Are local range expansions in southerly populations of *Aloidendron dichotomum* early indicators of a future range shift?

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Abstract

Aloidendron dichotomum is predicted to undergo a south and south-eastern range shift in response to anthropogenic climate warming. Despite this, its range is suggested to be limited by low temperature extremes at the cool range edge and no such range shift has yet been confirmed. However, eco-physiological knowledge necessary to predict and detect this range shift is lacking. This study aimed to determine whether physiological performance of *A. dichotomum* differs at a regional and local scale, and if so, whether regional and local temperatures explain physiological differences. Performance metrics and temperature data were collected during summer at 14 microsites in two sites at the cool range edge of *A. dichotomum*. Microsites were stratified by aspect and elevation. This study confirmed that *A. dichotomum* is performing CAM photosynthesis at its cool range edge. Further, the results provide evidence for the temperature dependence of *A. dichotomum*'s physiological performance. Higher performance was associated with warmer nights and the warmer north-facing slopes. This is in keeping with our hypothesis that *A. dichotomum* individuals at the cool range edge would take advantage of higher temperatures more typical of their core range areas. Opposing this, higher carbon gain was found at Gannabos – the cooler of the two study sites. This may be due to the less variable rainfall pattern at Gannabos. Furthermore, performance was higher on the flats where temperatures were also cooler. This may be due to lower water runoff on the flats compared to the slopes. As a result, I suggest that the cool range edge of *A. dichotomum* is limited both by low temperatures and rainfall variability and that, with warming, constraints on these populations are being removed. This is substantiated by the observed high levels of juvenile recruitment in these populations relative to others, and in time, is likely to lead to range expansion in the region.

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Plagiarism declaration

I, Kerry-Anne Grey, understand the meaning of plagiarism and declare that the work presented in this dissertation, apart from that which is properly acknowledged, is my own.

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Thesis formatting

This thesis was formatted according to the stylistic guidelines of the journal *Conservation Biology*. This journal requires the following sections: abstract, key words, introduction, material and methods, results, discussion and conclusion. It requires that each section be numbered, with subheadings numbered accordingly.

Referencing style used in this thesis:

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1. *Single author:* the author's name (without initials, unless there is ambiguity) and the year of publication;
2. *Two authors:* both authors' names and the year of publication;
3. *Three or more authors:* first author's name followed by 'et al.' and the year of publication.

In-text citations are listed chronologically and then alphabetically.

Example: (Foden 2002; Guo et al. 2016; Males & Griffiths 2017)

Reference list:

1. *Reference to a journal publication:*
   Van der Geer J, Hanraads JAJ, Lupton RA. 2010. The art of writing a scientific article. Journal of Science Communication. **163**:51–59. Available at https://doi.org/10.1016/j.Sc.2010.00372.
2. *Reference to a book:*
   Strunk Jr W, White EB. 2000. The Elements of Style, fourth ed. Longman, New York.
3. *Reference to a chapter in an edited book:*
   Mettam GR, Adams LB. 2009. How to prepare an electronic version of your article, in: Jones BS, Smith RZ (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.
4. *Reference to a website:*
   Cancer Research UK, 1975. Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/ (accessed 13 March 2003).

References listed alphabetically and then chronologically.

List of abbreviations

CAM	Crassulacean Acid Metabolism
CO_2	Carbon dioxide
PEPC	Phosphoenolpyruvate carboxylase
NADH	Nicotinamide adenine dinucleotide
WRZ	Winter-rainfall zone
SRZ	Summer-rainfall zone
ΔpH	Nocturnal change in pH
GiLM	Generalised linear model
AIC_c	Second-order Akaike's information criterion
n	Sample size
K	Number of estimable parameters in a model
Tris-HCl	Tris(hydroxymethyl)aminomethane hydrochloride
EDTA	Ethylenediaminetetraacetic acid
DDT	Dichlorodiphenyltrichloroethane
PVPP	Polyvinylpolypyrrolidone
$MgCl_2$	Magnesium chloride
$NaHCO_3$	Sodium bicarbonate
MDH	Malate dehydrogenase
ANOVA	Analysis of variance
OCGH	Optimal carbon gain hours
GLMM	Generalised linear mixed-effects model

1. Introduction

Second to land-use change, the adverse effect of anthropogenic climate change is projected to have the greatest effect on global biodiversity this century (Sala et al. 2000; Dawson et al. 2011; IPCC 2014). With an increase in global mean temperatures of up to 1.06°C since the preindustrial era, the current four decades have been the warmest of the last 1400 years (IPCC 2014). These temperature increases have been accompanied by increases in extreme weather events, such as droughts and heat waves, increasing the vulnerability of many species and ecosystems (IPCC 2014). There is high confidence that temperatures will continue to rise at an unprecedented rate into the future, with further increases of up to 0.7°C by 2035 for all emissions scenarios and exceeding 1.5-2°C by the end of this century for the higher emissions scenarios (IPCC 2014).

Responses of wildlife to climate change include adaptation (via phenotypic plasticity or selection), shifting geographic ranges or dieback. Many long-term monitoring studies have already shown evidence for physiological, phenological and distributional changes of species in response to recent atmospheric and climatic trends (Hughes 2000; Parmesan & Yohe 2003; IPCC 2014; Settele et al. 2014; Parmesan & Hanley 2015) and mortality has been observed in some tree species (Breshears et al. 2009; Allen et al. 2015). Globally, a significant fraction of endemic species have been assessed as at risk of local extinction (Settele et al. 2014).

Range shifts and distribution changes typically result in species establishing in areas where climate conditions were previously unfavourable (Hughes 2000; Thomas et al. 2001; Jump & Peñuelas 2005; Chen et al. 2011; Dawson et al. 2011; Settele et al. 2014). Such range shifts often approximate the “uphill and poleward” pattern, whereby species’ ranges shift to higher latitudes and altitudes to track their climatic envelope, resulting in the formation of novel communities (Graham & Grimm 1990; Cannone & Pignatti 2014; Settele et al. 2014). This directional response of species to warming temperatures is due to the autocorrelated nature of temperatures in space, with warmer conditions linked to lower elevations and latitudes and cooler conditions linked to higher elevations and latitudes (Lenoir & Svenning 2015). Because the climatic factors that limit distributions are known for very few species, precise attribution of range shifts to specific aspects of climate is lacking.

Species are furthermore predicted to respond differently to anthropogenic climate change at different parts of their ranges (Loarie et al. 2009). Mortality and local extinction are predicted in the warmer, drier parts of species' ranges (often referred to as "trailing edge"), accompanied by range expansions into areas that were previously too cool and/or wet for the species to establish – the "leading edges" (Huntley 1995; Hughes 2000; Parmesan & Yohe 2003; Pearson et al. 2013). This trend has been recorded for several, predominantly Northern Hemisphere species (Midgley & Thuiller 2011; Lenoir & Svenning 2015), and evidence indicates that there is a strong climate signal present in many of these recent range shifts (Settele et al. 2014).

However, it is increasingly apparent that the assumption of a uni-directional response of species to anthropogenic climate change may be too simplistic to fully capture the multiple interacting variables involved in the response of species range edges to local climate change (Lenoir et al. 2010; Lenoir & Svenning 2015). Furthermore, it has been noted that range shifts are generally individualistic, with different species or growth forms tending to respond differently to the changing climate (Chen et al. 2011). In a large-scale review of species range shifts, Chen et al. (2011) concluded that the extreme diversity of range shifts reported in the literature may be the result of one of two things. Firstly, they note that different species have different physiological constraints defining their distribution (Lenoir et al. 2010) and sensitivities to temperatures may differ at different life stages, further complicating the response of individual taxa to climate change. Secondly, they note that species responses to local changes in climate will differ due to inherent differences in life history characteristics.

For many plant species, especially long-lived, slow-growing species, natural migration rates are likely to be too slow to track changes in local climates due to inherent slow rates of establishment and dispersal, increasing their extinction risk (Pitelka 1997; Foden et al. 2007; IPCC 2014). Moreover, local extinctions should be more prominent in species living in harsh environments (such as deserts) where stresses related to climate conditions control the distributions and physiological processes of species (Jordan & Nobel 1979). Because of these taxon-specific responses to changing climate, Chen et al. (2011) suggest that when determining the potential response of a species to climate change, detailed environmental, ecological and physiological data are required.

One plant species that may be undergoing a range shift is the iconic desert-adapted tree-aloe *Aloidendron dichotomum* (Masson) Klopper & Gideon, commonly known as the quiver tree (Foden 2002; Foden et al. 2007; Guo et al. 2016; van der Merwe & Geldenhuys 2017). *A. dichotomum* grows on inselbergs and rocky outcrops in the Succulent and Nama Karoo biomes of South Africa, as well as in much of arid Namibia (Burke 2004; van der Merwe & Geldenhuys 2017). Several studies have shown population declines in the warmer, drier sites, where lower elevation populations show high mortality and reduced recruitment (Foden 2002; Foden et al. 2007; Hoffman et al. 2010; Jack et al. 2016; van der Merwe et al. 2017). Foden et al. (2007) attributed patterns of mortality to anthropogenic climate change, in a first example of such a response for a Southern Hemisphere plant species.

This suggestion has been questioned by researchers who propose that mortality may be due to local or historical factors, such as windthrow; prevailing rainfall regimes (Jack et al. 2014, 2016); herbivory; fungal infection; and an assortment of other variables (Midgley et al. 1997). However, Foden (2002) tested a multitude of variables that may be contributing to the mortality of this species and found that only climatic variables correlated with mortality. Furthermore, Guo et al. (2016) suggest that, because of the aridity of the western parts of southern Africa, climate factors are the main determinants of the distribution of *A. dichotomum*. These studies suggest that populations at warmer, drier (often but not always equatorward) sites are currently near their threshold for climate tolerance and that regional climate change has likely caused these thresholds to be exceeded, resulting in severe declines of these populations (Foden et al. 2007). In contrast to high rates of mortality at the trailing edge of the range of *A. dichotomum*, evidence exists for positive population growth rates in the southern and south-eastern populations (Foden et al. 2007; Hoffman et al. 2010; van der Merwe & Geldenhuys 2017), which could represent "leading edges" of an incipient range shift.

Studies using correlative species distribution modelling approaches predict a climate-driven southeast shift in the range of *A. dichotomum* into areas that were previously too cool for their establishment (Foden 2002; Guo et al. 2016). These projections support the notion that southern and south-eastern populations represent "leading edges". Foden et al. (2007) noted that, despite high rates of recruitment at the southern cooler edge of its distribution, migration of the species has not yet been documented. It is plausible that these poleward populations are at a threshold for climate tolerance and may begin to shift range, beginning with local scale population expansions in response to local shifts in climate (van Wilgen et al. 2016; Department of Environmental Affairs 2017) into microsites that were previously climatically unsuitable (Foden 2002; Kaleme 2003; Foden et al. 2007; Guo et al. 2016). If so, detailed knowledge of *A. dichotomum*'s local scale physiological functions and their relationship with micro-climatic factors would help to interpret such changes and help to detect and predict a future range shift.

Detailed knowledge of the physiological function of *A. dichotomum* is, however, lacking. It is held (but not yet confirmed) that the species performs Crassulacean Acid Metabolism (CAM; Foden et al. 2007). Evidence indicates that CAM plants are sensitive to certain environmental conditions, with their daily carbon gain and assimilation dependent on the abiotic conditions of their habitat (Flexas et al. 2012; Males & Griffiths 2017). Drought and heat stress are particularly important for CAM-photosynthetic plants, as they may cause excessive stomatal closure and thus reduce the amount of carbon available to the plants for growth (Flexas et al. 2012; Males & Griffiths 2017). Low temperatures are also important for these plants and are typically a major constraint on the distribution of terrestrial plants (Flexas et al. 2012). It has been observed that CAM plants generally respond to low temperatures by decreasing their rates of carbon assimilation (Flexas et al. 2012). If *A. dichotomum* does indeed perform CAM photosynthesis, then the threshold limiting its range in the south may be a physiological threshold caused by low growing-season temperatures, currently preventing range expansion (Guo et al. 2016).

For this study, I therefore focused on the potential effect of temperature as a primary predictor of the physiological function of *A. dichotomum* in different microsites in two leading edge populations. If temperature is important for the function of this species at its range edge (Foden et al. 2007; Guo et al. 2016), then one would expect local scale aspect and elevation differences to play a significant role in controlling physiological function at the range limits represented by the selected sites. The main aim of this study was therefore to determine whether physiological performance differs at a regional (i.e. the two chosen sites) and local (i.e. different aspects and elevations) scale, and if so, whether regional- and local-scale temperature differences explain these patterns. In order to address this aim, I attempted to determine the effect of temperature on the *in-situ* physiology of *A. dichotomum* individuals in two southerly populations in South Africa. These data should also provide evidence regarding whether *A. dichotomum* is indeed using the CAM photosynthetic pathway, as expected.

