK grassland. Also, we evaluated whether plant productivity, soil chemistry and microclimate metrics could be used to explain changes in GHG fluxes. Although we found no interactive effects of the treatments on plant productivity, there were treatment interaction effects on GHG fluxes. Warming increased air temperature in both

Table 2
The effect of warming (warm), AGB removal and nitrogen addition (Nadd) on CO_2, N_2O and CH_4 fluxes over 2015 and 2016. Significance tests using likelihood ratio test (LRT) comparing models with or without parameter of interest where degree of freedom (d.f.) shows the difference in degrees of freedom between the models. Significant effects ($P < 0.05$) are shown in bold. Arrows represent the direction of the significance effects.

2015		CO_2 fluxes mg CO_2-C m^{-2} h^{-1}		N_2O fluxes µg N_2O-N m^{-2} h^{-1}		CH_4 fluxes µg CH_4-C m^{-2} h^{-1}	
	d.f.	LRT	*P*	LRT	*P*	LRT	*P*
warm	1	**12.35**	**0.0004**	2.91	0.09	0.94	0.33
Nadd	1	**16.37**	**0.0001**	**51.57**	**<0.0001**	**↓6.07**	**0.01**
AGB removal	1	**28.05**	**<0.0001**	2.62	0.10	2.02	0.15
Warm *x* Nadd	1	**4.18**	**0.04**	**4.24**	**0.04**	0.18	0.67
AGB removal *x* Nadd	1	2.74	0.10	**4.70**	**0.03**	0.00	0.99
warm *x* AGB removal	1	0.02	0.88	1.41	0.23	0.69	0.40
warm *x* Nadd *x* AGB removal	1	1.02	0.31	0.10	0.74	0.36	0.55
2016		CO_2 fluxes mg CO_2-C m^{-2} h^{-1}		N_2O fluxes µg N_2O-N m^{-2} h^{-1}		CH_4 fluxes µg CH_4-C m^{-2} h^{-1}	
	d.f.	LRT	*P*	LRT	*P*	LRT	*P*
warm	1	**11.77**	**0.0006**	**↑5.99**	**0.01**	**↑4.36**	**0.04**
Nadd	1	**23.97**	**<0.0001**	**↑19.09**	**<0.0001**	0.02	0.89
AGB removal	1	**46.16**	**<0.0001**	**↑3.77**	**0.05**	**↑5.11**	**0.02**
Warm *x* Nadd	1	0.79	0.37	0.11	0.73	1.70	0.19
AGB removal *x* Nadd	1	**4.27**	**0.04**	1.41	0.23	0.78	0.37
warm *x* AGB removal	1	1.94	0.16	0.01	0.92	1.30	0.25
warm *x* Nadd *x* AGB removal	1	1.28	0.26	2.19	0.14	0.09	0.76

