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RESEARCH PAPER

Latitudinal variation in ambient UV-B radiation is an important determinant of *Lolium perenne* forage production, quality, and digestibility

David Comont[1], Ana Winters[1], Leonardo D Gomez[2], Simon J McQueen-Mason[2] and Dylan Gwynn-Jones[1,*]

[1] Institute of Biological Environmental and Rural Sciences, Aberystwyth University, Ceredigion, SY23 3DA, Wales, UK
[2] CNAP, Biology Department, University of York, Heslington, York, YO10 5DD, UK

* To whom correspondence should be addressed. E-mail: dyj@aber.ac.uk

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Abstract

Few studies to date have considered the responses of agriculturally important forage grasses to UV-B radiation. Yet grasses such as *Lolium perenne* have a wide current distribution, representing exposure to a significant variation in ambient UV-B. The current study investigated the responses of *L. perenne* (cv. AberDart) to a simulated latitudinal gradient of UV-B exposure, representing biologically effective UV-B doses at simulated 70, 60, 50, 40, and 30° N latitudes. Aspects of growth, soluble compounds, and digestibility were assessed, and results are discussed in relation to UV-B effects on forage properties and the implications for livestock and bio-ethanol production. Aboveground biomass production was reduced by approximately 12.67% with every 1 kJ m^{-2} day^{-1} increase in biologically weighted UV-B. As a result, plants grown in the highest UV-B treatment had a total biomass of just 13.7% of controls. Total flavonoids were increased by approximately 76% by all UV-B treatments, while hydroxycinnamic acids increased in proportion to the UV-B dose. Conversely, the digestibility of the aboveground biomass and concentrations of soluble fructans were reduced by UV-B exposure, although soluble sucrose, glucose, and fructose concentrations were unaffected. These results highlight the capacity for UV-B to directly affect forage productivity and chemistry, with negative consequences for digestibility and bioethanol production. Results emphasize the need for future development and distribution of *L. perenne* varieties to take UV-B irradiance into consideration.

Key words: Carbohydrate, digestibility, forage quality, growth, phenolics, ultraviolet-B.

Introduction

Perennial ryegrass (*Lolium perenne* L.) is a temperate grassland species, widely distributed across Europe as a forage and turf grass (Hannaway *et al.*, 1999). The distribution and use of *L. perenne* is likely to increase due to rising global demand for meat and livestock (Speedy, 2003; Daniel *et al.*, 2010). Recently, high-sugar varieties of *L. perenne* have been developed because the accumulation of foliar water-soluble carbohydrates increases the available energy for early fermentation in ruminants. This improves capture of forage protein (Theodorou *et al.*, 2006), significantly increasing weight gain and milk production from livestock (Lee *et al.*, 2001; Miller *et al.*, 2001). These varieties are also ideal candidates for future bio-ethanol production (Charlton *et al.*, 2009; Farrar *et al.*, 2011), providing a significantly greater yield of ethanol than other *L. perenne* varieties (Charlton *et al.*, 2009; Murphy and Power, 2009). Due to such potential benefits, this species has increasingly been adopted for use as forage. However, with the exception of temperature constraints on growth (Hulke *et al.*, 2008; Höglind *et al.*, 2011) and the effect of elevated temperature and CO_2 concentrations (Nijs *et al.*, 1996, 1997), few studies have considered how climatic factors control or limit the growth, productivity, and forage chemistry of this species.

Ultraviolet-B radiation (UV-B, 280–320 nm) varies substantially over the latitudinal range where *L. perenne* is employed

(Seckmeyer *et al*., 2008). Although it is a small component of sunlight, UV-B can be disproportionally damaging to plants, affecting tissue chemical composition and reducing growth and yield (Rozema *et al*., 1997b; Hollosy, 2002; Mpoloka, 2008; Ballaré *et al*., 2011). UK-developed varieties of *L. perenne* previously distributed across North-Western Europe (Marshall and Wilkins, 2003; Wilkins and Lovatt, 2004; Conaghan *et al*., 2008) are now being increasingly distributed in Southern Europe and Australasia, where ambient UV-B levels are markedly higher. Previous work by Deckmyn and Impens (1999) showed that *L. perenne* was sensitive to UV-B radiation, finding that UV-B caused a decrease in plant height and an increase in tillering. Similarly, growth location of *L. perenne* has been shown to significantly affect biomass and UV-B absorbing compounds in this species (Comont *et al*., 2012). However, no known studies have looked at how variation in ambient UV-B will affect forage quality traits of this species including carbohydrates and digestibility. The implications of UV-B on subsequent employment of this species for forage and bioethanol production have therefore not been considered.

There are no consistent patterns in terms of plant carbohydrate response to UV-B. Exposure to UV-B significantly increased total soluble leaf carbohydrates in *Vaccinium uliginosum* during heath regeneration (Phoenix *et al*., 2000). Similarly UV-B led to increased fructose concentrations in *Chenopodium quinoa* seedlings (Hilal *et al*., 2004), although the effect on total carbohydrates was negated by a uniform reduction of sucrose concentrations over a range of UV-B exposures. Further studies have either found no effect of UV-B on carbohydrates (Gehrke *et al*., 1995; Rozema *et al*., 1997a; Barsig and Malz, 2000) or found UV-B to cause decreased concentrations (Yue *et al*., 1998; Gwynn-Jones *et al*., 1999; Lindroth *et al*., 2000; Ghisi *et al*., 2002). However, given the importance of soluble carbohydrates for bioethanol production (Charlton *et al*., 2009) and grazing efficiency (Theodorou *et al*., 2006), the effect of UV-B on these compounds demands consideration.

More consistent patterns are reported for soluble foliar phenolics which usually increase in plants in response to UV-B (Agati and Tattini, 2010), and this has been demonstrated in grasses and other monocots (Liu *et al*., 1995; Burchard *et al*., 2000; van de Staaij *et al*., 2002). The accumulation of soluble phenolic monomers in forage may be beneficial for animal consumers as such chemicals protect protein from degradation in the rumen (Theodorou *et al*., 2006) and have been shown to suppress methane emissions during ruminant digestion (Leiber *et al*., 2012). However, phenolic monomers induced for UV-B defence may form intermediates in the synthesis of structural components (including lignin) and insoluble cell-wall-bound phenolics (Tu *et al*., 2010), which may negatively affect decomposition and digestibility (Hartley and Ford, 1989; Rozema *et al*., 1997a; Buanafina *et al*., 2006; Austin and Ballaré, 2010). A reduction in digestibility would negatively affect this species' utility as forage and would also impair second-generation bioethanol production which utilizes digestion of the lignocellulose to release further sugars for conversion to ethanol (Gomez *et al*., 2010).

The current study investigates aboveground and belowground growth, digestibility, and soluble foliar metabolites associated with forage quality of *L. perenne,* in response to a simulated latitudinal gradient of UV-B. Concentrations of soluble hydroxycinnamic acids, flavonoids, and carbohydrates are examined to determine how a UV-B gradient affects the accumulation of these metabolites in this high-sugar variety of *L. perenne* (cv. AberDart). The total foliar metabolomic fingerprint is also assessed using Fourier transform infrared spectroscopy (FT-IR), and wavenumbers associated with forage quality traits are examined for their contribution to the overall responses observed. Additionally, the effect of the UV-B gradient on biomass saccharification has been determined, and results are discussed in relation to forage quality and bio-ethanol production. This will address the hypotheses that growth and foliar carbohydrate concentrations will be negatively affected by UV-B, while foliar phenolics will accumulate under UV-B irradiance. These changes in the foliar soluble chemistry, coupled with changes in structural metabolites, will lead to a reduction in digestibility by UV-B with implications for the utility of this species as forage at high ambient UV-B.