The two sites chosen for this study are located in different rainfall zones, experiencing contrasting climatic conditions. Jack et al. (2016) suggest that populations found in winter and summer rainfall zones would be distinguishable, proposing a rainfall seasonality-based division into populations, presumably with distinct physiology. My first hypothesis was therefore that physiological performance of *A. dichotomum* individuals would differ between the two study sites.

Because substantial, detectible distribution changes at leading edges could take years or decades to manifest, it seems more feasible to explore population responses at these sites at a local scale, in order to anticipate such changes that may not be detectable in large-scale analyses (Thomas 2010). Detection of a response of *A. dichotomum* to environmental variables at the local scale would provide invaluable insight into the patterns, direction and magnitude of its current and future predicted responses to anthropogenic climate change (Cannone & Pignatti 2014). Moreover, when looking at local-scale responses of *A. dichotomum* to temperature variables, one may gain better understanding of its ability to track its environmental requirements (Randin et al. 2009; Cannone & Pignatti 2014). Furthermore, the presence of local-scale differences in physiological performance is expected to be more apparent in these two range edge sites

(Sexton et al. 2009). Typically, range edge populations are characterised by variable performance, with individuals generally being maladapted to the environmental conditions at and beyond the range edge (Sexton et al. 2009). However, these populations at the range edge may have individuals that perform optimally in microsites of favourable climate (Sexton et al. 2009). My second hypothesis was therefore that *A. dichotomum* individuals would perform differently in different microsites (i.e. different aspects and elevations).

Until recently, poleward populations of *A. dichotomum* have predominantly established on the equator-facing (north-facing) slopes of hills and mountains. However, these populations now extend onto all aspects in many sites, as well as onto the flats below the slopes. I therefore predicted that physiological performance would be highest on the north-facing slopes where establishment has previously been favoured. Furthermore, in the past, *A. dichotomum* populations have typically established on hillsides, with establishment being very low, if at all, on the flats below the slopes (Burke 2004; van der Merwe & Geldenhuys 2017). I therefore predicted that physiological performance would be superior on the slopes than on the flats, as establishment on the flats is a recent observation in these sites. The clear correlation of higher tree mortality at lower elevations at the warmer reaches of *A. dichotomum*’s range (Foden et al. 2007), indicates a link between performance and elevation for this species. I therefore predicted that physiological performance would also be dependent on elevation.

While topographic factors have a direct effect on the amount of solar and reflected radiation received by plants (Måren et al. 2015), slope aspect is known to influence ambient air temperature. Furthermore, because of the auto-correlation between elevation and temperature (Lenoir et al. 2010), elevation is expected to control ambient air temperature in these microsites. Based on this, my third hypothesis is that the differences in physiological performance of *A. dichotomum* are related to temperature differences at the regional and local scale. Indeed, through correlative modelling, Guo et al. (2016) found that the range of *A. dichotomum* is primarily determined by temperature. Past studies further highlight the importance of temperature (Foden 2002; Foden et al. 2007) in determining the range of *A. dichotomum*.

2. Material and methods

2.1 *Study species*

A. dichotomum (Aspholedaceae) is an iconic tree Aloe of the Succulent Karoo and surrounding arid regions (Jacobsen 1960). This arborescent stem- and leaf-succulent grows to 10m tall in scattered to dense stands (sometimes referred to as "forests"), in areas that rarely support trees (Foden et al. 2002). It has been suggested that *A. dichotomum* is a keystone species in these areas, acting as vantage points for raptors, nesting spots for sociable weavers, as well as providing a critical food source to sunbirds in some regions (Midgley et al. 1997; van der Merwe & Geldenhuys 2017). *A. dichotomum* individuals grow on many inselbergs in the arid Succulent- and Nama-Karoo regions of South Africa (Burke 2004; van der Merwe & Geldenhuys 2017). Their distribution stretches from the Brandberg in the arid southern parts of Namibia, southwards into South Africa's Namaqualand and Bushmanland (van Wyk & Smith 2014; Guo et al. 2016). The growth pattern of these trees is characterised by a relatively high growth rate in the first 50 years of life, followed by a strong decrease further on (Kaleme 2003). It has been suggested that the observed growth rate and the species' physiological performance is influenced by climate, disease, habitat substrate, altitude and aspect (Kaleme 2003).

A. dichotomum is in all likelihood an obligate CAM-photosynthetic plant, based on its taxonomic classification and growth form. In obligate CAM plants the initial fixation of carbon dioxide (CO_2) into malic acid occurs during the cooler night-time hours – the dark period – thus lowering tissue acidity, and subsequent incorporation of this CO_2 via the C3 pathway occurs during the day – the light period (Yamori et al. 2014; Taiz et al. 2015). The initial fixation of CO_2 is mediated by phosphoenolpyruvate carboxylase (PEPC) and the CO_2 is converted to oxaloacetate (Yamori et al. 2014; Taiz et al. 2015), and then converted to malate by NAD(P)-malate dehydrogenase (MDH) using nicotinamide adenine dinucleotide (NADH) as a source of energy (Yamori et al. 2014; Taiz et al. 2015). The malate is then stored in the vacuole of the mesophyll tissue (Kluge et al. 1979; Yamori et al. 2014; Taiz et al. 2015). The anatomy of the leaves consists of a sturdy outer epidermis, underneath which lies the mesophyll tissue where photosynthesis takes place

(Kluge et al. 1979). The water storage tissue is located at the centre of the leaves (Kluge et al. 1979).

2.2 *Study site*

Data were collected at two sites, Gannabos and Keikamspoort (Fig. 1). Gannabos is located approximately 30km north-east of Nieuwoudtville in the Northern Cape of South Africa and is situated in the winter-rainfall zone (WRZ) Succulent Karoo (Mucina et al. 2006; van der Merwe & Geldenhuys 2017). This is the most southerly population of *A. dichotomum* (Fig. 1). Keikamspoort is located approximately 30km south-east of Prieska, also in the Northern Cape. This site is further east than Gannabos and is in the Nama-Karoo – part of the summer rainfall zone (SRZ) of South Africa (Mucina et al. 2006; van der Merwe & Geldenhuys 2017). This is the most south-easterly population of *A. dichotomum* (Fig. 1). Keikamspoort is considerably higher above sea level than Gannabos (Fig. 1).

The WRZ has lower inter-annual rainfall variability than the SRZ, with predictable rainfall in the winter months and sporadic rainfall in the summer months (Kaleme 2003). The SRZ on the other hand rarely has rainfall during the winter months, with much more variable rainfall in the late summer months (Kaleme 2003; Mucina et al. 2006). These differing rainfall patterns may have a significant effect on the physiological functioning of *A. dichotomum* individuals in the field and may therefore affect recruitment of juveniles (Kaleme 2003). Both sites have thriving populations of *A. dichotomum* with a large cohort of juveniles (<2 m) and young adults (<50 years), suggesting that the populations are young and expanding (Foden personal observation; Hoffman et al. 2010; van der Merwe & Geldenhuys 2017).

Data were collected specifically at 14 microsites across these two sites to further address the local-scale hypotheses. Microsites were stratified by aspect and elevation and were predominantly situated on one chosen inselberg at each site (Fig. 1). There were five microsites at Gannabos. Four of the microsites were situated at the top of the north-, south-, east- and west-

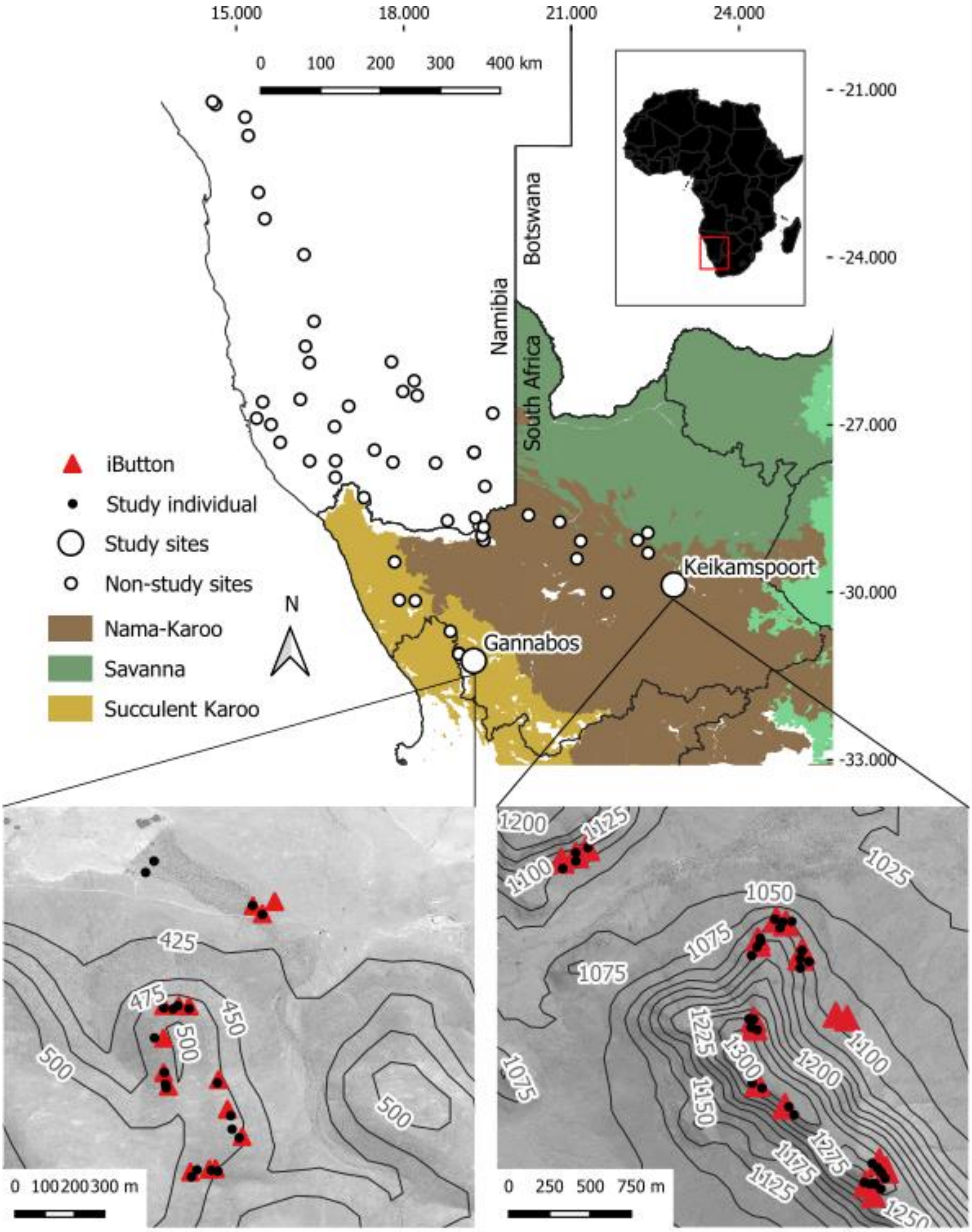

Figure 1. Map of the range of *Aloidendron dichotomum* and the sites used in this study. *A. dichotomum* populations are indicated with black outlined white circles. The two populations used in this study are indicated by larger circles. Longitude and latitude included along the top and right-hand side, respectively, of the full range map. Google satellite imagery and contour lines included for the site maps. Black circles in the site maps indicate the trees sampled during this study. Red triangles indicate locations of iButtons.

facing slopes on the inselberg, and the fifth was situated on the flats below the north-facing slope. There was *ca.* 100m difference in elevation between the flats and the other four aspects (Fig. 1).

There were nine microsites at Keikamspoort. Four of the microsites were situated at the top of the chosen inselberg on the north-, south-, east- and west-facing slopes – hereafter referred to as the “high elevation microsites”. Four were situated at the bottom of the inselberg on the north-, south-, east- and west-facing slopes – hereafter referred to as the “low elevation microsites”. The low elevation south-facing microsite was on a neighbouring hillside as the low elevation south-facing slope of the chosen inselberg was too far a distance to access within the sampling periods (Fig. 1). The last microsite was situated on the flats at the base of the east-facing slope. There was *ca.* 200m difference in elevation between the low elevation microsites, as well as those on the flats, and the high elevation microsites (Fig. 1). Physiological data were not collected for the flats in Keikamspoort as there were too few individuals scattered too widely to sample replicates effectively.

2.3 *Field data collection*

2.3.1 *Plant performance data*

2.3.1.1 *Nocturnal carbon gain*

As a powerful indication of physiological performance, the difference in pH before and after a given dark period (the nocturnal change in pH; ∆pH) of *A. dichotomum* individuals was measured. ∆pH indicates total nocturnal CO_2 gain as an integrated measurement for photosynthetic performance. The night-time process of storing CO_2 as malic acid substantially increases the overall acidity of the mesophyll layer. ∆pH, therefore, gives a measurement of the total CO_2 gain during that dark period (Flexas et al. 2012; Taiz et al. 2015).

