

## RESEARCH ARTICLE

# Interactive effects of climate warming and management on grassland soil respiration partitioning

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## Abstract

Grassland ecosystems are important for the provision of food, fuel and fibre. They represent globally important carbon (C) reservoirs that are under pressure from intensive management and ongoing climate change. How these drivers of change will interact to affect grassland soil C and nitrogen (N) cycling and heterotrophic and autotrophic respiration remains uncertain. Roots and mycelia in grassland soil are important regulators of ecosystem functioning and likely to be an influential determinant of CO<sub>2</sub> fluxes responses to global change. The aim of this study was to investigate the interactive effect of climate warming and grassland management on soil respiration originating from roots rhizosphere, mycelia and free-living microbes. The experiment used a block design to measure the interactive effects of warming, nitrogen addition, aboveground biomass (AGB) removal on belowground respiration in a temperate grassland ecosystem. An in-growth core method using cores with different mesh sizes was used to partition belowground respiration due to its simplicity of design and efficacy. We found that basal respiration (free-living microorganisms) was the highest (58.5% of the total emissions), followed by that from roots (22.8%) and mycelia (18.7%) across all treatments. Warming reduced basal respiration whilst AGB removal increased it. An antagonistic interaction between warming and nitrogen addition reduced root respiration, and a three-way interaction between warming, nitrogen addition and AGB removal affected mycelial respiration. The results show different contributions of belowground biota to soil respiration, and how interactions between climate change and grassland management may influence effects on soil respiration.

## KEYWORDS

autotrophic and heterotrophic respiration, cutting, grassland ecosystem, interactive effects, nitrogen addition, warming

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## 1 | INTRODUCTION

Soil contains 4.5 times as much carbon (C) as biotic pools (Lal, 2004) with biological processes significantly contributing to the accumulation of this carbon stock (Davidson et al., 2002; Giardina et al., 2004). Specifically, roots and fungal mycorrhizae are important regulators of nutrient cycling and the availability of nitrogen (N) and other elements essential for plant productivity (Johnson et al., 2006; Rillig, 2004). In grasslands, different soil biotic components may also affect nutrient cycling with feedbacks to greenhouse gas emissions (Dijkstra, Carrillo, et al., 2013; Dijkstra, Morgan, et al., 2013; Paterson, 2003; van Groenigen et al., 2015) and C storage belowground (Büscher et al., 2012; Johnson et al., 2006). However, studies on the contribution of specific belowground biotic components to grassland C and N cycling, in particular the contribution of mycorrhizae fungi, are limited (Heinemeyer et al., 2012; Johnson et al., 2002). Studies rarely consider the effects of interactions between climate change and grassland management (grassland management  $\times$  drought; Moinet et al. 2019), determining its effect related to only one driver of change (e.g., temperature/moisture; Heinemeyer et al., 2007, liming; Johnson et al., 2002).

In general, intensive management of temperate grasslands, for example, N fertilisation and mowing/grazing, increases plant productivity and nutrient inputs and off-take (Giese et al., 2013; McSherry & Ritchie, 2013). These changes might directly increase belowground C and N storage, by increasing belowground biomass (Bai et al., 2015; Gao et al., 2008, 2011). Climate warming might further emphasise these effects by accelerating nutrient cycling and enhancing respiration from the belowground components. Thus, it is crucial to understand how these belowground components will be affected by interactions between climate change and management as there is a high likelihood that multiple drivers operate concurrently and not only as single drivers.

Production of CO<sub>2</sub> in the soil occurs via multiple pathways. Heterotrophic respiration results from soil organic matter decomposition by microbes while autotrophic respiration is related to the respiratory activity of roots and associated microbes. The former primarily controls soil C storage and nutrient dynamics whereas the latter reflects plant activity and the supply of organic compounds to roots from the plant canopy (Hanson et al., 2000). Soil respiration is derived from roots, fungal mycelium (mostly found as arbuscular mycorrhizal (AMF) fungi in grasslands, Johnson et al., 1992) and free-living microorganisms. There is a large uncertainty associated with the amount of respiration originating

### Highlights

- Unravelling uncertainties in grassland soil carbon and nitrogen cycling under climate change and management
- Partitioning CO<sub>2</sub> fluxes from belowground compartments under interactions between climate and management
- Basal respiration enhances CO<sub>2</sub> fluxes, with warming decreasing it and aboveground biomass removal increasing
- Understanding the interplay between climate change and management is crucial for predicting soil respiration

from each of these sources due to variations in ecosystem properties and the interactive effects of climate change and management/land use (Jones et al., 2009; Nguyen, 2003). There are some studies which evaluate the partitioned soil respiration into roots, mycorrhizae and microbes (e.g., in forest: Heinemeyer et al., 2007 and Zhu et al., 2023; in barley field: Moyano et al., 2007), but there are only a few to date in grasslands ecosystems due to difficulty in separating root from mycorrhiza hyphal respiration. Heinemeyer et al. (2012) in a grassland study found that mycelium respiration contributed to 27% of soil respiration, while root respiration contributed to 11%, with considerable variation across the experimental period. Considering mycorrhizae and root together, Graham et al. (2014) and Zhang, Niu, et al. (2014) both found that heterotrophic respiration formed the largest contribution to grassland overall soil respiration, and this was due to microbial communities being able to access both old (C resident in the soil for decades, Trumbore, 2000) and recent soil C (Dijkstra, Morgan, et al., 2013). Yet the assessment of each belowground component (in particular mycorrhizae and roots) as distinct sources is required given that they may respond somewhat independently to environmental changes (Alberton et al., 2005).

