

THE FUTURE OF SOUTH AFRICAN GRASSLAND COMMUNITIES

An experimental analysis on plant trait responses to fire frequency and warming



Utrecht University



Master Thesis

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An experimental analysis on plant trait responses to fire frequency and warming

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Abstract

Fire has historically been an important natural disturbance factor structuring African mesic grasslands and is nowadays a widely used management tool for preserving soils and maintaining grassland structure. With predicted climatic changes ahead of us, increased temperatures and more frequent and intensified wildfires will affect vegetation communities, especially in grasslands that are vulnerable to shifts in disturbance regimes. A major challenge lies in the understanding of plant responses to the climatic and human-induced disturbance changes in order to conserve biodiversity and secure ecosystem functioning. This study investigates the effect of fire frequencies and warming on African grasslands communities, using a trait-based approach. Data on plant functional traits was gathered at two study locations in South Africa which are home to long-term fire and warming experiments using open top warming chambers: Ukulinga Research Farm (altitude: 840m) and Brotherton Research Trail (altitude: 1890m). Comparisons were made between trait data from plots that experienced different disturbance treatments (different frequencies of burning and the presence or absence of passive warming) to study patterns of variation (both communal and species-specific) of six growth-related traits (leaf table height, specific leaf area, leaf dry matter content, culm height, biomass-ratio and number of inflorescences). Results indicate that fire affects plant traits to a greater extent than warming, selecting for slow-growing and tall grass species with the decrease of fire disturbance. Increase in leaf table height was both the result of compositional changes as species-specific changes with less frequent burns, whereas specific leaf area decreases were found to be the result of solely compositional changes. Different responses of community and species-specific leaf dry matter content to fire were found between study locations and an interactive effect for fire and warming was only found for community leaf dry matter content at Brotherton. Results of this study help to understand vegetative changes due to fire disturbances, either natural or human induced, and climatic changes and are valuable for successful conservation strategies.

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INTRODUCTION

Natural disturbances have a regulatory effect on a wide range of ecosystems across the globe (Kirkman et al., 2014). Due to human induced global climate change and increasing climate variability, temperature is expected to continuously rise and extreme weather events are expected to occur more frequently (Steffen et al., 2015). In order to predict the effect that disturbances may have on ecosystem structures and communities in the future, it is important to understand the associated response of plant communities. Especially in plant communities that are disturbance dependent, like African mesic grasslands, knowledge of the response of plant species to disturbances provides insights for effective conservation strategies, upon which the threatened mesic grasslands depend (Kirkman et al., 2014).

Mesic grasslands (>500 mm mean annual precipitation) cover about 40% of the global terrestrial surface, and in South Africa mesic grasslands are characterized by a complex disturbance regime with fire being an important maintaining factor of plant diversity and community structure (Forrestel, Donoghue, & Smith, 2014; Gordijn, Everson, & O'Connor, 2018; Kirkman et al., 2014). Mesic grasslands and savannas are biodiversity hotspots and are generally dominated by C4 grasses with high productivity rates promoting fire occurrence (Forrestel et al., 2014; Fynn, Morris, & Edwards, 2005; Gordijn et al., 2018). The evolution of ecological strategies of plants in this biome led to increased tolerance to fire events, historically caused by lightning ignition (Gordijn et al., 2018). However, due to global changes and human alteration, disturbance regimes worldwide are changing. Increased occurrence of fire events and increased temperatures potentially have substantial effects on the structure and composition of these grasslands (Kirkman et al., 2014; Osborne et al., 2018). The vulnerability of mesic grasslands to changing disturbance regimes and the response of vegetation is substantially important to understand, because of the dependence of local societies on mesic grasslands as natural resources within livelihoods (Thomas, Twyman, Osbahr, & Hewitson, 2007).

The response of plant species and grassland communities to environmental disturbances can be predicted by studying the plant features (traits) that reflect the ecological strategies (Bjorkman et al., 2018; Pérez-Harguindeguy et al., 2013). The most popular method to measure the response of vegetation to environmental changes is using plant functional traits, either for individual species or for communities, as plant traits are the functional markers of the performance and strategies of plants (Hudson, Henry, & Cornwell, 2011; Lavorel & Garnier, 2002). For the investigation of plant response to fire disturbances and increased temperature, plant traits have been widely used amongst researchers in different biomes (Bjorkman et al., 2018; Forrestel et al., 2014; Kirkman et al., 2014). The distribution of trait values in communities reflect different pathways to which a disturbance can alter plant traits. Community traits are affected by niche differentiation among species and species-specific traits are affected by phenotypic plasticity or genetic adaptation (Bjorkman et al., 2018; Forrestel et al., 2014; Gordijn et al., 2018; Roche, Díaz-Burlinson, & Gachet, 2004). Response of species can either be measured using long term data records, or disturbance manipulation experiments. The latter provides insight in how species respond to isolated disturbance events by directly changing an ecological factor like fire frequency or temperature at a small scale (Wolkovich et al., 2012). However, for an experimental manipulation method to be reliable, it should be conducted over a longer time frame, as it may take decades for disturbances to affect community composition (Marion et al., 1997).

Although extended literature is available covering plant responses to altered fire frequencies, most studies focus on woody species or the transition between grasslands and shrublands (Archibald et al., 2018; Brigss et al., 2005; Wolkovich et al., 2012). Studies conducted on grassland biomes are less common and often only focus on community-level responses to disturbances. Forrestel et al. (2014) & Kirkman et al. (2014) investigated herbaceous species in a comparative study on the response of grassland communities to manipulated fire regimes between different continents. Furthermore, the response of grass species to the combination of two manipulated disturbance variables has been investigated at several grassland locations, with Gordijn et al. (2018) focusing on fire regimes and atmospheric CO₂ concentrations, Abdalla et al. (2016) and Wolkovic et al. (2012) focused on temperature and atmospheric CO₂ concentrations and Fynn, Morris & Edwards (2005) focusing on fire regimes and mowing. However, what has not yet been investigated is the effect of different fire frequencies and warming, including their interactive effect, on plant response at both community and species-specific level.

Knowledge on the response of mesic grasslands to disturbances is crucial for the implementation of appropriate conservation practices (Gordijn et al., 2018; Kirkman et al., 2014). In turn, this importance is illustrated by the effect that plant responses can have on ecosystems and their services, affecting entire food webs, hydrological cycles and carbon and nutrient cycling and storage (Abdalla et al., 2016; Briggs et al., 2005; Wolkovich et al., 2012). This study will provide insights on the response of particular plant traits that are affected by fire and temperature, which enables applicability of specific knowledge to a large range of environments (Fynn et al., 2005).