Materials and methods

Growth conditions and UV-B treatment

Seed of perennial ryegrass (*L. perenne* L. cv. AberDart) sourced from IBERS (Aberystwyth University, UK) were germinated and grown in individual 7 × 7 cm pots containing pre-fertilized vermiculite in a glasshouse for the duration of experimentation. Vermiculite was fertilized using a 0.8% solution containing an NPK 36:6:6 fertilizer (Miracle Grow, UK) and air dried before use. Plants were watered from below to minimize nutrient leaching, and no visible signs of nutrient deficiency were observed in plants over the experiment. Whilst it is accepted that plants grown in a non-soil-based medium may differ from those grown in natural soil conditions, vermiculite was specifically chosen for the current study as it allowed for easier extraction and more accurate analysis of root material. Seedlings were germinated for 2 weeks prior to treatment. UV-B treatments were then initiated at this early developmental stage and maintained for a further 5-week experimental period. Temperature and humidity were not controlled but remained on average 15 °C and 59% relative humidity over the course of the experiment, and minimum temperature did not drop below 5 °C (monitored using Helios Dataloggers, Skye Instruments, Llandrindod Wells, Wales).

The aim of this study was to simulate a latitudinal gradient of biologically effective UV-B doses ($UV\text{-}B_{BE}$) within the experimental glasshouse, simulating 70, 60, 50, 40, and 30 °N latitudinal treatment conditions, as well as a zero UV-B control. Target UV-B doses were determined using a UV radiation software model (the Atmospheric Chemistry Division Tropospheric Ultraviolet and Visible (ACD TUV) calculator, available at http://cprm.acd.ucar.edu/Models/TUV/), set to model changes in UV-B radiation under clear sky and overcast conditions over 5 weeks, from 29 June to 3 August). In the glasshouse, UV-B supplementation was provided using lamp-holders containing two Q-panel 313 UV-B bulbs (Q-Panel, Cleveland, Ohio, USA). Lamps were wrapped in cellulose diacetate (0.13 mm, Courtaulds, Derby, UK) to attenuate UV-C, and UV-B was provided using a square-wave approach over 8 hours daily (centred at noon). Lamp height was adjusted to vary the UV-B within each treatment to match the target daily UV-B dose rates modelled using the UV radiation software model. UV-B was assessed using a Fibre Optic Spectroradiometer (EPP2000, StellarNet, Tampa, Florida, USA) and weighted with the

Caldwell generalized plant damage action spectrum (Caldwell *et al*., 1986). Treatments represented doses of 2.3, 3.2, 4.1, 5.0, and 5.7 kJ m^{-2} day^{-1} UV-B_{BE} in the simulated 70, 60, 50, 40, and 30 °N latitudes respectively, and total UV-B at plant height remained stable over the experimental duration, monitored using a broadband UV-B sensor (Spectrosense 2^{+}, Skye Instruments). Supplementary photosynthetically active radiation (PAR) was also provided (200 µmol m^{-2} s^{-1}) for 8 hours daily so that shade events caused by movement of the sun were less pronounced.

Harvesting of material

An initial ($t = 0$) harvest was performed before treatments were initiated. Plants were then split between five replicated trays per treatment, each tray containing five individual plants. A further harvest was subsequently taken at the end of the 5-week experimental treatment period. To ensure sufficient material for chemical analysis, the five plants per tray were grouped as one replicate by taking the mean of their growth parameters and bulk grinding their aboveground material. This provided an overall replication per treatment of $n = 5$. Plants were separated into aboveground and belowground material, and root systems were washed in running water to remove vermiculite. Root systems were placed onto glass Petri dishes with gridded (0.5 cm^2) paper and photographed using a fixed camera mounted 1 m above. Total root length was assessed using the line intersect method (Tennant, 1975). Total plant leaf area (cm^2) was assessed using a leaf area measurement system (Delta-T devices, Cambridge, England). Leaf material was next flash frozen in liquid nitrogen and stored at –80 °C. The total aboveground and belowground biomass was recorded following freeze drying (48 hours at –50 °C) using a vacuum freeze drier (Lyotrap, LTE Scientific, Oldham, England). The growth parameters leaf area ratio, leaf weight ratio, specific leaf area, net assimilation rate, relative growth rate, and root:shoot ratio were assessed using formulae given by Hunt *et al*. (2002).

Assessment of soluble phenolics and carbohydrates

Soluble foliar phenolics and sugars were assessed by HPLC. Freeze-dried leaf material (20 mg) was ground and extracted twice, first in 70% methanol then in water, with the two supernatants combined. Supernatants were pelleted in a vacuum centrifuge (Savant SpeedVac SPD121P, Thermo-Scientific, Asheville, USA), then re-suspended in 500 µl water and split into two 250 µl aliquots. One aliquot was partially purified on a Waters Sep-pak (500 mg) C^{18} reversed-phase extraction cartridge (Waters, Elstree, UK) before analysis using a Waters HPLC system. A Nova-pak (4 µm, 8 × 100 mm) C^{18} column was used as the solid phase, while the mobile phase consisted of 5% acetic acid (solvent A) and 100% methanol (solvent B) with a linear gradient from 5–70% B in A, over 35 min. The injection volume was 25 µl.

The second 250 µl aliquot was used for assessment of soluble sugars, with 50 µl added to 950 µl buffer comprising 5 µM H_2SO_4, which contained 5 µM crotonic acid as an internal standard. These samples were analysed by HPLC (Jasco, Essex, UK). A Rezex ROA-Organic acid column (150 × 7.8 mm) was used, with a mobile phase of 5 mM H_2SO_4 at 0.6 ml min^{-1}. Peaks were identified using EZChrom Elite HPLC software (Jasco), and quantified by comparison with standard samples of fructan, sucrose, glucose, and fructose (Sigma Aldrich, St Louis, Missouri, USA).

FT-IR assessment of total metabolite fingerprint and structural material

Freeze-dried leaf samples (10 mg) were processed and analysed using FT-IR as a measurement of their metabolomic fingerprint. Samples were ground and suspended in de-ionized water at a concentration of 100 µg ml^{-1}. Each sample (5 µl) was pipetted onto an FT-IR plate and dried at 50 °C for 30 min before analysis using a Vertex 70 FT-IR spectrometer (Bruker Optics, Ettlingen, Germany). Absorbance spectra were collected at 1762 wavenumbers over the range 600–4000 cm^{-1}. The spectra were assessed for separation between UV-B treatments, to explore the linearity of response to the UV-B gradient over the whole metabolomic fingerprint. Additionally, FT-IR was used as a tool to assess the effect of the UV-B_{BE} gradient on leaf structural material. Characteristic spectral regions associated with cellulose, hemicellulose, and lignin were identified from the literature (Table 1) and total absorbance over these regions used as a proxy for measurement of these compounds.

Assessment of digestibility

Samples of freeze-dried leaf material (50 mg) were assessed using an automated enzymic digestion system, as described by Gomez *et al*. (2010). After an alkaline pre-treatment (0.5 mol NaOH l^{-1} at 90 °C for 30 min), samples were hydrolysed for 8 hours using an enzyme cocktail designed for biomass (Celluclast/Novozyme 188, 4:1, Novozymes, Bagsvaerd, Denmark). Following digestion, a 75 µl aliquot of the digestate was removed and added to a mixture comprising 25 µl NaOH (1 mol l^{-1}), and 50 µl of a solution containing (ml^{-1}) 0.43 mg 3-methyl-2-benzothiazolinonehydrazone and 0.14 mg dithiothreitol in a PCR plate. This mixture was incubated at 60 °C for 20 min before an aliquot was removed, placed into an optical plate, and 100 µl of an oxidizing reagent containing 0.5% $FeNH_4(SO_4)_2$, 0.5% sulphamic acid, and 0.25 mol l^{-1} HCL was added. Quantification of reducing sugars was performed calorimetrically at 620 nm. Four machine replicates of each individual sample were performed and the mean result used for analysis.

Statistical analysis

All parameters (excluding FT-IR data) were analysed using one-way ANOVA followed by Tukey's multiple-range test ($n = 5$, $P < 0.05$) in Minitab version 14 (Minitab, Coventry, UK). FT-IR spectra

Table 1. Characteristic FT-IR absorbance of the plant structural components cellulose, hemicellulose, and lignin. Data were taken from tables compiled by Mascarenhas *et al*. (2000) and Lammers *et al*. (2009).

