



Grasslands feeling the heat: The effects of elevated temperatures on a subtropical grassland



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Background: Tropical and subtropical Africa is predicted to experience a rise in temperature. The effects of rising temperatures on temperate grasslands have been studied using open-top chambers (OTCs) but reports for tropical/subtropical grasslands are scarce. This study used OTCs to investigate the effects of elevated temperatures on a threatened subtropical grassland type, namely KwaZulu-Natal Sandstone Sourveld (KZNSS).

Objectives: To assess the effects of OTCs on selected abiotic parameters and plant productivity within KZNSS.

Methods: Five OTC and control plots were randomly distributed at the same altitude within a patch of KZNSS. Air and soil temperature, relative humidity (RH), soil moisture content and light intensity were monitored in all plots in spring, summer, autumn and winter. Biomass production and plant density were measured in each season, for each life form (graminoid, forb and shrub), separately and combined.

Results: The OTCs resulted in a rise in average, maximum and minimum day and night, air and soil temperatures. This increase, the degree of which differed across seasons, was accompanied by a decline in RH and soil moisture content. Elevated temperatures led to a significant increase in combined, graminoid and shrub above-ground productivity (AGP) and a decrease in forb density, but in certain seasons only. Below-ground biomass production was unaffected by elevated temperatures.

Conclusions: OTCs can simulate realistic increases of air temperature in subtropical grasslands. Graminoids and shrubs appear to benefit from elevated temperatures whilst forbs decrease in abundance, possibly through competition and/or direct physiological effects.

Introduction

Extreme weather events, changes in precipitation, considerable variation in temperature and sea level rise provide evidence for global climate change (Dhillon & Wuenhlish 2013). Climate change, suggested to be largely a consequence of anthropogenic activities (Dhillon & Wuenhlish 2013), has impacted negatively on plant biodiversity in terms of both distribution and diversity (Trisurat, Shrestha & Kjellgren 2011).

Increasing temperatures, in particular, are likely to have a number of effects on plant communities globally (De Boeck *et al.* 2007). Global surface temperatures have risen by approximately 0.8 °C over the last century and are predicted to increase by 1.4–5.8 °C during the 21st century (Intergovernmental Panel on Climate Change [IPCC] 2007). In Africa, this increase in temperature is predicted to result in longer growing periods, higher fecundity, higher biomass allocation towards roots, and a possible shift towards tree-dominated biomes (Scheiter & Higgins 2009).

Predicting ecosystem responses to climate change is therefore becoming increasingly important, particularly in tropical regions, where species occupy narrow ranges owing to thermal specialisation (Laurance *et al.* 2011). These areas are likely to experience the greatest loss in biodiversity with an increase in temperature (Perez, Stroud & Feeley 2016). The effects of rising temperatures on grasslands is an important consideration given their high biodiversity (Boval & Dixon 2012), particularly in the tropics, where they occupy approximately 20% land cover (Parr *et al.* 2014). Grasslands play a particularly important role in carbon sequestration and nutrient recycling (Boval & Dixon 2012), and are threatened in many parts of the world by land-use change, poor management and climate change (Jewitt 2011; Parr *et al.* 2014; Sala *et al.* 2000).

The grassland biome in South Africa boasts high levels of endemic mammals, reptiles, butterflies, and very high levels of plant species diversity (Reyers *et al.* 2005). However, an estimated 35% of South African grassland has been either transformed or degraded (Egoh *et al.* 2011). An understanding of how grassland vegetation types will respond to, or be impacted on, by increased temperatures can inform their future conservation and management (Thuiller *et al.* 2008). Various climate change models and numerous *in* and *ex situ* experiments have attempted to predict and demonstrate the effects of rising temperatures within temperate grasslands (Flanagan & Johnson 2005; Godfree *et al.* 2011; Ojima *et al.* 1993), but effects on tropical and subtropical grasslands have received less attention (Godfree *et al.* 2011). Nevertheless, one of the major effects of rising temperatures on grassland and other biomes is predicted to be altered plant productivity (Guoju *et al.* 2005). Plant productivity, or more correctly net primary productivity (NPP), is the net rate of carbon (C) gain incorporated into plant vegetation over a given time period (Girardin *et al.* 2010; Long & Hutchin 1991). It represents a large portion of organic matter consumed by animals and microbes. Changes in NPP can therefore impact the quantity and quality of food available to animals, including humans (Potter, Klooster & Genovese 2012). In regions where plant carbon assimilation is limited because of low temperatures, increased temperatures could increase NPP (De Boeck *et al.* 2007); however, these responses are likely to be species and life form dependent (Lattanzi 2010; Luo *et al.* 2013; Wand *et al.* 1999).

Plants undergo physiological changes such as altered carbon assimilation rates when subjected to increased temperatures (Ahuja *et al.* 2010; Saxe *et al.* 2001). The effects of increased temperature are not universal – for example, C₃ and C₄ plants show different physiological responses to elevated temperatures (Horton & Murchie 2000; Sage 2000). C₄ plants have a carbon fixation pathway that is far less sensitive to an increase in temperature than C₃ types (Gowik & Westhoff 2011). In fact, C₄ crops and grasses are 40% more efficient at converting photosynthetically active radiation (PAR) into biomass than C₃ types in general (Long 1999). In warmer climates, the greater efficiency of C₄ photosynthesis at higher temperatures is likely to result in higher NPP in C₄ relative to C₃ species, particularly in low-latitude grasslands, deserts and coastal zones (Sage 2000). These differences in NPP between C₃ and C₄ species can impact on species composition and richness by altering competitive interactions (Wand *et al.* 1999).

Increased temperatures, in altering the standing biomass, can also impact on fire regimes, which are an essential part of grassland ecology (Trollope, Trollope & Hartnett 2002). Global warming is therefore expected to impact the carbon budget as a consequence of changes to plant photosynthesis and growth, as well as soil respiration (Schindlbacher, Zechmeister-Boltenstern & Jandl 2009; Wan *et al.* 2005). More specifically, changes in carbon cycling induced by global warming, as a result of changes in NPP and heterotrophic respiration, can influence whether terrestrial ecosystems act

as carbon sources or sinks (Wan *et al.* 2005). Increased evaporative cooling in the leaves, another predicted consequence of increased temperature, can also induce water stress in certain plant species (Beerling, Osborne & Chaloner 2001) and impact on ecosystem hydrology (Williams & Scott 2009). An increase in temperature is usually accompanied by a subsequent increase in water deficit, restricting plant growth and photosynthesis, which leads to varied effects in different ecosystems (De Boeck *et al.* 2007; Wan, Luo & Wallace 2002). This implies that both stresses, temperature and water deficit, and their interactive effects need to be considered when assessing plant responses to increased temperature.

The use of open-top chambers (OTCs) for studying the effects of simulated elevated temperatures on grassland (and other) vegetation has recently gained more interest, but predominantly in temperate and polar regions (Flanagan & Johnson 2005; Flanagan, Sharp & Letts 2013; Molau & Mølgaard 1996). However, the utility of these chambers in studying the effects of elevated temperatures on tropical and subtropical vegetation has yet to be established. This, and the likelihood that Africa will experience some of the greatest increases in temperature (IPCC 2007), prompted the present study, which used OTCs to assess the *in situ* growth and community structure responses of subtropical grassland vegetation to elevated temperatures. This study was conducted in a remnant patch of KwaZulu-Natal Sandstone Sourveld (KZNSS) in Durban, South Africa. This grassland type is located along the coastal belt of KwaZulu-Natal and is home to a number of endemic species (Mucina & Rutherford 2006). It is highly transformed and critically endangered (Jewitt 2011). The study assessed whether OTCs can simulate realistic increases (i.e. increases in line with predictions for this century) in temperatures in a subtropical grassland. More importantly, the effects of OTCs on soil and air temperature and a range of abiotic parameters (relative humidity, soil moisture content and light intensity) are related to seasonal and annual plant productivity and density responses across different life forms.