The aim of this study is to measure the response of South African grassland communities to experimental disturbances of fire and temperature. The following research question is formulated:

What is the effect of fire frequencies and passive warming on growth-related plant traits in South African grasslands?

Sub-questions and hypotheses are described following the theoretical background. This study uses long-term fire manipulation experiments and passive warming experiments at two locations in the mesic grasslands of South Africa: Ukulinga research farm (URF) at 840m altitude and Brotherton research trail (BRT) at 1890m altitude. The effect of different fire frequencies (no burn, annual burn, biennial burn and >3 years interval burn) and the presence or absence of passive warming, using open top warming chambers (OTCs), will be investigated on six plant traits (leaf table height (LTH), specific leaf area (SLA), leaf dry matter content (LDMC), culm height (CH), number of inflorescences (NI), biomass ratio (BR)) on both community as species-specific level.

THEORETICAL BACKGROUND

DISTURBANCE REGIMES IN GRASSLANDS

In ecosystems across the planet, ecological diversity and productivity are regulated by natural disturbances and in South African grasslands, the most prevailing disturbance is fire (Kirkman et al., 2014; Pausas & Bradstock, 2007). Historically, solely lighting ignition determined fire regimes to which mesic grasslands and its diversity is adapted (Archibald, 2013; Pausas & Bradstock, 2007). Nowadays, fire regimes are to a great extent determined by human management through manipulation of fire frequencies and intensities, often in contrast with the natural fire variability (Kirkman et al., 2014).

Burning is a common practice in maintaining mesic grasslands, to eliminate woody species and increase fodder production and quality (Abdalla et al., 2016). Grasslands are vulnerable for shifts in disturbance regimes and the response of the grassland vegetation is an important factor to understand. Due to feedback mechanisms, a thin line separates mesic grasslands and the domination of more woody species under different disturbance regimes (Archibald et al., 2018; Forrestel et al., 2014). The conservation of grasslands thus depends on managing disturbance regimes, since small changes in e.g. fire frequency and intensity, temperature, atmospheric carbon dioxide (CO₂) concentration, and their interactions, could offset an inevitable shift to shrubland (Archibald et al., 2018; Briggs et al., 2005).

Current and expected climate change, and its induced temperature increase, is expected to severely affect global fire regimes. Fire seasons are expected to increase in intensity and duration over the coming decades, paired with longer periods of extreme droughts (Jolly et al., 2015). Increasing temperatures and changing patterns of rainfall potentially affect plant growth and mortality, and therefore directly and indirectly changing the properties of fuel load (Osborne et al., 2018). Enhanced temperatures and related water deficits lead to a higher probability of intense wildfires with higher temperatures, having an increased devastating effect on plant communities (Werf et al., 2010) because root systems that are often fire-resistant are vulnerable to drought (Pausas & Bradstock, 2007).

EFFECT DISTURBANCES ON MESIC GRASSLANDS

Fire is an important management tool in South Africa for preserving soils and maintaining grassland structure (Archibald et al., 2018). Fire events influence grasslands by reducing aboveground biomass and altering the distribution of resources like nutrients, light and moisture, as increasing fire frequencies induce high nutrient turnover rates (Keeley, 2018; Smit et al., 2010). Although fire does not directly influence the productivity of grasslands, the altered distribution of resources generally lead to a greater C:N ratio with increasing burning frequencies, leading to soils that are limited in N (Manson, Jewitt, & Short, 2007). Fire can also alter the local and global climate directly through the emissions of greenhouse gasses and aerosols and indirectly by altering surface albedo and primary productivity (S Archibald et al., 2018).

Furthermore, fire has a great impact on the vegetation structure and composition (Pausas & Bradstock, 2007), controlling the ecosystem diversity and functional traits (Archibald, 2016). Fire affects the

species diversity, richness and heterogeneity, either directly by increasing mortality rates, or indirectly by altering the structure and composition of the community (Gordijn et al., 2018; Kirkman et al., 2014). Functional traits of grassland vegetation can be affected by fire regimes following several pathways (figure 1). Disturbances like fire can trigger interspecific selection due to the successional replacement of species by species that are better adapted to the particular disturbance, resulting in alteration of species composition and thus community traits (Lavorel & Garnier, 2002). The diversity of community traits tends to be greater in undisturbed communities. This indicates that fire acts as an environmental filter, selecting for species that have a fast post-fire regeneration (Forrestel et al., 2014).

Besides compositional changes that affect the community traits, several mechanisms can cause species-specific trait changes as an effect of a disturbance. Phenotypic plasticity allows species to respond to abiotic changes on a short term. On a longer term, (epi)genetic adaptation allows for intraspecific changes in traits that are better adapted to a particular disturbance regime (figure 1) (Bjorkman et al., 2018; Read, Moorhead, Swenson, Bailey, & Sanders, 2014).



Figure 1. Schematic overview of the pathways from fire regime changes to plant trait changes, and the interaction with human influences and climate change. In the field, plant traits can be measured either as community or species-specific traits. There are several pathways to which a change in fire regime (in this case fire recurrence) can lead to changes in plant traits. On the (relatively) short term, compositional changes may occur due to interspecific interactions leading to altered community traits and phenotypic plasticity can result in species-specific trait changes. On the (relatively) long term, (epi)genetic adaptation of species can lead to a change in species-specific traits. The yellow box on the bottom entails drivers that influence the processes in the green boxes. Dark orange lines in the figure indicate interactions directly related to this research.

FUNCTIONAL PLANT TRAITS

In order to assess plant responses to certain environmental variables, plant traits can be used as measurable units, either morphological, physiological or phenological, that describe the functioning and performance of a plant (Jolly et al., 2015; Pérez-Harguindeguy et al., 2013). Plant functional traits can be measured at different scales, ranging from cellular, individual to communal.

Plants may develop certain strategies to optimally profit from their local environment as a response to disturbances and the constant struggle to obtain resources and produce offspring (Ordoñez et al., 2010). The evolution of certain plant traits indicates different plant strategies, showing differences in morphological and physiological traits amongst species in different environment (intraspecific variation), or between species in the same local environment (interspecific variation) (Ordoñez et al., 2010; Wright et al., 2004). Strategies may include investment in all traits that eventually influence fitness, like growth rate investments (e.g. high SLA) but could also include phenological traits like seed production or root economics, depending on the local environment and disturbance regime (Wright et al., 2004). Fire tolerance is related to survival and regrowth after fire or the avoidance of fire damage to basal meristems which in turn is related to the behavior of fire (Lavorel & Garnier, 2002). For example, the distribution of biomass and the projection of seedbanks above the ground (culm height) are traits that influence the behavior of a fire and may indicate fire-related plant strategies.