Wavenumber cm^{-1}	Functional group	Compound	Reference
1705–1720	Carbonyl/carboxyl stretch	Lignin	Boeriu *et al*. (2004)
1215–1220	C–C, C–O, C=O	Lignin	Boeriu *et al*. (2004)
1370–1375	Phenolic OH	Lignin	Boeriu *et al*. (2004)
1610	–	Lignin	Boeriu *et al*. (2004)
1510, 1514, 1595	Phenylpropanoid/aromatic ring	Lignin	Mascarenhas *et al*. (2000)
			Boeriu *et al*. (2004)
1050–1130	Polysaccharide	Cellulose	Stewart (1996)
1150–1170	C–O–C stretch	Cellulose	Michell (1990)
1240, 1732	Ester C=O	Hemicellulose	Stewart and Morrison (1992)

were analysed using principal components analysis, multivariate analysis of variance (MANOVA), and canonical variates analysis (CVA) as described in Johnson *et al*. (2007), using Minitab version 14 (Minitab inc., Coventry, UK), Matlab version 6.5 (Mathworks Inc, Natick, massachusetts, USA), and PyChem 3.0.5f beta (Jarvis *et al*., 2006). The minimum number of principal components (PCs) explaining >99% of the data's variability was chosen as the threshold for PC selection. No spectral pre-processing was performed as all spectra recorded very similar maximum and minimum absorbance, with a high signal to noise ratio. Mean centering of the spectra was initially performed but was found to have no effect on the analysis.

Results

UV-B modelling and simulation of the UV-B_{BE} gradient

To identify the target UV-B_{BE} doses required for an ecologically relevant latitudinal gradient (30–70 °N) of UV-B_{BE}, a UV radiation software model (the ACD TUV calculator) was first used to model changes in UV-B radiation (280–320 nm) over this range (Supplementary Fig. S1, available at *JXB* online). As expected, UV-B_{BE} doses over the modelled time frame were greatest at 30 °N and lowest at 70 °N. Maximal dose rates per hour were approximately 0.4 and 1.5 kJ UV-B_{BE} m^{-2} h^{-1} at the modelled 70 and 30 °N latitudes respectively. Increasing the modelled cloud optical depth caused a substantial reduction in the modelled UV-B_{BE}.

In the glasshouse, lamp heights were adjusted so that the biologically effective daily dose rates provided a gradient of daily UV-B_{BE} in five UV-B supplementation treatments representing 70, 60, 50, 40, and 30 °N latitude. Biologically weighted hourly UV-B_{BE} dose rates did not exceed the hourly maxima modelled for each latitude. The weighted daily UV-B_{BE} doses provided a gradient of UV-B_{BE}, with values falling between the clear sky and overcast conditions modelled using the tropospheric UV-B modelling software (TUV) (Supplementary Fig. S2) and constituted doses of 2.3, 3.2, 4.1, 5.0, and 5.7 kJ UV-B_{BE} m^{-2} day^{-1}. A control (zero UV-B_{BE} treatment) was also established providing a standard against which the effects of the UV-B_{BE} gradient could be tested.

Effects of the UV-B_{BE} gradient on aboveground and belowground growth

Growth of *L. perenne* was significantly inhibited by UV-B exposure (Fig. 1, Supplementary Fig. S3). Negative effects of UV-B were observed both above ground in tiller length, biomass and leaf area, and below ground in total root length and biomass (Fig. 1). In all cases, the greater the UV-B_{BE} dose the more negative the effects, as most clearly shown in assessment of leaf area and relative growth rate (Fig. 1E and F). Acclimation to UV-B was observed at low UV-B irradiance via a reduction in leaf area ratio relative to the zero UV-B control, representing a reduced resource allocation to leaf area production. At higher UV-B irradiance, leaf area ratio was unaffected but net assimilation rate was reduced (Fig. 1G and H). This suggests that alteration of morphology under low UV-B irradiance allows productivity in relation to leaf area (i.e. net assimilation rate) to be maintained, but a comparable response was not observed at greater UV-B irradiance causing net assimilation rate to be adversely affected.

Effects of the UV-B gradient on soluble foliar carbohydrates and phenolics

Fructan was found to be the dominant soluble foliar carbohydrate present, but there was a significant reduction in concentration in samples from higher UV-B exposure regimes relative to control and 70 °N treatments (Table 2). However, no significant UV-B effect was found in the foliar concentrations of glucose, sucrose, or fructose (Table 2). In contrast, total foliar hydroxycinnamic acids (HCAs) and flavonoids were significantly increased by UV-B (Fig. 2). Chlorogenic acid was the dominant HCA in leaf extracts, and concentrations increased proportionally with the UV-B_{BE} dose, resulting in a monotonic increase in total HCA concentrations with UV-B_{BE} (Fig. 2A). Total flavonoids were increased by approximately 76% over all UV-B treatments relative to controls, although the 40 and 60 °N treatments could not be separated from controls due to large variability between samples (Fig. 2B).

Chemometric analysis of FTIR spectra in relation to UV-B

PC-CVA and MANOVA was applied to the FT-IR spectra according to Johnson *et al*. (2007). The first eight PCs were found to contain >99% of the FT-IR spectra's variability, and MANOVA identified a significant treatment effect within them ($P < 0.001$). CVA of these eight PCs identified significant separation between the canonical treatment means according to the UV-B_{BE} dose rate (Fig. 3A). Analysis of the PC-CVA loadings identified peaks on both axes between 1550 and 1800 cm^{-1} (Supplementary Fig. S4). Absorbance in this region is dominated by amides (Allwood *et al*., 2008), suggesting separation between groups on both axes may be caused by changes in protein. Wavenumbers around 3500 cm^{-1} also show strong weighting on CV2, and are usually characteristic of hydroxyl groups, possible related to alcohols, phenols, or sugars (Nakanishi and Solomon, 1977).

Further targeted analysis of wavenumbers associated with cellulose, lignin, and hemicellulose (Table 1) was performed, but showed no significant overall treatment effects ($P > 0.05$). Determination of treatment effects in specific wavenumber groupings such as this may be hampered by potential co-absorbance from other metabolites present. Nevertheless, in each case there was a strong trend for change in FT-IR absorbance with UV-B (Fig. 3B–D). Sample groups were therefore pooled and re-analysed to further highlight these pronounced trends within the data. Total absorbance over wavenumbers associated with cellulose showed a distinct increase between the 60 and 50 °N treatments. When the data were analysed as two groups ($n = 15$) according to low UV-B (control, 70°, 60°) and high UV-B (30°, 40°, 50°), a significant result is observed