Methods

Site selection and experimental approach

The study site was a remnant patch of KZNSS located within Tanglewood Nature Reserve, in the eThekweni Municipal Area (Durban), South Africa (29°48'37"S, 30°49'18"E). Five experimental (OTC) and five control, hexagonally shaped plots, approximately 2.012 m², were randomly distributed along a south-east facing slope at approximately the same altitude (425 m above sea level). Each experimental plot was paired with a control plot, located within 2 m of each other. The vegetation in all plots was cut to ground-level and the soil was disked to promote seedling recruitment through softening the soil and splitting of grass roots (Farooq *et al.* 2011; van Acker, Bullied & du Croix Sissons 2004). Once the plots were prepared, hexagonal polycarbonate OTCs (described below) were installed in all experimental plots. The OTCs were installed and left in place for 2 months during

each of the four seasons (spring: 1 September – 30 November; summer: 1 December – 28/29 February; autumn: 1 March – 31 May; winter: 1 June – 31 August).

Several antelope species (e.g. blesbok [*Damaliscus pygargus phillipsi*], bushbuck [*Tragelaphus sp.*] and zebra [*Equus quagga*]) occur on the reserve, and to prevent the potential confounding effects of herbivory, the control plots were enclosed by 1.5 m high wire fence.

Chamber construction

The OTCs used in this study are based on a design modified from the International Tundra Experiment (ITEX) (Molau & Mølgaard 1996). The chambers were hexagonal in shape (see Figure 1 for dimensions) and constructed using clear 2 mm thick Naxel polycarbonate sheeting (Maizey Engineering Plastic Products, New Germany, South Africa), which has a light transmittance of $\pm 90\%$. The frame supporting the polycarbonate was constructed from 20 mm plastic conduit tubing. The OTCs were secured to the ground using steel pegs.

Measurement of environmental parameters

Air and soil temperatures were monitored across all plots (control and treatment) throughout the experimental period using digital temperature loggers (Thermochron iButton Device – DS1921G, Maxim Integrated™, San Jose, USA), placed 5 cm below ground level for soil temperature and 10 cm above ground level for air temperature. Four temperature loggers were used in each control ($n = 20$) and six ($n = 30$) within each experimental plot. The Thermochrons were programmed to record temperatures on an hourly basis throughout the experimental period. Their positioning within the control and OTC plots is shown in Figure 1c.

Additionally, air temperature and relative humidity (RH) were measured in all plots 25 cm above ground level using a 4500 Pocket Weather Tracker (Kestrel, Birmingham, UK) weekly (at midday). Soil moisture content and light intensity were also measured weekly (at midday) using a Soil Moisture Meter (HH2 Moisture Meter, Delta-T Devices, Cambridge, UK) and a Digital Light Meter (ESR-1, PP Systems, Amesbury, USA). The positions at which these measurements were carried out within the OTC plots are shown in Figure 1c. Weekly rainfall data were collected using a standard non-recording rain gauge. Seasonal rainfall was calculated by summing the weekly rainfall data.

Plant growth measurements

At the start of each season, immediately before installing the OTCs, soil cores were taken along the immediate periphery of (but external to) all plots, using a soil corer (18 cm long by 4.75 cm wide). A total of three cores were taken for each plot ($n = 15$ for control and OTC) in each season and these were stored at -18°C . After the 2-month experimental period, soil cores were performed again for each plot ($n = 15$ for control and OTC), but this time within each plot. Soil cores were

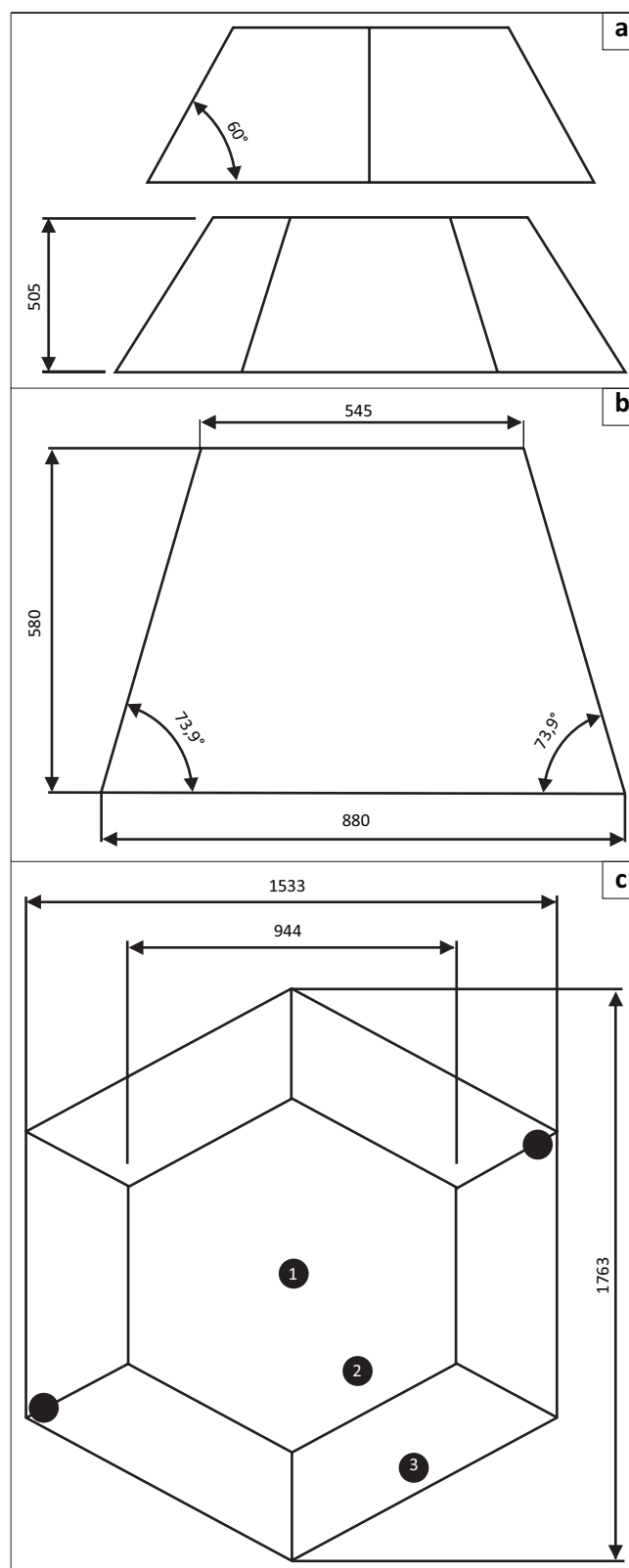


FIGURE 1: Open-top chamber design modified from Molau and Mølgaard (1996). Side view (a), individual panel of chamber (b), and top view (c). The black circles indicate locations at which light intensity, relative humidity and temperatures measurements were conducted at midday ($n = 5$), whilst the numbered black circles indicate where digital temperature loggers were positioned.

rinsed under running water with a sieve to free roots of soil and dead roots were removed. The total (indiscriminate) root dry mass was determined by drying the roots collected at

75 °C for 72 h, and then weighing these using a three-place balance (Mettler Toledo, Columbus, USA).

