The responses of three C_4 grasses to elevated temperature and CO_2 in the field

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Abstract

A series of field experiments were carried out to assess the impact of two important global change factors (increased temperature and elevated atmospheric CO_2) on the germination and growth of three C_4 grass species found in New Zealand pastures: *Digitaria sanguinalis* (summer grass), *Paspalum dilatatum* (paspalum) and *Pennisetum clandestinum* (kikuyu). The early spring seedling emergence of all species combined was decreased by elevated CO_2. Spring growth of paspalum and kikuyu transplants was unaffected by temperature or CO_2 concentration. Kikuyu showed a positive growth response to warming in autumn that was associated with increased soil N mineralisation. However, elevated CO_2 dampened the mineralisation response to temperature and reduced the stimulatory effect of increased temperature on growth. Due to this secondary feedback, C_4 grass spread under future environmental conditions (elevated CO_2) may be less than anticipated when considering temperature responses alone.

Keywords: *Digitaria*, FACE, global change, *Paspalum*, *Pennisetum*

Introduction

Pastoral agriculture, producing about 40% of New Zealand's exports by value, is vulnerable to climate change (MAF 2007) by virtue of its overwhelming reliance on climatically dependent forage supply. The industry is founded on pastures dominated by species which use the C_3 photosynthetic pathway, making them eminently suited to a historically temperate climate. However, projected future global change scenarios for New Zealand indicate warmer temperatures combined with less rainfall in the north and east of the country and more rainfall in the south and west (Mullan *et al.* 2008). Warmer temperatures, especially when combined with drier conditions, are likely to shift the competitive balance toward subtropical grasses having the C_4 photosynthetic pathway (e.g. kikuyu and paspalum, Collatz *et al.* 1998). These types of grasses are already present in northern New Zealand (Field 1989) but low winter temperatures generally restrict their growth elsewhere. Given the projections of warmer temperatures and drier summers, there is an expectation that the C_4 species will spread and/or increase in dominance over large areas of the North Island as a result of climate change (Field & Forde 1990). Potentially this could negatively affect productivity and profitability of farming enterprises currently based on C_3 pasture species, through feed quality and seasonality of growth issues (Crush & Rowarth 2007).

Climatic "envelopes" for occurrence (but not growth and dominance) in relation to selected parameters have been determined for a number of C_4 species in New Zealand (Campbell *et al.* 1999). However, a lack of information meant that elevated CO_2 was not able to be included in generating the envelopes. Recent work, ranging from single leaf to meta-analyses, has shown that C_4 species can respond to elevated CO_2 under warmer conditions, more so than C_3 species (Wand *et al.* 1999; Sage & Kubien 2003). To forecast C_4 dominance in response to changing climate and elevated CO_2, the interactions between factors must be considered.

The objective of this study was to investigate the effect of elevated CO_2 and increased temperatures on the germination, emergence and early growth of selected C_4 subtropical grasses, within grazed pastures currently dominated by C_3 species.

Methods

Site

The study was conducted at the New Zealand FACE (Free Air CO_2 Enrichment) facility, located at Flock House, 12 km southwest of Bulls in the Manawatu. The system, management and measurement protocols are described by Newton *et al.* (2006). The facility consists of six circular grazed pasture plots of 12 m diameter ("rings"), three of which have the atmospheric CO_2 content elevated from current ambient levels (385 ppm) to 475 ppm during daylight. CO_2-enriched air is delivered around the perimeter, controlled by solenoid valves in response to monitoring of CO_2 concentration and wind velocity at the centre. All rings have a passive warming system installed, consisting of a long wave radiation blocking cover located 500 mm above the soil surface at night on a 3 × 3 m area of the ring. This reduces the heat lost by infra-red radiation and elevates soil and air temperatures while retaining local temperature variation. The cover is retracted during rain and the warmed sub-plot is matched with an un-warmed sub-plot with a similar frame but no cover. Thus the experimental design consists of 12 experimental units (two CO_2 levels × two warming levels × three replicates).

Autumn growth experiment

Seed of the C_4 species *Digitaria sanguinalis* (summer grass), *Paspalum dilatatum* (paspalum) and *Pennisetum clandestinum* (kikuyu) were germinated in incubators in December 2008 for transplanting into the FACE in January 2009 (Table 1). Despite the application of alternating diurnal temperatures (30/20°C) and KNO_3 solution to the seed lots, insufficient seeds of paspalum and summer grass germinated and hence these species were not transplanted into the autumn experiment.