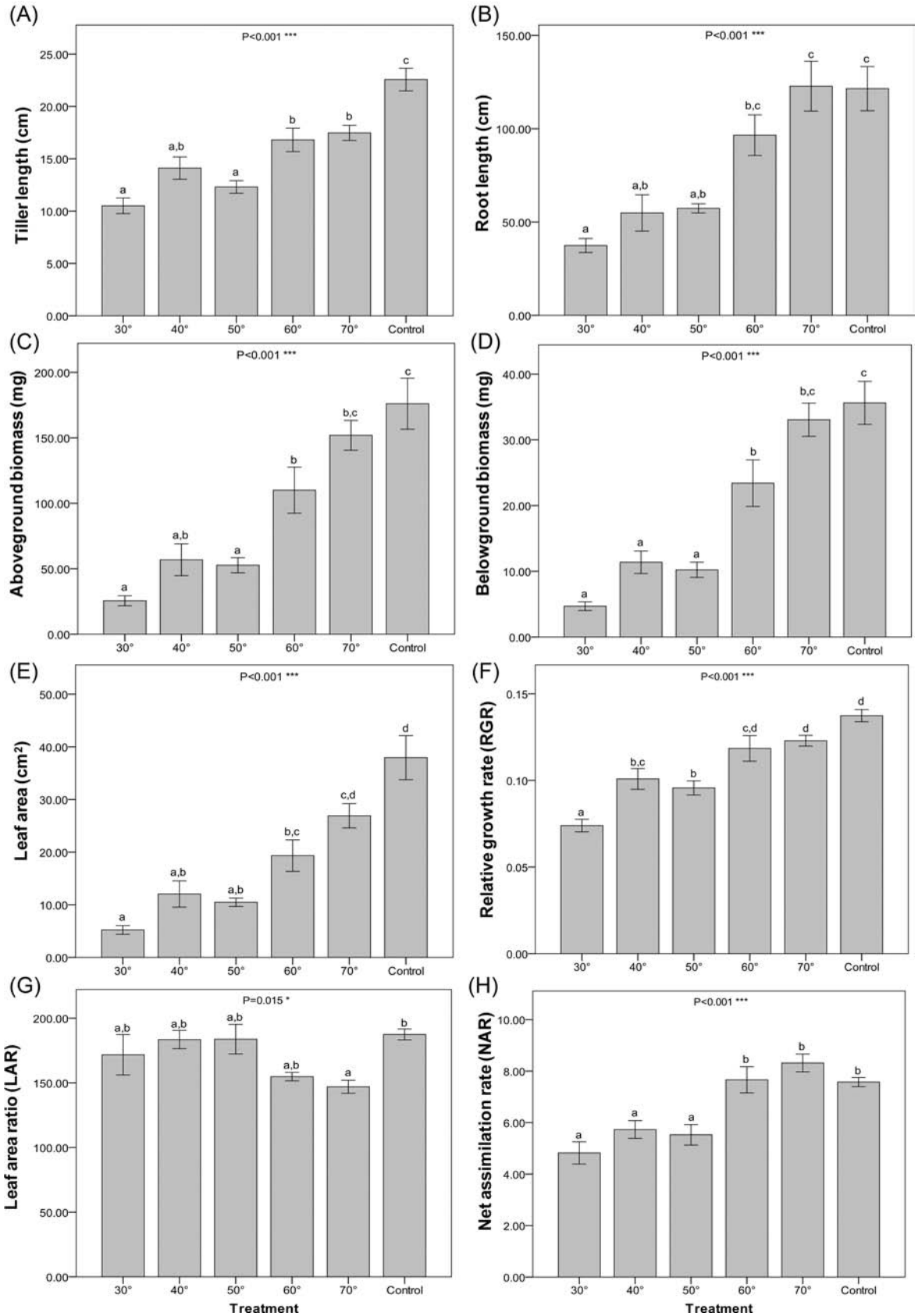

Fig. 1. Results of the simulated UV-B_{BE} gradient on aboveground and belowground plant measurements. Relative growth rate (RGR) is measured as biomass accumulation in g g^{-1} day^{-1}, while net assimilation rate (NAR) has units of g m^{-2} day^{-1}. *P*-values are the result of analysis using a one-way ANOVA (* <0.05, ** <0.01, *** <0.001). Letters denote differences between treatments as shown by a Tukey's multiple-range test ($n = 5$, $P < 0.05$). Values are mean ± SE.

Table 2. Foliar sugar concentrations extracted from leaf material (mg g^{-1}) assessed by HPLC. Values are mean ± SE. *P*-values show the result of a one-way ANOVA (* <0.05, ** <0.01, *** <0.001). Letters denote significant differences following a Tukey's multiple-range test ($n = 5$, $P < 0.05$).

	P-value	Treatment (UV-B_{BE} dose kJ m^{-2} day^{-1})					
		Control (0)	70° (2.3)	60° (3.2)	50° (4.1)	40° (5.0)	30° (5.7)
Fructans	0.003**	116.1±18.1[a]	116.0±13.6[a]	87.0±12.5[a,b]	68.7±13.5[a,b]	36.1±13.3[b]	52.5±18.1[b]
Sucrose	0.586	31.8±5.24	36.9±4.15	37.4±4.66	42.2±10.5	34.5±2.94	32.2±1.95
D(+)-Glucose	0.143	14.9±2.78	22.1±1.90	17.9±2.00	18.2±1.45	20.6±1.04	18.5±0.62
D(–)-Fructose	0.505	37.1±3.83	42.9±5.30	38.3±4.20	37.5±3.07	37.1±2.62	34.5±2.50

($P = 0.007$). Similarly, hemicellulose and lignin absorbances showed a consistent increase with increasing UV-B over the control up to the 50 °N treatment. When these treatments alone are assessed, the treatment effect becomes more pronounced at $P = 0.086$ (hemicellulose) and $P = 0.021$ (lignin). This suggests UV-B may cause accumulation of these compounds, although not at all UV-B doses, and the current FT-IR analysis can only serve as a guideline for assessment of these components due to possible co-absorbance from other metabolites.

Digestibility

Saccharification analysis showed that higher UV-B irradiance caused a significant ($P = 0.003$) reduction in the quantity of sugars released during enzymic digestion of the aboveground biomass (Fig. 4). This clearly demonstrates the negative effect of UV-B irradiance on the digestibility of this material. There was also some indication that the response did not increase further at the highest UV-B doses (40–30 °N), although generally the greater the UV-B dose the greater the reduction in

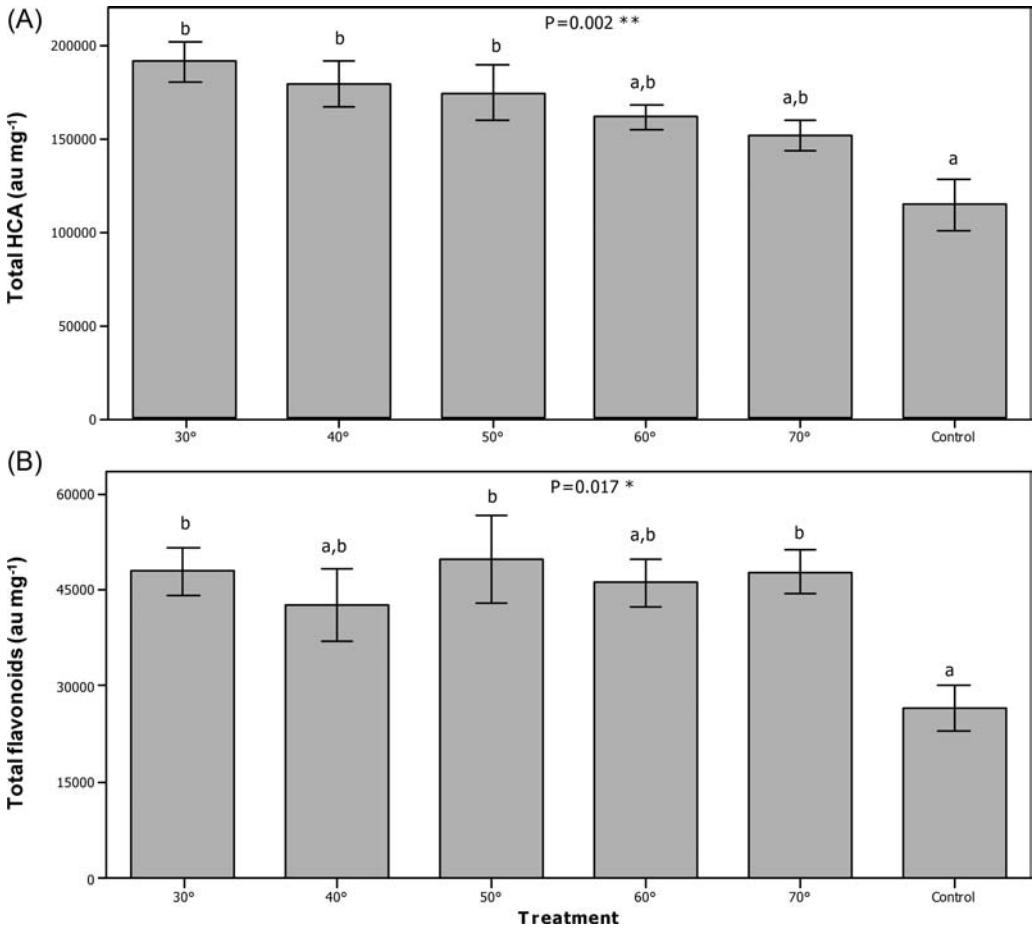

Fig. 2. Effect of the UV-B_{BE} gradient on (A) total soluble hydroxycinnamic acid (HCA) content, and (B) total soluble flavonoid content from methanolic extracts measured by HPLC. HCA and flavonoids are expressed as the total absorbance at 340 nm (mg dryweight leaf)$^{-1}$. Statistical analysis was performed using one-way ANOVA (* <0.05, ** <0.01, *** <0.001). Letters denote significant differences following a Tukey's multiple-range test ($n = 5$, $P < 0.05$). Values are mean ± SE.