I measured ∆pH of four plants within a 10 m radius of at least one of the temperature loggers (detailed in the *temperature data* section below) at the five microsites in Gannabos and the four high elevation microsites in Keikamspoort. The low elevation trees in Keikamspoort could not be sampled for pH change due to time and logistical constraints. However, the physiological performance of trees in the low elevation microsites was assessed using a complementary technique detailed in the *PEPC activity* section below. Nocturnal change in pH was therefore measured for 20 trees in Gannabos – north, south, east, west and flats – and 16 in Keikamspoort – north high, south high, east high and west high. None of the selected plants exceeded the rapid growth phase of under 50 years old, based on their heights (Kaleme 2003).

I measured the pH of the mesophyll layer of the plants using a micro pH sensor attached to a Mettler Toledo Seven2Go Portable pH Metre. I inserted the pH sensor at an angle of ~45 degrees into the leaf through a hole that I made immediately previously with a sharp needle. This was repeated before and after the dark period on different leaves for three consecutive days at each site. A two-point calibration was done on the pH sensor at the beginning of every measurement cycle. The sensor was calibrated to a pH of 4 and 7 using two buffer solutions and checked before and during the measurement process to allow *post hoc* pH adjustment to account for sensor drift. In order to correct the pH values, I fitted a straight line to the actual and measured pH of both buffer solutions for every measurement cycle and used this to correct the measured pH values *post hoc*.

2.3.1.2 *PEPC activity*

I collected leaf cuttings for PEPC assays to quantify the activity of PEPC through the *in vitro* consumption of NADH (Tovar-méndez et al. 2000). Leaf cuttings of approximately 2 mm thick were taken across the width of a single leaf during a single dark period at each site. Cuttings were taken at the beginning of the dark period (~7pm) and then again from the same leaf at the end of the dark period (~7am the following morning). Thereafter, they were stored in dry ice while in the field and later stored in a -22°C portable freezer containing dry ice. On return from the field, the samples were moved to a -80°C freezer at Stellenbosch University, where they were kept for analysis.

As with the pH measurements, leaf cuttings were taken from four trees at each microsite in Gannabos and four trees from the low elevation microsites in Keikamspoort. This time leaf cuttings were not taken at the top of the slopes in Keikamspoort due to logistical constraints. Leaf cuttings were therefore taken from 20 trees in Gannabos – north, south, east, west and flats – and 16 in Keikamspoort – north low, south low, east low and west low.

2.3.2 *Temperature data*

Temperature data were collected using 33 Maxim Thermochron Hi Res iButtons (DS1922L) installed across the 14 microsites. The iButtons recorded local air temperature every 15 minutes from 20 October to 17 November 2018. The iButtons were housed in Styrofoam cases used as radiation shields, with the measuring surface permanently shaded but exposed to the air. Cases were fastened to 1 m steel rods that were erected in the field. All the iButtons were mounted at a height of 70 cm above the ground surface, a height selected to avoid steep temperature gradients that may occur below 0.5m above ground level (Nobel 1984).

Fifteen iButtons were installed at Gannabos across the five microsites (Fig. 1). Three were installed on each aspect – north, south, east and west, and on the flats. Eighteen iButtons were installed at Keikamspoort across the nine microsites (Fig. 1). Two were installed at each aspect and elevation – north high and low, south high and low, east high and low, west high and low and two on the flats.

2.4. Data analysis

All data were analysed in R v. 3.5.2 (R Core Team 2018) and all figures were constructed using the GGplot2 package (Wickham 2016).

2.4.1. *Plant performance*

2.4.1.1. *Nocturnal carbon gain*

Generalised linear models (GiLM; Gaussian-identity distribution-link) were used to identify the key variables that determine nocturnal carbon gain of *A. dichotomum* individuals. I initially fitted multiple candidate models using combinations of the following predictor variables: site (Gannabos or Keikamspoort), aspect, elevation, the initial pH as recorded at the beginning of the dark period, as well as the interaction between site and aspect. In addition to these explanatory variables, I included two temperature variables – the total number of hours in the day for optimal carbon gain and the total number of hours at night for optimal carbon gain of *A. dichotomum* – these will be further explained in the *temperature data analysis* section below. There was a total of nine candidate models.

I then performed model selection using the *aictab* function in the AICcmodavg package (Mazerolle 2019) on the nine candidate models based on the second-order Akaike's information criterion (AIC_c). AIC_c was used as it accounts for over-parameterisation that occurs when using AIC values on a small data set (Burnham & Anderson 2002). Burnham & Anderson (2002) suggest that AIC_c be used if the ratio of the sample size (n) to the number of estimable parameters in the model (K) is smaller than 40. This was the case for this analysis.

The GiLMs with the lowest AIC_c values that did not differ from each other by more than 2 AIC_c values were assumed to have substantial support (Burnham & Anderson 2002). These models were then averaged using the *model.avg* function in the MuMIn package (Barton 2018). Models were averaged because it is assumed that they vary according to the specific data set used in their construction and therefore inference is improved when using all the models rather than one alone (Burnham & Anderson 2002). When interpreting the coefficients of the averaged models, the *full average* was used. This method substitutes a zero for any parameter values that do not appear in the respective models (Burnham & Anderson 2002). This method of parameter shrinkage removes a large amount of model selection bias (Burnham & Anderson 2002). Residual plots were used to assess whether the assumptions of the model were met. The assumptions were met, with the choice of model and distribution being suitable for the data.

2.4.1.2. *PEPC Activity*

The frozen leaf cuttings were homogenised in liquid nitrogen using a pestle and mortar. Proteins from the leaves were then extracted according to Ocaña et al. (1996), altered to a ratio of 0.250 g of ground tissue to 1 ml of extraction cocktail consisting of 100 nM Tris-HCl (pH 7.8), 1 nM EDTA, 5 nM DDT dissolved into 500 µl water, 20% (v/v) ethylene glycol, 2% (m/v) insoluble

PVPP, and one Complete Protease Inhibitor Cocktail tablet (Roche Diagnostics, Randburg, South Africa) per 50 ml buffer solution. I then clarified the homogenate by 8-minute centrifugation (up to 3 500g) at 4°C. The supernatant was then transferred to a second Eppendorf and was further clarified by 40-minute centrifugation (up to 15 000g).

Immediately thereafter the PEPC assay was initiated according to the methods used by Stevens et al. (2019) by adding 30 µl crude extract in a total volume of 250 µl per well. The enzyme assay mixture consisted of 100 mM Tris (pH 8.5), 5 mM $MgCl_2$, 5 mM $NaHCO_3$, 4 mM PEP, 0.20 mM NADH, and 5 units of MDH (Ocaña et al. 1996). Blanks contained the reaction medium but lacked PEP (Hurley et al. 2010; Stevens et al. 2019). Each sample was replicated three times and each plate had three blanks.

NADH oxidation (i.e. loss of NADH – a direct indication of PEPC activity) was then measured spectrophotometrically at 340 nm at 25°C for 5 min. Thereafter, rate of loss of NADH was calculated for each sample. Because the loss of NADH was linear for most samples, the slope of the line was used as the rate of loss of NADH. GiLMs (Gaussian-identity distribution-link) were then used to determine the key predictors of PEPC activity in the *A. dichotomum* individuals at each microsite.

I constructed six candidate models using combinations of the following predictor variables: site (Gannabos or Keikamspoort), aspect, elevation, and time when the cutting was taken (PM or AM), as well as the mean dark and light period temperatures for the given day. Model selection was performed in the same way as described in the *nocturnal carbon gain* section above. AIC_c was used again as the ratio of n/K in this analysis was less than 40. Using the car package (Fox & Weisberg 2011), a post-hoc analysis of variance (ANOVA) was run to determine which variables significantly explained the variance in the PEPC activity. Using the *r.squaredLR* function I obtained the pseudo-R^2 value for the best-fit model. Residual plots were used to assess whether the assumptions of the model were met. The assumptions were met, with the choice of model and distribution being suitable for the data.

2.4.2. *Temperature data analysis*

Once the temperature data had been downloaded from the iButtons, I removed all half days of recording that were a result of start- and end-days. Data from two sensors required further cleaning, as one of the iButtons was found to have been dislodged, an event that was detectable in the data, allowing data removal after the event. A second sensor was removed entirely from the dataset as it had abnormally large fluctuations in temperature and flat-lined for a few days and was therefore assumed to have been malfunctioning. A third sensor did not read in the iButton reader and the data could therefore not be downloaded. There was therefore no replication for temperatures at the low elevation north-facing, low elevation west-facing, and high elevation south-facing slopes in Keikamspoort.

2.4.2.1. *Site-level differences*

In order to determine if the two sites, Gannabos and Keikamspoort, followed the same regional pattern of ambient temperature, I correlated the mean hourly temperatures at the two sites using the Pearson's product moment correlation coefficient (referred to as Pearson's correlation). Pearson's correlation method gives a measurement of the direction and strength of association between the temperatures at the two sites and provides a t-statistic to derive the significance of the correlation coefficient, *r*. For this I offset the times in Keikamspoort by 10 minutes to account for the earlier sunrise and sunset in comparison to Gannabos.

2.4.2.2. *Optimal carbon gain hours (OCGH)*

I analysed the temperature data to determine the number of hours available to the *A. dichotomum* individuals for optimal carbon gain during the recording period at each microsite (hereafter referred to as OCGH). Using a dataset collected previously (Grey 2017), I constructed a predictive model for the optimal temperature for carbon gain for quiver trees. For this I used ΔpH data for 11 nights collected from juveniles grown under different warming and cooling conditions in a semi-controlled greenhouse experiment. Temperature data from the greenhouse were used to calculate the mean daytime temperature (light period; 7am-7pm) and mean night time temperature (dark period; 7pm-7am) for each ΔpH value in the data set. The mean daytime and night time temperatures and their corresponding ∆pH values were fit with second-order polynomials using the *polynom* function in R (Venables et al. 2016) – one for ΔpH against mean daytime temperature and the other for ΔpH against mean night time temperature. The equations of the two second-order polynomials were then used to predict the maximum nocturnal carbon gain of the plants, as well as the daytime and night time temperatures at which this maximum carbon gain occurs. Thereafter the ranges for the mean daytime and night time temperatures associated with 90% of optimal nocturnal carbon gain for each predictive model were obtained. The temperature ranges at which optimal carbon gain (within 10% of maximum carbon gain) occurred in the *A. dichotomum* individuals grown in the semi-controlled greenhouse conditions were 14.07-24.93°C and 22.99-33.53°C for the dark and light periods, respectively.

I then used the temperature data from the current study to determine the number of hours at each microsite that fell within these two temperature ranges. Once I had calculated the total number of hours within each temperature range for each microsite, I ran a Chi-squared goodness-of-fit test to determine if there was a significant difference in the number of optimal carbon gain hours per microsite. The null hypothesis for this analysis assumed equal frequencies of OCGH at each microsite. Thereafter I ran a post-hoc pairwise comparison on the data using the RVAideMemoire package (Hervé 2019) to see which microsites were significantly different from the others. As mentioned above, the total number of OCGH for the day and night were used in the GiLM for nocturnal carbon gain.

2.4.2.3. *Microsite-level differences*

In addition to characterising the microsites by the number of hours within the day and night temperature ranges that should allow for optimal carbon gain, I also characterised them using their mean hourly temperatures, as well as their daily maximum and minimum temperatures for the study period.

Generalised linear mixed-effects models (GLMMs) were used to identify the major determinants of these three temperature variables at Gannabos and Keikamspoort. I constructed five candidate models using combinations of the following predictor variables: site (Gannabos or

Keikamspoort), aspect, elevation, and time when the recording was taken, as well as the interaction between aspect and site. Time was included in the model as a random effect as it would have had a strong effect on the minimum and maximum temperatures, but it was not a variable of interest for this study. All other variables were included as fixed effects. All GLMMs were fit using the *lmer* function in the lme4 package (Bates et al. 2015) with Gaussian-identity distribution-links.

Model selection was performed in the same way as described in the *nocturnal carbon gain* section above. AIC_c was used again. No model averaging was needed for any of the mixed-effect models as all best-fit models were more than 2 AIC_c values smaller than the rest of the candidate models. Using the *r.squaredLR* function I obtained the pseudo-R^2 value for the best-fit models to assess how much of the variability in the data was explained by each model. The effects package (Fox & Weisberg 2018) was used to plot and make inferences for the significant interactions. Finally, I used the *emmeans* function in the emmeans package (Lenth 2019), which uses the Tukey post-hoc method, to obtain pairwise comparisons for the aspects at each site. Using the car package (Fox & Weisberg 2011), post-hoc ANOVAs were run to determine which variables significantly explained the variance in the three temperature variables. Residual plots were used for all models to assess whether the assumptions were met. The assumptions were met, with the choice of models and distributions being suitable for the data.

