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The climate benefits of high-sugar grassland may be compromised by ozone pollution

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HIGHLIGHTS

- Exposure to elevated ozone had large impacts on growth and quality of high-sugar pasture.
- Negative effect of ozone on below-ground biomass and clover nodulation
- Ozone impacts on forage quality parameters include reducing sugar content.

GRAPHICAL ABSTRACT

Ozone impacts on climate-smart grass pasture.

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ABSTRACT

High sugar ryegrasses (HSG) have been developed to improve the uptake, digestion and nitrogen (N)-utilisation of grazing stock, with the potential to increase production yields and benefit climate by reducing methane (CH_4) and nitrous oxide (N_2O) emissions from livestock farming. In this study, the effects of tropospheric ozone pollution on the seasonal growth dynamics of HSG pasture mesocosms containing *Lolium perenne* cv. AberMagic and *Trifolium repens* cv. Crusader were investigated. Species-specific ozone (O_3) dose-response relationships (seasonal means: 35, 41, 47, 51, 59 & 67 ppb) based on the Phytotoxic Ozone Dose (PODy) were constructed for above and below ground biomass, injury, N-fixation and forage quality. The dynamics of effects of ozone exposure on HSG pasture changed over the course of a season, with the strongest responses occurring in the first 4–8 weeks. Overall, strong negative responses to ozone flux were found for root biomass, root nodule mass and N-fixation rates, and ozone adversely impacted a range of forage quality parameters including total sugar content and relative and consumable food values. These results indicate that increasing ozone pollution could decrease the N-use efficiency and reduce the sugar content of managed pasture, and thereby partially detract from some of the suggested benefits of HSG.

1. Introduction

Concentrations of tropospheric ozone have increased worldwide since the start of the industrial era (IPCC, 2013) and ground-level ozone is presently thought to be the most important air pollutant that directly affects vegetation (Royal Society, 2008). The largest increases in background ozone have occurred over the industrialised northern

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hemisphere, which reports an annual average of 30–40 ppb over the mid-latitudes (Royal Society, 2008) with additional increases likely in the coming decades (IPCC, 2013). In Europe, ambient surface concentrations frequently exceed critical thresholds/levels for plant protection (EEA, 2011, Mills et al., 2011a). Despite sustained efforts to control the emission of ozone precursor compounds, decreased cloud cover and increasing temperature are expected to increase the potential for ozone formation in the European region during this century (Katragkou et al., 2011; IPCC, 2013).

In recent years, environmental and economic concerns have led to the development of high-sugar ryegrass (HSG), novel cultivars designed to improve the uptake, digestion and nitrogen (N)-utilisation of grazing stock in managed pasture, and suggested as having the potential to increase production yields and reduce methane (CH_4) and nitrous oxide (N_2O) emissions from livestock farming (Ellis et al., 2011; Staerfl et al., 2012; IBERS, 2015). In parallel, rising fertiliser costs, the low N-use efficiency of traditional fertilisers in pasture, and climate change, have also spurred the development of new clover varieties for varied functions in pasture and fodder (British Grassland Society, 2014). In Europe, open-pasture grasslands constitute ~35% of the agriculturally utilised area (Smit et al., 2008), and are of high value in terms of biodiversity and food production. The importance of grassland as a large continental sink for the deposition of ozone, coupled with evidence of significant ozone effects on grassland species and communities, highlights the need to quantify the risks of rising ozone (Ashmore et al., 2007). However, historically, most studies concerning the effects of ozone have focused on the impacts on arable crops and forests (Richards et al., 1968; Roper and Williams, 1989), though in recent years more attention has been paid to the responses of natural and semi-natural grassland communities (Danielsson et al., 2013; Hayes et al., 2016; Wagg et al., 2013; Wyness et al., 2011). Amongst other effects, ozone induces premature senescence (Hayes et al., 2010a) and decreases the forage quality (Gonzalez-Fernandez et al., 2008; Gilliand et al., 2012; Gilliand et al., 2016; Hayes et al., 2016), gross primary production (GPP) (Calvete-Sogo et al., 2014) and yield of grassland vegetation (Danielsson et al., 2013). Grasslands may contain species both tolerant and sensitive to ozone and grassland legumes are thought to be particularly sensitive (Hayes et al., 2007). However, intact mature and long-established grassland may display a large degree of inertia to ozone stress (Stampfli and Fuhrer, 2010; Bassin et al., 2013; Volk et al., 2014).

Perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) are globally-important components of annually seeded grasslands and yet the effects of ozone on modern cultivars of both *L. perenne* and *T. repens* are virtually unknown (Gonzalez-Fernandez et al., 2008; Hewitt et al., 2014). Several older studies have considered the effects of ozone on the functioning of *Lolium*/*Trifolium* pasture, with most having focused on the response of above-ground biomass (Fuhrer et al., 1994; Nussbaum et al., 1995; Wilbourn et al., 1995). More modern cultivars, bred for higher productivity, may display increased sensitivity to ozone as has been found in wheat (Velissariou et al., 1992; Pleijel et al., 2006) and soybean (Osborne et al., 2016). Despite their important potential future role in reducing greenhouse gas (GHG) emissions and N excretion from grazing ruminants, no previous study has considered the effects of ozone on the digestibility of HSG cultivars including on sugar content, a rationale central to the development of HSG (IBERS, 2015).

The phytotoxic ozone dose above a threshold of Y nmol m^{-2} s^{-1} (POD_Y) is an exposure index based on the modelled flux of ozone into the plant via the stomatal pores. Stomatal conductance of ozone is estimated using the multiplicative Jarvis-type conductance model (Jarvis, 1976), which requires the parameterisation of biotic and abiotic modifying factors such as phenology, temperature and light (Emberson et al., 2000). The more biologically relevant POD_Y provides a better fit to effect-data than concentration-based exposure-based indices (Mills et al., 2011a), and flux-based critical levels of ozone have been developed for key agricultural and forest species (Mills et al., 2011b; Danielsson et al., 2013). For productive grasslands, and grasslands of high conservation value, an accumulated 3-month POD_1 of 2 mmol m^{-2} has been provisionally determined as the critical level, representing the ozone flux required to induce a 10% reduction in the shoot biomass yield of *T. repens* (Mills et al., 2011a). This provisional critical level is currently only based on UK and Swiss data, and dose-response relationships suitable for derivation of critical levels for several other aspects of managed pasture, such as N-fixation, below ground biomass and forage quality, remain undeveloped. In addition, there is a longstanding need to develop multi-species, multi-layer dose-response relationships, which are required to better simulate the complexity of pasture systems.

In this study, we investigated the impact of a range of possible future ozone scenarios on pasture mesocosms containing a variety of HSG (*L. perenne* cv. AberMagic) and a modern cultivar of white clover (*T. repens* cv. Crusader) introduced in 2012 and 2009 respectively. Through the use of multiple ozone exposure treatments, we tested the hypothesis that ozone reduces above and below-ground biomass production, nodulation, and N-fixation, and increases leaf injury. We also tested the hypothesis that ozone reduced the forage quality of mixed-species pasture, including the total sugar content, a factor targeted by breeders (IBERS, 2015).

2. Materials and methods

2.1. Pasture mesocosms

In early April 2013, *L. perenne* cv. AberMagic, a variety recommended for long-term pasture (British Grassland Society, 2014), was sown as seed at a density of 4.7 g/m^2 directly into 10 L pots (27.5 cm diameter × 22 cm height) filled with compost (John Innes No. 2; J. Arthur Bowers, Lincoln, UK). *T. repens* cv. Crusader plants, recommended for general use in grassland leys (British Grassland Society, 2014), were also propagated from seed, grown in the same compost in plug-plant trays in an unheated glasshouse. After 4 weeks of growth, 3 clover plants were transferred to each 10 L pot, with one clover plant in each third of the pot. A plastic collar (6 cm diameter, 5 cm depth) was inserted to a depth of 4 cm within the centre of each pot for subsequent acetylene reduction assays. Seeds were obtained from a commercial seed supplier (Wynnstay Seeds; UK). To introduce a soil microbe population, pots were inoculated with 400 ml of a soil slurry mixture made from 5 kg of soil from agricultural grassland (Abergwyngregyn, North Wales, UK, 53°14′N, 4°01′W) and 14 L water. Mesocosms were grown for a further 4 weeks in ventilated greenhouses under optimum watering conditions. On 07/06/2013, 24 pots, of equal size and distribution of clover and grass, were transferred to each of 6 'solardomes' (ventilated hemispherical glasshouses; 3 m diameter, 2.1 m high) at the CEH solardome facility near Bangor, North Wales.

2.2. Ozone system and treatments

Ozone exposure was based on an episodic profile recorded at a rural ozone monitoring site (Aston Hill, Wales, UK, 52°50′N, 3°03′W 15/07/2006 to 22/07/2006) with a unique, weekly repeating treatment in each solardome as described previously (Hewitt et al., 2014). In summary, the Aston Hill profile was applied as the highest treatment, and for the 5 other treatments the concentration was reduced by 10 ppb during the peaks and 3 ppb during periods with background O_3. Ozone treatments were applied to the solardomes randomly. In one solardome, ambient air temperature, photosynthetically active radiation (PAR) and relative humidity were continuously monitored by an automatic weather station (Skye Instruments Ltd, Llandridod Wells, UK) and soil moisture content was continuously monitored using Theta Probes (Delta-T Devices Ltd, Cambridge, UK). Plants were rotated within each dome weekly and watered twice-weekly, with additional watering when necessary to maintain soil moisture content at or near field capacity. Plants