Increases in temperature, due to climate change, may enhance both autotrophic and heterotrophic soil respiration, affecting, directly and indirectly, ecosystem respiration (Chen, Luo, et al., 2016; Graham et al., 2014; Peng et al., 2015). Directly, warming can affect mycorrhiza and its colonisation (Heinemeyer & Fitter, 2004), and indirectly the C allocation from the host plant (Heinemeyer et al., 2006; Rillig, Wright, et al., 2002). According to Smith and Read (2008) autotrophic respiration is highly dependent on substrate supply from photosynthesis; therefore, it will influence C inputs and cycling

in the soil. Additionally, warming is expected to accelerate nutrient mineralisation, and fine root growth could decrease due to less C needing to be allocated to belowground under increased biomass productivity (Dieleman et al., 2012). Heterotrophic respiration, in turn, can be affected by warming due to an increase in microbial biomass (Lu et al., 2013) and decomposition of stable organic matter (Hopkins et al., 2012).

Increasing biomass productivity through the addition of N-fertiliser as part of intensified grassland management can influence soil respiration and its components. However, studies indicate significant variability and a lack of consistent results in this regard (Tu et al., 2013; Zhang, Han, et al., 2014; Zhou et al., 2014). The application of N-fertiliser may decrease (Treseder, 2008) or increase (Dong et al., 2022) enzyme activity and soil organic matter decomposition, resulting in corresponding changes in heterotrophic respiration. Likewise, N addition may stimulate autotrophic respiration due to increased plant growth and root biomass (Cleveland & Townsend, 2006) or suppress it by reducing belowground C allocation (Giardina et al., 2004; Kuzyakov et al., 2002; Wang, Zhang, et al., 2017) resulting in changes in autotrophic respiration. Additionally, increases in cutting/grazing or harvesting for biomass have been shown to negatively affect soil respiration and its components (Bremer et al., 1998; Wei et al., 2016) especially in the short-term (Bahn et al., 2006). Autotrophic respiration might be less affected due to existing carbohydrate reserves which sustain root metabolism whilst heterotrophic respiration strongly responded negatively to short-term changes in assimilate supply (Bahn et al., 2006). Grazing is also suggested to affect mycorrhizae fungi due to C limitation (Sonnemann et al., 2016; van der Heyde et al., 2017) by the reduction of aboveground biomass (AGB).

Although studies of the effects of single drivers (abiotic or biotic) on soil functions are valuable, ecological drivers occur simultaneously in the real world interacting in ways that may be difficult to predict and rarely tested experimentally. For example, studies have shown that interactions between warming and N addition showed no changes on the partitioned respiration in a grassland ecosystem (Graham et al., 2014) whereas changes in nitrification processes were recorded in a forest ecosystem (Liu et al., 2011). In other grassland experiments, warming interacted with AGB removal and did not result in significant changes in partitioned soil respiration (Zhou et al., 2007), whereas Zong et al. (2017) discovered that N and cutting increased belowground biomass with no changes in partitioned soil respiration.

A range of methods have been used to partition soil respiration, and these include the use of stable isotopes

(such as  $^{13}\text{C}$  natural abundance discrimination and isotopic  $^{14}\text{C}$  mass balance), root removal, gap analysis techniques and in-growth cores (Chin et al., 2023; Hanson et al., 2000). All of these methods have caveats leading to over- and underestimations of soil respiration (Neill, 1992). The isotopic technique may promote minor soil disturbance (Vargas et al., 2011), but requires a complex experimental setup including specific expertise and expensive analysis. In-growth mesh-cores are the most widely used, because of their simplicity of design (Chen, Lin, et al., 2016; Chen, Luo, et al., 2016; Milchunas, 2009), allowing the free movement of water, bacteria and nutrients through the mesh (Moyano et al., 2007). These cores also have the advantage of enabling the partition of soil respiration from the root and mycorrhiza fungi soil components (Han et al., 2021; Heinemeyer et al., 2007; Johnson et al., 2001), although soil disturbance may increase transient  $\text{CO}_2$  fluxes.

The aims of this study were (i) to investigate the relative contribution of soil microbes, roots and mycorrhizae to total soil respiration in grasslands, and (ii) to understand how climate and grassland management interact to affect soil respiration and the relative contribution of soil microbes, roots and mycorrhizae.

We hypothesised that: (H1) Mycorrhizal and root respiration comprised the majority of belowground respiration; (H2) warming and N addition will interact synergistically, increasing both autotrophic and heterotrophic respiration and plant productivity, due to an increase in root biomass; (H3) a synergistic interaction between warming and AGB removal will increase both autotrophic and heterotrophic respiration mainly due to the effect of warming on the root biomass and the effect of C limitation for soil microbes; and (H4) N addition will synergistically interact in response to AGB removal, increasing both heterotrophic and autotrophic respiration, due to increased N availability in the soil. To test these hypotheses a field experiment was conducted with a full factorial design including interactions between warming, N addition and AGB removal, and three in-growth cores (with three different mesh sizes). The interactive effects of these treatments on plant-soil C and N cycling were determined through the measurement of above- and belowground plant productivity, soil properties and heterotrophic and autotrophic respiration over 60 days.

## 2 | MATERIALS AND METHODS

### 2.1 | Site description

The experimental site was located at Lancaster University, Lancaster, the United Kingdom ( $54^\circ 1'50''\text{N}$ ,  $2.7^\circ 46'30''\text{W}$ , 94.1 m a.s.l.) adjacent to Hazelrigg Weather Station. This site is a 61 ha area of permanent unfertilised

grassland which is owned and managed by Lancaster University and has been intermittently grazed by sheep and used as a hay meadow. The site is under maritime temperate climatic conditions, and the mean annual air temperature was 13°C between 1981 and 2010 with January being the coldest month (average of 7°C) and July the warmest (average of 19°C). The mean annual precipitation is 1049 mm. The soil is semi-permeable, seasonally wet, acidic, loamy and clayey according to the National Soil Resources Institute, UK soil classification survey (Farewell et al., 2011), and classified as Stagnosols according to FAO classification (FAO, WRB). Initial analyses of the properties of the upper 10 cm of the soil profile are total N content 0.3%, total C content 3.5% (inorganic C was negligible), C/N ratio of 12, pH of 5.3 and bulk density of 1.06 g cm<sup>-3</sup>.