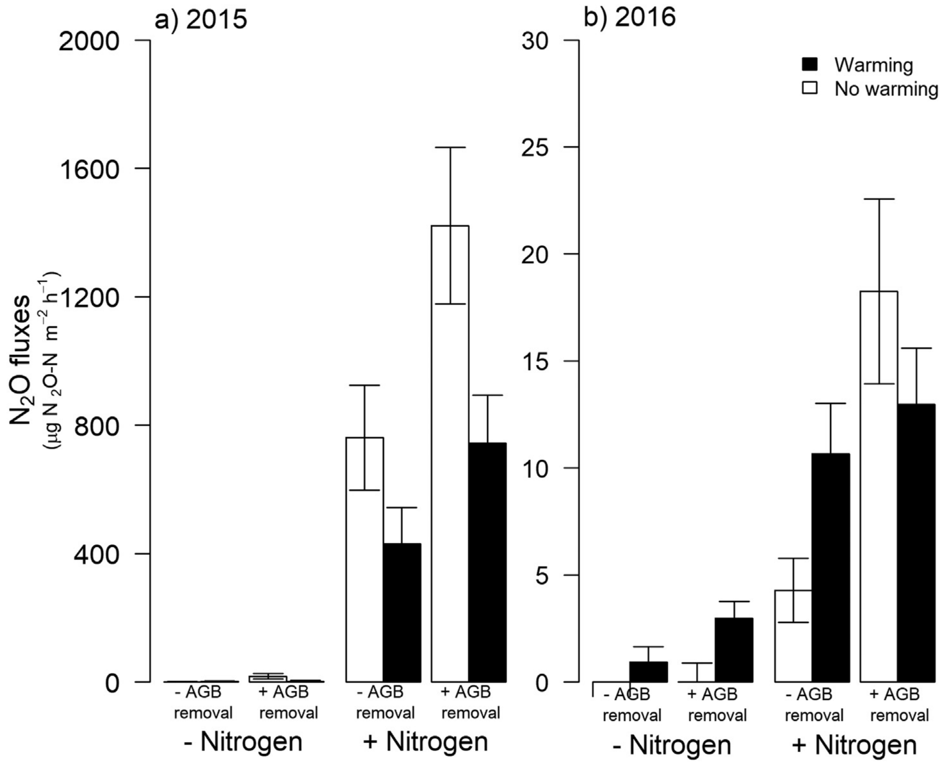

Fig. 4. N_2O fluxes in response to warming, nitrogen addition and AGB removal treatment over a) 2015 and b) 2016. Data are means (μg N_2O-N m^{-2} h^{-1}) for all sampling dates ± SE (n = 14). Statistical analysis reported in Table 2.

experimental years, increasing above-ground productivity in the first year with a small decrease in below-ground biomass in the second year. N addition increased above-ground productivity in both years. AGB removal reduced the AGB in both years, with an increase of below-ground biomass in the first year. Warming and N-fertiliser addition increased CO_2 fluxes and decreased N_2O fluxes in 2015. The N addition treatment interacted with AGB removal increasing N_2O fluxes in 2015 and reducing CO_2 fluxes in 2016.

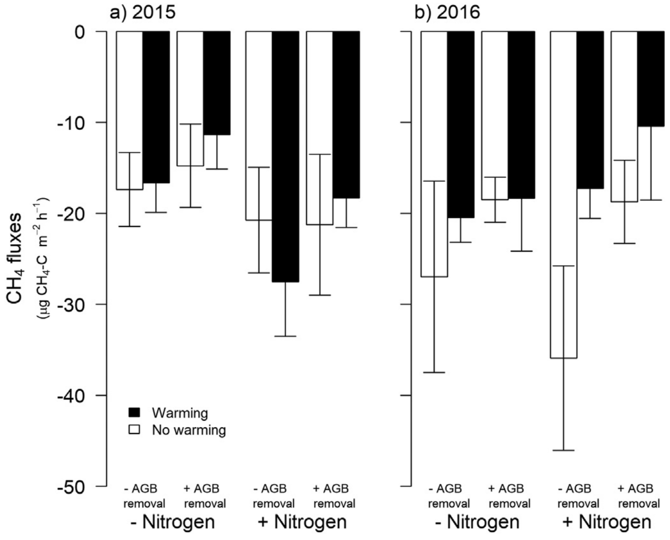

Fig. 5. CH_4 fluxes in response to warming, nitrogen addition and AGB removal treatments over a) 2015 and b) 2016. Data are means (μg CH_4-C m^{-2} h^{-1}) for all sampling dates ± SE (n = 14). Statistical analysis reported in Table 2.

4.1. Effect of warming, N-addition and AGB removal treatments on plant productivity

Warming increased air and soil temperature by 2 °C and 0.5 °C, respectively. As in other studies, climate warming promoted an increase in AGB (Fig. 2) (Graham et al., 2014; Rustad et al., 2001). This may be a direct

Table 3
P-values obtained from multiple linear regressions constructed with significant predictors for CO_2, N_2O and CH_4 fluxes. AGB = above-ground biomass; BGB = below-ground biomass; LDMC = leaf dry matter content; LNC = leaf N content; LCC = leaf carbon content; SRL = specific root length; RDMC = root dry matter content; RNC = root nitrogen content; RCC = root carbon content.