The above-ground biomass of each plant, which was categorised based on life form (graminoid, forb or shrub), within all plots was harvested and placed in separate paper bags. The plant material was dried at 75 °C for 72 h, and then weighed using a three-place balance (Mettler Toledo, Columbus, USA). Biomass production (grams of dry mass [g]) was compared between control and OTC plots, within life forms for above-ground biomass production (AGP). Within life form comparisons were not possible for below-ground biomass production (BGP) as roots could not be separated into individual species/life forms.

Statistical analysis

All data were tested for normality using either the Kolmogorov-Smirnov or Shapiro-Wilk test, depending on sample size. Percentage data were arcsine transformed prior to any analyses. As initial analyses revealed no significant differences in temperature across different locations within individual plots (control and OTC), these data were pooled for all subsequent analyses. Significant differences between control and OTC plots for all environmental parameters (midday temperature, RH, soil moisture content and light intensity) were tested within seasons via a Wilcoxon signed-rank test, as all data were non-parametric. For the Thermochron readings, differences between control and OTC plots were tested for within 3-hour intervals (00:00–03:00; 03:00–06:00; 06:00–09:00; 09:00–12:00; 12:00–15:00; 15:00–18:00; 18:00–21:00; 21:00–24:00) using a Wilcoxon signed-rank test within each season. The maximum and minimum temperatures for each Thermochron within OTC plots and within control plots were averaged seasonally to determine the average maximum and minimum temperatures. The highest and lowest temperature reading for each season (in OTCs and control plots) was labelled absolute maximum and absolute minimum temperature.

Seasonal plant densities were determined for each life form by dividing the number of individual plants by the plot size (2.012 m²). Above-ground biomass allocation was calculated by expressing the AGP for each life form as a percentage of the total AGP for all life forms combined. Comparisons of AGP, BGP, above-ground biomass allocation and plant densities between control and OTC plots were made within and across life forms using a Wilcoxon signed-rank test. Pearson's correlation was used to determine the relationship between the abiotic parameters (temperature, RH and soil moisture content) and AGP, above-ground biomass allocation and plant density. Where applicable, non-parametric data were tested with a Spearman's rank correlation. All statistical analyses were performed using IBM SPSS Statistics 21 (SPSS IBM, New York, USA) and differences were considered significant at the 0.05 level.

Results

The results presented below reflect the effects of open-top chambers (OTCs) on *in situ* temperatures, other abiotic

factors, and plant productivity and community structure. Measurements were conducted in each of the four seasons.

Diurnal air and soil temperature in control and OTC plots

Diurnal patterns of variation in temperature were similar in OTC and control plots in all seasons (Figure 2), with the highest temperatures occurring between 11:00 and 12:00 and lowest between 04:00 and 06:00. The air and soil OTC temperatures were significantly ($p < 0.05$ in all cases) higher than the control temperatures for all day and night 3-hour intervals, in all seasons.

Air temperature differences between OTC and control plots were most marked during the day between 12:00 and 14:00, except for summer when differences were at a maximum between 09:00 and 11:00. At night these differences were most marked between 21:00 and 23:00 in autumn, 18:00 and 20:00 in winter and spring, and 03:00 and 05:00 in summer. These differences across time intervals were less apparent for soil temperatures.

Additionally, the OTCs increased the diurnal absolute maximum air and soil temperatures, ranging between 4.4 °C and 5.7 °C and 0.9 °C and 1.8 °C, respectively, and also increased absolute minimum air and soil temperatures, ranging between 0.3 °C and 2.0 °C and 0.3 °C and 0.8 °C, respectively (Table 1). The OTCs had significantly ($p < 0.05$) higher average day and night, air and soil maximums in all seasons, except for winter night air temperature, and had significantly higher average day and night, air and soil minimums in all seasons, except for winter day air temperature (data not shown). When data on the degree of temperature increase induced by OTCs were compared within seasons, the OTCs significantly ($p < 0.05$) increased the day air (by 1.7 °C–2.4 °C; Figure 3a) and soil (by 0.7 °C–1.0 °C; Figure 3a) temperatures, and night air (by 0.3 °C–0.6 °C; Figure 3b) and soil (by 0.5 °C–1.2 °C; Figure 3b) temperatures in all seasons relative to the control, with the exception of night air temperature in winter.

In spring, the OTCs experienced the greatest increase in day air temperature and smallest increase in day soil temperature, whilst autumn was characterised by the smallest increase in day air temperature and highest increase in day soil temperature (Figure 3a and 3b). In autumn, the OTCs experienced the greatest increase in night air and soil temperature, whilst spring and summer had the smallest increase in night air and soil temperature, respectively.

When temperature data for the different seasons were pooled within day and within night for analysis, referred to as 'annual' data henceforth, it was evident that the OTCs increased air and soil temperatures for both day and night significantly ($p < 0.05$; Figure 3a and 3b respectively). The OTC plots increased the annual day and night air temperatures