For this study traits will be measured on the individual level, weighed to community traits, to identify different responses to different disturbance regimes. Although the community level is a good measure to compare plant response to different disturbances, groups of species that respond similar to a particular environmental factor, so called functional response groups, can exist within communities as a result of individual adaptation of certain species (Lavorel & Garnier, 2002). This means that if a community trait describes a certain strategy as a response to a certain disturbance regime, still a large variation in strategies between species in this community may exist. Thus, this interspecific or speciesspecific variation, may result in counterbalanced community trait values and is of substantial importance when measuring plant responses to disturbance regimes. Additionally, intraspecific variation, described as the natural variation within species populations due to local adaptation, may have effects on community dynamics. These different trait variations together determine the distribution of trait values within a community, also called functional diversity (FD) (Albert et al., 2012; Bolnick et al., 2011). For this study, the community responses are calculated and compared between disturbance regimes to identify a net community response. Hereafter, an analysis will be done on species-specific trait variation on a number of species that exists throughout all the disturbance treatments, to assess species-specific strategies.

GROWTH RELATED TRAITS

As a proxy for plant size leaf table height (LTH) will be measured, which describes the height under which 80% of the plant leaves are estimated to occur (O'Reagain, 1993). Plant size is an important trait to measure as it reflects the competitive ability with respect to light capture (Hudson et al., 2011). Furthermore, two leaf traits, specific leaf area (SLA) and leaf dry matter content (LDMC) will be measured. These traits map altered components of the carbon and nitrogen cycles at leaf, plant and community level and therefore describe the functioning of plant species and communities (Lavorel & Garnier, 2002; Wright

et al., 2004). In plant ecology, the most common traits to measure on species level are leaf traits, as these represents the most important organ of a plant, determining its productivity (Jin et al., 2011).

SLA is defined as the ratio of leaf area to dry mass [cm² g⁻¹] and often positively relates to the relative growth rate (RGR) of a plant (Pérez-Harguindeguy et al., 2013). Furthermore, SLA relates positively to photosynthetic rate and leaf nitrogen (N) content, and negatively to leaf longevity and investment in quantitatively important secondary (hydro-carbonate) compounds like tannins or lignin (Pérez-Harguindeguy et al., 2013). Thus, a high SLA allows an early successional plant to efficiently produce leaf area at a low cost for construction and support (Poorter, Van De Plassche, Willems, & Boot, 2004). Lower SLA reflects an effective strategy in environments where resources are low (Pérez-Harguindeguy et al., 2013).

LDMC is defined as the grams of leaf dry matter per unit leaf fresh mass $[g g^{-1}]$ and is, unlike SLA, often negatively correlated with the relative growth rate of a plant and positively with leaf lifespan (Hudson et al., 2011). LDMC is a good predictor of resource capture, usage and availability. Leaves with high LDMC tend to be relatively tough and decompose slower compared to leaves with a low LDMC (Pérez-Harguindeguy et al., 2013). LDMC is a good trait to measure when assessing disturbances, as this trait is assumed to be related to physical hazards. The flammability of a plant is partly determined by the LDMC and species with low LDMC tend to be associated with productive and highly disturbed environments (Pérez-Harguindeguy et al., 2013). Early successional plants often have a low LDMC, as they invest more in leaf area than in leaf thickness. LDMC increases as a strategy for leaf defense against natural disturbances ranging from fires, winds, drought or herbivory (Garnier, Shipley, Roumet, & Laurent, 2001).

CULM HEIGHT AND NUMBER OF INFLORESCENCES

Traits that are related to the reproduction potential and strategy are assumed crucial in response to disturbances and are strongly depended on the timing of biological events, the phenology. Changes in reproduction potential might have significant implications for community dynamics (Dorji et al., 2013). An extended research over several growing cycles on flowering phenology is crucial to understand the change of timing due to disturbances. However, the functional trait: number of inflorescences (NI) can give insight in the reproduction potential and productivity in relation to post fire regrowth. It is predicted that both the NI will be lower with a higher burning frequency, due to the reduced abundance of individuals that participate in each phenophase and the limited time for growth (Alvarado et al., 2014).

The culm height (CH), the average height at which flowers grow per individual, is a functional trait related to reproduction potential and may indicate growth strategies in response to changes in fire frequencies, without taking into account the phenology. The number and projection of inflorescences above the vegetative part of a plant may indicate a strategy in relation to light capture and fire behavior and spread (Pérez-Harguindeguy et al., 2013). Increased heights of the culm and erect life forms may facilitate protection from fire and influence fire behavior by preventing lateral spreading (Pérez-Harguindeguy et al., 2013).

BIOMASS RATIO

Biomass ratio is a trait that is described as the ratio of canopy biomass above 10 cm to the biomass below 10 cm and is related to grass canopy architecture and flammability (Gao & Schwilk, 2018). The distribution of biomass at either the upper or lower part of a plant can indicate architectonical strategies (erect or prostrate architecture) in relation to competition for resources such as light and space (Archibald, Hempson, & Lehmann, 2019). Furthermore, this trait is related to fire behavior as it explains how the architecture of a grass influences the spread of a wildfire. During fires, plants with more biomass above 10 cm negatively influence fire temperatures at the soil surface, increasing the likelihood of plant survival.

Hypothesis

To answer the following research question: What is the effect of fire frequencies and passive warming on growth-related plant traits in South African grasslands? The following sub-questions are formulated:

- 1. What is the effect of different fire frequencies on grass traits in South Africa?
- 2. What is the effect of passive warming on grass traits in South Africa?
- 3. What is the interactive effect of fire and warming on grass traits in South Africa?

BURNING FREQUENCY

Firstly, it is predicted that LTH will increase when burning frequencies decrease, as plants have a longer lifespan and fuel accumulation will trigger tall and upright tufts to retain photosynthetic ability (Hempson, Archibald, Donaldson, & Lehmann, 2019). Secondly, it is expected that the growth rate will increase once burning frequencies increase, resulting in grasses with higher SLA and lower LDMC. When burning frequencies decrease, the strategy of efficient nutrient use will result in grasses with lower SLA and higher LDMC.

The phenology will be affected by fire, as fire directly affects mortality and thus lifespan. It is predicted that both the NI will be lower with a higher burning frequency, due to the reduced abundance of individuals that participate in each phenophase, and the limited time for growth (Alvarado et al., 2014). On the other hand, it can be hypothesized that with increasing fire frequencies, more annual grass species will occur, that invest in a higher reproduction by producing more flowers, thus increasing NI. Additionally, It is expected that culm height will increase with and increasing FI (Pérez-Harguindeguy et al., 2013).