Group	Predictor metrics	CO_2	N_2O	CH_4
Above-ground	AGB	8.09e-14	–	0.038
	LDMC	0.026	–	–
	LNC	–	–	–
	LCC	–	–	–
Below-ground	BGB	–	–	–
	Root diameter	0.162	–	–
	SRL	–	0.006	0.027
	RDMC	–	–	0.016
	RNC	0.001	–	–
	RCC	–	–	–
Soil chemistry	Soil N	–	–	–
	Soil C/N	–	–	–
	Soil mineral-N	–	7.28e-06	0.050
	Net Mineralisation rate	–	0.001	–
	Net Nitrification rate	–	–	–
Microclimate	Air temperature	0.011	–	0.0002
	Soil temperature	0.065	–	–
	Soil moisture	0.024	0.004	0.0003
	Model significance	*P* < 2.2e-16 Adj R^2 = 0.776 AIC = 786.61	*P* = 3.10e-11 Adj R^2 = 0.495 AIC = 1135.95	*P* = 0.00020 Adj R^2 = 0.240 AIC = 647.14

result of by higher photosynthesis rates or due to indirect effects through increased nutrient availability (Rustad et al., 2001) or higher soil moisture (Xue et al., 2015). In year two, soil mineral N was 50% lower than in year one and soil moisture was 68% lower (in May, after N addition) which may have been a limiting factor for plant growth. Furthermore, a warming effect reduced below-ground biomass (in the second year) probably as part of ecosystem acclimation (Zhou et al., 2011) which may be related to changes of root exudation of organic C compounds (Williams and de Vries, 2020).

In this grassland ecosystem experiment, a positive effect of N addition was observed with increasing primary productivity in both years (Fig. 2). Numerous other studies have demonstrated that N addition stimulates plant growth (Högberg et al., 2006) and plant productivity (Kidd et al., 2017) thus enhancing C inputs to the soil and promoting increased respiration rates (Davidson et al., 2004). The AGB removal treatment reduced the AGB by 21% and 31% in both years, therefore significantly reducing C inputs to the soil, limiting substrate availability to microbes and respiration (Wan and Luo, 2003).

4.2. Interactive effects of warming and grassland management on GHG fluxes

4.2.1. Interactive effect of N addition and warming

Many studies have demonstrated the effects of warming and N addition in diverse ecosystems, however, interactive effects in grasslands are rarely investigated (Graham et al., 2014; Zhu et al., 2015). In partial agreement with our hypothesis H1, during the first year of the study N interacted synergistically with warming increasing CO_2 fluxes and antagonistically reducing N_2O fluxes from soil compared to warming and N-only.

Warming in a non N limited ecosystem was expected to promote greater N_2O fluxes due to the acceleration of microbial activity and nutrient cycling (Hoyle and Murphy, 2011) leading to an increase of mineral N transformation in the soil. However, the results showed that warming reduced the effect of N addition, reducing N_2O fluxes (Fig. 4). This might be related to a reduction of soil water content due to increased temperature (Table S1), limiting denitrification processes that favour anaerobic conditions (Ussiri and Lal, 2012). Denitrification is generally considered as the major process driving N_2O fluxes from soils (Saggar et al., 2013; Saggar et al., 2009); however, in this study, the conditions of WFPS lower than 60% may favour nitrification more often than denitrification (Bateman and Baggs, 2005; Davidson et al., 2000). Besides denitrification, N not released may be processed in two ways: i) allocated in the leaves and roots, which showed an increased N uptake in warmer conditions (Tables S3, S4, Bai et al. (2013), or ii) increased N uptake by microbes (increase N immobilisation, data not measured). Warming may lead to increased competition for N between plants and microbes (Hodge et al., 2000; Kaye and Hart, 1997), which was limiting in our study, although soil microbes are highly limited by C sources (Bai et al., 2013). Grassland is known to be less affected by warming due to its indirect effect on soil moisture, offsetting the temperature effect (Bai et al., 2013). These mechanisms together or separately may be driving the observed reduction in N_2O fluxes after N addition in warmed plots in this study. This could be a climate change positive feedback with N less likely to be released as N_2O.