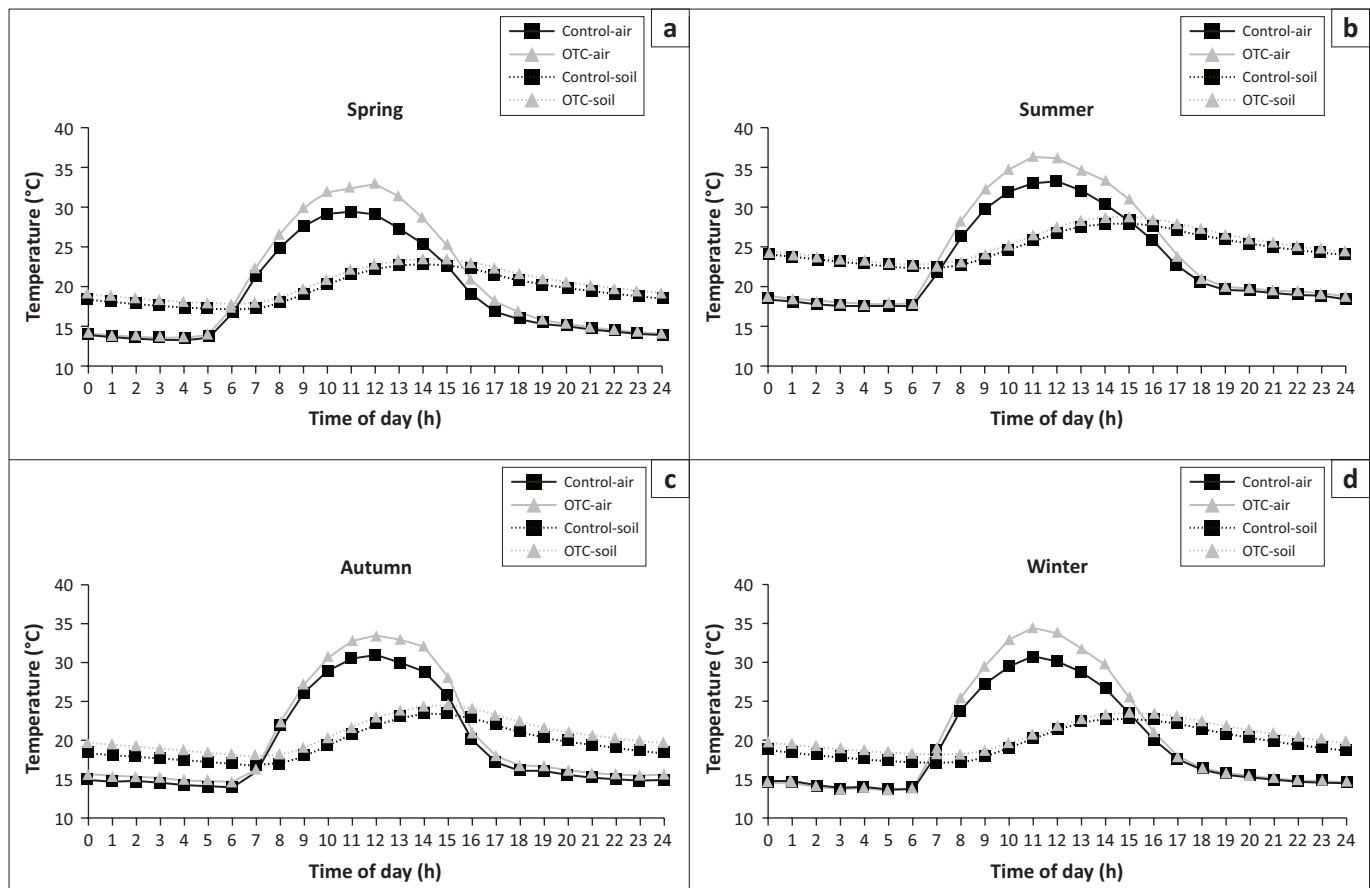


FIGURE 2: Diurnal variation in air and soil temperature in OTC and control plots in spring (a), summer (b), autumn (c) and winter (d). Air temperature values represent means, with $n = 10\,240$ for spring, summer and autumn; and $n = 9665$ for winter. For soil temperature values represent means, with $n = 10\,240$ for spring, summer and autumn; $n = 9665$ for winter. $P < 0.05$ when air and soil temperatures were compared within three hour intervals in each the four seasons (Wilcoxon signed-rank test). Standard deviations ranged from 2.16–9.98 °C for air temperatures and 2.09–3.97 °C for soil temperatures.

TABLE 1: Seasonal and annual mean, absolute maximum and absolute minimum temperatures for OTC and control plots.

		Annual		Spring		Summer		Autumn		Winter	
		Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
Mean	Air	20.1 ± 7.6	21.3 ± 8.9	19.2 ± 7.6	20.5 ± 9.4	23.2 ± 7	24.5 ± 8.4	18.2 ± 7.5	19.3 ± 8.3	19.5 ± 7.3	20.5 ± 8.7
	Soil	20.8 ± 4.3	21.7 ± 4.3	19.7 ± 3.4	20.4 ± 3.4	24.9 ± 3.3	25.5 ± 3.7	18.2 ± 3.3	19.4 ± 3.2	19.7 ± 3.2	20.6 ± 3.1
Absolute maximum	Air	45.4	51.1	45.2	49.6	44.1	49.5	45	50	45.4	51.1
	Soil	35.9	37.7	30.7	31.9	35.9	37.7	30.5	31.4	29.9	30.8
Absolute minimum	Air	5.9	6.2	6.8	7.7	10.3	12.3	7.1	8.7	5.9	6.2
	Soil	11.5	12.1	11.8	12.1	17.2	17.6	13.2	14	11.5	12.1

Values in second row represent means ± SD.

$n = 10240$ in spring, summer and autumn; $n = 9665$ in winter.

$P < 0.05$ (Wilcoxon signed-rank test) when means were compared within seasonal categories between temperature treatments.

by 2.1 °C and 0.3 °C (Figure 3a), and soil temperatures by 0.8 °C and 0.8 °C, respectively (Figure 3b).

Midday temperature, RH and soil moisture content, differed significantly ($p < 0.05$) between OTC and control plots in all seasons (Table 2). Average midday OTC temperature readings were significantly ($p < 0.05$) higher than those in control plots; however, the OTC plots exhibited significantly ($p < 0.05$) lower RH and soil moisture content in all seasons (Table 2). Light intensity was significantly ($p < 0.05$) lower in OTC plots in spring, summer and winter; however, these differences were minimal, ranging from 70.59–110.39 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (Table 2). When control and OTC data for all seasons were pooled for analysis (annual data), there was a strong negative

correlation ($r = -0.745 - -0.910$, $p < 0.05$) between temperature and RH and within seasons there was a strong positive correlation between RH and soil moisture content in spring ($r = 0.818$, $p < 0.05$) and summer ($r = 0.811$, $p < 0.05$).

There was a large variation in the total rainfall across seasons: 313 mm in spring, 198.5 mm in summer, 46.5 mm in autumn and 13.5 mm in winter.

Growth responses

In both OTC and control plots, the highest annual AGP (i.e. all seasons summed) occurred in graminoids, followed by forbs and shrubs (Figure 4). Biomass production for

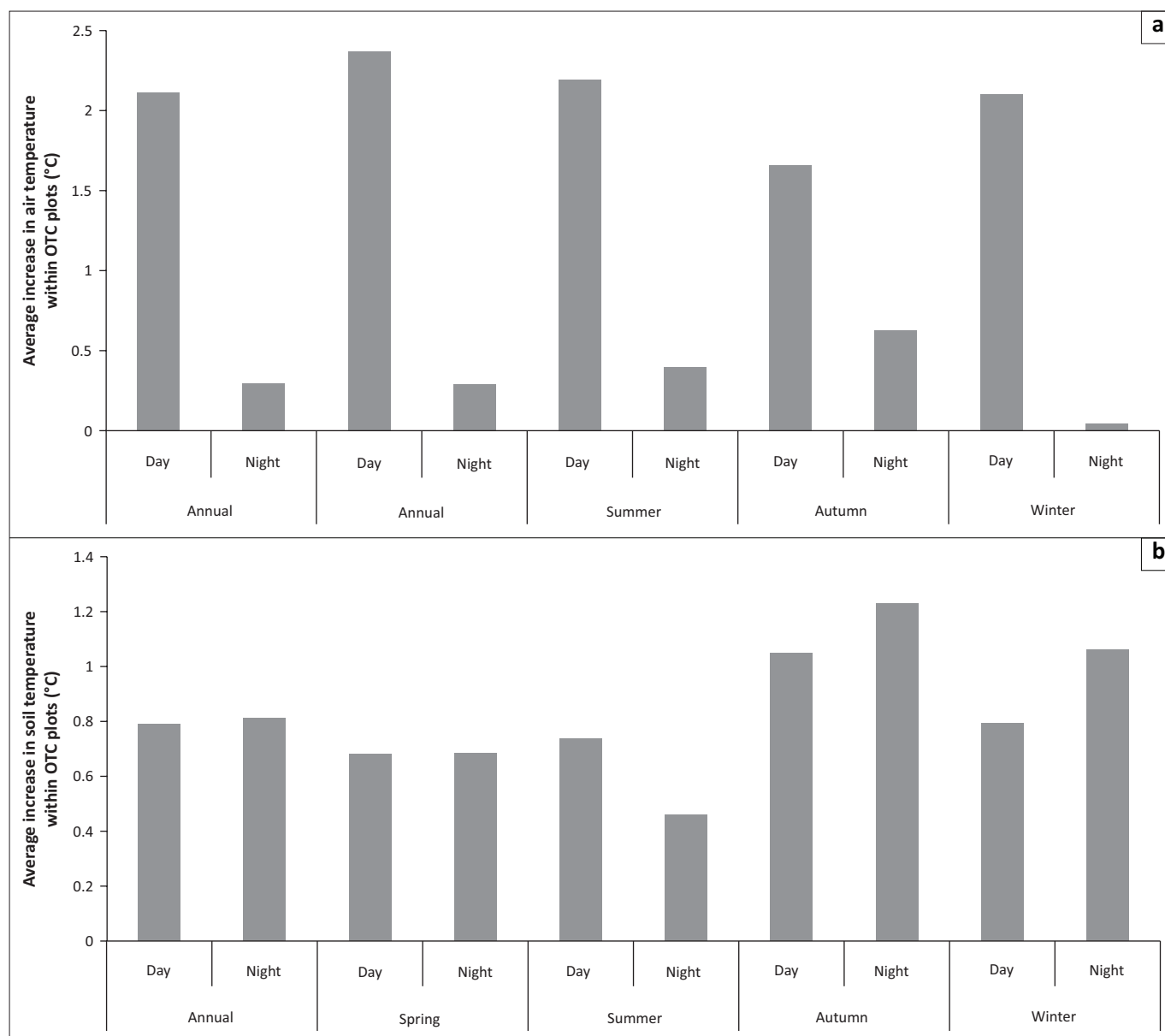


FIGURE 3: Average increase in temperature (annual and seasonal) within OTC plots, relative to control plots, for air (a) and soil (b). For air and soil measurements $n = 40\,385$ for annual; $n = 10\,240$ for spring, summer and autumn; $n = 9665$ for winter. Standard deviations ranged from 1.56–1.82 °C for day, and 0.29–0.53 °C for night air temperature; and from 0.38–0.49 °C for day, and 0.34–0.47 °C for night soil temperature.