Furthermore, it is hypothesized that biomass ratios will increase in communities with a high frequency of hot fires, indicating a plant strategy to aggregate its biomass above the ground to minimize damage to the main basal meristems and allowing the plant to survive and resprout after fires (Gao & Schwilk, 2018; Hempson et al., 2019).

TEMPERATURE

LTH and SLA are expected to increase when temperatures increase, due to stimulated plant investment in new tissue by exploiting stored resources (Hudson et al., 2011). Subsequently, LDMC is expected to

be negatively related to temperature (Bjorkman et al., 2018). Furthermore, it is predicted that the NI and CH both increase with increasing temperatures. As a result of temperature increase, longer growing and flowering seasons are expected as well, although this will not directly be measured in this study, it might influence CH and NI positively. Lastly, it is expected that temperature enhancement, either indirect or direct, will influence plant biomass productivity positively, resulting in higher BR ratios.

Table 1. Hypothesis prediction table. Positive or negative indicates the predicted relation between the relevant response and effect variable. with: LTH: leaf table height, CH: culm height, NI: number inflorences, SLA: specific leaf area, LDMC: leaf dry matter content, BR: biomass ratio.

		BURNING FREQUENCY	TEMPERATURE					
RESPONSE	Predicted		Predicted					
VARIABLES	relation	Mechanism	relation	Mechanism				
LTH	Negative	Frequent burning result in low LTH due to limited growth time	Positive	Increased temperature				
СН	Negative	Increased mortality due to burning	Positive	catalyzes plant growth				
NI	Negative /Positive	leading to less reproductive individuals, thus low CH, but more annual species will occur that invest in high reproduction, leading to high NI.	Positive					
SLA	Positive	Increasing relative growth rate due to	Positive	Temperature stimulates				
LDMC	Negative	frequent burning will result in high SLA values and subsequently, low LDMC values	Negative	plant investment in new tissue, resulting in a high SLA, and a low LDMC.				
BR	Positive	Growth strategy: aggregate biomass above ground	Positive	Expected increases in LTH and will result in higher BR.				

INTERACTIVE EFFECT FIRE AND WARMING

Interaction effects of fire and warming occur when temperature accelerate the effect of fire on plants, or vice-versa. With enhanced temperature, growth rates increase (increased LTH, SLA) and more biomass is produced in a certain timeframe, especially of species in early successional stages. This increased fuel load will strongly affect the fire properties. Furthermore, as enhanced temperatures, and related water deficits lead to a higher probability of intense wildfires with higher temperatures, it is expected that the combination of fire and warming has an increased devastating effect on plant communities (Werf et al., 2010). Drought poses a greater threat for post-fire generating plants, compared to plants in a later successional state (Pausas & Bradstock, 2007). The consideration of other environmental variables such as water availability and the range of temperature change is therefore important in investigating the combined effect of temperature and fire. In general, it is expected that the effect of fire on plant traits will be amplified with warming.

METHODS

STUDY AREAS

In order to test the effect of fire and warming on plant functional traits related to growth strategy, data was gathered at two locations where vegetation is dominated by grass communities and where long-term fire- and temperature manipulation experiments have been set up. The first study site, Ukulinga research farm (URF) of the University of KwaZulu-Natal, is located near Pietermartitzburg (29°40' S, 30°24' E) and was designed to test the effect of various combinations of mowing and burning, at different frequencies and in different seasons (Abdalla et al., 2016; Kirkman et al., 2014). Fire manipulation experiments have been established in 1950 and OTCs are placed in august 2019 (Kirkman et al., 2014).

The second study site, Brotherton research trial (BRT), is located on the Brotherton plateau at Cathedral Peak in the uKhahlamba-Drakensberg Park (29°00'S,29°15'E) and was initiated with a similar aim: monitoring of botanical composition in response to fire-return interval (FI) and burn season treatments (Gordijn et al., 2018; Manson et al., 2007). Fire manipulation experiments were established in 1980 and open top warming chambers have been established in Jan 2017. Both study locations are dominated by C4 grasses such as such as *Themeda triandra* and *Heteropogon contortus* (Gordijn et al., 2018; Kirkman et al., 2014; Manson et al., 2007), with precipitation and primary growing season in summer (Dec-Mar) (Forrestel et al., 2014). Additional site characteristics of both study locations can be found in table 2.



Figure 2. Left: position of province Kwazulu-natal. right: position of study locations in kwazulu-natal: BRT: Brotherton research trail, URF: Ukulinga research farm

SITE CHARACTERISTICS	UKULINGA RESEARCH FARM	BROTHERTON RESEARCH TRAIL
ALTITUDE	840m ¹	1890m ⁴
MEAN ANNUAL PRECIPITATION	790 mm ⁵	1380mm ⁴
SUMMER MEAN MONTHLY TEMPERATURE (FEBRUARY)	26.4 °C ^{2,5}	17.1°C ³
WINTER MEAN MONTHLY TEMPERATURE (JULY)	13.2 °C ²	10°C ³
UNDERLYING GEOLOGY	Shales ^{1,2}	Basalt ⁴
SOIL TYPE	Silty clay loam ¹	Hutton ⁴
TOPOGRAPHY	Flat	Flat to gently rolling ⁴

Table 2. Study site characteristics (¹Abdalla et al., 2016; ²Fynn et al., 2005; ³GOrdijn et al., 2018; ⁴Manson et al., 2007; ⁵Kirkman et al., 2014)

EXPERIMENTAL DESIGN

To investigate the effect of fire and temperature on functional plant traits, data was gathered at the two study locations from plots with a combination of fire and warming treatments. This study focuses on six key functional traits, that are commonly used and enable data comparability and transfer among different study sites (Hudson et al., 2011). From the long-term experiments at both study locations, four burning treatments were used for this study: every year (annual), 2-yr FI (biennial), long term FI (triennial at Ukulinga and quinquennial at Brotherton) and unburned control (no burn), with burning taking place in spring (August). At Ukulinga, plots are 251 m² and replicated three times according to slope position in a randomized blocks design (Abdalla et al., 2016; Kirkman et al., 2014). At Brotherton, burning treatments are applied to a plot of 625m² and replicated four times in a randomized blocks design (Manson et al., 2007; Gordijn et al., 2018)(figure 3). As a result of the absence of disturbances since the initiation of the experiment, the no-burn plots at Ukulinga are densely covered with woody species (predominantly *Acacia siberiana*) and could not be used to test the response of grass species in this study (Abdalla et al., 2016).