3. Results

3.1. *Plant performance*

3.1.1. *Nocturnal carbon gain*

Three models (Table 1) did not differ from one another by more than 2 AIC_c values and were therefore considered to have substantial support (Burnham & Anderson 2002).

Based on inferences across the three best-fit models, site was a significant predictor of nocturnal carbon gain (Table 2). Overall, nocturnal carbon gain was lower at Keikamspoort than at Gannabos (Table 2). The initial pH at the beginning of the dark period was a significant predictor

Table 1. The three models that best predict (with $\Delta AIC_c < 2$) the nocturnal carbon gain (measured as nocturnal change in pH; ΔpH) of *Aloidendron dichotomum* individuals at two sites in the Northern Cape of South Africa. K represents the number of fitted parameters, ΔAIC_c is the difference between the AICc value of the best model and the AICc value for each of the other models and the weight is the probability that the given model is the best approximating model (Symonds & Moussalli 2010; Burnham & Anderson 2002).

Predictor variables	K	AICc	ΔAICc	AICc Weight
site+basevalue+aspect+elevation+nighthours+dayhours	11	-11.325	0.000	0.491
site+basevalue+aspect+elevation+nighthours+dayhours+site:aspect	12	-9.785	1.541	0.227
site+basevalue+aspect+elevation+site:aspect	12	-9.785	1.541	0.227

Table 2. Summary of model averaged coefficients across the three best-fit Generalised Linear Models of nocturnal carbon gain (measured as nocturnal change in pH; ΔpH) of *Aloidendron dichotomum* individuals in two populations in the Northern Cape of South Africa. Parameter estimates, Z-values and p-values are given. Significance given as $^*p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$. The reference categories were Gannabos for site, east for aspect, day 1 for day, and the interaction between east and Keikamspoort.

Explanatory variable	Estimate	z-value	p-value	
(Intercept)	6.991	2.774	0.006	**
Site – Keikamspoort	-15.579	2.768	0.006	**
Initial pH value	0.475	21.429	< 2e-16	***
Aspect – Flat	1.679	3.034	0.002	**
Aspect – North	1.001	1.262	0.207	
Aspect – South	0.609	1.856	0.064	.
Aspect – West	0.470	1.377	0.168	
Aspect – North: Site - Keikamspoort	0.267	0.695	0.487	
Aspect – South: Site - Keikamspoort	-0.015	0.221	0.825	
Aspect – West: Site - Keikamspoort	-0.053	0.468	0.640	
Elevation	8.770	3.149	0.002	**
Night hours	1.343	1.310	0.190	
Day hours	0.499	1.321	0.186	

of nocturnal carbon gain, with a higher initial pH resulting in higher nocturnal carbon gain (Table 2). At Gannabos the nocturnal carbon gain on the flats was higher than that on the east-facing slopes (Table 2). Furthermore, the nocturnal carbon gain on the north-facing slopes was higher at both sites than on the east-facing slopes (Table 2). Elevation was also a significant predictor of nocturnal carbon gain, with carbon gain increasing with elevation (Table 2).

3.1.2. *PEPC activity*

The model with the lowest AIC_c value (lower than all the others by more than 2 AIC_c values) had a weight of 1.00 and therefore a probability of 1.00 of being the best-fit model of all five candidate models. It included site, aspect, elevation, the mean night temperature, the mean day temperature, the time of day and the tree from which the cutting was taken. This model had a pseudo-R^2 of 0.424 and therefore explained 42.4% of the variance in this data set.

Table 3. Results of the post-hoc analysis of variance for the generalised mixed-effects model weight for rate of loss of NADH of *Aloidendron dichotomum* individuals in two populations in the Northern Cape of South Africa. The Chi-squared value (X^2), degrees of freedom and p-value are given. Significance levels given as *p<0.05, **p<0.01 and ***p<0.001.

Predictor variable	X^2	Df	p-value	
Site	1.356	1	0.244	
Aspect	42.190	4	0.000	***
Elevation	4.089	1	0.043	*
Mean night temperature	5.212	1	0.022	*
Mean daytime temperature	0.466	1	0.495	
Time	55.880	1	0.000	***

There was no significant difference in the rate of loss of NADH between the two sites (Table 3 & 4). Mean night time temperature was a significant predictor of the rate of loss of NADH, with higher night time temperatures resulting in a greater rate of NADH loss (Table 3 & 4). Because loss of NADH is a negative linear relationship, a greater rate of loss corresponds with lower PEPC activity. Therefore, PEPC activity is higher when the mean night time temperatures are lower. The time of day at which the cuttings were taken was also a significant predictor of the rate of loss of NADH, with the rate being greater in the evenings (Table 3 & 4). Therefore, the PEPC activity in the evenings was lower than in the mornings.

Elevation was also a significant predictor of the rate of loss of NADH, with higher elevations being associated with higher PEPC activity (Table 3 & 4). Overall, aspect explained significant variance in the rate of loss of NADH (Table 3). Being positioned on the north-facing slopes or the flats was a significant predictor of the rate of NADH loss, with the rate being lower in both positions than the east-facing slopes (Table 4). Therefore, PEPC activity was higher on the north-facing slopes and flats than the east-facing slopes.

Table 4. Summary of the generalised linear model with the highest AICc weight for rate of loss of NADH of *Aloidendron dichotomum* individuals in two populations in the Northern Cape of South Africa. Parameter estimate, t-values and p-values are given. Significance given as *p<0.05, **p<0.01 and ***p<0.001.

Explanatory variable	Estimate	t-value	p-value	
(Intercept)	-0.037	-2.174	0.031	*
Site – Keikamspoort	0.024	1.164	0.246	
Aspect – Flat	-0.008	-3.706	0.000	***
Aspect – North	-0.001	-2.520	0.013	*
Aspect – South	0.000	0.091	0.928	
Aspect – West	0.000	0.611	0.542	
Elevation	-0.018	-2.022	0.045	*
Mean night temperature	0.002	2.283	0.024	*
Mean daytime temperature	0.000	-0.683	0.496	
Time – PM	0.002	7.475	0.000	***

3.2. *Temperatures*

3.2.1 *Site-level differences*

Mean hourly temperatures were significantly positively related, with a Pearson's correlation coefficient (*r*) of 0.806 (t_{670} = 35.228, p < 0.0001; Fig. 2). However, Gannabos was cooler than Keikamspoort, with lower hourly (Fig. 4), maximum (Fig. 5) and minimum (Fig. 6) temperatures.

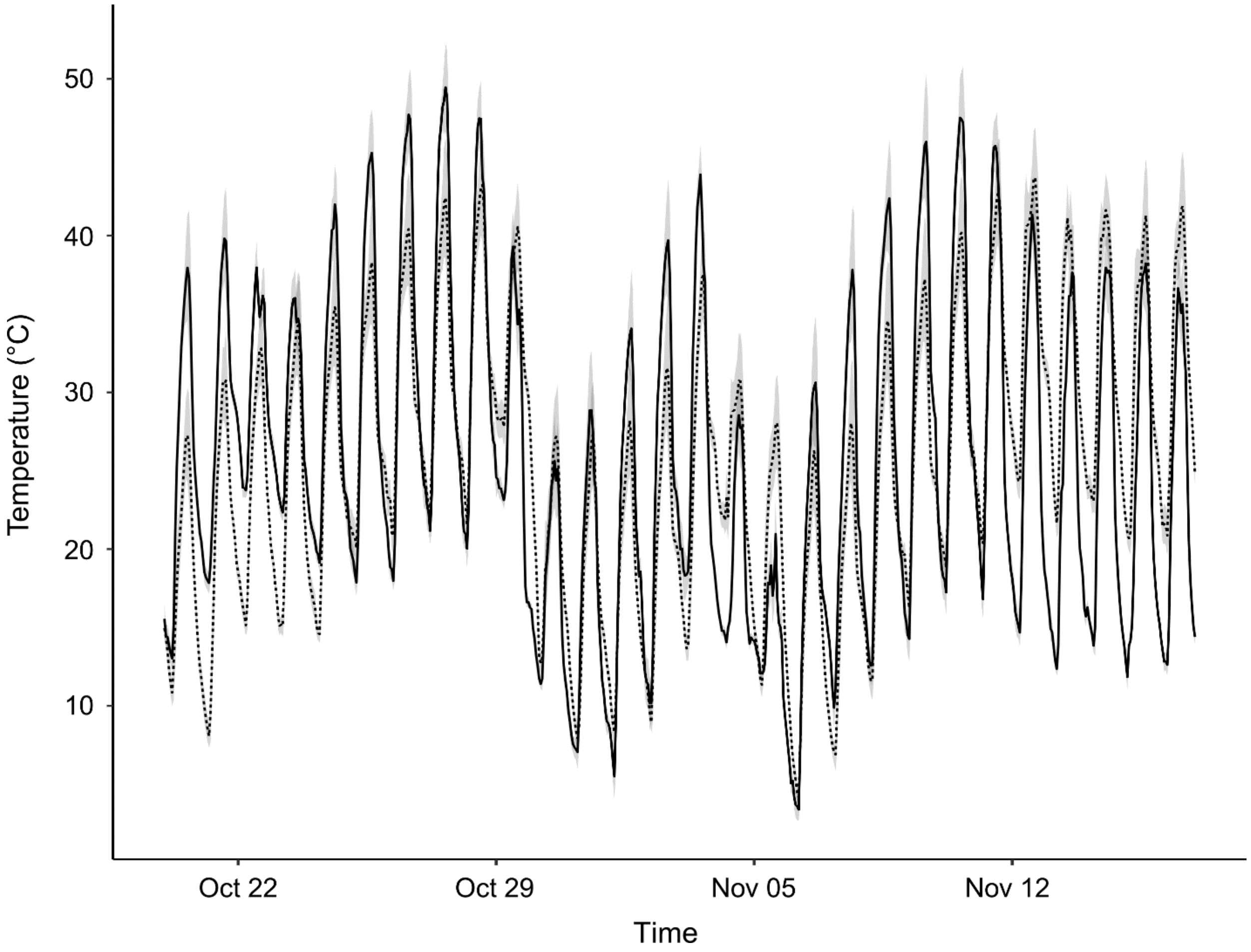

Figure 2. Mean hourly temperatures (°C) for the Gannabos (solid line) and Keikamspoort (dotted line) populations of Aloidendron dichotomum for the period of 20 October to 17 November 2018. Error ribbons correspond to the standard errors for the data

3.2.2. *Optimal carbon gain hours*

The OCGH during the period 20 October to 17 November are shown in Figure 3. The OCGH did not differ at any of the microsites (Fig. 3a). In general, the OCGH were slightly higher for Keikamspoort than Gannabos (Fig. 3b). The OCGH did not differ between the microsites at Gannabos or Keikamspoort (Fig. 3b). Both the high and low elevation east-facing slopes at Keikamspoort had significantly higher OCGH than the south- and west-facing slopes at Gannabos, as well as the flats at Gannabos (Fig. 3b). Similarly, all microsites at Keikamspoort apart from the low elevation south-facing slopes and the flats, had significantly higher OCGH than the south-facing slopes in Gannabos (Fig. 3b).