## 2.2 | Experimental in-growth core design

The in-growth core experiment was nested within the main experiment of Barneze et al. (2022) totalling eight treatment combinations with five replicates (one within each experimental block). The treatments were soil control (soil only), warming, N addition and AGB removal, and the interactions AGB removal + warming, N addition + warming, AGB removal + N addition and AGB removal + N addition + warming. Each block was comprised of 25 plots (9 m<sup>2</sup>) 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 (Figure S1).

The warming treatment was accomplished using open-top passive conical chambers, which increased efficiently the air temperature for 2°C. N addition was applied in May (spring) as ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>) at a rate of 100 kg N ha<sup>-1</sup> y<sup>-1</sup> (consistent with typical grassland management recommendations for hay meadows in the United Kingdom). 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). More detailed information can be found in Barneze et al. (2022).

In each main treatment, three soil in-growth manipulation cores were inserted to allow either ingress of roots and mycelial, mycelial only or no mycelial or root inputs. The soil in-growth cores were made based on the design of Johnson et al. (2001), which consists of plastic drainage pipe (6.8 cm diameter and 15 cm depth) with two slots (5 and 10 cm) cut into the sides to adhere exclusion mesh for the given in-growth

treatments. For excluding roots or mycelium, or both, nylon closed-bottom mesh bags (Plastok Associates Ltd, Birkenhead, Wirral, United Kingdom) were adhered to the pipe (Figure S2). The three types of in-growth cores (40 of each type) were filled with approximately 500 g of fresh (soil moisture approx. 49%), sieved (through 4 mm mesh) and root-free soil taken from within each main treatment (120 in-growth cores in total). Root/mycelia cores allowed root and mycelial in-growth using a 2 mm mesh; mycelia cores excluded roots but allowed mycelial in-growth (35 µm mesh) and no in-growth was achieved through using 1 µm mesh (Figure S3). One core of each mesh size was inserted in each plot to 15 cm depth in the soil and nylon top-covered mesh. The cores were installed in May 2015 and allowed to settle for 1 year before gas measurements were made during May and June 2016.

## 2.3 | Heterotrophic and autotrophic respiration measurements

Measurements of soil respiration were made on each root in-growth core in May and June 2016. Gas sampling lids were made using drainage pipe (6.8 cm diameter and 9 cm depth, Screwfix, United Kingdom) fitted with a lid and septum for gas sampling (Figure S4). For each flux measurement, the lid was secured to the in-growth core and 5 mL gas samples (t<sub>0</sub>) were taken immediately and then after 30 min (t<sub>30</sub>) using a 10 mL syringe. Gas samples were transferred to 3 mL pre-evacuated exetainer vials (Labco, Lampeter, United Kingdom) for storage until analyses. Gas samples were analysed using a PerkinElmer Autosystem XL Gas Chromatograph (GC) (PerkinElmer, Waltham, MA, United States) with a Flame Ionisation Detector (FID) fitted with a methaniser and Electron Capture Detector (ECD) operating at 130°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. Three calibration gas standards (500, 1000, 4000 ppm CO<sub>2</sub>) (Air Products, Waltham-on-Thames, United Kingdom) were run every 14 samples (Case et al., 2012). Linearity of the gas concentration in each in-growth core was tested regularly (Chadwick et al., 2014). Gas fluxes were calculated by the difference in (t<sub>0</sub>) and (t<sub>30</sub>) gas concentrations corrected for air temperature and barometric pressure following the ideal gas law (Holland et al., 1999). Air and soil temperature were taken using a Tiny Tag temperature logger with integral stab probe (Gemini Data Loggers, United Kingdom) and soil moisture was taken using ML2x Theta Probe (Delta T Devices, United Kingdom) at each gas sampling date inside each main treatment (and outside the exclusion



cores). Following N additions in May 2016, CO<sub>2</sub> measurements were made between 3rd June and 23rd June, for a total of 11 sampling occasions.

## 2.4 | Soil and root analyses

Soil cores were removed from each plot for physical and chemical analyses at the end of the gas measurement period. Soil gravimetric moisture content was determined after drying 5 g of soil at 105°C for 24 h. Soil bulk density and water-filled pore space (WFPS) were calculated. Ammonium (NH<sub>4</sub><sup>+</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>) were extracted with 1 M KCl in a 1:5 (soil weight:extractant volume) and analysed with a spectrophotometer (AutoAnalyser 3 Digital colorimeter BRAN + LUEBBE). Soil C and N were determined on dried (60°C), finely ground soil samples, using an elemental analyser (TruSpec<sup>®</sup> CN, St. Joseph, MI) with furnace temperature at 950°C. From the remaining soil, the below ground biomass was determined after washing all root and dried at 60°C.

## 2.5 | Calculations and statistical analyses

Linear mixed effects models (LME) were used to test for treatment effects on soil properties (i.e., to account for the overall effect of the main treatments and in-growth cores,  $n = 11$ ) to the responses to warming, N addition, AGB removal and in-growth cores. Fixed effects were warming, N addition, AGB removal, in-growth cores 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 residual plots method and log-transformed where necessary before statistical 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). Being the fixed term 'IG' (in-growth core) significantly different (i.e., bulk density, WFPS and soil NO<sub>3</sub><sup>-</sup>-N), Tukey post-hoc analyses were carried out and a significant effect was determined at  $p \leq 0.05$ . All statistical analyses and graphs were made using R 3.4.3 (R Development Core Team, 2017) using the additional packages *nlme* (Pinheiro et al., 2009) and *plyr* (Wickham, 2011).