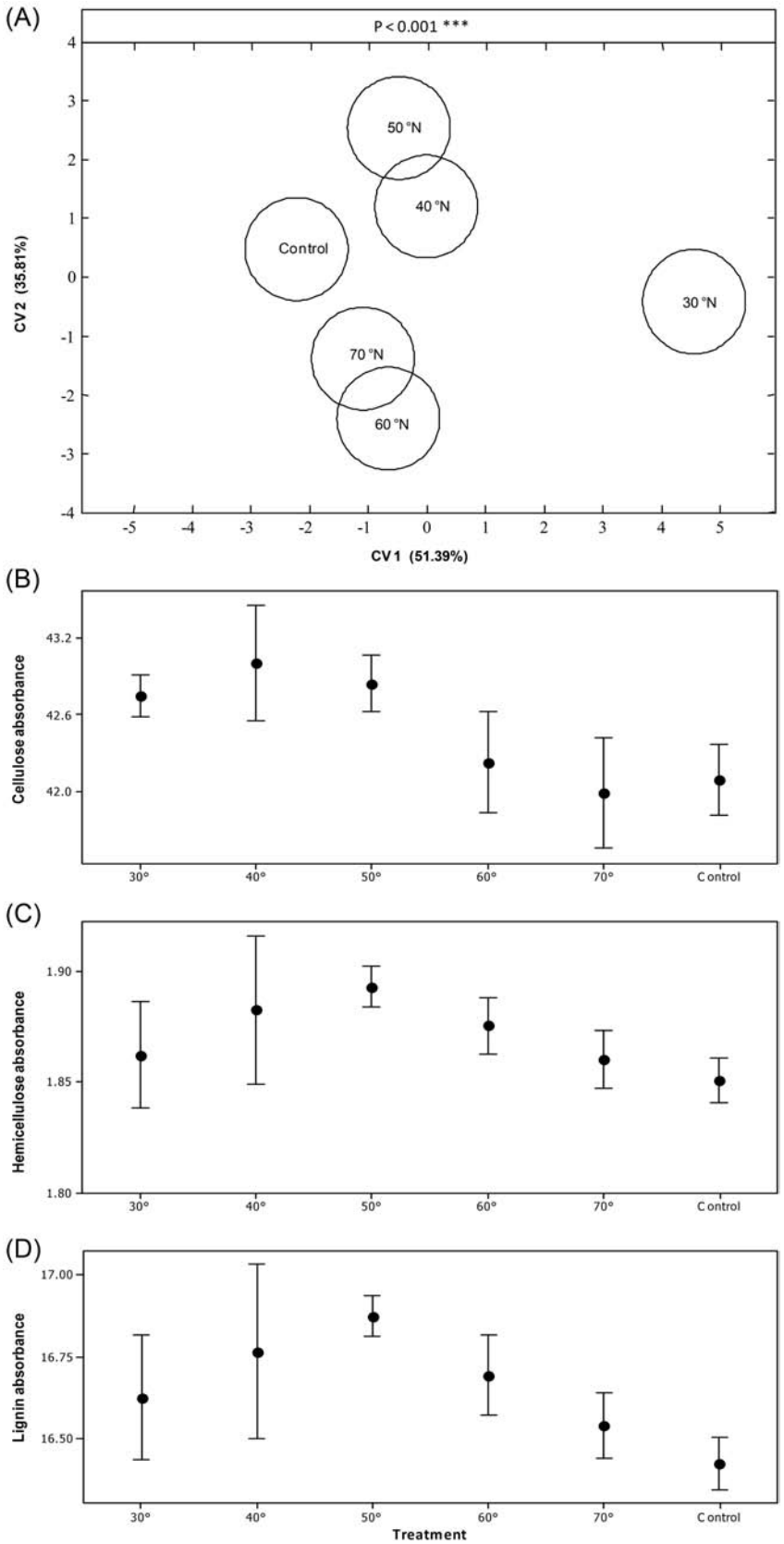

Fig. 3. Analysis of FT-IR spectra of *L. perenne* leaf material raised over a UV-B_{BE} gradient. (A) Separation of the treatments over the first two canonical axes (CVs) including their 95% confidence circles following PC-CVA analysis. The *P*-value represents the result of a MANOVA using the first eight principal components (>99% of the variability within the spectra) (* <0.05, ** <0.01, *** <0.001). (B–D) Total absorbance over the FT-IR wavenumbers shown to be characteristic of cellulose (B), hemicellulose (C), and lignin (D) (see Table 1 for specific wavenumbers). Values are mean ± SE.

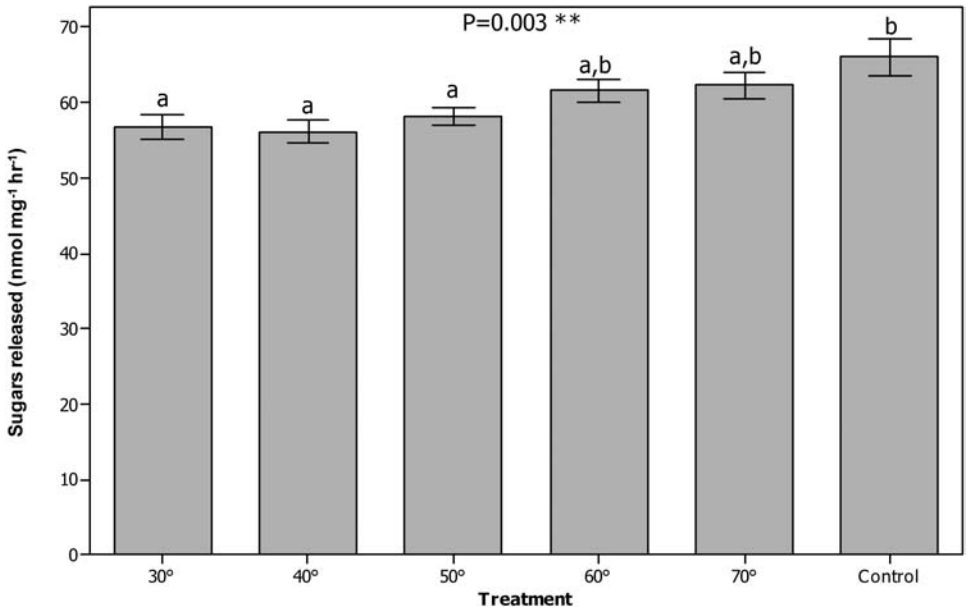

Fig. 4. Concentration of sugars released per hour during an enzymic saccharification analysis of aboveground *L. perenne* biomass exposed to different UV-B regimes. The total concentration of reducing sugars was assessed calorimetrically following 8 hours of enzymic digestion. The *P*-value shows the result of a one-way ANOVA (* <0.05, ** <0.01, *** <0.001). Letters denote differences between treatments as shown by a Tukey's multiple-range test ($n = 5$, $P < 0.05$). Values are mean ± SE.

digestibility, as shown by the significant reduction in sugars released during enzymic digestion (Fig. 4).

Discussion

Previous work has shown that exposure to UV-B can adversely affect plant growth (Rozema *et al.*, 1997b; Mpoloka, 2008), but by far the commonest observed response to UV-B is an effect on tissue chemistry (Searles *et al.*, 2001; Newsham and Robinson, 2009), with implications for other trophic processes including decomposition and digestibility (Rozema *et al.*, 1997a; Austin and Ballaré, 2010). This study targeted UV-B effects on the soluble leaf chemistry, plant growth, and subsequent digestibility of the commercially important and internationally distributed forage species *L. perenne*. The scale of latitudinal gradient studied (30 to 70 °N) meant that a glasshouse experimental system was employed, and it is accepted that lamp UV-B supplementation cannot entirely represent an ambient UV-B regime. Similarly, the PAR:UV-B ratio will not necessarily represent that found under true field conditions (Krizek, 2004). Nevertheless, care was taken to ensure that the weighted UV-B_{BE} doses were comparable to those which occur at latitudes between 30 and 70 °N. Furthermore, following the work of Flint *et al.* (2009) and Krizek (2004), this study was particularly careful to minimize shading from the lamp holders, ensuring a high PAR environment at all times via supplementary lighting.