In each experimental unit, an area of 0.5 × 0.5 m was sprayed with glyphosate (10 ml/L of Roundup Transorb® in a knapsack sprayer) in mid-January 2009. On February 16 the dead shoot residue was removed and 21 kikuyu seedlings were transplanted into the sprayed plots. Ten survivors were tagged with plastic rings around the main tiller/stolon on March 19 as resident vegetation recovered. Leaves on the marked stolon were counted weekly from April 9 until May 21 and mean weekly leaf appearance rates were calculated.

Spring germination/emergence experiment

Seeds of four accessions of three C_4 species present near the FACE (Table 1) were subjected to viability tests (tetrazolium stain, ISTA 2003).

Within each experimental unit, four soil cores of 50 mm diameter and 120 mm depth were taken from the pasture, the plant material removed and the soil replaced to create a gap in the sward. Sowing lots of each accession, sufficient to include 20 viable seeds, were hand-sown into the gaps on June 16, 2009. The small plots of sown seed were protected from sheep grazing by cages and the resident vegetation harvested to the grazing residual height with electric shears at the same time as the grazing events.

Seedling emergence was recorded every 7 days from August 6 until November 3, 2009. Seedlings of non-sown species were removed. Germination curves were developed based on cumulative seedling emergence over time.

Spring growth experiment

Seven genotypes of kikuyu and five of paspalum were selected for transplanting into the FACE in spring 2009. The kikuyu genotypes were sourced from plant material collected in Northland in 1997 and subsequently maintained in pots outdoors at AgResearch Grasslands, Palmerston North. The paspalum genotypes were sourced from plant material collected from pastures at AgResearch Grasslands, Palmerston North. Rooted stolon (for kikuyu) and rhizome (for paspalum) cuttings were transplanted into plastic sleeves of 50 mm diameter and 120 mm depth and the sleeves transferred to excavated holes in the experimental units of the FACE on August 20. Each experimental unit included one plant of each genotype (i.e. 12 plants per experimental unit without duplication).

At weekly intervals from September 2 until November 3, each plant was measured for the number of growing points and emerged leaves on a marked tiller (the oldest tiller). On November 3, all green tissue was harvested from each plant, dried for 24 h at 65ºC and weighed for dry matter accumulation.

Soil moisture, temperature and plant available nitrogen measurements

Soil moisture, air temperatures and soil mineral nitrogen (N) were regularly measured in each control and warmed plot in all ambient and elevated CO_2 rings. Volumetric soil moisture to 120 mm depth was measured weekly using time domain reflectometry (TDR 300, Spectrum Technologies Inc., Plainfield, Illinois, USA). Air temperature was measured every 5 minutes at 20 cm above the soil surface using a solar radiation shielded USB temperature/humidity datalogger. To determine the level of warming, the hourly differences in temperature between the warmed and control plots were calculated and averaged for the ambient and elevated CO_2 rings. Soil mineral N was sampled to 50 mm depth every fortnight using eight ion exchange resins on plastic tags (Bowatte *et al.* 2008) in each experimental unit.

Table 1 Species/accessions used in the study and their viability (tetrazolium test) and mean field emergence in spring.

Botanical name	Common name	Life form	Accession[1]	Viability (%)	Mean total seedlings emerged (20 viable seeds)
D. sanguinalis	summer grass	annual	BZ4724	68	3.5
P.dilatatum	paspalum	perennial	cv. 'Raki'	63	10.3
P. dilatatum	paspalum	perennial	BO445	12	10.0
P.clandestinum	kikuyu	perennial	cv. 'Whittett'	92	7.2

[1] Margot Forde Germplasm Centre, Palmerston North

Statistical analysis

Where plants were measured at intervals the data were analysed as a split-plot repeated measures design; CO_2 concentration was taken as the whole plot and warming as the sub-plot. Mixed models were used in REML variance components analysis using Genstat (Payne *et al.* 2008). Seedling emergence data were square root transformed, modelled with a logistic equation and the three model parameters analysed for differences in the R statistical package (R Development Core Team 2010). Statements about non-significance refer to P>0.05.