In agreement with our hypothesis H1, a synergistic interaction between warming and N addition enhanced CO_2 fluxes but did not affect AGB productivity. In contrast, Gill (2014) reported no effect on respiration rates

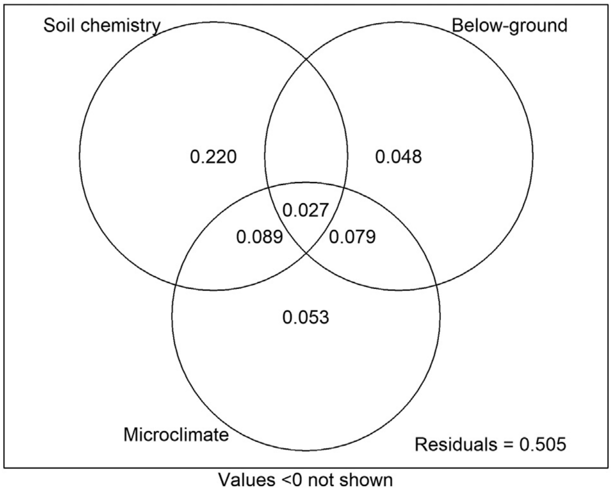

Fig. 7. Multiple model approach to predict N_2O fluxes based on plant productivity (above- and below-ground), plant and root traits, soil chemistry, and microclimate metrics. Soil chemistry group is represented by soil mineral N and net mineralisation rate; below-ground group is represented by SRL and microclimate group is represented by soil moisture. Residuals are variables not measured in this study.

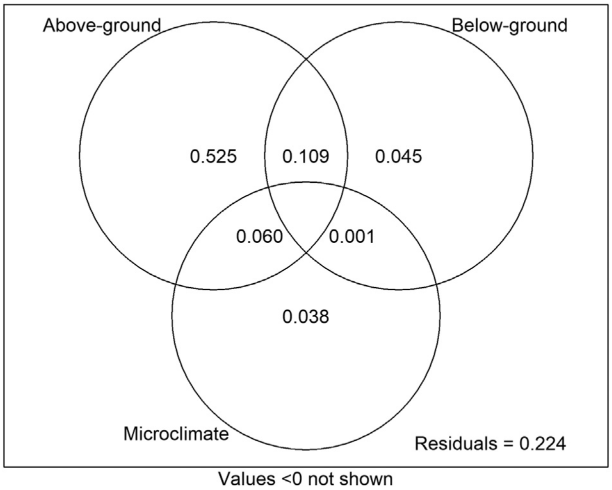

Fig. 6. Multiple model approach to predict CO_2 fluxes based on plant productivity (above- and below-ground), plant and root traits, soil chemistry, and microclimate metrics. Above-ground group is represented by AGB and LDMC; below-ground group is represented by root N content and microclimate group is represented by air and soil temperature and soil moisture. Residuals are variables not measured in this study.

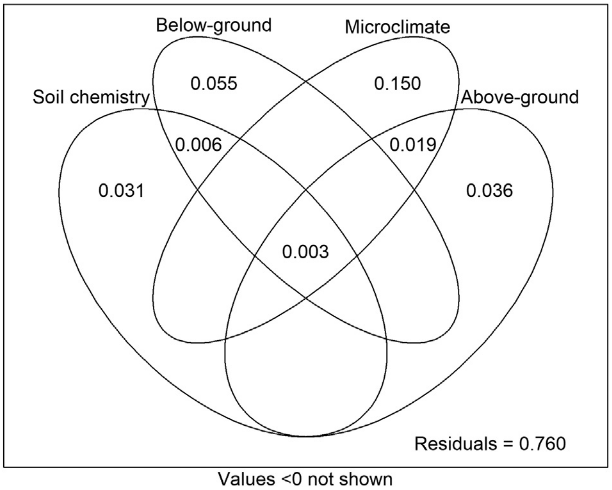

Fig. 8. Multiple model approach to predict CH_4 fluxes based on plant productivity (above- and below-ground), plant and root traits, soil chemistry, and microclimate metrics. Soil chemistry group is represented by soil mineral N; below-ground group is represented by SRL and RDMC; microclimate group is represented by soil moisture and air temperature and above-ground group is represented by AGB. Residuals are variables not measured in this study.