Additional to the fire manipulation experiments, passive warming experiments were set up at Ukulinga and Brotherton, in the year 2019 and 2017 respectively. Warming chambers passively increase the air temperature and subsequently minimize unwanted ecological effects compared to active warming systems (Marion et al., 1997). However, inevitable alterations of other factors in the field need to be considered, like changes in wind, pollination potential and altered soil moisture and relative humidity (Marion et al., 1997). Various studies performed on the ecological response to passive temperature increase using OTCs show an approximate relative air temperature increase ranging between $\pm 0.8 - \pm 1.7$ °C (Hudson, Henry, & Cornwell, 2011; Marion et al., 1997; Zhang et al., 2015). For this study, hexagon open top warming chambers (OTCs) are situated in the spring burn plots including all replicate plots. Figure 3 shows the experimental treatment design used in this study, involving a combination of burning and passive warming treatments, leading to 6 and 8 treatments at Ukulinga and Brotherton respectively.



FIRE TREATMENT: BURNING RETURN INTERVALS

Figure 3. Schematic overview of experimental treatment design for both study locations, Ukulinga contains 6 treatments, 3 levels of fire treatments (annual, biennial and triennial) and 2 nested warming treatments (ambient and warming), replicated 3 times. Brotherton contains 8 treatments, 4 levels of fire treatments (annual, biennial, quinquennial & no-burn) and 2 nested warming treatments (ambient and warming), replicated 4 times.

SPECIES SELECTION

Two selection procedures were undertaken, one selection of species for the calculation of community traits and one selection of species for species-specific trait data analysis. Firstly, per location and per treatment, a selection of dominant grass species was made (table 3) to calculate the community traits in order to identify net community responses to disturbances. Hereby, data on species composition for both locations (2018 data for Brotherton, 2018 data for Ukulinga) was used. This selection was made aiming on choosing species with the highest relative abundance per plot to allow calculation of community traits. Species were selected that collectively make up for 80% of the cumulative relative abundance per plot, following guidelines of Lavorel & Garnier (2002) and Pérez-Harguindeguy et al., (2013). This procedure aligns with the 'biomass ratio hypothesis' stating that the response of a community to environmental changes is equivalent to the response of the most dominant species present in the community (Grime, 1998). Secondly, species were selected that were present in all the fire treatments, to assure the possibility of species-specific trait comparison, since not all species are present in all treatment plots (table 3).

Of the selected species, the cumulative relative abundance in each replicate plot per fire treatment (annual, biennial, triennial/quinquennial, no burn) was calculated and averaged over the replicate plots (Appendix A.1 & A.2). Hereby, it was assumed that the most dominant species inside and outside the OTCs are similar. The cumulative relative abundance of the selected species per fire treatment per location is found in Appendix A.3 and on average make up 88,87 % of total relative abundance. In total, 7 and 6 species were sampled at Ukulinga and Brotherton respectively (table 3, Appendix figure 24).

UKULINGA RESEARCH FARM		BROTHERTO	NRESEARCH TRAIL
Code	Scientific name	Code	Scientific name
Eracur	Eragrostis curvula *	Thetri	Themeda triandra *
Thetri	Themeda triandra	Trileu	Trystachya leucothrix *
Ariju	Aristida junciformis *	Harfal	Harpochlia falx
Hetcon	Heteropogon contortus *	Коесар	Koeleria capensis
Trileu	Trystachya leucothrix *	Hetcon	Heteropogon contortus
Cymval	Cymbopogon validus	Stibalo	Stiburus alopecuroides *
Diham	Diheteropogon amplectens		

Table 3. List of sampled species per study location, sorted from most dominant to least dominant based on cumulative relative abundance per location. (*) indicate species that were used for species-specific trait analysis and are present at all 6 and 8 treatment levels for Ukulinga and Brotherton respectively.

TRAIT MEASUREMENTS

Plant trait data was collected in January and February 2020 on both study locations. At least 5 tufts per selected species were sampled randomly across the replicate plots for each of the six and eight treatments at Ukulinga and Brotherton respectively (figure 3). Tufts were sampled from reproductively mature, healthy looking individuals (Pérez-Harguindeguy et al., 2013). In the field, for each tuft the following traits were measured: the leaf table height (LTH) measured as the height under which 80% of the plants leaves are estimated to occur (O'Reagain, 1993) and culm height (CH) measured as the distance of ground level to the tip of the inflorescences (Solofondranohatra et al., 2018), both measured with a tape measure in cm. Additionally, the number of inflorescences (NI) was counted per tuft.

Of each tuft, five leaves were collected, cooled and analyzed in the lab. It was assured that collected leaves were healthy looking and unshaded (Pérez-Harguindeguy et al., 2013). In total, 1120 and 1075 leaves were collected and analyzed at Ukulinga and Brotherton respectively. In the lab, the leaf area (LA), of each leaf was measured with a leaf area analyzer (LI-COR, LI-3000C). Furthermore, the fresh weight (FW) and dry weight (DW) of the five leaves per individual tuft were measured using a precision balance. Leaves were dried in the oven (60°C) for at least 48h, following University of Kwazulu-Natal's (UKZN) procedure (Pérez-Harguindeguy et al., 2013). Specific leaf area (SLA), one-sided area of fresh leaf/oven dry mass of leaf, and the leaf dry mass content (LDMC), oven-dry mass of leaf/ fresh leaf mass, were calculated and averaged for each tuft.

Due to time limitation, the trait biomass ratio was only calculated for four dominant species at URF. Four dominant species were selected according to their presence in all the three fire treatments: *H. concortus, T. Leucothrix, E. curvula and A. juinciformis.* Per species, aboveground biomass of 5 tufts was clipped and separated into all material above 10 cm and material between the soil surface and 10 cm (Gao & Schwilk, 2018). Tuft material was then cooled and transported to the lab, where the samples were dried in the oven (60°C) for at least 48h, following UKZN procedure. Biomass ratio per tuft was calculated as the ratio of dry mass of material above 10 cm to the dry mass of material below 10 cm.

STATISTICAL ANALYSES

Functional trait data from both locations were analyzed separately following the same two procedures for community trait data comparisons and species-specific comparisons, in order to asses functional shifts in response to fire frequencies and warming. For both locations, community aggregated trait values were calculated per trait and per treatment using community composition data (appendix A). Hereby, for each trait and per species, mean values present in the community were weighed according to the relative abundance of a species in that community, resulting in 5 community traits values (LTH, CH, NI, SLA, LDMC) per treatment (Lavorel & Garnier, 2002; Pérez-Harguindeguy et al., 2013).