This study hypothesized that increased UV-B doses would cause inhibition of growth and soluble carbohydrates and accumulation of soluble phenolic monomers. Aboveground biomass production was reduced by 12.67% with every 1 kJ m^{-2} day^{-1} increase in biologically weighted UV-B_{BE}. As a result, plants grown in the highest UV-B treatment had a total biomass of just 13.7% of control (zero UV-B treatment). UV-B is also known to reduce root growth (Zaller *et al.*, 2002), and in the current study both root biomass and root length were reduced by the UV-B gradient, likely as a consequence of the pronounced reduction in plant leaf area by UV-B. Ballaré *et al.* (2011) have argued that growth of plants in the field is generally less sensitive to UV-B in comparison to controlled environment studies such as this one, nevertheless, the current results agreed closely with the previous work of Deckmyn and Impens (1999), who also demonstrated that *L. perenne* showed considerable sensitivity to UV-B.

Fructan concentrations were significantly reduced by elevated UV-B causing lowered total soluble carbohydrate concentrations, although sucrose, glucose, and fructose were unaffected. Similar results have been observed in a number of commercially important monocots (Yue *et al.*, 1998; Ghisi *et al.*, 2002; Quaggiotti *et al.*, 2004), demonstrating the potential for UV-B to negatively affect soluble carbohydrates. A study by Parsons *et al.* (2004) identified that water-soluble carbohydrate production was less pronounced in high-sugar *L. perenne* cultivars, including AberDart, when grown in New Zealand compared to the UK. The authors attributed this to higher growth temperatures, but the results of the current study suggest variation in UV-B may also play an important role. Conversely, an increasing UV-B intensity resulted in higher concentrations of soluble foliar phenolics, comparable with results from Deckmyn and Impens (1999). In the current study, HCAs were the dominant phenolic monomers present, although both total HCAs and flavonoids were significantly increased by elevated UV-B, as reported in a number of other species (Agati and Tattini, 2010).

However, as a determinant of forage quality it is the structural and cell-wall-associated components which contribute most to digestibility. It was hypothesized that UV-B exposure would cause an increase in cell structural metabolites, thereby reducing digestibility. For example, structural metabolites accumulate under UV-B exposure in a range of species (Rozema *et al*., 1997a; Laakso *et al*., 2000; Hilal *et al*., 2004; Yamasaki *et al*., 2007) and can influence cell-wall digestibility in forage grasses (Buxton and Marten, 1989; Smith and Flinn, 1991; Moore and Jung, 2001). Chemometric assessment of FT-IR wavenumbers associated with lignin, cellulose, and hemicellulose suggested increases with UV-B exposure. However, FT-IR analysis can only be used to imply changes in these components due to possible co-absorbance from other metabolites. Saccharification was therefore used as a more accurate determination of the forage digestibility. UV-B irradiance was shown to cause a significant reduction in digestibility, as demonstrated by a 15% reduction in release of sugars during enzymic digestion of material from the highest UV-B treatment. Consequently, these results could have significant implications for forage quality and bioethanol production.

It is the water-soluble carbohydrates in this high-sugar variety of *L. perenne* that, when used as forage, contributes to the increased weight gain and milk production of livestock (Lee *et al*., 2001; Miller *et al*., 2001). Therefore, a negative effect of UV-B on fructan concentration as observed in the current study may have deleterious consequences for forage quality in areas where ambient UV-B is high. However, the presence of increased soluble phenolics at higher UV-B may partially alleviate this, as mounting evidence suggests that such phenolic monomers serve as a substrate for polyphenol oxidase and cross link to dietary protein. This protects protein from degradation in the rumen and thereby increases the efficiency of conversion from plant to animal protein (Theodorou *et al*., 2006), as well as suppression of methane emissions (Leiber *et al*., 2012). Nevertheless, the observed reduction in digestibility could potentially have the greatest impact upon forage quality. It is estimated that an increase in perennial ryegrass digestibility of only 5–6% may increase summer milk production in southern Australia by up to 27% (Smith *et al*., 1998). Therefore conversely, reduction in digestibility of up to 15% by UV-B as observed in the current study would likely have a pronounced negative impact upon ruminant grazing efficiency. First-generation methods for bio-ethanol production are dependent upon the extractable foliar sugars for conversion to bioethanol (Charlton *et al*., 2009; Farrar *et al*., 2011), and fructan is the dominant water-soluble storage carbohydrate in *L. perenne* (McGrath, 1988; Farrar *et al*., 2011). As a result, the reduction in soluble fructan concentration by UV-B observed would likely reduce the yield of bioethanol produced. First-generation biofuels are now being phased out to make way for second-generation biofuels, which will utilize digestion of lignocellulose to provide additional sugars for the production of bioethanol (Gomez *et al*., 2010). Nevertheless, the current study highlights that UV-B irradiance may also reduce the efficiency of this process, limiting the release of sugars from biomass, and thereby reducing the efficiency of conversion to bioethanol. It is the high bioethanol yield per hectare which makes this high-sugar *L. perenne* variety so attractive for future bio-ethanol production (Charlton *et al*., 2009; Farrar *et al*., 2011). However, when the negative effects of UV-B on biomass production, soluble sugars, and digestibility are considered together, the significant potential for UV-B to limit the efficiency of such bioethanol production is demonstrated.

The current study only utilized one *L. perenne* variety and under glasshouse conditions. Also, some discrepancy in the PAR:UV-B ratio between these treatments and those found at each latitude is expected. This may affect UV-B responses (Krizek, 2004), for example a higher PAR:UV-B ratio could potentially reduce UV-B responses to some extent. Nevertheless, the strength of this study is in its demonstration of the pronounced effects of a UV-B gradient under otherwise standardized conditions in this species. The pronounced negative impacts of UV-B observed, and the previously documented sensitivity of this species to UV-B (Deckmyn and Impens, 1999), suggest the need to consider UV-B tolerance as an important breeding trait. UV-B tolerance has largely been ignored, yet potentially co-occurring factors have been considered, including water stress (e.g. Wang and Bughrara, 2008; Foito *et al*., 2009; Li *et al*., 2010). Previous research on drought tolerance in this species could represent an important basis for UV-B tolerance, as application of drought and UV-B in other species, either consecutively or simultaneously, has been shown to ameliorate responses to either factor in isolation (Schmidt *et al*., 2000; Alexieva *et al*., 2001; Ren *et al*., 2007; Cechin *et al*., 2008; He *et al*., 2011). Such findings beg the question of whether plant breeding for UV-B resistance could also provide beneficial impacts for drought tolerance and vice versa, and highlight the importance of considering both factors together when developing cultivars with increased environmental resistance.

In conclusion, the observed UV-B induced increase of soluble phenolics and reduction of aboveground biomass, soluble fructan concentrations, and digestibility provide clear evidence that UV-B can reduce the forage quality of this species. This may have important considerations for grazing efficiency, as well as both first- and second-generation bioethanol production in areas of high ambient UV-B. Given the pronounced responses to UV-B in the current study, a large-scale field trial is warranted with other varieties of *L. perenne* to identify both the extent of UV-B response and possible mechanisms of UV-B tolerance in this species under field conditions.

Supplementary material

Supplementary data are available at *JXB* online.

Supplementary Fig. S1. Modelled biologically effective UV-B (UV-B_{BE}) irradiance data created using the ACD TUV model.

Supplementary Fig. S2. Measured biologically effective UV-B (UV-B_{BE}) irradiances from the UV-B treatments under glasshouse conditions.

Supplementary Fig. S3. Representative *L. perenne* plants from each of the six UV-B treatments.

Supplementary Fig. S4. Wavenumber loadings on the first and second CV axes following PC-CVA of FT-IR spectra of *L. perenne* leaf material raised over the simulated UV-B_{BE} gradient.

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References

Agati G, Tattini M. 2010. Multiple functional roles of flavonoids in photoprotection. *New Phytologist* **186,** 786–793.

Alexieva V, Sergiev I, Mapelli S, Karanov E. 2001. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant, Cell and Environment* **24,** 1337–1344.

Allwood JW, Ellis DI, Goodacre R. 2008. Metabolomic technologies and their application to the study of plants and plant-host interactions. *Physiologia Plantarum* **132,** 117–135.

Austin AT, Ballaré CL. 2010. Dual role of lignin in plant litter decomposition in terrestrial ecosystems. *Proceedings of the National Academy of Sciences, USA* **107,** 4618–4622.

Ballaré CL, Caldwell MM, Flint SD, Robinson SA, Bornman JF. 2011. Effects of solar ultraviolet radiation on terrestrial ecosystems. Patterns, mechanisms, and interactions with climate change. *Photochemical and Photobiological Sciences* **10,** 226–241.