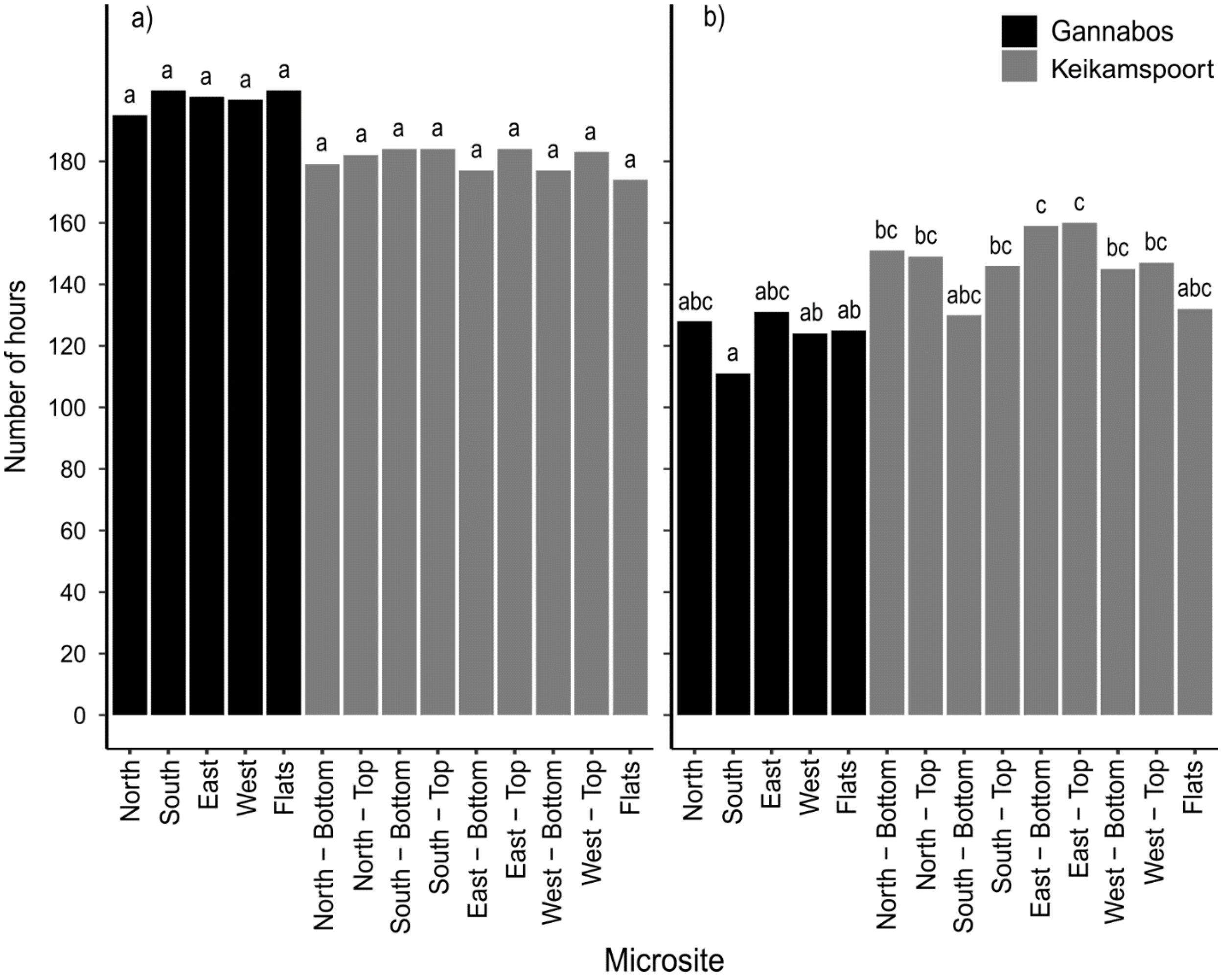

Figure 3. The total number of optimal carbon gain hours (OCGH) during the (A) dark (7pm-7am) and (B) light (7am-7pm) periods at 14 microsites in two populations of *Aloidendron dichotomum* - Gannabos (black) and Keikamspoort (grey) - in the Northern Cape of South Africa during the study period of 20 October to 17 November 2018. OCGH are defined as those that fall within the temperature ranges of (A) 14.07-24.93°C and (B) 22.99-33.53°C allowing for optimal nocturnal carbon gain of A. dichotomum. Optimal nocturnal carbon gain is defined here as carbon gain within 10% of the maximum carbon gain obtained from greenhouse experiments. Microsites with the same letters were significantly the same ($\alpha=0.05$), for which results were obtained from chi-squared goodness-of-fit analyses for both the dark ($X^2=7.663$, df=13, p=0.865) and light ($X^2=19.862$, df=13, p=0.099) period. Letters are only applicable within respective panels.

3.2.3. *Microsite temperatures*

For each temperature variable there was one best supported model that differed from all the others by more than 2 AIC_c points (Burnham & Anderson 2002). For the mean hourly temperatures (Table 5a & 6a) and the daily maximum temperatures (Table 5b & 6b) the best-fit models included the same predictor variables – site, aspect, elevation and the interaction between aspect and site. For the daily minimum temperatures, however, elevation was not included in the best fit model with only site, aspect and the interaction between aspect and site being predictor variables (Table 5c & 6c).

Table 5. Results of the post-hoc analysis of variance for the generalised linear mixed-effects models for the (a) mean hourly temperatures, (b) daily maximum temperatures and (c) daily minimum temperatures in two populations of *Aloidendron dichotomum* in the Northern Cape of South Africa. The Chi-squared value (X^2), degrees of freedom and p-value are given. Significance levels given as *p<0.05, **p<0.01 and ***p<0.001.

Response variable	Predictor variable	X^2	Df	p-value	
a) Mean hourly temperature	Site	231.480	1	0.000	***
	Aspect	43.405	4	0.000	***
	Aspect:Site	39.227	4	0.000	***
	Elevation	247.009	1	0.000	***
b) Daily maximum temperature	Site	16.757	1	0.000	***
	Aspect	62.599	4	0.000	***
	Aspect:Site	76.129	4	0.000	***
	Elevation	26.440	1	0.000	***
c) Daily minimum temperature	Site	53.736	1	0.000	***
	Aspect	13.579	4	0.009	**
	Aspect:Site	6.417	4	0.170	

For the mean hourly temperatures, the best-fit model had an AICc weight of 0.999 and therefore had a probability of 0.999 of being the best-fit model of the five candidate models. This model had a pseudo-R^2 of 0.820 and therefore explained 82.0% of the variance in this data set. For the daily maximum temperatures, the best-fit model had a probability 0.998 of being the best-fit model of the five candidate models, explaining 71.2% of the variance in this data set. The best-fit model for the daily minimum temperatures had a probability of 0.742 of being the best-fit model of the five candidate models, explaining 72.0% of the variance in this data set.

In all three models, site was a significant predictor variable (Table 5), with Gannabos having lower temperatures than Keikamspoort (Table 6; Figs 4-6). For the mean hourly and daily maximum temperatures, elevation was also a significant predictor variable (Table 5a & b), with lower mean and maximum temperatures at higher elevations (Table 6a & b). For all three temperature variables, aspect explained significant variance in the data (Table 5). For both the mean hourly and maximum temperatures, the interaction between site and aspect was significant

(Table 5a & b), indicating that the effect of aspect on these two temperature variables was different for the two sites.

Table 6. Summary of the generalised linear mixed effects models for the (a) mean hourly temperatures, (b) daily maximum temperatures and (c) daily minimum temperatures in two populations of *Aloidendron dichotomum* in the Northern Cape of South Africa. Parameter estimates, t-values and p-values are given. Significance given as *p<0.05, **p<0.01 and ***p<0.001. The reference categories were Gannabos for site, east for aspect and the interaction between east and Keikamspoort.

Response variable	Predictor variable	Estimate	t-value	p-value	
a) Mean hourly temperature	(Intercept)	29.659	68.137	0.000	***
	Site – Keikamspoort	5.565	13.898	0.000	***
	Aspect – Flat	-0.904	-4.806	0.000	***
	Aspect – North	0.607	3.292	0.001	**
	Aspect – South	0.077	0.416	0.678	
	Aspect – West	-0.406	-2.201	0.028	*
	Aspect – Flat:Keikamspoort	0.808	3.294	0.001	**
	Aspect – North:Keikamspoort	-0.674	-2.980	0.003	**
	Aspect – South:Keikamspoort	0.164	0.725	0.469	
	Aspect – West:Keikamspoort	0.242	1.074	0.283	
	Elevation	-0.008	-15.717	0.000	***
b) Daily maximum temperature	(Intercept)	45.415	28.711	0.000	***
	Site – Keikamspoort	3.293	2.039	0.042	*
	Aspect – Flat	-2.378	-4.018	0.000	***
	Aspect – North	1.572	2.747	0.006	**
	Aspect – South	0.871	1.524	0.128	
	Aspect – West	-0.408	-0.713	0.476	
	Aspect – Flat:Keikamspoort	7.509	7.651	0.000	***
	Aspect – North:Keikamspoort	0.041	0.054	0.957	
	Aspect – South:Keikamspoort	2.640	3.360	0.001	***
	Aspect – West:Keikamspoort	0.346	0.441	0.660	
	Elevation	-0.011	-5.142	0.000	***
c) Daily minimum temperature	(Intercept)	14.170	15.115	0.000	***
	Site – Keikamspoort	1.373	3.556	0.000	***
	Aspect – Flat	-1.193	-2.890	0.004	**
	Aspect – North	0.167	0.404	0.686	
	Aspect – South	-0.940	-2.278	0.023	*
	Aspect – West	-0.662	-1.603	0.109	
	Aspect – Flat:Keikamspoort	1.351	1.911	0.056	
	Aspect – North:Keikamspoort	-0.175	-0.320	0.749	
	Aspect – South:Keikamspoort	0.734	1.298	0.194	
	Aspect – West:Keikamspoort	0.051	0.090	0.929	

The flats were significant predictors of all three temperature variables (Table 6) but because of the significant interaction between site and aspect for the flats (Table 6a & b), this effect can be interpreted differently for the different sites. When looking only at Gannabos, the flats always had lower temperatures than the north-facing slopes (Figs 4a, 5a & 6a). Furthermore, the flats had lower mean hourly and daily maximum temperatures than the east- and south-facing slopes (Table 6a & b; Figs 4a & 5a). The flats also had lower daily maximum temperatures than the west-facing slopes (Fig. 5a). When looking only at the Keikamspoort temperatures, the flats had higher maximum temperatures than the east-, north-, and west-facing aspects (Fig. 5b).

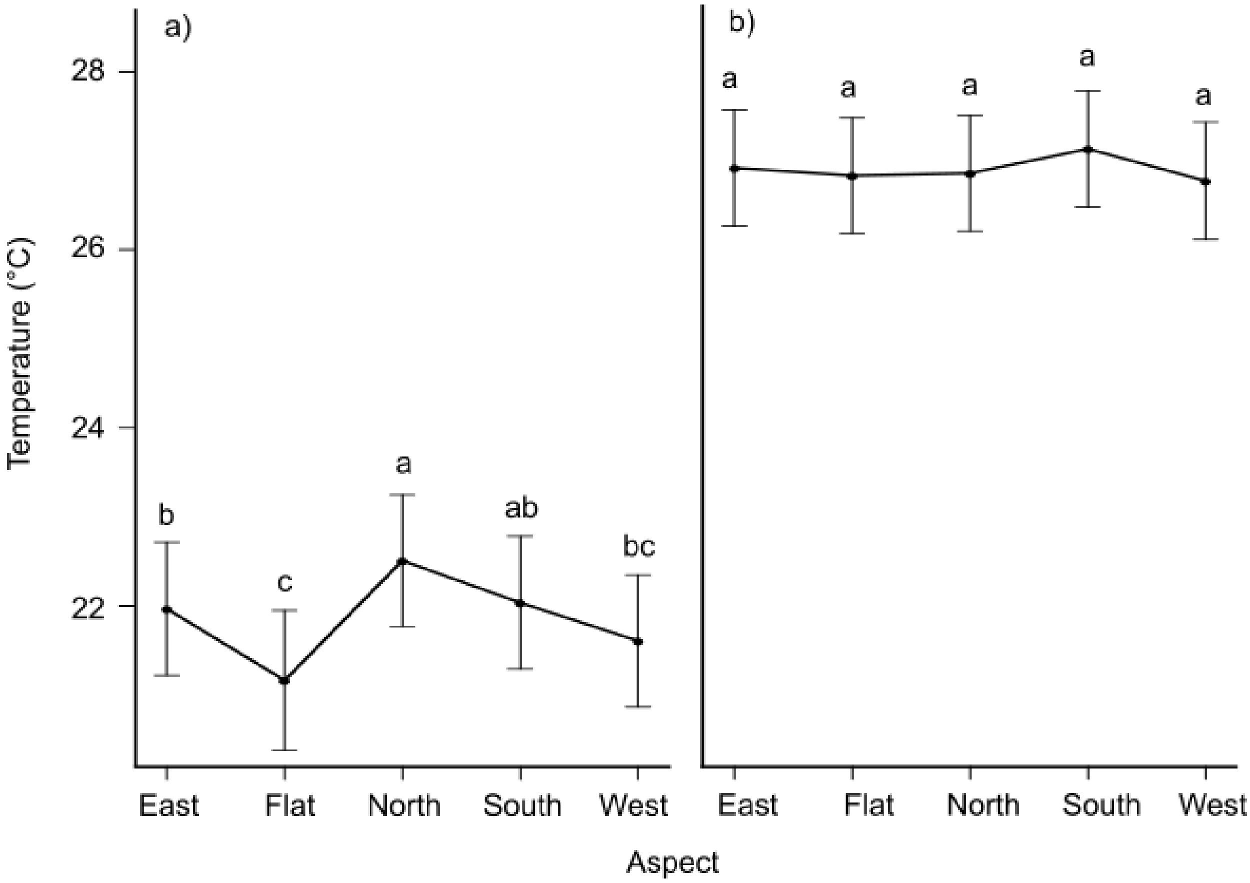

Figure 4. The mean hourly temperatures for (A) Gannabos and (B) Keikamspoort at different aspects in two populations of *Aloidendron dichotomum* in the Northern Cape of South Africa. Temperatures were recorded from 20 October to 17 November 2018. Plot represents output for the interaction between site and aspect from the generalised mixed-effects model for mean hourly temperature. Standard errors shown. For panels A and B separately, aspects with the same letters have temperatures that are not significantly different from each other ($\alpha = 0.05$).