When testing the response of plant communities to disturbances, manipulation experiments are widely used among researches and rest on the assumption that observational and experimental results are complementary (Wolkovich et al., 2012). Because of the experimental nature and to test for significant shifts in community and species-specific trait values associated with the fire frequencies, warming or an interactive effect, a factorial model approach was used to analyze results. Subsequently,

as fire frequency can be seen both as a factorial or continuous variable, also a continuous model approach was used. In the factorial design approach for the community data, a linear mixed effect model was used, with fixed-effects terms for fire and warming treatments, and random effects terms for block. The random effects term was included to account for the randomized-block sampling design, to allow site to site comparisons while not taking into account the natural variability of other environmental factors.

For community data analysis, a univariate two-way ANOVA model was used, and for the speciesspecific data, a three-way ANOVA analysis was done, adding species as a third fixed effect. For both the community as for species-specific data, pairwise group differences were identified using a *post hoc* Tukey HSD test. For the continuous model approach, linear regressions were used on both community and species-specific data.

Per location, species-specific trait data was used only of species that were present in all treatment plots (table 3). Statistical analyses were conducted in Mathworks MATLAB 2019b, specifying the type of model as 'interaction' to allow computation of statistics for the null hypotheses on the main effects (2 for community data and 3 for species-specific data) and the two- factor interactions (fire*temp). An overview of the methodology for this research is found in the technical design (figure 17 in the appendix).

GROWTH RELATED TRAITS: LTH, SLA & LDMC

UKULINGA

COMMUNITY TRAITS

Ukulinga ANOVA results indicate that fire significantly affects the community SLA and LDMC (table 4), with community SLA decreasing and LDMC increasing with an increased fire-return interval, independent of the temperature treatment (figure 4). No significant effect of warming, nor an interaction effect between fire*temp was found. There was a significant block effect for community SLA (p-value<0.001, F: 25.16) and LDMC (p-value: <0.001 & F:41.82) indicating an underlying environmental gradient that has a non-neglectable effect on the plant response to the treatment.



Table 4. ANOVA output for Ukulinga community trait data for the traits: leaf table height (LTH), specific leaf area (SLA) and leaf dry matter content (LDMC) with block as random effect. Significant effects are in bold, according to a 95% significance interval (P<0.005).

Figure 4. Boxplots of Ukulinga community trait data for LTH, SLA and LDMC Ukulinga. Boxplot range indicate the averages per block.

Although ANOVA results did not show any significant effect of fire on LTH, looking at the data with fire response as a continuous variable and temperature as a grouping variable in a linear regression, LTH in the ambient plots shows a significant positive relation with fire-return interval (figure 5). Corresponding to ANOVA output, linear regression found a significant negative relation for SLA ambient data, and a significant position relation for LDMC for both ambient and warming data (figure 5). In contrast to ANOVA results, linear regression output shows a different response to fire between warming treatments, as all traits show a significant effect for ambient data, but no significant effect for the warming data (figure 5).



Figure 5. Linear regression output showing 1. Scatterplot of Ukulinga mean community trait data per block, divided in groups ambient and warming, over the 3 different fire treatments and 2. Linear regression fit line with corresponding R² and p values.

SPECIES-SPECIFIC TRAITS

Species-specific trait analysis was done for the 4 most dominant species of Ukulinga, which are present at all the fire treatments: *T. triandra*, *H. contortus*, *T. leucotrix* & *E. curvula* (see table 5; figure 18 in appendix for boxplots of trait data per species).

Table 5. Summary table of Ukulinga species-specific trait data, with in bold the averages of all species and species with (*) are listed as most dominant and present in all fire treatments.

				SLA (cm²/g)				LDMC (mg/g)				
	Mean	Stdev	Min	Max	Mean	Stdev	Min	Max	Mean	Stdev	Min	Max
Ukulinga	43,82	18,10	12,50	102,00	123,87	36,83	38,86	302,00	423,21	81,17	30,11	840,62
A. junciformis	52,25	11,65	30,00	79,00	82,36	41,76	38,86	296,37	470,14	107,13	199,23	840,62
C. validus	75,36	11,72	54,00	102,00	117,08	19,00	80,51	144,70	377,61	42,32	294,40	461,29
D. amplectens	36,55	15,84	24,00	79,00	139,01	21,92	120,86	193,81	333,43	23,35	315,53	392,86
E. curvula*	58,03	14,46	38,00	101,00	115,69	14,85	86,46	142,20	484,92	48,35	362,51	579,48
H. contortus*	26,43	8,77	12,50	47,00	151,54	32,78	102,58	302,00	416,93	45,53	277,00	517,05
T. triandra*	36,20	6,53	25,00	50,00	145,22	31,59	62,56	233,12	443,70	65,87	283,66	718,67
T. leucothrix*	35,34	8,74	19,00	54,00	122,46	26,37	82,55	207,84	362,33	64,46	30,11	476,65

ANOVA results show a significant positive relation between the overall LTH and fire-return interval for the four most dominant species (table 6 & figure 6, first graph). A post-hoc test reveals that the mean LTH values for annual- and triennial burned plots are significantly different, although biennial does not significantly differ with annual or triennial burned plots (figure 6, graph *a*). Post hoc analysis also shows that the increase in LTH group means per fire treatment is significant only for the individual species *H. contortus*.

Table 6. ANOVA output for Ukulinga species-specific trait data for the traits: leaf table height (LTH), specific leaf area (SLA) and leaf dry matter content (LDMC) with block as random effect. Significant effects are in bold, according to a 95% significance interval (P<0.005).

		LTH			SLA	LDMC		
	Df	F	р	F	р	F	р	
fire	2	20.82	0.014	1.1	0.427	1.68	0.301	
temp	1	0.23	0.682	23.97	0.234	2.06	0.316	
species	3	37.8	<0.001	6.31	0.033	38.45	0.001	
fire*temp	2	1.07	0.347	1.46	0.237	0.35	0.706	
fire*species	6	1.62	0.146	5.3	<0.001	1.56	0.165	
temp* species	3	1.51	0.218	3.25	0.024	0.08	0.970	
residual	128							
total	159							

Furthermore, ANOVA analysis show that species affects all traits LTH, SLA and LDMC significantly, pointing out significant different responses between species (figure 6). For SLA, the effect of fire and temperature is significantly dependent on species (graph b & e figure 6). Post-hoc analysis shows that the mean SLA of *T. leucothrix* differs significantly between annual and triennial (p= 0.0315), but the other three species do not show a significant trend (graph *b* figure 6). For the effect of temperature, a post hoc test shows that only the mean SLA of *T. triandra* differs significantly between ambient and warming (p= 0.0247) (graph *e* figure 6). A significant block effect is found for all traits, and some interactions (LTH: temp*rep & species*rep, SLA: species*rep, LDMC: fire*rep), indicating spatial variability among environmental variables.