To account for the effect of different belowground components on ecosystem respiration rates, the absolute contribution of fine roots, mycelial and free-living soil

microbes was calculated and partitioned according to Moyano et al. (2007), that is,

Total belowground respiration flux = 2 mm mesh cores

Root rhizosphere respiration = (2 mm – 35 μm)

Mycelial respiration = (35 μm – control)

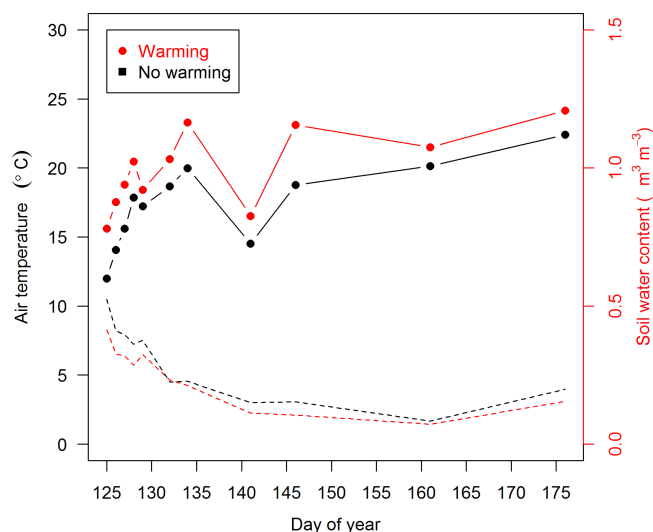
Basal respiration = control (1 μm mesh core)

Partitioned ecosystem respiration was analysed as described above for each belowground component separately. LME were used for each partitioned ecosystem respiration and microclimate data response to warming, N addition and AGB removal. Fixed effects were warming, N addition, 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 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 an LRT.

## 3 | RESULTS

### 3.1 | Treatment microclimate

During the experimental period, the absolute maximum air temperature observed was 29°C with a minimum of 8°C. Microclimate measurements were taken at each treatment plot to coincide with the 11 gas sampling occasions in May and June 2016. From this period, results showed that mean air temperature was raised by 2.5°C (LRT = 168,  $p < 0.0001$ , Table S1), and soil water content was reduced by 18% (LRT = 47,  $p < 0.0001$ , Table S1) in the warmed plots relative to the non-warmed plots (Figure 1). Mean soil temperature was reduced by N addition (LRT = 7,  $p = 0.01$ , Table S1), and increased by AGB removal (LRT = 10,  $p = 0.001$ , Table S1). Interactive effects showed that N addition increased soil moisture in the AGB removal plots only (LRT = 10,  $p = 0.001$ , Table S1), and the effect of this interaction was even higher in the non-warmed plots (three-way interaction, LRT = 47,  $p < 0.0001$ ; LRT = 6,  $p = 0.01$ ; LRT = 20,  $p < 0.0001$ , Table S1).



**FIGURE 1** Seasonal variation in the warmed and no-warmed plots. Mean air temperature (°C), and soil water content ( $\text{m}^3 \text{m}^{-3}$ ), at 100 mm depth. Solid lines represent air temperature, while dashed lines represent soil water content.

### 3.2 | Soil chemical and physical properties

Soil  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  concentrations were affected by a synergistic interaction between warming and N addition (LRT = 11,  $p = 0.0006$ ; LRT = 43,  $p < 0.0001$ , Figure 2, Table S2, Figure S6). There was an interactive effect of N and AGB removal, that is, N addition increased soil  $\text{NH}_4^+\text{-N}$  only in plots where AGB were not removed (LRT = 5,  $p = 0.02$ , Figure 2, Table S2, Figure S6). Warming increased both  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  concentrations (LRT = 6,  $p = 0.01$ ; LRT = 11,  $p = 0.001$ , Table S2).

The in-growth core approach was successful with minimal root biomass detected in the 35 and 1  $\mu\text{m}$  in-growth cores relative to the 2 mm mesh core (Table 1). In line with reduced root biomass, soil bulk density and WFPS were significantly higher in 2 mm in-growth core followed by 35 and 1  $\mu\text{m}$  mesh core (Table 1). Soil  $\text{NO}_3^-\text{-N}$  varied in relation to in-growth cores (LRT = 10,  $p = 0.002$ , Table S2) and was higher in the 35  $\mu\text{m}$  in-growth core, followed by 2 mm and 1  $\mu\text{m}$ , with no significant difference between 2 mm and 1  $\mu\text{m}$  in-growth core (Table 1). As a non-significant root biomass was found in the 35 and 1  $\mu\text{m}$  mesh cores, the interactive treatment effects on root biomass were only analysed in the 2 mm mesh core. Root biomass was decreased by 25% (LRT = 5.3,  $p = 0.02$ ) and 14% (LRT = 5.1,  $p = 0.02$ ) under N addition and warming treatment, respectively (Figure 3).