Barsig M, Malz R. 2000. Fine structure, carbohydrates and photosynthetic pigments of sugar maize leaves under UV-B radiation. *Environmental and Experimental Botany* **43,** 121–130.

Boeriu CG, Bravo D, Gosselink RJA, van Dam JEG. 2004. Characterisation of structure-dependent functional properties of lignin with infrared spectroscopy. *Industrial Crops and Products* **20,** 205–218.

Buanafina MMO, Langdon T, Hauck B, Alton SJD, Morris P. 2006. Manipulating the phenolic acid content and digestibility of Italian ryegrass (*Lolium multiflorum*) by vacuolar-targeted epession of a fungal ferulic acid esterase. *Applied Biochemistry and Biotechnology* **130,** 416–426.

Burchard P, Bilger W, Weissenböck G. 2000. Contribution of hydroxycinnamates and flavonoids to epidermal shielding of UV-A and UV-B radiation in developing rye primary leaves as assessed by ultraviolet-induced chlorophyll fluorescence measurements. *Plant, Cell and Environment* **23,** 1373–1380.

Buxton DR, Marten GC. 1989. Forage quality of plant parts of perennial grasses and relationship to phenology. *Crop Science* **29,** 429–435.

Caldwell MM, Camp LB, Warner CW, Flint SD. 1986. Action spectra and their key role in assessing biological consequences of solar UV-B radiation change. In: RC Worrest, MM Caldwell, eds, *Stratospheric ozone reduction, solar ultraviolet radiation and plant life*. Berlin: Springer-Verlag, pp 87–111.

Cechin I, Corniani N, de Fátima Fumis T, Cataneo A. 2008. Ultraviolet-B and water stress effects on growth, gas exchange and oxidative stress in sunflower plants. *Radiation and Environmental Biophysics* **47,** 405–413.

Charlton A, Elias R, Fish S, Fowler P, Gallagher J. 2009. The biorefining opportunities in Wales: understanding the scope for building a sustainable, biorenewable economy using plant biomass. *Chemical Engineering Research and Design* **87,** 1147–1161.

Comont D, Martinez Abaigar J, Albert A, *et al.*. 2012. UV responses of *Lolium perenne* raised along a latitudinal gradient across Europe: a filtration study. *Physiologia Plantarum* **145,** 604–618.

Conaghan P, O'Kiely P, Howard H, O'Mara F, Halling M. 2008. Evaluation of *Lolium perenne* L. cv. AberDart and AberDove for silage production. *Irish Journal of Agricultural and Food Research* **47,** 119–134.

Daniel CR, Cross AJ, Koebnick C, Sinha R. 2010. Trends in meat consumption in the USA. *Public Health Nutrition* **14,** 575–583.

Deckmyn G, Impens I. 1999. Seasonal responses of six *Poaceae* to differential levels of solar UV-B radiation. *Environmental and Experimental Botany* **41,** 177–184.

Farrar K, Bryant DN, Turner L, Gallagher JA, Thomas A, Farrell M, Humphreys MO, Donnison IS. 2011. Breeding for bio-ethanol production in *Lolium perenne* L.: association of allelic variation with high water-soluble carbohydrate content. *BioEnergy Research*, 1–9.

Flint SD, Ryel RJ, Hudelson TJ, Caldwell MM. 2009. Serious complications in experiments in which UV doses are effected by using different lamp heights. *Journal of Photochemistry and Photobiology B* **97,** 48–53.

Foito A, Byrne SL, Shepherd T, Stewart D, Barth S. 2009. Transcriptional and metabolic profiles of *Lolium perenne* L. genotypes in response to a PEG-induced water stress. *Plant Biotechnology Journal* **7,** 719–732.

Gehrke C, Johanson U, Callaghan TV, Chadwick D, Robinson CH. 1995. The impact of enhanced ultraviolet-B radiation on litter quality and decomposition processes in *Vaccinium* leaves from the Subarctic. *Oikos* **72,** 213–222.

Ghisi R, Trentin AR, Masi A, Ferretti M. 2002. Carbon and nitrogen metabolism in barley plants exposed to UV-B radiation. *Physiologia Plantarum* **116,** 200–205.

Gomez LD, Whitehead C, Barakate A, Halpin C, McQueen-Mason SJ. 2010. Automated saccharification assay for determination of digestibility in plant materials. *Biotechnology for Biofuels* **3,** 1–23.

Gwynn-Jones D, Lee JA, Johanson U, Pheonix GK, Calaghan TV, Sonesson M. 1999. The responses of plant functional types to enhanced UV-B radiation. In: J Rozema, ed, *Stratospheric ozone depletion: the effects of enhanced UV-B radiation*. Leiden: Backhuys Publishers, pp 173–185.

Hannaway D, Fransen S, Cropper J, Teel M, Chaney M, Griggs T, Halse R, Hart J, Cheeke P, Hansen D. 1999. *Perennial ryegrass (Lolium perenne L.)*. Corvallis: Pacific Northwest Extension Publishing, Oregon State University.

Hartley RD, Ford CW. 1989. Phenolic constituents of plant cell walls and wall biodegradability. In: NG Lewis, MG Paice, eds, *Plant cell wall polymers, biogenesis and biodegradation*, vol. **399**. Washington DC: American Chemical Society, pp 137–149.

He L, Jia X, Gao ZLR, Li R. 2011. Genotype-dependent responses of wheat (*Triticum aestivum* L.) seedlings to drought, UV-B radiation and their combined stresses. *African Journal of Biotechnology* **10,** 4046–4056.

Hilal M, Parrado MF, Rosa M, Gallardo M, Orce L, Massa EM, González JA, Prado FE. 2004. Epidermal lignin deposition in quinoa cotyledons in response to UV-B radiation. *Photochemistry and Photobiology* **79,** 205–210.

Höglind M, Hanslin HM, Mortensen LM. 2011. Photosynthesis of *Lolium perenne* L. at low temperatures under low irradiances. *Environmental and Experimental Botany* **70,** 297–304.

Hollosy F. 2002. Effects of ultraviolet radiation on plant cells. *Micron* **33,** 179–197.

Hulke BS, Watkins E, Wyse DL, Ehlke NJ. 2008. Freezing tolerance of selected perennial ryegrass (*Lolium perenne* L.) accessions and its association with field winterhardiness and turf traits. *Euphytica* **163,** 131–141.

Hunt R, Causton DR, Shipley B, Askew AP. 2002. A modern tool for classical plant growth analysis. *Annals of Botany* **90,** 485–488.

Jarvis RM, Broadhurst D, Johnson H, O'Boyle NM, Goodacre R. 2006. PYCHEM: a multivariate analysis package for python. *Bioinformatics* **22,** 2565–2566.

Johnson HE, Lloyd AJ, Mur LAJ, Smith AR, Causton DR. 2007. The application of MANOVA to analyse *Arabidopsis thaliana* metabolomic data from factorially designed experiments. *Metabolomics* **3,** 517–530.

Krizek DT. 2004. Influence of PAR and UV-A in determining plant sensitivity and photomorphogenic responses to UV-B radiation. *Photochemistry and Photobiology* **79,** 307–315.

Laakso K, Sullivan JH, Huttunen S. 2000. The effects of UV-B radiation on epidermal anatomy in loblolly pine (*Pinus taeda* L.) and Scots pine (*Pinus sylvestris* L.). *Plant, Cell and Environment* **23,** 461–472.

Lammers K, Arbuckle-Keil G, Dighton J. 2009. FT-IR study of the changes in carbohydrate chemistry of three New Jersey pine barrens leaf litters during simulated control burning. *Soil Biology and Biochemistry* **41,** 340–347.

Lee MRF, Jones EL, Moorby JM, Humphreys MO, Theodorou MK, MacRae JC, Scollan ND. 2001. Production responses from lambs grazed on *Lolium perenne* selected for an elevated water-soluble carbohydrate concentration. *Animal Research* **50,** 441–450.

Leiber F, Kunz C, Kreuzer M. 2012. Influence of different morphological parts of buckwheat (*Fagopyrum esculentum*) and its major secondary metabolite rutin on rumen fermentation in vitro. *Czech Journal of Animal Science* **57,** 10–18.