TABLE 2: Seasonal midday air temperature (°C), relative humidity (%), soil moisture content (%), light intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$) and total rainfall (mm) for OTC and control plots.

Season	Elevated/Ambient	Air Temperature (°C)	Relative Humidity (%)	Soil Moisture Content (%)	Light Intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Total Rainfall (mm)
Spring	Elevated	32.07 ± 4.47	39.2 ± 12.46	7.76 ± 3.34	691.41 ± 165.84	313
	Ambient	28.2 ± 3.69	46.52 ± 14.55	9.85 ± 4.10	762 ± 84.14	
Summer	Elevated	35.3 ± 3.13	49.89 ± 5.02	14.57 ± 4.14	713.67 ± 166.12	198,5
	Ambient	31.45 ± 3.53	54.81 ± 4.99	18.23 ± 3.41	824.05 ± 81.76	
Autumn	Elevated	31.91 ± 4.68	42.71 ± 8.91	6.1 ± 2.71	490.52 ± 171.64	46,5
	Ambient	28.74 ± 4.22	48.3 ± 9.18	8.33 ± 2.63	498.51 ± 89.44	
Winter	Elevated	31.58 ± 3.26	35.45 ± 8.63	3.49 ± 1.45	1120.73 ± 296.73	13,5
	Ambient	28.83 ± 2.88	39.08 ± 9.64	3.81 ± 0.84	1223.98 ± 158.10	

OTC, open-top chamber.

Temperature, relative humidity, soil moisture content and light intensity values represent mean ± SD. For air temperature, relative humidity, soil moisture content: $n = 275$ for spring; $n = 125$ for summer and autumn; $n = 175$ winter. For light intensity: $n = 125$ for spring, autumn and winter; $n = 75$ for summer. Except for autumn light intensity, $p < 0.05$ when values were compared between temperature treatments (Wilcoxon signed-rank test).

graminoids and all life forms combined was significantly higher in OTC plots ($p < 0.05$; Figure 4). In terms of annual data there was also a trend for shrub productivity to be higher in OTCs, whilst forb productivity was slightly higher in control plots.

When seasonal AGP was compared between OTC and control plots, there were also no significant differences ($p > 0.05$), except in autumn and spring, when graminoid productivity was higher ($p < 0.05$) in OTCs (Figure 5). Shrub productivity in summer was significantly ($p < 0.05$) higher in OTC plots. Additionally, combined productivity was significantly ($p < 0.05$) higher in OTCs in summer and autumn. Combined productivity was significantly positively correlated ($r = 0.794$ – 0.956 ; $p < 0.05$) with graminoid productivity in all seasons and forb productivity in winter

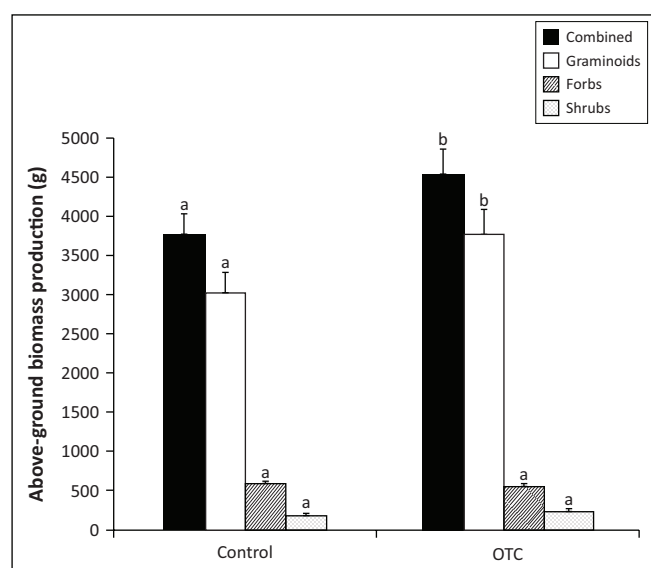


FIGURE 4: Annual above-ground biomass production (g) for individual and all life forms combined. Values represent mean \pm SD ($n = 20$). Values labelled with different letters are significantly different ($p < 0.05$, Wilcoxon signed-rank test) when compared within life forms between temperature treatments.

($r = 0.830$, $p < 0.05$). In summer, there was a strong negative correlation between graminoid and forb productivity ($r = -0.706$, $p < 0.05$). There were no significant differences ($p > 0.05$) in below-ground biomass production between OTC and control plots in individual and combined seasonal scenarios (data not shown).

Maximum temperature was significantly positively correlated with combined AGP (data for all life forms combined) in summer and autumn ($r = 0.818$ and $r = 0.661$, respectively; $p < 0.05$). Maximum temperature was also significantly positively correlated with graminoid AGP in summer and autumn ($r = 0.903$ and $r = 0.661$, respectively; $p < 0.05$). Maximum temperature was also significantly positively correlated with shrub AGP in summer ($r = 0.673$; $p < 0.05$). Minimum temperature was only significantly positively correlated with combined ($r = 0.840$; $p < 0.05$) and graminoid ($r = 0.850$; $p < 0.05$) AGP in autumn.

During autumn, there was a significant negative correlation between combined AGP and both RH ($r = -0.650$; $p < 0.05$) and soil moisture content ($r = -0.840$; $p < 0.05$), as well as between soil moisture content and graminoid AGP ($r = -0.765$; $p < 0.05$). Similarly, in spring there was a strong negative correlation between graminoid AGP and both RH ($r = -0.655$; $p < 0.05$) and soil moisture content ($r = -0.714$; $p < 0.05$).

Summer and annual biomass allocation to forbs was significantly ($p < 0.05$) lower in OTC plots (Figure 6). Additionally, biomass allocation to shrubs was significantly ($p < 0.05$) higher in the OTC plots in winter. Excluding summer, there were significant negative correlations between above-ground biomass allocation to graminoids and forbs ($r = -0.908$ to -0.995 , $p < 0.05$). During summer there was a significant negative correlation ($r = -0.671$, $p < 0.05$) between above-ground biomass allocation to graminoids and shrubs.