### 3.3 | Partitioned soil respiration

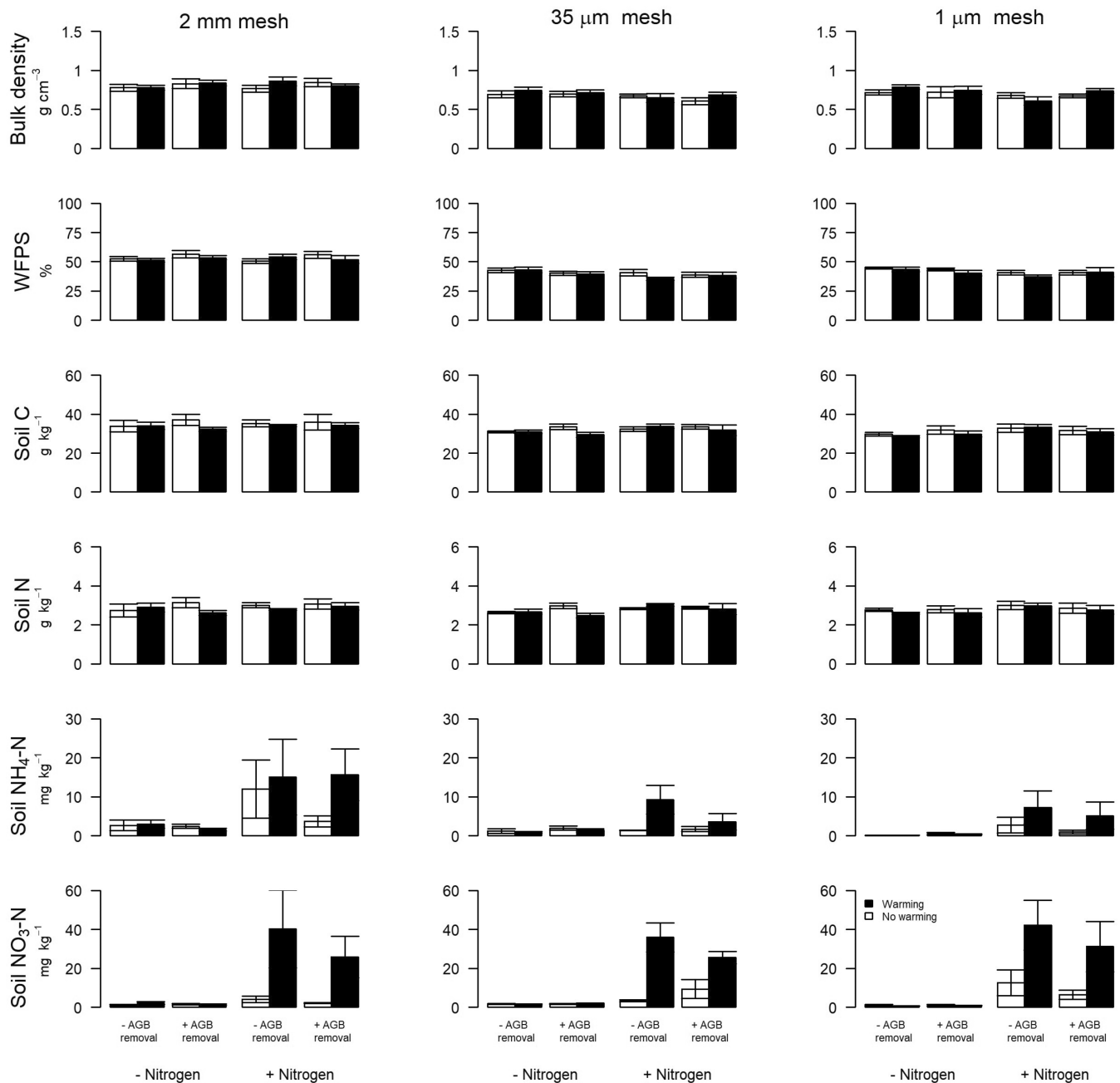
Respiration fluxes from the belowground components varied over time with higher emissions from soil only compared to mycelial and root cores (Figure S5). The partition of soil respiration across all treatments showed that  $58.5\% \pm 2.3$  ( $56.4 \text{ mg m}^{-2}$ ) of the ecosystem respiration was due to the basal contribution,  $22.8\% \pm 1.3$  ( $22 \text{ mg m}^{-2}$ ) from roots, and  $18.7\% \pm 2.0$  ( $18 \text{ mg m}^{-2}$ ) from mycelia (Figure 4). Basal respiration was higher, and root and mycelial respiration were not statistically different from each other ( $p > 0.05$ ), that is, basal > root rhizosphere = mycelial respiration.

Main treatments had a range of effects on partitioned soil respiration (Figure 5). Root respiration was increased by N addition only in the non-warmed plots, in a synergistic interaction (LRT = 4,  $p = 0.04$ , Table 2). Mycelial respiration was marginally affected by the three-way interaction between warming, AGB removal and N addition (LRT = 4,  $p = 0.04$ , Table 2). N addition increased mycelial respiration in plots which had AGB removed, but warmed plots had higher respiration irrespective of N addition and AGB removal. Basal respiration was reduced by warming (LRT = 5,  $p = 0.03$ , Table 2) and increased by AGB removal (LRT = 4,  $p = 0.03$ , Table 2).

## 4 | DISCUSSION

### 4.1 | Partitioned soil respiration from grassland

Mycorrhizae have been widely studied, however, their importance for terrestrial ecosystem functions is still not fully understood (Pendall et al., 2004), despite evidence of their key role, for example, on the soil C and N cycling (Hawkins et al., 2023; Heinemeyer et al., 2007; Nottingham et al., 2010). Mycorrhizae also mediate changes in soil structure and plant nutrient foraging (Rillig et al., 2003; Staddon et al., 2002). This work showed that mycelial respiration contributed 20% of total grassland soil respiration during the growing season, similar to root respiration (22% of total respiration). As these in-growth cores were left in the ground for 1 year prior to commencing  $\text{CO}_2$  flux measurements (mycelia growth rate is  $10 \text{ mm day}^{-1}$ ; Donnelly et al., 2004; Leake et al., 2004), it is likely that hyphae would have sufficiently grown to reflect mycelial respiration of undisturbed soils (Nottingham et al., 2010). There are only a few studies, which partition soil respiration in grasslands, and they found similar contribution of mycorrhizae and roots (27% and 11% of mycorrhizal and root respiration, respectively, Heinemeyer et al., 2012). A recent



**FIGURE 2** Interactive effect of warming, nitrogen addition, aboveground biomass removal and in-growth cores (2 mm, 35  $\mu\text{m}$  and 1  $\mu\text{m}$ ) on bulk density, water-filled pore space (WFPS), soil C, soil N, soil  $\text{NH}_4^+ - \text{N}$  and  $\text{NO}_3^- - \text{N}$ . Data are mean  $\pm$  SE ( $n = 5$ ). Stats can be found in Table S2 and S6.