Li X, Cheng X, Liu J, Zeng H, Han L, Tang W. 2010. Heterologous expression of the *Arabidopsis* DREB1A/CBF3 gene enhances drought and freezing tolerance in transgenic *Lolium perenne* plants. *Plant Biotechnology Reports* **5,** 61–69.

Lindroth RL, Hofman RW, Campbell BD, McNabb WC, Hunt DY. 2000. Population differences in *Trifolium repens* L. response to ultraviolet-B radiation: foliar chemistry and consequences for two lepidopteran herbivores. *Oecologia* **122,** 20–28.

Liu L, Gitz iii DC, McClure JW. 1995. Effects of UV-B on flavonoids, ferulic acid, growth and photosynthesis in barley primary leaves. *Physiologia Plantarum* **93,** 725–733.

Marshall AH, Wilkins PW. 2003. Improved seed yield in perennial ryegrass (*Lolium perenne* L.) from two generations of phenotypic selection. *Euphytica* **133,** 233–241.

Mascarenhas M, Dighton J, Arbuckle GA. 2000. Characterization of plant carbohydrates and changes in leaf carbohydrate chemistry due to chemical and enzymatic degradation measured by microscopic ATR FT-IR spectroscopy. *Applied Spectroscopy* **54,** 681–686.

McGrath D. 1988. Seasonal variation in the water-soluble carbohydrates of perennial and Italian ryegrass under cutting conditions. *Irish Journal of Agricultural Research* **27,** 131–139.

Michell AJ. 1990. Second-derivative FT-IR spectra of native celluloses. *Carbohydrate Research* **197,** 53–60.

Miller LA, Moorby JM, Davies DR, Humphreys MO, Scollan ND, Macrae JC, Theodorou MK. 2001. Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.): milk production from late-lactation dairy cows. *Grass and Forage Science* **56,** 383–394.

Moore KJ, Jung HJG. 2001. Lignin and fiber digestion. *Journal of Range Management* **54,** 420–430.

Mpoloka SW. 2008. Effects of prolonged UV-B exposure in plants. *African Journal of Biotechnology* **7,** 4874–4883.

Murphy JD, Power NM. 2009. An argument for using biomethane generated from grass as a biofuel in Ireland. *Biomass and Bioenergy* **33,** 504–512.

Nakanishi K, Solomon P. 1977. *Infrared absorption spectroscopy*. San Francisco: Holden-Day.

Newsham KK, Robinson SA. 2009. Responses of plants in polar regions to UVB exposure: a meta-analysis. *Global Change Biology* **15,** 2574–2589.

Nijs I, Ferris R, Blum H, Hendrey G, Impens I. 1997. Stomatal regulation in a changing climate: a field study using free air temperature increase (FATI) and free air CO_2 enrichment (FACE). *Plant, Cell and Environment* **20,** 1041–1050.

Nijs I, Teughels H, Blum H, Hendrey G, Impens I. 1996. Simulation of climate change with infrared heaters reduces the productivity of *Lolium perenne* L. in summer. *Environmental and Experimental Botany* **36,** 271–280.

Parsons AJ, Rasmussen S, Xue H, Newman JA, Anderson CB, Cosgrove GP. 2004. Some 'high sugar grasses' don't like it hot. *Proceedings of the New Zealand Grassland Association* **66,** 265–271.

Phoenix GK, Gwynn-Jones D, Lee JA, Callaghan TV. 2000. The impacts of UV-B radiation on the regeneration of a sub-arctic heath community. *Plant Ecology* **146,** 67–75.

Quaggiotti S, Trentin AR, Dalla Vecchia F, Ghisi R. 2004. Response of maize (*Zea mays* L.) nitrate reductase to UV-B radiation. *Plant Science* **167,** 107–116.

Ren J, Dai W, Xuan Z, Yao Y, Korpelainen H, Li C. 2007. The effect of drought and enhanced UV-B radiation on the growth and physiological traits of two contrasting poplar species. *Forest Ecology and Management* **239,** 112–119.

Rozema J, Tosserams M, Nelissen HJM, Van Heerwaarden L, Broekman RA, Flierman N. 1997a. Stratospheric ozone reduction and ecosystem processes: enhanced UV-B radiation affects chemical quality and decomposition of leaves of the dune grassland species *Calamagrostis epigeios*. *Plant Ecology* **128,** 285–294.

Rozema J, van de Staaij J, Björn LO, Caldwell M. 1997b. UV-B as an environmental factor in plant life: stress and regulation. *Trends in Ecology and Evolution* **12,** 22–28.

Schmidt AM, Ormrod DP, Livingston NJ, Misra S. 2000. The interaction of ultraviolet-B radiation and water deficit in two *Arabidopsis thaliana* genotypes. *Annals of Botany* **85,** 571–575.

Searles PS, Flint SD, Caldwell MM. 2001. A meta-analysis of plant field studies simulating stratospheric ozone depletion. *Oecologia* **127,** 1–10.

Seckmeyer G, Pissulla D, Glandorf M, Henriques D, Johnsen B, Webb A, Siani AM, Bais A, Kjeldstad B, Brogniez C. 2008. Variability of UV irradiance in Europe. *Photochemistry and Photobiology* **84,** 172–179.

Smith KF, Flinn PC. 1991. Monitoring the performance of a broad-based calibration for measuring the nutritive value of two independent populations of pasture using near infrared reflectance (NIR) spectroscopy. *Australian Journal of Experimental Agriculture* **31,** 205–210.

Smith KF, Kearney GA, Culvenor RA. 1998. The use of repeated measurements analysis for the evaluation of seasonal variation in the dry matter yield and nutritive value of perennial ryegrass (*Lolium perenne* L.) cultivars. *Australian Journal of Experimental Agriculture* **38,** 145–154.

Speedy AW. 2003. Global production and consumption of animal source foods. *The Journal of Nutrition* **133,** 4048–4053.

Stewart D. 1996. Fourier transform infrared microspectroscopy of plant tissues. *Applied Spectroscopy* **50,** 357–365.

Stewart D, Morrison IM. 1992. FT-IR spectroscopy as a tool for the study of biological and chemical treatments of barley straw. *Journal of the Science of Food and Agriculture* **60,** 431–436.

Tennant D. 1975. A test of a modified line intersect method of estimating root length. *The Journal of Ecology* **63,** 995–1001.

Theodorou MK, Kingston-Smith AH, Winters AL, Lee MRF, Minchin FR, Morris P, MacRae J. 2006. Polyphenols and their influence on gut function and health in ruminants: a review. *Environmental Chemistry Letters* **4,** 121–126.

Tu Y, Rochfort S, Liu Z, *et al.*. 2010. Functional analyses of caffeic acid O-methyltransferase and cinnamoyl-CoA-reductase genes from perennial ryegrass (*Lolium perenne*). *The Plant Cell* **22,** 3357–3373.

van de Staaij J, De Bakker NVJ, Oosthoek A, Broekman R, Van Beem A, Stroetenga M, Aerts R, Rozema J. 2002. Flavonoid concentrations in three grass species and a sedge grown in the field and under controlled environment conditions in response to enhanced UV-B radiation. *Journal of Photochemistry and Photobiology B* **66,** 21–29.

Wang JP, Bughrara SS. 2008. Evaluation of drought tolerance for atlas fescue, perennial ryegrass, and their progeny. *Euphytica* **164,** 113–122.

Wilkins PW, Lovatt JA. 2004. Recent gains from forage grass breeding. *IGER Innovations* **8,** 18–21.

Yamasaki S, Noguchi N, Mimaki K. 2007. Continuous UV-B irradiation induces morphological changes and the accumulation of polyphenolic compounds on the surface of cucumber cotyledons. *Journal of Radiation Research* **48,** 443–454.

Yue M, Li Y, Wang X. 1998. Effects of enhanced ultraviolet-B radiation on plant nutrients and decomposition of spring wheat under field conditions. *Environmental and Experimental Botany* **40,** 187–196.

Zaller JG, Caldwell MM, D. Flint S, Scopel AL, Salo OE, Ballaré CL. 2002. Solar UV-B radiation affects below-ground parameters in a fen ecosystem in Tierra del Fuego, Argentina: implications of stratospheric ozone depletion. *Global Change Biology* **8,** 867–871.