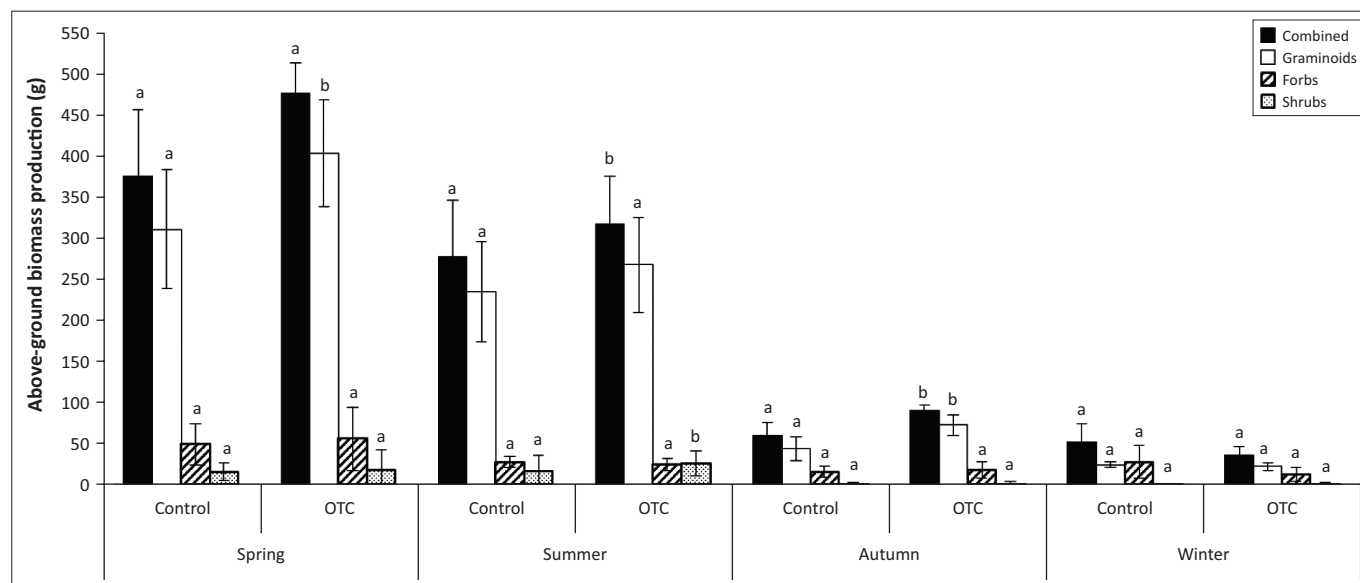


FIGURE 5: Above-ground biomass production (g) for individual and combined life forms in OTC and control plots. Values represent mean \pm SD ($n = 5$ for individual seasons). Values labelled with different letters are significantly different ($p < 0.05$, Wilcoxon signed-rank test) when compared within life forms between temperature treatments.

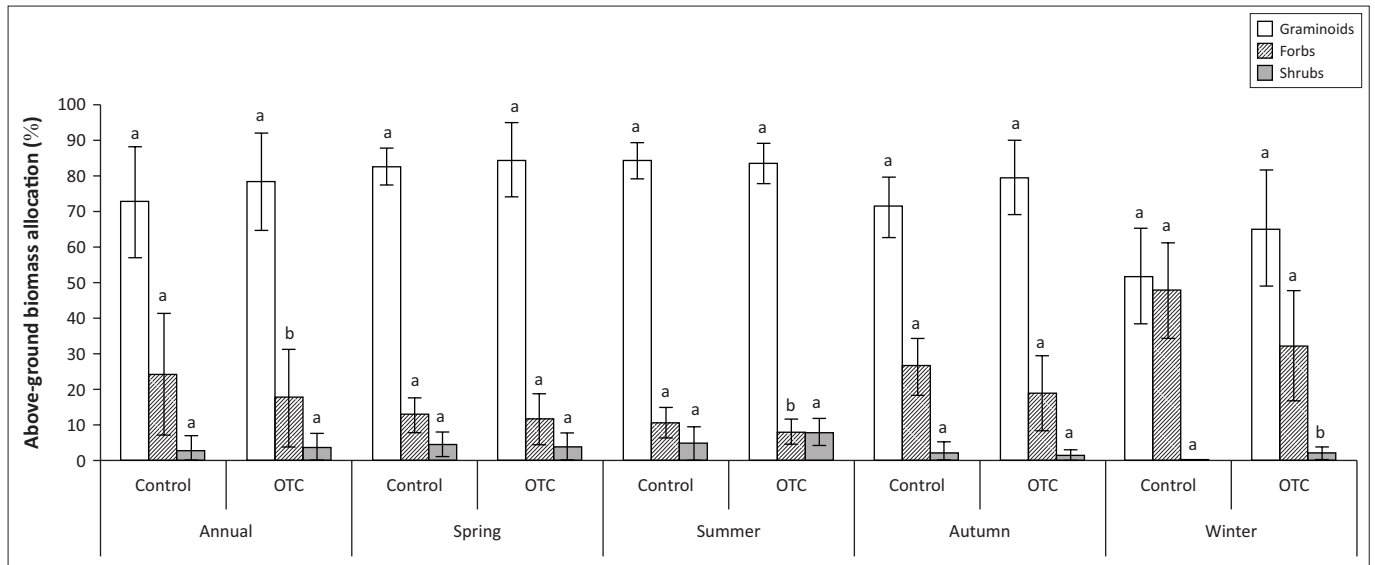


FIGURE 6: Above-ground biomass allocation (%) for individual life forms in OTC and control plots for each season and for all seasons combined (annual). Values represent mean \pm SD ($n = 5$ for individual seasons and $n = 20$ for all seasons combined) and when labelled with different letters are significantly different ($p < 0.05$, Wilcoxon signed-rank test) when compared within season and life form categories, between temperature treatments.

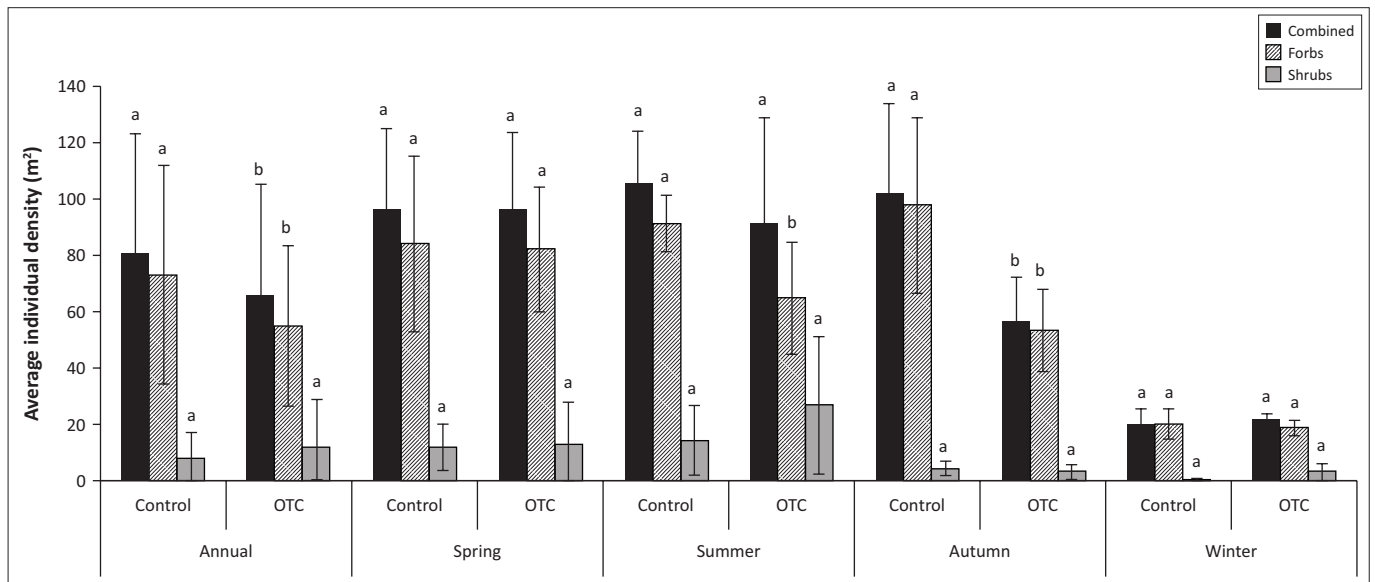


FIGURE 7: Plant density for forbs and shrubs for each season and for all seasons combined (annual). Values represent mean \pm SD ($n = 20$ for combined and $n = 5$ for individual seasons). Values labelled with different letters are significantly different ($p < 0.05$, Wilcoxon signed-rank test) when compared within season and life form categories, between temperature treatments.