after three years of warming and N addition in a sub-alpine meadow, although plant productivity above-ground was increased. The authors suggest that three years was not sufficient time to change the soil organic matter pools under temperate conditions. Also, the effect might be dependent on quantity and forms of N-fertilised applied in the soil (Cardenas et al., 2010). In this study, it is possible that N addition increased N uptake by roots, requiring greater maintenance respiration (Scheurwater et al., 2000). This effect was only observed in the first year, perhaps because soil moisture was reduced in association with N addition in the second year, limiting N diffusion and cycling in the soil.

Although there was no interactive effect between warming and N addition on CH_4 fluxes, these factors individually affected CH_4 fluxes in the grassland plots. Warming was expected to increase CH_4 uptake (Blankinship et al., 2010; Dijkstra et al., 2013; Zhu et al., 2015) primarily due to a reduction in soil moisture (increase aerobic conditions) (Jones et al., 2005; Livesley et al., 2009), while N addition was expected to supress it (Jang et al., 2011; Liu and Greaver, 2009; Zhang et al., 2017). Conversely, in this study, warming reduced CH_4 uptake in the second year, and N increased it in the first year. Studies have suggested that factors other than soil moisture may also affect CH_4 fluxes including soil texture, soil nutrients, physical diffusion, microbial activity, and the duration of N addition (Carter et al., 2011; Dijkstra et al., 2013). For instance, increased temperature may cause lower methanotrophic activity, decreasing CH_4 uptake from the soil (Blankinship et al., 2010; Horz et al., 2005). During the first year, N increased NO_3^- concentrations (data not shown) in the soil, which could have contributed to an increase of soil CH_4 uptake as suggested by Jang et al. (2011). In addition, other studies found that under low CH_4 concentrations (~2 ppmv), inorganic N did not reduce CH_4 uptake (Jang et al., 2011; Steinkamp et al., 2001). These findings can be explained by different methane oxidising bacteria being active under different CH_4 concentrations (Jang et al., 2011), i.e. N affects type I methane oxidising bacteria thus if soil is more dominant by Type II methane oxidising bacteria, N addition will not affect CH_4 uptake. In this study we did not analyse the microbial community structure, but this analysis could, in future, help determine how, and why N addition affects CH_4 uptake in grassland soils.

4.2.2. Interactive effect of N addition and AGB removal

The interactive effect of N addition and AGB removal partially confirmed our hypothesis H2 as CO_2 fluxes was antagonistically diminished in the second year, however, N_2O fluxes were synergistically raised in the first year, with an increased root/shoot ratio. In an alpine meadow, the effect of cutting, N-fertilisation and warming also increased N_2O fluxes with the magnitude varying across years (Zhu et al., 2015). In addition, Wang et al. (2015) observed that mowing and N addition increased ammonification and net N mineralisation rates, which could also affect N release to the atmosphere.

We found after two years of experimental treatments, AGB removal reduced the effect of N addition on CO_2 fluxes (Fig. 2). We hypothesise that this is due to biomass removal (Fig. 5) increasing soil moisture (Table S1) and therefore reducing soil oxygen availability, promoting a decrease CO_2 fluxes. It is possible that cutting limited the N effect on soil microbial communities by reducing labile C sources and root exudation, and reducing microbial activity (Wang et al., 2015). More studies are needed to confirm the interactive effect of AGB removal and N addition on microclimate to relate it back to CO_2 fluxes from grassland soils.

In contrast with our hypothesis H2, AGB removal increased the effect of N addition during the first year, enhancing N_2O fluxes from the soil. It has been proposed that grazing triggers and accelerates plant growth promoting nutrient uptake from soil and increases root exudation (Hamilton and Frank, 2001; Yoshitake et al., 2015). This could stimulate microbial mineralisation of soil organic matter and liberate mineral N, leading to greater N_2O fluxes. Cutting stimulates an increase in N in the plant leaf due to rapid use of N to restructure the plant. However, in the presence of excess soil N, AGB removal decreases plant leaf and root N content (Tables S3, S4) promoting a release of N by microbial N_2O production.