The north-facing slopes were also a significant predictor of mean hourly (Table 6a) and daily maximum (Table 6b) temperatures. The interaction between the north-facing slopes and site was, however, only significant for the mean hourly temperatures (Table 6a). Therefore, the difference in mean hourly temperatures between the east-facing and north-facing slopes was different for each site. In Gannabos, the mean hourly temperatures were higher on the north-facing slopes than the east-facing slopes (Fig. 4a), while in Keikamspoort there was no difference in mean hourly temperature between these two aspects (Fig. 4b).

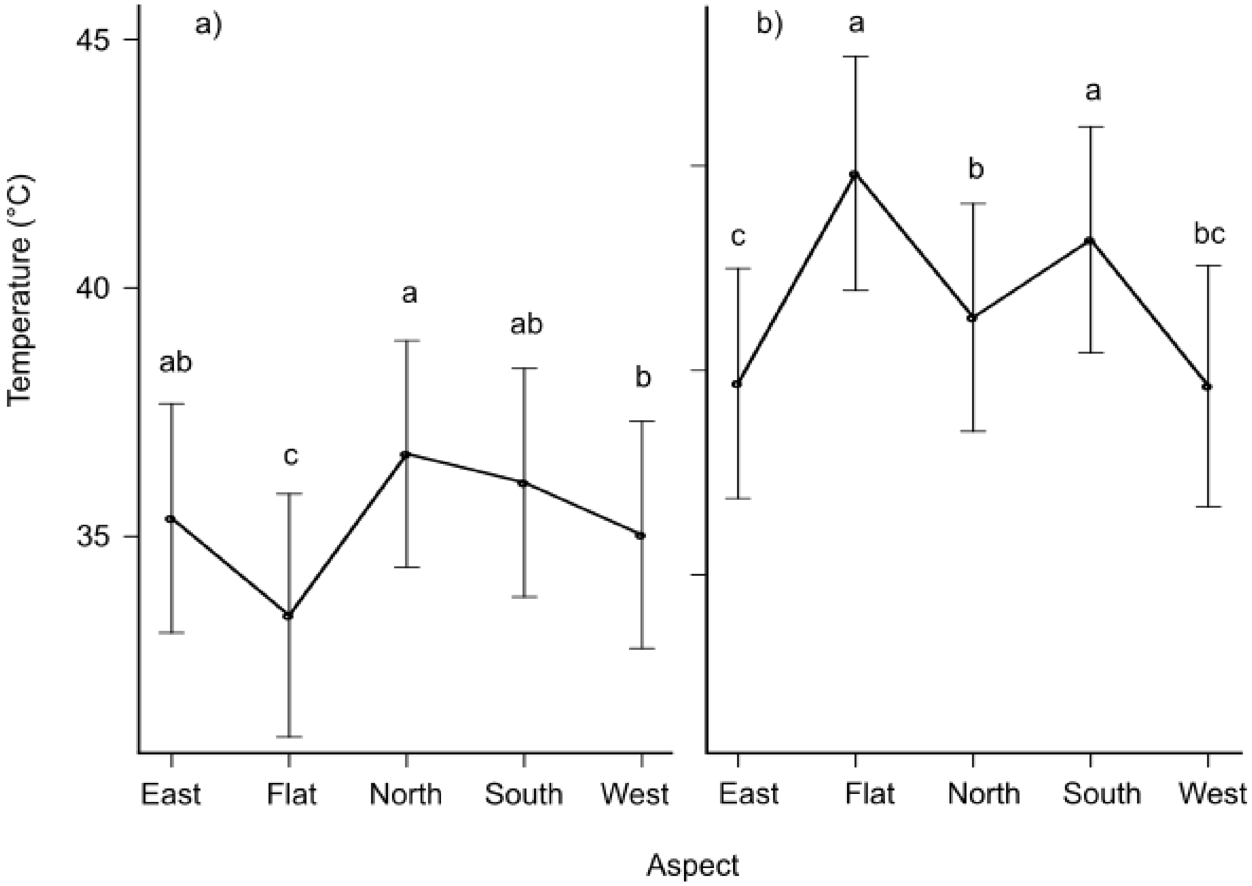

Figure 5. The daily maximum temperatures for (A) Gannabos and (B) Keikamspoort at different aspects in two populations of *Aloidendron dichotomum* in the Northern Cape of South Africa. Temperatures were recorded from 20 October to 17 November 2018. Plot represents output for the interaction between site and aspect from the generalised mixed-effects model for daily maximum temperatures. Standard errors shown. For panels A and B separately, aspects with the same letters have temperatures that are not significantly different from each other ($\alpha = 0.05$).

The west-facing slopes were a significant predictor of the mean hourly temperatures (Table 6a), however within each site the temperatures at this aspect did not differ from those on the east-facing slopes (Fig. 4). This significance was due to the model detecting differences in the mean hourly temperatures between the west-facing slopes at one site and the east-facing slopes at the other site – a comparison that I was not interested in for this study. In Gannabos, the west-facing slopes had lower mean hourly and daily maximum temperatures than the north-facing slopes (Figs 4a & 5a).

The south-facing slopes were a significant predictor of minimum temperatures (Table 6) but again this was a result of the model detecting differences between the south- and east-facing slopes between the two sites. In Keikamspoort, similarly to the flats, the daily maximum temperatures on the south-facing slopes were also higher than at the north-, east- and west-facing slopes were lower than the south-facing slopes and the flats (Table 5; Fig. 5b). Furthermore, the east-facing slopes also had lower daily maximum temperatures than the north-facing slopes (Table 5; Fig. 5b).

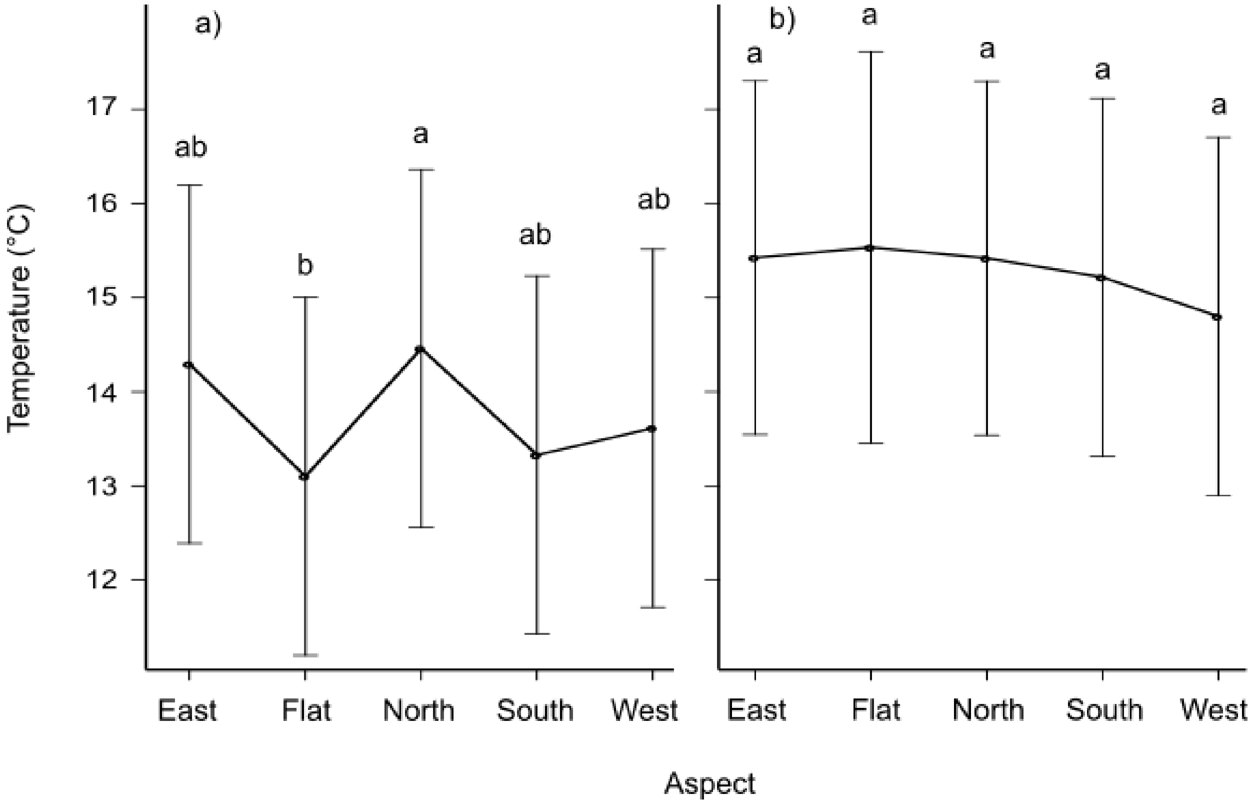

Figure 6. The daily minimum temperatures for (A) Gannabos and (B) Keikamspoort at different aspects in two populations of *Aloidendron dichotomum* in the Northern Cape of South Africa. Temperatures were recorded from 20 October to 17 November 2018. Plot represents output for the interaction between site and aspect from the generalised mixed-effects model for daily minimum temperatures. Standard errors shown. For panels A and B separately, aspects with the same letters have temperatures that are not significantly different from each other (α = 0.05).

The mean hourly and daily minimum temperatures at Keikamspoort did not differ between the different aspects (Figs 4b & 6b). Positioning on the flats was a predictor of the minimum temperatures experienced (Table 6) but this again was a result of between-site comparisons.

4. Discussion

Elucidating the factors that act to limit the geographic ranges of species is challenging, given that these may be manifold. Yet such an effort is needed if conservation efforts to understand and predict how populations and species of interest respond to anthropogenic climate change are to succeed. This study has attempted to further the understanding of the role of eco-physiological aspects in determining the range limits of *A. dichotomum*, but this effort is developed from a low knowledge base for this species.

This work has shown that *A. dichotomum* is indeed performing CAM photosynthesis under field conditions and that, as a result, it is subject to a daily photosynthetic capacity determined largely (but not exclusively) by acid accumulation. Further to this, my results strongly indicate that the species is performing CAM-photosynthesis at similar rates at both its southern (winter rainfall) and south-eastern (summer rainfall) range limits. This is supported firstly by the clear repeated occurrence of a nocturnal change in pH of the mesophyll layer in the leaves of the *in-situ* individuals at both sites, confirming a diurnal malate rhythm typical of CAM plants (Kluge et al. 1979). As a CAM-photosynthetic plant, *A. dichotomum* is expected to undertake carbon fixation and storage (as malic acid) during the cool night time hours (Yamori et al. 2014; Taiz et al. 2015). Secondly, the results of the PEPC assay indicated a clear diurnal cycle for PEPC activity; with NADH loss, and thus PEPC activity, being higher in the mornings than in the evenings. This substantial difference in PEPC activity during these indicative periods of the day is also a diagnostic characteristic of CAM plants (Flexas et al. 2012; Taiz et al. 2015), and together with diurnal acidity fluctuation suggests that this species is indeed CAM-photosynthetic.

A further finding in this study is the importance of the pH value at the beginning of the dark period in predicting the net nocturnal carbon gain of *A. dichotomum* individuals. This may indicate the presence of a maximum carbon storage capacity of *A. dichotomum*’s succulent leaves, and thus a maximum photosynthetic capacity of this species. It has also been shown that mesophyll factors limit the rate of nocturnal carbon gain in CAM-photosynthetic plants (Winter 1985). If there is a maximum storage capacity in the mesophyll, then increases in temperatures may differentially affect different life stages of this species. Under excessively cold conditions, the photosynthetic pathways of CAM plants are significantly slowed (Flexas et al. 2012). Warming at the cool range edge should relax the effects of unfavourable cold conditions and allow for increased carbon gain during the night and increased photosynthetic rates during the day. However, with a maximum storage capacity, juveniles would not be able to exploit these increased temperatures to the extent to which larger trees could. As a result, population growth of *A. dichotomum* at the cool range edge resulting from increases in temperatures may be age-dependent.

Further, the work identifies several factors relating to the temperature dependence of photosynthetic performance in *A. dichotomum* which may have relevance in explaining the recent shifts of this species into new microsites at the leading edge of its range. With respect to eco-physiological performance at the local scale, my results show firstly that the activity of PEPC is positively associated with mean night temperature. By contrast, I found that higher carbon gain was associated with the cooler site, namely Gannabos. At local scale, greater physiological performance was associated with both the warmer northern-facing slopes, as well as the cooler flats. Higher carbon gain was also associated with the cooler higher elevations. These findings are discussed in detail below.