Figure 6. Interaction plots for Ukulinga species-specific trait data. Graph (a), (b) & (c) shows the interaction plots of fire for LTH, SLA and LDMC over the three fire treatments for the four most dominant species. Graph (a) shows the significant effect of fire on LTH (*). Graphs (b) shows the significant interaction effect of fire*species. All graphs show the significant effect of species. Single (*) shows significant post hoc results per species. Graph (d), (e) & (f) show the interaction plot of warming for LTH, SLA and LDMC over the two warming treatments for the four most dominant species. All graphs show the significant effect of species, graph (e) shows the significant interaction of temp*species. Single (*) show significant post hoc results per species.

Corresponding to ANOVA results, linear regression output shows significant positive relations between the LTH and fire-return interval of each of the 4 most dominant species, looking at the ambient data (figure 7). Furthermore, the linear regression shows a positive significant effect for SLA and fire-return interval measured on *T. leucothrix* for warming data and a negative significant effect for SLA and FI measured on *H. contortus* for warming data (figure 7).



Figure 7. Linear regression output Ukulinga species-specific data for the traits LTH and SLA, showing the four most dominant species (Thetri: *T. triandra*, Hetcon: *H. contortus*, Trileu: *T. leucothrix*, Eracur: *E. curvula*) divided into warming treatments groups: ambient and warming. Each graph shows trait data over the 3 blocks (scatter) including a linear regression fit-line with R² and P-values.

BROTHERTON

COMMUNITY TRAITS

Brotherton ANOVA output shows a positive significant effect between community LTH and fire-return interval and both community SLA and community LDMC show an overall significant negative relation with fire-return interval (table 7, figure 9). Both SLA and LDMC increase from annual to biennial and decrease from biennial to no burn. Furthermore, warming shows to significantly affect the community LDMC (table 7). The significant effect of fire on community SLA is also evident in the post-hoc analysis, as most group means differ significantly from each other over the different fire treatments, although the group means do not seem to differ between temperature treatments (figure 9). Furthermore, post hoc analysis reveal that the difference between community LDMC group means over fire treatments is only significantly for the ambient data. A significant block effect is found for the effects of fire and temperature on community LTH data (fire*rep: F-value:41.1 p-value: <0.001, temp*rep: F-value:25.46 p-value: 0.001).

	-	-		-	-			
		LTH			SLA	LDMC		
	Df	F	р	F	р	F	р	
fire	3	8.06	0.016	35.34	<0.001	36.13	<0.001	
temp	1	0.09	0.794	0.08	0.802	22.45	0.042	
fire* temp	3	39.18	<0.001	0.42	0.745	2.82	0.129	
residual	6							
total	23							

Table 7. ANOVA output for Brotherton community trait data for the traits leaf table height (LTH), specific leaf area (SLA) and leaf dry matter content (LDMC) with rep as random effect. Significant effects are in bold, according to a 95% significance interval (P<0.005).



Figure 8. Boxplot visualization of Brotherton community traits over all fire treatments, divided in ambient and warming. Boxplot range indicate averages per block. Compact letter displays indicate the significance of group mean pair-wise comparisons: means sharing a letter are not significantly different.

ANOVA results show a significant interaction effect of fire*temp for community LTH, meaning that the effect of fire is dependent on the warming treatment (table 7, figure 8). Post-hoc Tukey test shows that the LTH differences between fire treatments are all significant for the warming data, whether ambient data only shows a significant relation between annual burned plots and the groups biennial, quinquennial and no-burn plots (see figure 8 & 9).



Figure 9. Interaction plot of Brotherton community LTH. The first figure shows the response of LTH to fire the 4 fire treatments, with the data grouped in ambient and warming data. The second figure shows the response of LTH to temperature treatments, grouped per fire treatment.

Similar to ANOVA results, linear regression outputs show significant positive relations between the community LTH and fire-return interval for both ambient and warming data (figure 10). For SLA and LDMC, linear regression analysis indicate different responses to fire treatments between the temperature treatment groups. SLA decreases significantly with increasing fire-return intervals only for the warming data, ambient data shows a similar trend albeit not significant (figure 10). The opposite is seen for LDMC, as LDMC decreases with increasing fire-return intervals only for the ambient data (figure 10).



Figure 10. Linear regression output showing 1. Scatterplot of Brotherton mean community trait data per block, divided in groups ambient and warming, over the 3 different fire treatments and 2. Linear regression fit line with corresponding R² and p values.

SPECIES-SPECIFIC TRAITS

Species-specific trait data analysis is done on the 3 most dominant species of Brotherton, which are present at all the fire treatments: *T. triandra, T. leucothrix & S. alopecuroides* (see table 8; figure 19 in appendix for boxplots of trait data per species).

	Mean	Stdev	Min	Max	Mean	Stdev	Min	Max	Mean	Stdev	Min	Max
Brotherton	22,40	8,75	7,00	49,00	118,18	51,94	3,75	313,52	420,09	79,24	13,88	873,91
H. flax	32,24	9,61	13,00	49,00	46,53	10,32	3,75	58,60	335,65	57,91	70,98	374,15
H. contortus*	14,35	3,05	9,00	21,50	135,18	34,04	91,76	232,97	438,49	36,25	363,64	500,00
K. capensis	12,21	4,38	7,00	20,00	56,59	11,19	41,40	80,00	387,81	135,77	13,88	490,00
S. alopecuroides	19,39	6,05	8,00	33,00	124,42	30,45	43,88	256,17	432,89	93,31	200,00	592,50
T. triandra*	26,21	6,41	14,00	39,00	174,01	49,39	85,12	313,52	453,11	71,53	376,92	873,91
T. leucothrix*	24,96	7,49	12,00	47,00	96,36	19,53	52,76	172,07	413,05	49,89	264,29	516,67

Table 8. Summary table of Brotherton species-specific trait data, with in bold the averages of all species and species with (*) are listed as most dominant and present in all fire treatments

For Brotherton, ANOVA output shows that fire has a significant positive effect on the LTH of all three most dominant species (table 9; figure 11, first graph *a*).

Table 9. ANOVA output Brotherton species-specific data for the trait leaf table height (LTH), specific leaf area (SLA) and leaf dry matter content (LDMC) with rep as random effect. Significant effects are in bold, according to a 95% significance interval (P<0.005).