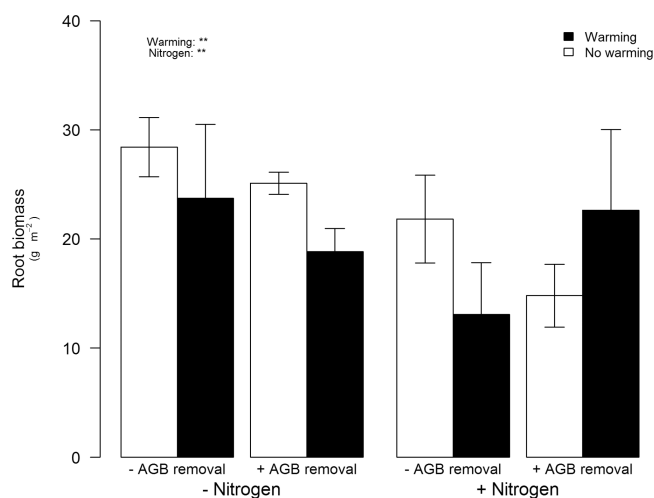
metanalyses showed that the contribution of mycorrhizal to soil respiration ranged between 2% and 48% (being 14% for grassland ecosystems), and 38% of autotrophic respiration on average (Han et al., 2021). Experiments in forests, using the in-growth core method, found a higher mycelial contribution of 25% (ectomycorrhizal:  $26 \text{ mg C m}^{-2} \text{ h}^{-1}$ , Heinemeyer et al., 2007) and 14% of total soil respiration (AMF:  $17.3 \text{ mg C m}^{-2} \text{ h}^{-1}$ , Nottingham et al., 2010). Our results are in line with these studies despite being made in different ecosystems with mycorrhiza contributing to

respiration in equal measure to roots. Estimates of mycelial and root-rhizosphere respiration may be subject to several sources of error, for example, mycelia (especially AMF in grasslands) are also found inside roots (counting for an estimated 20% of root weight, Smith & Read, 2008). Moreover, for the same reason, root respiration might be overestimated. In addition, the in-growth core method does not account for differences in the presence of any mesofauna (e.g., earthworms, mites, collembolans) in the 2 mm mesh, which could

**TABLE 1** Bulk density, water-filled pore space, root biomass and soil  $\text{NO}_3^- - \text{N}$  for in-growth cores measured at the end of the experiment.

In-growth cores	Bulk density $\text{g cm}^{-3}$	Water-filled pore space %	Root biomass $\text{g m}^{-2}$	Soil $\text{NO}_3^- - \text{N}$ $\text{mg kg}^{-1}$
2 mm	$0.81 \pm 0.015^a$	$53.33 \pm 0.85^a$	$21.04 \pm 1.63^a$	$9.08 \pm 3.06^b$
35 $\mu\text{m}$	$0.68 \pm 0.014^b$	$39.89 \pm 0.77^b$	$0.71 \pm 0.15^b$	$9.76 \pm 2.27^b$
1 $\mu\text{m}$	$0.71 \pm 0.016^b$	$41.46 \pm 0.77^b$	$0.81 \pm 0.21^b$	$12.05 \pm 3.26^a$

Note: Significant differences between treatments based on Tukey test of significance are indicated by different lowercase letters ( $p < 0.05$ ). Data are means  $\pm$  SE ( $n = 5$ ).



**FIGURE 3** Interactive effect of warming, nitrogen addition and aboveground biomass removal on root biomass. Bars indicate mean  $\pm$  SE ( $n = 5$ ). The significance of effects indicated by \*\* =  $p < 0.05$ .

promote respiration. Even so, in-growth cores are recognised to be an accurate and simple approach to make these measurements. These results demonstrate the importance of mycorrhizae fungi on the C cycle, emitting  $\text{CO}_2$  approximately at roughly the same amount as root-only treatments.

Basal respiration (microbial or heterotrophic respiration) represented the largest proportion of respiration accounting for 58.5% of total respiration. Other studies found similar findings; Graham et al. (2014) and Zhang, Niu, et al. (2014) studying a grassland ecosystem found 71% and 50%, respectively. In a forest ecosystem, Heinemeyer et al. (2007) found a contribution of 65% of total respiration, while Subke et al. (2018) found 55.2% of the total emissions. Microbes can be responsible for the decomposition of both recent and older C in soil organic matter and by rhizosphere priming (heterotrophic respiration) (Dijkstra, Carrillo, et al., 2013; Ryan & Law, 2005; Trumbore, 2000). Again, our measures of basal respiration may be overestimated as we assumed that our control core (1  $\mu\text{m}$  mesh) contained microbes exclusively.