Mean night time temperature was shown to be an important predictor of PEPC activity in *A. dichotomum*. This finding supports past evidence for the importance of night time temperatures for the net carbon gain, and thus photosynthesis, of CAM plants (Nobel & Hartsock 1978). Within the chosen two populations, warmer nights resulted in higher PEPC activity. These two range edge sites should be characterised by cooler temperatures than other sites found in the core areas of the range of *A. dichotomum* – temperatures to which this species is best adapted (Hughes 2000; Thomas et al. 2004; Jump & Peñuelas 2005; Chen et al. 2011). Because of the monotonic response of the carbon gain of CAM plants to temperature (Berry & Björkman 1980; Nobel 1984; Yamori et al. 2014), it would be expected that *A. dichotomum* would perform better when night time temperatures in these cooler range edge sites are closer to that of the core sites, as these would allow for relaxation of cold stress (Flexas et al. 2012).

At regional scale, this study found evidence for higher nocturnal carbon gain in Gannabos than Keikamspoort, supporting an *a priori* ecological or functional division between these populations (Jack et al. 2016). There was, however, no significant difference in the PEPC activity between the sites. This suggests that conditions during the study period at Gannabos may be more suitable for *A. dichotomum* than in Keikamspoort. Whether this difference can be attributed to differences in temperatures between these two sites was a further question of this study and is discussed below.

This study indicated that the measured air temperatures at Gannabos were cooler than those at Keikamspoort (Figs 4-6), and that these were significantly positively correlated between the two sites (Fig. 2). This suggests that they were exposed to a common weather system during the study period that affected diurnal temperature fluctuations at both sites in the same way. In addition to higher temperatures, Keikamspoort had more OCGH during the day (Fig. 3b). In keeping with the above assumption that *A. dichotomum* individuals would take advantage of higher temperatures at the cooler range edge, one would expect nocturnal carbon gain to be higher where temperatures are warmer.

However, during the study period, Keikamspoort experienced an extreme heat wave in the days directly before the nocturnal carbon gain measurements were taken. The data indicated that these high temperatures extended into the early hours of the night when carbon gain is typically highest for CAM plants (Taiz et al. 2015). As a result, the trees at Keikamspoort may have limited carbon gain during this time in order to conserve water (Foden et al. 2007; Flexas et al. 2012). The cooler temperatures experienced by the Gannabos population would have allowed for continued physiological functioning and thus higher performance of these trees. The finding that PEPC activity (for which data was collected before the heat wave) did not differ between the sites indicates that prior to the heat wave, the two sites had similar physiological performance. This further supports the suggestion that the heat wave had a severe effect on the physiological performance of the *A. dichotomum* individuals at Keikamspoort.

An alternative argument for the higher carbon gain at Gannabos is the effect of long-term rainfall patterns and rainfall extremes at these two sites (Kaleme 2003; Guo et al. 2016; Jack et al. 2016). It is a long-standing belief that water availability is the key predictor of plant performance in desert systems (Nobel 1984). The southern WRZ, in which the Gannabos population is found, has a less variable rainfall regime than the SRZ, in which the Keikamspoort population is found. On this basis, it has been suggested that WRZ and SRZ populations can be distinguished, with substantially higher recruitment and lower mortality in the WRZ than in the SRZ populations as a result of the contrasting climatic conditions (Kaleme 2003; Jack et al. 2016). Furthermore, although adult *A. dichotomum* individuals are well-adapted to survive long periods of drought, with shallow root systems and large succulent stems and leaves (Kaleme 2003; Jack et al. 2014, 2016), juveniles may not be as resistant to the unpredictable rainfall in the SRZ.

At the local scale, physiological performance differed at different aspects and elevations. In keeping with my prediction, physiological performance was higher on the north-facing slopes than the east-facing slopes. The results of this study indicated that the local-scale differences in physiological performance on the north-facing slopes may be explained by local-scale temperature differences. In Gannabos, the mean hourly temperatures on the north-facing slope were higher than those on the east-facing slope (Fig. 4a) and in Keikamspoort the daily maximum temperatures on the north-facing slopes were higher than those on the east-facing slopes. In keeping with the assumptions about the range edge populations of *A. dichotomum*, the warmer temperatures on the north-facing slopes may allow for escape from the cold temperatures associated with the cool range edge, allowing for better physiological performance here. However, for Keikamspoort the nocturnal carbon gain on the north-facing slopes should have been negatively affected by the heat wave but it did not seem to be. This may be because the microsite temperature differences at Keikamspoort were not as expected, with the flats and south-facing slopes being warmer than the north-facing slopes. As a result, the north-facing slopes here were most likely buffered from the extreme heat wave by some microsite characteristic not identified in this study. Furthermore, from observations in the field, the density of quiver trees at the two sites is noticeably higher on the north-facing slopes than the other slopes. This further suggests that this aspect may have growing conditions that are more suitable for *A. dichotomum*.

I also found that at the local scale, carbon gain (and not PEPC activity) was higher at higher elevations. This seemed to contradict my prediction that trees at lower elevations would perform better. However, the higher nocturnal carbon gain of the higher elevations only applied to Gannabos as carbon gain was only measured at one elevation in the Keikamspoort population. Therefore, the higher carbon gain at higher elevations in Gannabos is likely driven by the higher physiological performance on the north-facing slopes.

However, in contrast to my temperature-driven prediction, performance was higher on the flats than on the east-facing slopes. Because no physiological data were collected from the flats in Keikamspoort, this finding once again only applies to the Gannabos *A. dichotomum* population. The association of higher temperatures with better physiological performance suggested for the north-facing slopes was not apparent for the flats at Gannabos. Rather, the flats were cooler than the east-facing slopes at Gannabos (Fig. 4a & 5a). Therefore, something in addition to the temperature patterns recorded during this study period may be responsible for the observed local-scale differences in physiology.

It has been strongly suggested that the recruitment and percentage change of *A. dichotomum* populations is explained by climatic variables (Foden 2002; Kaleme 2003; Foden et al. 2007; Guo et al. 2016). It therefore follows that physiological performance of *A. dichotomum* should be most affected by climatic variables. In keeping with the above suggestion that rainfall may also be affecting the physiological performance, it may be that the flats have higher physiological performance than the east-facing slopes because water runoff here is lower than on the slopes. As a result, the trees on the flats would have been able to absorb more rain water over the winter months in Gannabos and would have more water stored in their succulent stems and leaves, allowing for prolonged stomatal opening and higher rates of photosynthesis.

Furthermore, although the flats do experience cooler temperatures than the other aspects, recent warming of the entire western region of southern Africa (Foden et al. 2007; van Wilgen et al. 2016), in combination with greater access to water may explain the higher physiological performance on the flats than on the east-facing slopes in Gannabos. The complexity of the combined effect of temperature and water availability (Foden et al. 2007; Guo et al. 2016) may therefore be apparent in this study. Although temperature conditions may be becoming more suitable for establishment at these two cool range edge sites, the dependence of *A. dichotomum* on water availability may in some cases override the effect of temperature increases. The overriding importance of rainfall may further suggest that the higher carbon gain at Gannabos was due to the difference in rainfall regimes between these two sites.

The local-scale differences in *A. dichotomum* densities observed at the two sites may be a result of the life history of this species or a climatic threshold acting outside of my study period. Because of the winged nature of its seeds *A. dichotomum* is capable of dispersing considerable distance (Cousins & Witkowski 2012). However, *A. dichotomum* has episodic germination and recruitment, relying on variable climatic events (Kaleme 2003; Van der Merwe & Geldenhuys 2017). Recruitment in arid environments is typically event driven because suitable climatic conditions for recruitment are rare (Midgley et al. 1997). This is typical of sessile, long-lived, arid-adapted species (Jordan & Nobel 1979; Pitelka 1997; Foden et al. 2007). It is suggested that this species recruits successfully in intervals of 15 years on average (Foden 2002; Foden et al. 2007; Hoffman et al. 2010). Therefore, the lack of a coherent pattern of physiological performance linked to temperatures at the local scale may indicate that the climate conditions at the local scale have already become suitable for establishment and these range edge sites of *A. dichotomum* may be undergoing a range-filling process (Cannone & Pignatti 2014).

Alternatively, it has been suggested that temperature extremes during winter may limit the distribution of species at the cool range edge (Graham & Grimm 1990), and it has been found that one of the most important factors determining the current range of *A. dichotomum* is the minimum temperatures of the coldest month (Guo et al. 2016). There was no consistent evidence for differences in minimum temperatures at the different aspects and elevations but with PEPC activity being favoured on warmer nights, cooler nights should limit photosynthesis. This effect was already apparent in the early summer – the time of year where plant growth is favoured in South Africa but temperatures are substantially warmer than the other seasons. The effect of low temperature extremes may therefore be even more pronounced during the cold winter months. When CAM plants experience sub-optimal temperatures their photosynthesis generally decreases due to changes in their photosynthetic processes (Flexas et al. 2012).

Are the juveniles that may be establishing further south able to survive the cold winter months? This may be what is restricting the range expansion of these cool range edge populations. Since the period of this study did not span the important winter months, confirming this requires further studies that perform the same protocol for more populations of *A. dichotomum* over a full year period in order to determine if the species is in fact affected by very low winter temperatures at the cool range edge.

Moreover, it has been suggested that assuming that temperature is the only driver impacting the distribution of a species may result in underestimating the full effect of climate change (Lenoir & Svenning 2015). Without the incorporation of other variables, such as rainfall extremes and patterns, the increasing reports of unexpected range shifts, such as downhill movement (observed for *A. dichotomum*) or expansion across longitudes (predicted for *A. dichotomum*), may be inexplicable (Lenoir & Svenning 2015). I therefore suggest that future studies include other variables in addition to temperature. Chief amongst these are likely to be drought-related indices, which would represent stress relating to evaporative water demand and the inherent drought tolerance of different life stages (Foden et al. 2007).

5. Conclusion

This study contributes to understanding the eco-physiology of *A. dichotomum* and has provided valuable insight into the effects of temperature on the physiological performance of this species. It confirms that at its cool range edge, *A. dichotomum* is performing CAM photosynthesis and should therefore be sensitive to certain environmental changes. In addition, there may be evidence for a maximum storage capacity of malic acid in the mesophyll tissue of *A. dichotomum*, suggesting that the benefit of warming at the cool range edge may be greater for older and larger individuals. Furthermore, PEPC activity in *A. dichotomum* is temperature dependent, with higher night time temperatures resulting in higher activity. This provides further support for the suggestion that night time temperatures are more important than daytime temperatures for nocturnal carbon gain in CAM plants.

At regional scale, *A. dichotomum* had higher carbon gain at Gannabos, a result that may be a result of a local heat wave in Keikamspoort or the more predictable rainfall pattern and more recent winter rainfall of Gannabos. At the local scale, *A. dichotomum* had higher physiological performance on the north-facing slopes where temperatures were higher. This was predicted as individuals at the cool range edge of *A. dichotomum* should favour warmer temperatures that more closely approximate those at the core of its range. However, performance was also higher on the flats where temperatures are cooler than those on the slopes. This was not in keeping with my predictions and may suggest that the combined effect of recent regional warming, as well as lower water runoff on the flats, may allow for higher physiological performance of *A. dichotomum*.

In conclusion, I suggest that the cool range edge of *A. dichotomum* is determined both by cold temperature extremes and rainfall variability. However, with the current rate of warming in this region (Mackellar et al. 2007), constraints on these populations are likely being removed. This is substantiated by the observed high levels of juvenile recruitment in these populations relative to others (Foden 2002; Foden et al. 2007; Hoffman et al. 2010), and in time, is likely to lead to range expansion in the region.

As with most species, the response of *A. dichotomum* to anthropogenic climate change is, indeed, complex and individualistic (Lenoir et al. 2010; Chen et al. 2011). Because of this, detailed environmental, ecological and physiological data are required when predicting its potential response climate change (Chen et al. 2011). Environmental and ecological data for *A. dichotomum* is becoming abundant (Foden 2002; Foden et al. 2007; Hoffman et al. 2010; Jack et al. 2014, 2016), and now, as a result of this study, more is known about its eco-physiology. Such knowledge will prove vital in making predictions about the impacts of anthropogenic warming on the distribution of *A. dichotomum*.

References

Allen C, Breshears D, McDowell N. 2015. On underestimation of global vulnerability to tree mortality and forest die- off from hotter drought in the Anthropocene. Ecosphere **6**:1–55. Available from https://doi.org/10.1890/ES15-00203.1.

Barton K. 2018. MuMIn: Multi-model inference. Available from https://cran.r-project.org/package=MuMIn.

Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software **67**:1–48.

Berry J, Björkman O. 1980. Photosynthetic response and adaptation to temperature in higher plants. Annual Review of Plant Physiology **31**:491–543.