The clonal propagation strategy adopted by many of the graminoid species sampled prevented the quantification of the graminoid density, thus density was calculated for forbs and shrubs only (Figure 7). Annual and autumn combined density was significantly lower ($p < 0.05$) in OTC plots. Annual, autumn and summer forb densities were also significantly ($p < 0.05$) lower in OTC plots, but shrubs were unaffected (Figure 7). Additionally, during autumn forb density was negatively correlated with maximum ($r = -0.669$; $p < 0.05$) and minimum ($r = -0.674$; $p < 0.05$) temperatures. In summer, there was also a significant negative correlation between maximum temperature and forb density ($r = -0.661$; $p < 0.05$). Additionally, there was a significant positive correlation between forb density and RH in autumn ($r = 0.643$; $p < 0.05$). All other correlations between life forms and abiotic parameters were not significant ($p > 0.05$).

Discussion

This study assessed the utility of OTCs in simulating elevated temperatures in a subtropical grassland, and the subsequent vegetation responses to these temperatures in terms of productivity and community structure.

Chamber design and effects on abiotic parameters

The OTCs used in this study are classified as passive systems, as they primarily rely on solar energy being trapped inside the chamber, allowing no direct controlled manipulation of temperature (Marion *et al.* 1997). Studies on elevating temperatures with passive OTCs include many different variations on their design, which in many cases aim to reduce

unwanted chamber effects that do not correspond to past or predicted environmental changes associated with global warming (Aronson & McNulty 2009). The effectiveness of OTCs is based on their ability to simulate realistic elevated temperatures, i.e. temperatures that are in line within predictions for the near future. There are other methods that can be employed to elevate temperatures in plant/ecological studies (e.g. infrared heaters [Dukes *et al.* 2005; Sherry *et al.* 2008; Wan *et al.* 2005], in-line heaters when combined with blowers [Norby *et al.* 1997] and thermal inertia (water filled pipes) [Flanagan *et al.* 2013; Godfree *et al.* 2011]). However, these can be costly and cumbersome when installed *in situ*.

The OTC design used in this study, adapted from Molau and Mølgaard (1996), increased air and soil temperatures significantly relative to the control plots, irrespective of the time of day or season (Figure 2). More specifically, mean day temperature increased by ± 2.1 °C for air and ± 0.3 °C for soil, and night temperature by ± 0.8 °C for air and ± 0.8 °C for soil in KZNSS vegetation (when annual data were considered; Figure 3). The degree of air temperature increase relative to the control plots ranged from 1.6 °C to 2.4 °C during the day and 0.0 °C to 0.6 °C during night across the four seasons (Figure 3a). Midday temperatures measured using a precision thermistor (Kestrel 4500 Pocket Weather Tracker) (Table 2) support these findings. Further analysis of the temperature data revealed that the OTCs increased absolute maximum air and soil temperatures (Table 1). For air, this increase ranged between 4.4 °C and 5.7 °C for absolute maximum and between 0.3 °C and 2.0 °C for absolute minimum. Godfree *et al.* (2011) experienced similar increases in maximum air temperature in their 70 cm high hexagonal chambers, with a ± 4.1 °C increase in spring-summer, and ± 5.6 °C in summer. Given that temperatures in southern Africa are likely to increase by 3 °C to 4 °C within the century (studies cited in IPCC 2007), these data suggest that the OTC design used in this study can simulate realistic and consistent increases in temperature when installed in subtropical grasslands. Additionally, average, maximum and minimum day and night soil temperatures were significantly ($p < 0.05$) higher in OTCs in all seasons; the absolute maximum was increased by 0.9 °C – 1.8 °C whilst the absolute minimum was increased by 0.3 °C – 0.8 °C (Table 1). Rustad *et al.* (2001) conducted a meta-analysis on 32 various experimental projects and found that an increase in soil temperature between 0.3 °C and 6.0 °C significantly increased above-ground biomass production and soil respiration. A study in Canada revealed that OTCs with an opening of 1.0 m can increase mean daily soil temperatures (at 3.0 cm below ground level) by 0.58 °C (Marion *et al.* 1997).

Marion *et al.* (1997) noted that the internal air and soil temperatures of OTCs are directly influenced by the ratio of the external chamber surface to the internal volume, together with the ratio of the size of the opening at the top of the chamber to the height of the chamber. A smaller internal volume and top opening of a chamber will result in increased temperatures. Additionally, Godfree *et al.* (2011) examined

the increase in temperatures induced by OTCs with a height of 50 cm and 70 cm in a temperate grassland. When compared with the control plot, the 50 cm and 70 cm high OTCs had a mean day temperature increase of 1.2 °C and 3.2 °C, respectively, with a 0.3 °C increase in night temperature. The chamber height used in the present study was 50 cm but the increases in day and night temperatures observed here were both higher than that quoted for 50 cm high OTCs by Godfree *et al.* (2011). So, whilst the chamber design used in the present study may have yielded a realistic increase in temperature for the study site selected, a larger internal volume, larger top opening and lower chamber height may have to be employed when these OTCs are installed in subtropical grasslands characterised by relatively lower present-day mean daily temperatures.

Open-top chambers are prone to numerous ‘chamber effects’, which include, but are not limited to, a reduction in RH, light intensity (Flanagan *et al.* 2013; Godfree *et al.* 2011) and soil moisture content (Aronson & McNulty 2009). Similar decreases in RH, soil moisture content and light intensity were observed within OTCs in this study, in all seasons. There was also a strong negative correlation between RH and diurnal temperature in all seasons, and a positive correlation between soil moisture content and RH in the warmer seasons (i.e. spring and summer). Flanagan *et al.* (2013) report that in OTCs a reduction in soil moisture content may be a result of an increased vapour pressure deficit (VPD) triggered by the elevated temperatures and decreased RH. Furthermore, Rustad *et al.* (2001) and Wan *et al.* (2002) have shown that with warming there is generally always a reduction in the soil moisture content. Whilst the interaction amongst temperature, RH and soil moisture within OTCs are difficult to disentangle, the results obtained here and elsewhere (Aronson & McNulty 2009; Flanagan *et al.* 2013; Flanagan & Johnson 2005; Godfree *et al.* 2011) suggest that OTCs offer the opportunity to investigate the combined effects of elevated temperatures and reduced soil moisture content in grasslands – both of which have been predicted for subtropical grasslands (IPCC 2007). The decline in light intensity in the OTCs was marginal ($70.59 - 110.39 \mu\text{mol m}^{-2} \text{s}^{-1}$), given that maximum light intensities measured ranged from 900 to $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$. Reference to the literature suggests that a significant decline in light intensity can reduce graminoid productivity in grassland vegetation (Olf 1992). In the present study, graminoid productivity increased within the OTCs, implying that the reduction in light intensity within the chambers was not significant enough to override the effects of temperature.

The modifications to the ITEX design made here (e.g. increased chamber size and improved support frame) were to allow the chambers to withstand the heavy summer rains, unpredictable hail storms and strong wind gusts associated with the study area. Even with these modifications, each chamber could be manufactured for less than \$70, making them a very affordable experimental tool for studies focused on grassland responses to climate change.