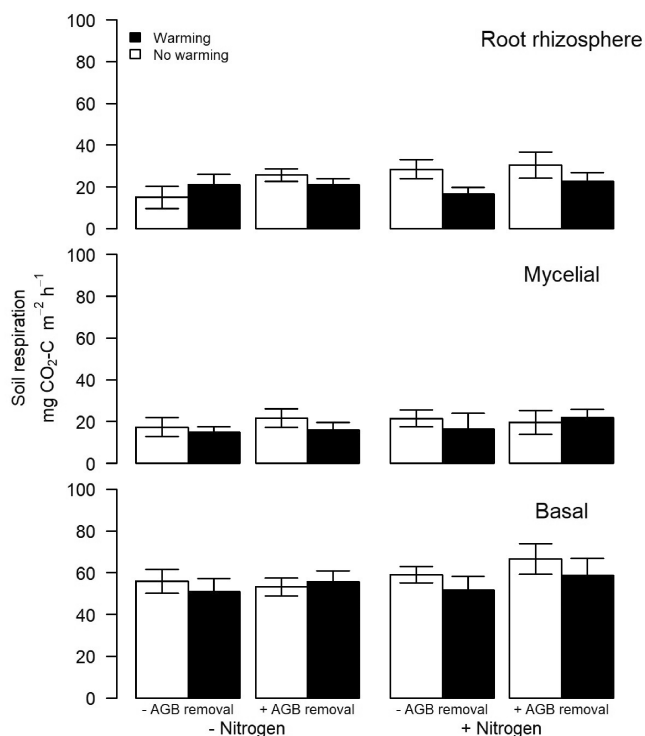
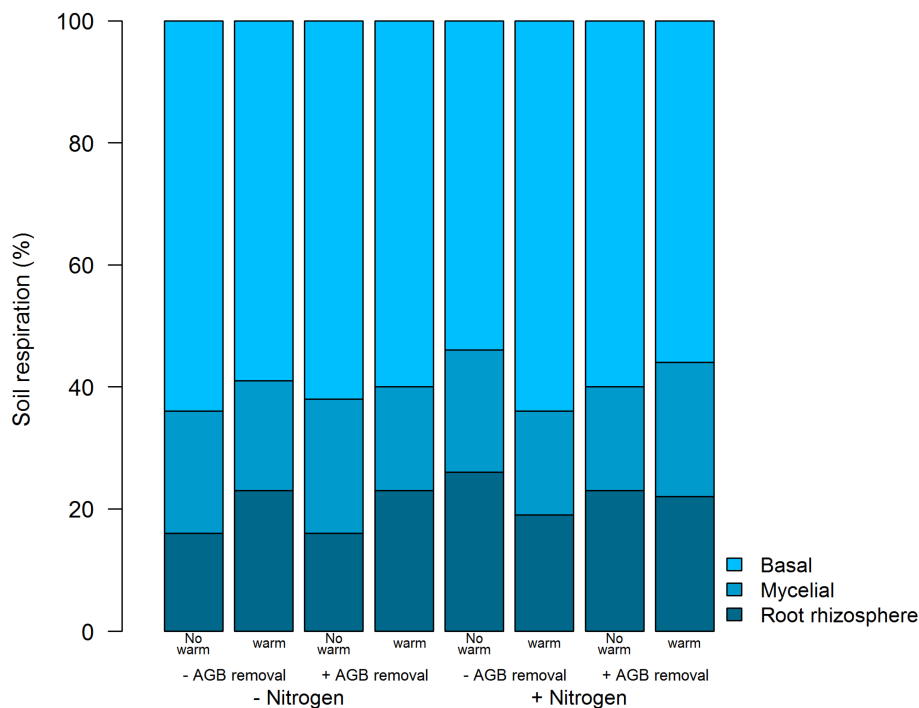
While short-term studies (in this case a one-year study) may not be expected to increase soil C and consequently soil C sequestration, other studies had similar findings suggesting that mycorrhizae make a large contribution on the C sequestration in the soil in the long-term, due to the production of chitin, and in particular in AMF fungi, glomalin production (approx. 30%–60% of C in soil) (Treseder & Allen, 2000). Johnson et al. (2002) also showed a highly dependency of AMF on photosynthetic activity, approximately 4%–6% of photo-assimilates were from the mycelium respiration. As atmospheric  $\text{CO}_2$  concentration rises, plants will face a growing limitation in terms of nutrients rather than carbon, potentially leading to an increased allocation of C to external mycelia for nutrient uptake (Terrer et al., 2016; Vicca et al., 2012). Nevertheless, the short duration of the experiment may be the reason for a lack of significant differences on the total soil C and N.

## 4.2 | Effect of warming, nitrogen addition and aboveground biomass removal on soil respiration partitioning

A significant climate effect was observed with warming decreasing basal respiration, whilst diminishing soil  $\text{NO}_3^- - \text{N}$  availability and root biomass. Several studies suggest an increase in soil respiration and component autotrophic and heterotrophic sources under warming scenarios (Rustad et al., 2001). There are several explanations for the reduction of soil basal respiration in our study. Firstly, the warming effect might be transient (Luo et al., 2001; Melillo et al., 2002), with longer-term ecosystem acclimation explained by reduction of the root respiration rate (Burton et al., 2008) due to a reduced root biomass (Zhou et al., 2011). Secondly, an indirect effect of warming leading to drier soil (confirmed by our measurements of soil water content), limiting soil respiration (Pendall et al., 2004). Thirdly, soil warming may have led to an increase in N-mineralisation and higher  $\text{NO}_3^-$  leaching or immobilisation affecting basal respiration due to limited labile C supply (Hillstrom et al., 2010).



**FIGURE 4** Relative contribution of root rhizosphere, mycelial and basal respiration on the total soil respiration in response to the interactive effect of warming, nitrogen addition and aboveground biomass removal. Bars represent the percentage of total below ground respiration flux for each main treatment. No warm = no warming, warm = warming.



**FIGURE 5** Interactive effect of warming, nitrogen addition and aboveground biomass removal on the root rhizosphere, mycelial and basal respiration. Data are means for all sampling dates  $\pm$  SE ( $n = 11$ ). Stats can be found in Table 2.

Lastly, warming could have forced the conversion of a portion of the CO<sub>2</sub> to CH<sub>4</sub> (Pendall et al., 2004), explaining a reduction of respiration from the soil, although it is an unlikely mechanism, unless the soil were very wet.

Although evidenced in some studies (Rustad et al., 2001) and in one of our hypotheses, increases in temperature did not affect mycorrhizal respiration. In a review by Mohan et al. (2014), warming showed a decrease in mycorrhizae activity (71% of studies), although mycorrhizae abundance was found to be increased in 63% of the evaluated studies. The lack of response on the mycorrhizae respiration could be attributed to an indirect effect of warming. Warming is supposed to increase net N mineralisation (Melillo et al., 2011; Rillig, Treseder, & Allen, 2002; Rillig, Wright, et al., 2002), thus causing a warming-induced indirect 'fertilisation effect' (Mohan et al., 2014). In this way, AMF fungi can be 'inhibited' by fertilisation (Blanke et al., 2012), as N availability increases in the soil, grassland plant hosts became less dependent on mycorrhizae for N acquisition (Mohan et al., 2014). Again, contrary to our hypothesis, N addition did not affect soil respiration in either of the soil components. Lilleskov et al. (2011) suggest that AMF abundance is not consistently affected by increased N availability, although N addition is highly related to increase of plant productivity. The direct role of mycorrhizae on increases in productivity is not clear. Nevertheless, a three-way interaction between warming, N addition and AGB removal was found in our study, suggesting that warming in some way interacted with grassland management, affecting mycorrhizal respiration.