Results

Treatment effects on environmental conditions

During autumn, passive night-time warming increased air temperatures at 20 cm above ground level by 1.0-1.3 °C (Table 2). Warming was slightly greater and more consistent in the autumn (+1.18 °C) than in the spring (+ 0.93 °C), due to extensive periods of wet, cloudy conditions making the passive warming less effective.

The warming treatment reduced soil moisture in autumn by ~4 v/v percentage points (Table 2), but there was no significant effect on soil moisture of the elevated CO_2, nor any warming by CO_2 interaction or time interaction. In spring, soil moisture increased from ~30% v/v to ~45% v/v, without any significant treatment effects.

Soil mineral N declined during April and increased briefly during May. There was a significant effect of warming which led to increased soil mineral N, but also a significant interaction whereby this effect was not observed under elevated CO_2 (Table 2). This pattern was also observed in spring, but at much lower mean resin N levels (10-30 µg N per resin).

Autumn growth

Weekly mean leaf appearance rates of kikuyu varied from 0.25 to 1.3/week during April and May. The pattern of leaf appearance showed an initial decline during April, a brief resurgence in late April-early May before declining later in May. There was a significant CO_2×warming effect (P<0.01) with the fastest rate of appearance under warming in ambient CO_2 concentration and no impact of warming at elevated CO_2 (Table 3). However, there was no significant interaction between climate treatments and time. By the end of May few new leaves were appearing and many plants showed extensive senescence. There was no evidence that the different CO_2 and warming treatments had any effect on the maintenance of the growth of kikuyu into the cool season, and all plants died over the winter.

Spring germination and emergence

Viability according to the tetrazolium test was high for the kikuyu cultivar 'Whittett', moderate for the paspalum cultivar 'Raki' and the summer grass, and low for the paspalum accession (Table 1). Total cumulative seedling emergence was highest in the two paspalum accessions, followed by kikuyu and low numbers for summer grass (Table 1). These relativities were similar throughout the measurement period. There was an effect of the CO_2 enrichment treatments, but not of the warming treatments, on the pattern of emergence

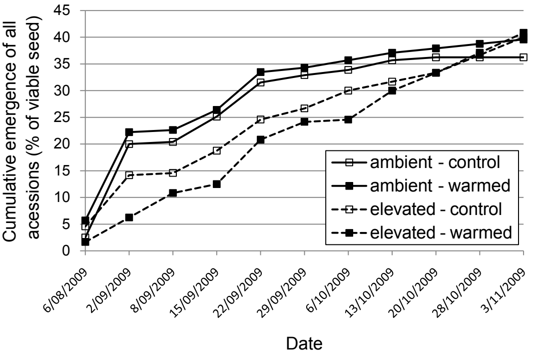
Table 2 Effect of elevated CO2 and passive warming treatments on mean soil moisture (%v/v), mean overnight minimum air temperature (°C) and mean soil mineral N (µg N/resin) in autumn (March-May) and spring (August-October) of 2009 at the FACE facility. Letters denote significant treatment differences within season.

CO_2+warming treatment	Soil moisture (%v/v)		Air temperature (°C)		Soil mineral N (µg N/resin)	
	Autumn	Spring	Autumn	Spring	Autumn	Spring
ambient+control	21.2 a	36.2 a	4.49 b	4.50 b	22 c	12.4 b
ambient+warmed	17.6 b	35.1 a	5.50 a	5.15 a	74 a	20.9 a
elevated+control	20.5 a	36.4 a	4.61 b	4.57 b	35 b	16.6 b
elevated+warmed	16.7 b	33.6 a	5.96 a	5.58 a	41 bc	17.7 b

Table 3 Effect of elevated CO_2 and passive warming treatments on mean leaf appearance rate of kikuyu in autumn (March-May), kikuyu and paspalum in spring (August-October) of 2009 at the FACE facility. Letters denote significant treatment differences within season.