Plant productivity and community structure

Studies have shown that increased temperature can influence floristics and community structure in temperate grasslands (Saleska *et al.* 2002; Shaver *et al.* 2000). Elevated temperatures can alter recruitment patterns, influencing community composition and species richness, both of which have a bearing on ecosystem health (De Boeck *et al.* 2007; Laurance *et al.* 2011). Studies in temperate systems have also shown that elevated temperatures can alter productivity in grasslands, which has implications for ecosystem functioning (Dukes *et al.* 2005).

According to Wan *et al.* (2005), elevated temperatures could have both a positive and a negative effect on the carbon sequestration in grasslands, depending on the season, as well as an indirect effect by extending the growing season and altering soil nitrogen mineralisation and availability. This may explain reports of mixed responses of carbon sequestration to elevated temperatures in grasslands (Jones & Donnelly 2004).

In the present study, elevated temperatures increased annual graminoid AGP by $\pm 19.9\%$ and decreased forb AGP by $\pm 9.0\%$ (Figure 4). In terms of annual combined AGP, levels in OTC plots were also $\pm 16.9\%$ higher compared to control plots (Figure 4). Additionally, at elevated temperatures graminoid AGP was significantly higher in spring and autumn, shrub AGP was significantly higher in summer, and combined AGP was significantly higher in summer and autumn (Figure 5). These results are largely understandable as spring and summer represent the major growing seasons in subtropical grasslands. The increase in graminoid and combined AGP within OTCs in autumn (Figure 5) suggests that elevated temperatures may also increase biomass production during periods of low productivity and low rainfall. This study was conducted in a summer rainfall region in southern Africa, where precipitation can vary between 640 and 1800 mm (Neumann, Botha & Scott 2014); the highest precipitation occurs in spring and summer under increased RH and temperature, and rainfall deficit during autumn and winter under increased evaporation (Neumann *et al.* 2014). The potential interactions between elevated temperatures and rainfall deserve further investigation.

Graminoid AGP was positively correlated with diurnal, maximum and minimum temperatures in autumn, and with maximum temperatures in summer when correlated. Furthermore, during summer graminoid AGP was significantly negatively correlated with forb AGP; this also was evidenced by the significant decline in biomass allocation to forbs at elevated temperatures (for annual and summer, Figure 6). This suggests that in KZNSS, elevated temperatures in promoting graminoid productivity may compromise forb AGP. Interestingly, shrub AGP was significantly enhanced at elevated temperatures in summer (Figure 5). Del-Val and Crawley (2005) observed a reduction in forb and shrub AGP with increased grass biomass. Those authors also showed monocotyledonous species to be stronger competitors within

grasslands than forbs or shrubs, and that removal of grasses led to a dramatic increase in forb and shrub AGP. They suggested that competition for light could be the main driver in these competitive interactions. Carlsen, Menke and Pavlik (2000) also suggest that grasses, in forming dense swards, could possibly cause a reduction in the quality and quantity of light reaching forb species below. However, these studies did not manipulate temperature, and at the time of this study there were no recently published data on *in situ* responses of subtropical grassland species to elevated temperature. Nevertheless, Morgan *et al.* (2011) showed that C_4 grass responded positively to elevated temperatures in terms of AGP; however, small population sizes and yearly variation reduced detectable effects on forbs. This may have also contributed to the lack of significant differences observed in the present study.

Typically, C_4 grasses dominate tropical and subtropical grasslands (Still *et al.* 2003). These C_4 species have higher thermal thresholds than C_3 species such as forbs, largely as a consequence of differences in the enzyme they employ for carbon fixation (De Boeck *et al.* 2007; Hatfield & Prueger 2015; Wan *et al.* 2002). The ability of graminoids (mainly C_4 species) to enhance their productivity at elevated temperatures is well documented (Horton & Murchie 2000; Sage 2000), whereas at these temperatures, forbs (mainly C_3 species) could be reaching their thermal maximum temperatures, triggering a reduction in their photosynthetic efficiency and hence, productivity (De Boeck *et al.* 2007; Hatfield & Prueger 2015; Wan *et al.* 2002). Whilst graminoid AGP was enhanced at elevated temperatures in the present study (as described above), forb AGP was not correlated with temperature, RH or soil moisture content. On the other hand, graminoid AGP was negatively correlated within both RH and soil moisture content in spring, the season of maximum growth. This implies that the increase in grass productivity at elevated temperatures in KZNSS may have led to a decrease in soil water availability. This is worrying because forbs and shrubs have an inferior water-use-efficiency to graminoids (Sherry *et al.* 2008; Wan *et al.* 2005).

It is also worth noting that winter biomass allocation percentages in control plots were more comparable between forbs and graminoids than in OTC plots (Figure 6). This difference appears to be the result of a decrease (18.2% relative to control) in forb AGP in the OTC plots in winter and suggests that elevated temperature effects may manifest during times of minimal growth within KZNSS as well. This suggestion is reinforced by the fact that forb density within the OTCs was lower than the control plots for autumn, summer and annual data (Figure 7). Additionally, there was also a strong negative correlation between forb density and both maximum and minimum temperatures in autumn and between forb density and maximum temperature in summer. Alward *et al.* (1999), in examining 23 years of correlation data between temperature (minimum, maximum and average), AGP and abundance, showed that native and exotic forbs in cold temperate grasslands exhibited increased net primary productivity (NPP) and density at elevated temperatures.

However, they stated that this result may be a consequence of the decreased annual NPP of a dominant C_4 grass, resulting in increased space, nutrients and water availability, rather than the effects of elevated temperatures. It should be noted that although graminoid densities were not recorded in the present study, they displayed 90%–100% coverage in most control and OTC plots. Carlsen *et al.* (2000) showed that high density grasslands provided a poor habitat for native forbs. Furthermore, Sternberg *et al.* (1999) predicted that an increase in graminoid productivity will result in decreased availability of gaps for forb recruitment or growth, and consequently a reduction in the forb species richness and density. This is very concerning because many of the forb and shrub species endemic to KZNSS are taxa of conservation concern (Mucina & Rutherford 2006).

Concluding remarks and recommendations

The effects of climate change are becoming increasingly evident and the need to predict ecosystem responses to increased temperatures, in particular, is of paramount importance. This is particularly true for grasslands, which are threatened worldwide. The present study has shown that the effects of elevated temperatures on subtropical grasslands can be successfully investigated *in situ* using OTCs. The elevated temperatures simulated by these OTCs appear to be in line with predictions for the next century and accompanying 'chamber effects', such as decreased soil moisture content, may allow for the simultaneous investigation of different climate change scenarios.

The results suggest that the responses of KZNSS vegetation to elevated temperatures will vary across life forms, with C_4 graminoids appearing to benefit in terms of AGP. There were also signs that this enhanced graminoid productivity at elevated temperatures may lead to the displacement of C_3 forbs, possibly as a result of competition. Shrubs, on the other hand, appear to be unaffected (both directly and indirectly) by elevated temperatures. The effects of elevated temperatures on KZNSS are likely to manifest during periods of maximum and minimum growth. This has implications for KZNSS conservation planning efforts.

We, therefore, recommend the use of OTCs in future investigations of the effects of elevated temperatures on subtropical grasslands. Apart from productivity, such studies should investigate how elevated temperatures are likely to influence species composition and abundance, which will yield more informed recommendations for the conservation of threatened grassland types like KZNSS.

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Competing interests

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors' contributions

R.D.B. carried out the field work, processed and analysed the data and wrote the manuscript. The study was supervised by S.N., S.R. and N.W.P., who also made contributions to the manuscript.

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