In this study, higher N_2O fluxes corresponded with higher specific root length (SRL), suggesting that the system had lower nutrient availability due to its release to the atmosphere as N_2O. SRL can explain about 5% of the variance in N_2O fluxes according to the multiple regression (Fig. 7). This suggests that this metric could be used in the future to predict N_2O fluxes in grasslands. As SRL usually describes the economic aspects of the root system, it is linked to root-nutrient uptake efficiency (Eissenstat, 1992; Eissenstat et al., 2000). Ostonen et al. (2007), Du et al. (2013) and Siebenkäs et al. (2015) implies that fertiliser application increases nutrient availability and then reduces explorative root growth, decreasing SRL. Almost half of the variation was unaccounted for by our study variables. Some of the unexplained variation might be related to changes in microbial communities of nitrifiers and/or denitrifiers and/or nitrifier denitrifies (Kool et al., 2011; Selbie et al., 2015).

4.2.3. Interactive effect of AGB removal and warming

Contrary to our hypothesis and in agreement with Zhu et al. (2015), there was no interactive effect of AGB removal and warming on GHG fluxes (Table 1), showing that changes in plant traits and soil chemical properties did not influence ecosystem GHG fluxes. Differences in plant traits were greater during the first year of the experiment likely due to differences in rainfall (89 mm more in 2015 after N addition), increasing water content, microbial activities and other soil properties.

The interactive effect of cutting and warming on GHG fluxes is likely influenced by the balance of: i) the rate of AGB removal and labile C inputs to the soil which affect soil and microbial respiration (Cao et al., 2004; Raiesi and Asadi, 2006) and N_2O fluxes (Dijkstra et al., 2012); and ii) the increase in soil temperature due to cutting (Dijkstra et al., 2012; Luo et al., 2010) (Table S1).

In terms of plant-soil properties, AGB removal could lead to increased N availability in the soil (Hamilton et al., 2008), while warming led to an increase in N mineralisation (Rustad et al., 2001) leading to reduced N availability (evidenced by the reduction of leaf C/N ratio, Table S3). It also could have affected the root system, increasing SRL (Table S4) which is known to be increased in N limiting ecosystem, by which roots invest in their structure to acquire N in the soil (Ostonen et al., 2007).

5. Conclusions

In conclusion, our two years field experiment showed that the interactive effects of climate warming and management practices are significant for short-term nutrient cycling in temperate grasslands. This suggests that there is potential to mitigate warming effects with management approaches that improve C sequestration and reduce GHG fluxes. It is expected that further increases in large-scale N fertilisation to increase productivity will accelerate plant-soil N cycling, thereby increasing the risk of N losses to the environment. Reduced cutting frequency and lower N addition rates are, according to our findings, possible options to keep C and N cycles tight and lower net GHG fluxes. In view of the significant effect of the interannual variability in rainfall in our study, management plans must be multi-year. Cutting treatment in this experiment did not simulate livestock completely (there were no urine/feaces deposition and animal trampling), so caution is needed when drawing overall conclusions about livestock effect in this experiment. More studies are needed to confirm the findings of this study especially in other climate conditions and soil types, different N-fertiliser rates, frequency of cutting and by using varies plant community compositions. Overall, our work demonstrates the importance of interactions between climate and management as determinants of short-term grassland GHG fluxes. These results show that reduced cutting combined with lower inorganic N additions would constrain grassland C and N cycling and GHG fluxes in warmer climatic conditions. This has implications for strategic grassland management decisions to mitigate GHG fluxesin a warming world. The study showed that, despite the fact that single factors showed strong effects, their interactions are crucial to understanding grassland acclimation to future warming.

CRediT authorship contribution statement

Arlete S. Barneze: Conceptualization, Data curation, Formal analysis, Writing – original draft. **Jeanette Whitaker:** Writing – review & editing. **Niall P. McNamara:** Writing – review & editing. **Nicholas J. Ostle:** Investigation, Writing – review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.155212.

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