CO_2+warming treatment	Kikuyu leaf appearance rate (new leaves/week)		Paspalum leaf appearance rate (new leaves/week)
	Autumn	Spring	Spring
ambient+contol	0.40 c	0.75 a	0.53 a
ambient+warmed	0.64 a	0.68 a	0.50 a
elevated+control	0.48 b	0.66 a	0.52 a
elevated+warmed	0.44 bc	0.69 a	0.47 a

Figure 1 Cumulative emergence (percent of viable seed) of seedlings for all four C_4 accessions combined. Statistical estimates not shown as they were based on a logistic curve fitting.



for all accessions combined. Emergence followed a typical asymptotic pattern in the ambient CO_2 rings but a more linear pattern in the elevated CO_2 rings (Fig. 1). Consequently, total percentage emergence was not different between treatments but was reached at an earlier stage in ambient CO_2 conditions (i.e. the half-maximum parameter in the logistic model was significantly lower, $P<0.05$). The patterns for all species combined were largely a reflection of the patterns for the two paspalum accessions.

Spring growth

Mean leaf appearance rates for kikuyu varied between 0.27 and 1.2/week during the spring, being highest in mid-September, declining from then until mid-October and increasing rapidly after that. Mean leaf appearance rates for paspalum varied much less, ranging between 0.2 and 0.6 leaves/week over most of the spring except for one week in late September when they averaged 1.2 leaves/week. There was no significant effect of the treatments on leaf appearance rate for either species, nor any significant treatment and time interactions (Table 3).

The relationship between N availability and leaf appearance was linear and strong for the autumn data (regression $P<0.001$; adjusted $R^2 = 0.58$). The data for spring showed no relationship but values for the resin N were much lower than those measured during autumn (mean < 20 µg N per resin).

Discussion

The two factors manipulated in the FACE facility clearly had interactive effects on the key drivers of plant growth - temperature, soil moisture and soil mineral N (Table 2). Consequently, it is difficult to untangle the proximal drivers of the growth responses seen in the C_4 grasses, and thus the following discussion focuses on the similarities between the effects of the two factors on drivers and on plant responses. However, the benefit of this experimental approach lies in documenting the actual net outcome in the field of these two factors that are definite features of global change.

Establishment of all C_4 grasses from seed was generally poor with a maximum of 40% emergence (Fig. 1). This response was not unexpected for the perennial species paspalum and kikuyu which are notoriously poor germinators (Hsu *et al.* 1985; Schrauf *et al.* 1995). Day-night soil surface temperature amplitudes (White *et al.* 2001) may have been insufficient at this site during the period studied. A more surprising result was that there was a CO_2 effect on emergence rate with elevated CO_2 suppressing early emergence (Fig. 1), an effect for which there is no clear explanation. This indirect effect of climate change on germination and emergence is potentially important as it has been shown at the FACE site that recruitment from seed is a powerful influence on species composition (Edwards *et al.* 2001). The result also influences the potential for the spread of C_4 species under climate change. A potential germination/establishment limitation mechanism under elevated CO_2 might mitigate the spread rate of those species for which spread via seed is important (e.g. summer grass and paspalum, as opposed to kikuyu).

There was a marked difference in treatment effects on vegetative growth between the autumn and the spring (Table 3). In autumn, leaf appearance was stimulated by warming at ambient but not elevated CO_2 levels, consistent with previous studies with kikuyu at ambient CO_2 (Ivory & Whiteman 1978) and *Andropogon* at ambient and elevated CO_2 (Kakani & Reddy 2007). The impact was aligned with treatment effects on soil N availability (rather than soil moisture or temperature, compare data in Table 3 and Table 2) and leaf appearance could be modelled as a linear function of N availability. The basis for a regulatory effect of elevated CO_2 on N mineralisation lies in the sequestration of N in organic matter i.e. the 'PNL effect' (Luo *et al.* 2004). In spring, this relationship was not evident despite a higher N availability in the ambient CO_2 + warmed treatment in autumn. As we have no *a priori* expectation of a different relationship between leaf appearance and N availability in autumn compared to spring it seem likely that the much lower levels of N found in spring, and the consequent smaller differences in absolute amounts between treatments, were not sufficient to drive differences in leaf appearance.

Our experimental data are the first to document climate change impacts on C_4 grasses in a grazed field experiment. Treatment effects, where they occurred, were largely interactions between CO_2 concentration and temperature showing how important it is to consider both of these aspects of our future environment rather than just temperature. The apparent mitigation of the temperature response in kikuyu by elevated CO_2

implies the need to revise our view of the likely impact of C_4 grasses and further investigation will allow us to make more robust predictions for pastoral agriculture across a range of systems in New Zealand.

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