As reported in many experiments, AGB removal or clipping negatively affects total soil respiration (Bremer et al., 1998; Wan & Luo, 2003; Zhou et al., 2007), however, its effect may differ in each belowground

**TABLE 2** Effects of warming (WARM), nitrogen addition (NADD) and aboveground biomass removal (AGB REMOVAL) on the root rhizosphere, mycelial and basal respiration.

	d.f.	Root rhizosphere respiration		Mycelial respiration		Basal respiration	
		mg CO <sub>2</sub> -C m <sup>-2</sup> h <sup>-1</sup>		LRT	p	LRT	p
WARM	1	2.18	0.14	0.68	0.41	<b>4.74</b>	<b>0.03</b>
AGB REMOVAL	1	2.50	0.11	0.99	0.32	<b>4.56</b>	<b>0.03</b>
NADD	1	1.75	0.18	0.42	0.51	2.44	0.12
WARM × AGB REMOVAL	1	0.24	0.62	1.84	0.17	0.05	0.81
WARM × NADD	1	<b>3.92</b>	<b>0.04</b>	0.17	0.67	1.02	0.31
AGB REMOVAL × NADD	1	0.02	0.90	0.15	0.69	1.06	0.30
WARM × NADD × AGB REMOVAL	1	1.67	0.19	<b>4.26</b>	<b>0.04</b>	0.41	0.52

Note: 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.

component. As hypothesised, the short-term effect of clipping is observed in the basal respiration rather than root-rhizosphere respiration. The main reason is that roots have more carbohydrate reserves to continue the metabolism under limited C supply to the system (Bahn et al., 2006), thus root-rhizosphere respiration may not be affected by clipping in a temperate grassland (Moinet et al., 2019). The effect of AGB removal on the basal respiration may be due to the availability of fresh C (derived from rhizodeposition) affecting microbial decomposition of soil organic matter (Fontaine et al., 2004; Kuzyakov et al., 2002; Subke et al., 2004) and/or due to an increase of soil respiration affected by clipping. Thus, according to McSherry and Ritchie (2013), grazing intensities, grazing duration or climatic conditions can be reasons of variable results of different studies.

Contrary to our hypothesis, an interaction between warming and N negatively affected root respiration in our experiment, with no effects for mycorrhizal and basal respiration. Graham et al. (2014) studying the effect of N and warming in grassland found an additive effect, as warming drove the interactive effect. In our study, warming could have induced the mineralisation of the N added to the system and increased root N uptake, lowering soil mineral-N. This could lead to a C limitation to the system, reducing root respiration. Similarly, warming might increase the demand for N in plants due to increased growth rates. However, if N availability is limited, this increased demand could lead to N stress, which may ultimately suppress root respiration. As discussed before, the increase of N availability in the soil leading by the interaction between warming and N could inhibit mycorrhizal respiration, showing no effect. Although a reduction in root respiration was found in the belowground component level, warming

and N increased total soil respiration in the ecosystem level (Barneze et al., 2022).

Warming interactions with AGB removal did not result in significant changes in partitioned grassland soil respiration, and our results agree with those of Zhou et al. (2007) who investigated the effect of warming (2°C) and yearly clipping over 5 years in tallgrass prairie ecosystem, and did not find an interaction effect between warming and cutting on the soil respiration contribution. However, our study found that this interaction reduced total soil C and N which might be explained by a reduction of canopy photosynthesis, slowing the translocation of C to the rhizosphere, counteracting the effect of warming. Recently, Wang, Liu, et al. (2017) found that grazing over the growing season (warmed conditions) did not affect soil respiration and its components over 5 years measurements, however, cold-grazing occurred on the non-growing season enhanced autotrophic (23.2%) and heterotrophic (4.9%) respiration. In this study, the authors propose that the reduction in aboveground biomass caused by grazing may potentially offset the increase in temperature during the growing season. This interaction did not affect soil moisture, which then may be buffered against significant changes in soil respiration.

The interaction between AGB removal and N addition did not affect either any of the soil component respiration. We found no study that evaluated this interaction in the field, thus it is very difficult to predict responses to these changes. Despite this, an increase of soil moisture under the interaction between AGB removal and N addition was observed. The interaction effect may have led to an imbalance in soil microbial communities, and thus soil respiration dynamics. Studies suggest that extremes in soil moisture, high or low, may result in a reduction of root and/or basal respiration, which consequently will

inhibit soil respiration (Wang et al., 2003; Xu et al., 2004). This may be the reason by which the interaction between AGB removal and N addition did not affect soil respiration in the component level. Besides, Kuzyakov et al. (2002) found that after cutting the CO<sub>2</sub> efflux was reduced in the fertilised compared to unfertilised plants in a 55 day incubation experiment. The authors then suggest that N fertilisation might lower the C losses, especially in the regrown plants after cutting (due to reduction of C assimilation), limiting soil respiration.

## 5 | CONCLUSIONS

This study demonstrates that basal respiration accounted for most of the grassland soil respiration, followed by mycorrhizal and root respiration. The contribution of soil respiration did not differ under interactions between climate warming and grassland management. Warming may promote the acclimation of soil respiration, due to the decrease of basal respiration, while also decreasing soil NO<sub>3</sub><sup>-</sup> availability. Warming interacted with N addition decreasing root-rhizosphere respiration and mineral-N in the soil. Warming and AGB removal treatments had opposite effects on basal respiration; warming reduced it, while AGB removal increased it. Future experiments will need to include longer temporal and larger spatial scales (world temperate grasslands) to evaluate the potential impact on different soil belowground components. Overall findings show that, despite important individual effects, interactive effects of climate warming and management practices are often complex and difficult to predict.

### AUTHOR CONTRIBUTIONS

**Arlete S. Barneze:** Investigation; conceptualization; writing – review and editing; writing – original draft; data curation; methodology; validation. **Jeanette Whitaker:** Investigation; conceptualization; writing – review and editing; supervision; methodology. **Niall P. McNamara:** Investigation; conceptualization; writing – review and editing; supervision; methodology. **Nicholas J. Ostle:** Conceptualization; investigation; writing – review and editing; supervision; methodology.

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### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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