Breshears DD, Myers OB, Meyer CW, Barnes FJ, Zou CB, Allen CD, McDowell NG, Pockman WT. 2009. Tree die-off in response to global change-type drought: Mortality insights from a decade of plant water potential measurements. Frontiers in Ecology and the Environment **7**:185–189.

Burke A. 2004. From plains to inselbergs: species in special habitats as indicators for climate change? Journal of Biogeography **31**:831–841.

Burnham K, Anderson D. 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach, Second Ed. Springer-Verlag, New York.

Cannone N, Pignatti S. 2014. Ecological responses of plant species and communities to climate warming: Upward shift or range filling processes? Climatic Change **123**:201–214.

Chen I, Hill JK, Ohlemüller R, Roy DB, Thomas CD. 2011. Rapid range shifts of species associated with high levels of climate warming. Science **333**:1024–1027.

Cousins SR, Witkowski ETF. 2012. African aloe ecology: A review. Journal of Arid Environments **85**:1-17. DOI: 10.1016/j.jaridenv.2012.03.022.

Dawson TP, Jackson ST, House JI, Prentice IC, Mace GM. 2011. Beyond predictions:

Biodiversity conservation in a changing climate. Science **332**:53–58.

Department of Environmental Affairs. 2017. South Africa’s Third National Communication under the United Nations Framework Convention on Climate Change. Pretoria. Available from https://www.environment.gov.za/sites/default/files/reports/draftsouthafricas3rdnationalcommunication_unfccc2017.pdf.

Flexas J, Loreto F, Medrano H. 2012. Terrestrial photosynthesis in a changing world. Cambridge University Press, Cambridge.

Foden WB. 2002. A Demographic Study of *Aloe dichotoma* in the Succulent Karoo: Are the Effects of Climate Change Already Apparent? MSc thesis, University of Cape Town, Cape Town.

Foden WB, Midgley GF, Hughes G, Bond WJ, Thuiller W, Hoffman MT, Kaleme P, Underhill LG, Rebelo A, Hannah L. 2007. A changing climate is eroding the geographical range of the Namib Desert tree *Aloe* through population declines and dispersal lags. Diversity and Distributions **13**:645–653.

Fox J, Weisberg S. 2011. An R Companion to Applied Regression Second Ed. Sage, Thousand Oaks CA.

Fox J, Weisberg S. 2018. Visualizing fit and lack of fit in complex regression models with predictor effect plots and partial residuals. Journal of Statistical Software **87**:1–27.

Graham RW, Grimm EC. 1990. Effects of global climate change on the patterns of terrestrial biological communities. Trends in Ecology and Evolution **5**:289–292.

Grey K-A. 2017. Going south: The changing geographical range of *Aloidendron dichotomum* (Quiver Tree) may be explained by ecophysiological constraints at the juvenile stage. Honours thesis, Stellenbosch University, Cape Town.

Guo D, Arnolds JL, Midgley GF, Foden WB. 2016. Conservation of Quiver Trees in Namibia and South Africa under a changing climate. Journal of Geoscience and Environment

Protection **4**:1–8.

Hervé M. 2019. RVAideMemoire: Testing and plotting procedures for biostatistics. Available from https://cran.r-project.org/package=RVAideMemoire.

Hoffman MT, Rohde RF, Duncan J, Kaleme P. 2010. Repeat photography, climate change, and the long-term population dynamics of tree *Aloes* in Southern Africa. Pages 133–142 in R. Webb, D. Boyer, and R. Turner, editors. Repeat photography: methods and applications in the geological and ecological sciences. Island Press, Washington DC.

Hughes L. 2000. Biological consequences of global warming: Is the signal already apparent? Trends in Ecology & Evolution **15**:56–61.

Huntley B. 1995. Plant species’ response to climate change: implications for the conservation of European birds. Ibis **137**:S127–S138.

Hurley B, Tran H, Marty N, Park J, Snedden W, Mullen R. 2010. The dual-targeted purple acid phosphatase isozyme AtPAP26 is essential for efficient acclimation of *Arabidopsis* to nutritional phosphate deprivation. Plant Physiology **153**:1112–1122.

IPCC. 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Page (Core Writing Team, Pachauri RK, Meyer LA, editors). Geneva, Switzerland.

Jack SL, Hoffman MT, Rohde RF, Durbach I. 2016. Climate change sentinel or false prophet? The case of *Aloe dichotoma*. Diversity and Distributions **22**:745–757.

Jack SL, Hoffman MT, Rohde RF, Durbach I, Archibald M. 2014. Blow me down: A new perspective on *Aloe dichotoma* mortality from windthrow. BMC Ecology **14**:1–12.

Jacobsen H. 1960. A handbook of succulent plants. Blandford Press, London.

Jordan PW, Nobel PS. 1979. Infrequent Establishment of Seedlings of *Agave deserti* (Agavaceae) in the Northwestern Sonoran Desert. American Journal of Botany **66**:1079–1084.

Jump AS, Peñuelas J. 2005. Running to stand still: Adaptation and the response of plants to rapid climate change. Ecology Letters **8**:1010–1020.

Kaleme P. 2003. Regional differences in the long-term population dynamics of a succulent tree, *Aloe dichotoma* in the semi-arid Karoo, South Africa as revealed by repeat photography. MSc thesis, University of Cape Town, Cape Town.

Kluge M, Knapp I, Kramer D, Schwerdtner I, Ritter H. 1979. Crassulacean acid metabolism (CAM) in leaves of *Aloe arborescens* Mill: Comparative studies of the carbon metabolism of chlorenchym and central hydrenchym. Planta **145**:357–363.

Lenoir J, Gégout JC, Guisan A, Vittoz P, Wohlgemuth T, Zimmermann NE, Dullinger S, Pauli H, Willner W, Svenning JC. 2010. Going against the flow: Potential mechanisms for unexpected downslope range shifts in a warming climate. Ecography **33**:295–303.

Lenoir J, Svenning JC. 2015. Climate-related range shifts - a global multidimensional synthesis and new research directions. Ecography **38**:15–28.

Lenth R. 2019. Emmeans: Estimated Marginal Means, aka Least-Squares Means. Available from https://cran.r-project.org/package=emmeans (accessed April 16, 2019).

Loarie SR, Field CB, Ackerly DD, Duffy PB, Asner GP, Hamilton H. 2009. The velocity of climate change. Nature **462**:1052–1055. Available from http://dx.doi.org/10.1038/nature08649.

Mackellar NC, Hewitson BC, Tadross MA. 2007. Namaqualand's climate: Recent historical changes and future scenarios. Journal of Arid Environments **70**:604–614.

Males J, Griffiths H. 2017. Stomatal biology of CAM plants. Plant Physiology **174**:550–560.

Måren IE, Karki S, Prajapati C, Yadav RK, Shrestha BB. 2015. Facing north or south: Does slope aspect impact forest stand characteristics and soil properties in a semiarid trans-Himalayan valley? Journal of Arid Environments **121**:112–123. Available from http://dx.doi.org/10.1016/j.jaridenv.2015.06.004.

Mazerolle M. 2019. Model selection and multimodel inference based on AICc. Available from https://cran.r-project.org/package=AICcmodavg.

Midgley GF, Thuiller W. 2011. Potential responses of terrestrial biodiversity in Southern Africa to anthropogenic climate change. Regional Environmental Change **11**:127–135.

Midgley JJ, Cowling RM, Hendricks H, Desmet PG, Esler K, Rundel P. 1997. Population ecology of tree succulents (*Aloe* and *Pachypodium*) in the arid western cape: Decline of keystone species. Biodiversity and Conservation **6**:869–876.

Mucina L, Jurgens N, le Roux A, Rutherford M, Schmiedel U, Esler K, Powrie L, Desmet P, Milton S. 2006. Succulent Karoo Biome. Pages 221–299 in L. Mucina and M. Rutherford, editors. The vegetation of South Africa, Lesotho and Swaziland. Strelitzia 19, Pretoria.

Nobel PS. 1984. Productivity of *Agave deserti*: Measurement by dry weight and monthly prediction using physiological responses to environmental parameters. Oecologia **64**:1–7.

Nobel PS, Hartsock T. 1978. Resistance analysis of nocturnal carbon dioxide uptake by Crassulacean acid metabolism succulent, *Agave deserti*. Plant Physiology **61**:510–514.

Ocaña A, Cordovilla MP, Ligero F, Lluch C. 1996. Phosphoenolpyruvate carboxylase in root nodules of Vicia faba: partial purification and properties. Physiologia Plantarum **97**:724–730. doi: 10.1111/j.1399-3054.1996.tb00537.x

Parmesan C, Hanley ME. 2015. Plants and climate change: Complexities and surprises. Annals of Botany **116**:849–864.

Parmesan C, Yohe G. 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature **421**:37–42.

Pearson RG, Phillips SJ, Loranty MM, Beck PSA, Damoulas T, Knight SJ, Goetz SJ. 2013. Shifts in Arctic vegetation and associated feedbacks under climate change. Nature Climate Change **3**:1–5. Available from http://dx.doi.org/10.1038/nclimate1858.

Pitelka L. 1997. Plant migration and climate change. American Scientist **85**:464–473.

Randin CF, Engler R, Normand S, Zappa M, Zimmermann NE, Pearman PB, Vittoz P, Thuiller W, Guisan A. 2009. Climate change and plant distribution: Local models predict high-elevation persistence. Global Change Biology **15**:1557–1569.

Sala OE, Chapin FS, Armesto JJ, Berlow R, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF, Jackson RB, Kinzig A, Leemans R, Lodge D, Mooney HA, Oesterheld M, Poff NL, Sykes MT, Walker BH, Walker M, Wall DH. 2000. Global biodiversity scenarios for the year 2100. Science 287:1770–1774.

Settele JR, Scholes R, Betts R, Bunn S, Leadley P, Nepstad D, Overpeck JT, Taboada MA. 2014. Terrestrial and inland water systems. Page (Field CB et al., editors) Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Sexton JP, Mcintyre PJ, Angert AL, Rice KJ. 2009. Evolution and ecology of species range limits. The Annual Review of Ecology, Evolution, and Systematics **40**:415–436.

Stevens GG, Pérez-Fernández MA, Morcillo RJL, Kleinert A, Hills P, Brand DJ, Steenkamp ET, Valentine AJ. 2019. Roots and Nodules Response Differently to P Starvation in the Mediterranean-Type Legume Virgilia divaricata. Frontiers in Plant Science **10**:73. doi: 10.3389/fpls.2019.00073

Taiz L, Zeiger E, Moller I, Murphy A. 2015. Plant physiology and development, 6th edition. Sinauer Associates, Inc., Sunderland.

Team RC. 2018. R: A language and environment for statistical computing. Available from http://www.r-project.org/.

Thomas CD. 2010. Climate, climate change and range boundaries. Diversity and Distributions **16**:488–495.

Thomas CD, Bodsworth EJ, Wilson RJ, Simmons AD, Davies ZG, Musche M, Conradt L. 2001.

Ecological and evolutionary processes at expanding range margins. Nature **411**:577–581.

Thomas CD, Cameron A, Green RE, Bakkenes M, Beaumont LJ, Collingham YC, Erasmus BFN. 2004. Extinction risk from climate change. Nature **427**:145–148.

Tovar-méndez A, Mújica-jiménez C, Muñoz-clares RA. 2000. Physiological implications of the kinetics of maize leaf Phosphoenolpyruvate Carboxylase. Plant Physiology **123**:149–160.

van der Merwe H, Geldenhuys C. 2017. Proposed long-term monitoring protocol and applications for *Aloidendron dichotomum* populations. South African Journal of Botany **109**:253–262. SAAB. Available from http://dx.doi.org/10.1016/j.sajb.2017.01.008.

van Wilgen NJ, Goodall V, Holness S, Chown SL, Mcgeoch MA. 2016. Rising temperatures and changing rainfall patterns in South Africa's national parks. International Journal of Climatology **36**:706–721.

van Wyk B, Smith G. 2014. Guide to the Aloes of South Africa, Third Ed. Briza Publications, Pretoria.

Venables B, Hornik K, Maechler M. 2016. Polynom: A collection of functions to implement a class for univariate polynomial manipulations. Available from https://cran.r-project.org/package=polynom.

Wickham H. 2016. Ggplot2: Elegant graphics for data analysis. Springer-Verlag, New York.

Winter K. 1985. Crassulacean acid metabolism. Pages 329–387 in J. Barber and N. Baker, editors. Photosynthetic Mechanisms and the Environment. Elsevier, Amsterdam.

Yamori W, Hikosaka K, Way D. 2014. Temperature response of photosynthesis in C3, C4, and CAM plants: Temperature acclimation and temperature adaptation. Photosynthesis Research **119**:101–117.