		LTH			SLA	LDMC		
	Df	F	р	F	р	F	р	
fire	3	22.5	0.001	0.63	0.626	0.75	0.567	
temp	1	0.32	0.630	3.38	0.437	0.09	0.806	
species	2	46.97	0.002	105.99	0.002	3.11	0.168	
fire*temp	3	1.81	0.151	0.54	0.657	1.73	0.164	
fire*species	6	0.37	0.898	0.31	0.932	1.56	0.166	
temp* species	2	0.1	0.905	0.04	0.963	0.28	0.759	
residual	110							
total	141							

Furthermore, species type affects the traits LTH and SLA significantly, pointing out different responses per species (table 9; figure 11, graph *a*). No interaction effect fire*temp was found for the Brotherton data. Furthermore, a significant block effect is found for temperature on LTH data (F-value:2.31 p-value: <0.039)



Figure 11. Interaction plots of Brotherton species-specific data. Graph (a), (b) & (c) show the effect of fire on LTH, SLA & LDMC for the three most dominant species. Graph (a) showing the significant effect of fire and species on LTH. Graph (b) showing the significant effect of species on SLA. Graphs (d), (e) & (f) show the effect of warming on LTH, SLA & LDMC. No effect is significant.

Supplementing ANOVA output, linear regression output shows significant positive relations between the LTH and fire-return interval of each of the three most dominant species for both the ambient and warming data (with all p-values: <0.002) (figure 12).



Figure 12. Linear regression output Brotherton species-specific data for the trait LTH, showing the three most dominant species (Thetri: *T. triandra*, Trileu: *T. leucothrix*, Stibalo: *S. alopecuroides*) divided into warming treatments groups: ambient and warming. Each graph shows trait data over the 3 blocks including a linear regression fit-line with R² and P-values.

BIOMASS RATIO

UKULINGA

SPECIES-SPECIFIC TRAITS

Biomass ratio was measured at Ukulinga, only on the ambient data, to investigate the effect of fire (figure 13). Linear regression output show that the biomass ratio of *A. junciformis* and *H. contortus* is positively and significantly related to fire-return interval (R2: 0.52 & p-value: 0.002 and R2: 0.265 & p-value: 0.04 respectively).



Figure 13. Ukulinga: Biomass ratio of *H. contortus, T. leucothrix, E. curvula & A. junciformis,* measured only in ambient plots. First graph is boxplot with variation of 5 tufts that were clipped per species per treatment. Second graph is linear regression output with A. junciformis and H. contortus showing significant positive relation with fire-return interval.

CULM HEIGHT & NUMBER OF INFLORESCENCES

UKULINGA

SPECIES-SPECIFIC TRAITS

No significant interactions were found in the community CH & NI data for Ukulinga (see Appendix table 19 for ANOVA output table), opposing the species-specific data. The culm height of Ukulinga's four most dominant species is significantly different for each species (see first graph figure 14). While the CH of *T. triandra*, *H. contortus* and *T. leucothrix* overall increases with an increased fire-return interval, the CH of *E. curvula* decreases. As an effect of warming, both traits decrease, albeit not significantly. Furthermore, the effect of fire and temperature on the CH and NI are significantly different for each species (table 10). A significant block effect is seen for fire with both traits (both with p-value<0.001).

Table 10. ANOVA output of Ukulinga species-specific data for traits: culm height (CH) and number of inflorescences (NI). Significant effects are in bold, according to a 95% significance interval (P<0.005).

				NI	
	Df	F	р	F	р
fire	2	1.6	0.313	1.17	0.401
temp	1	12.26	0.077	8.41	0.133
species	3	32.71	<0.001	3.31	0.105
fire*temp	2	0.82	0.446	0.34	0.714
fire*species	6	8.12	0	4.38	<0.001
temp* species	3	4.3	<0.001	6.15	0.003
erro	r 128				
tota	/ 159				



Figure 14. Ukulinga community traits interaction plot showing interaction species*fire & species*temp for CH and NI.

BROTHERTON

COMMUNITY TRAITS

For Brotherton's community NI data, the interaction fire*temp is significant (table 12). NI measured inside the OCTs have a larger variation over the fire treatments, driven by an explicitly high number of inflorescences in the biennial burned plots. NI measured on ambient data shows a steady trend across fire treatments (figure 15). Furthermore, for NI a significant block effect is seen for fire (p-value: 0.033).

Table 10. ANOVA output for Brotherton community trait data culm height (CH) & number	
of inflorescences (NI).	

		СН			NI
	Df	F	р	F	р
fire	3	2.58	0.149	1.45	0.318
temp	1	4.76	0.1608	0.93	0.436
fire* temp	3	0.52	0.6821	0.051	0.051
error	6				
total	23				



SPECIES- SPECIFIC TRAITS

ANOVA analysis shows a significant effect of species for the trait CH (table 13). Also, the interactions fire*species and temp*species are significant for CH, meaning that the effect of the treatments is dependent on the species type (figure 16). For NI, each species has a different response on fire treatments, as the interaction fire*species is significant (table 13).

		СН				NI
		Df	F	р	F	р
fire		3	7.66	0.077	0.61	0.636
temp		1	3.14	0.232	0.06	0.832
species		2	654.46	0.006	6.22	0.075
fire*temp		3	1.86	0.141	1.58	0.198
fire*species		6	19.87	0	3.15	0.006
temp* species		2	4.66	0.011	0.07	0.935
	error	109				
	total	140				

Table 11. ANOVA output for Brotherton of species-specific traits culm height (CH) & number inflorescences (NI).



Figure 16. Interaction plot of fire*species for species-specific data Brotherton

DISCUSSION

In this study, the effect of fire frequency and temperature on the growth of plants in South African grassland communities was investigated, using a trait-based approach. Plant traits were measured in two long-term fire experiments in combination with passive warming systems at two different locations: Ukulinga and Brotherton. For LTH it can be concluded that decreasing fire frequencies result in both compositional changes with a selection on larger species and an increase of the height of individual species at both study locations. Furthermore, at both study locations it was found that community SLA decreases with less frequent fires, although at Ukulinga, species-specific SLA changes over fire treatments were divergent and thus indicate a compositional shift to communities with slowgrowing species in the fire exclusion treatments. Fire frequency affected community LDMC differently at the two study locations. At Ukulinga, investment in carbon content in leaf to support for erect and upright tufts led to increasing LDMC trait values with decreasing fire frequencies. While at Brotherton, a significant decrease in community LDMC and divergent narrow range changes in species-specific LDMC were detected. Taking into account results from both study locations, it is indicated that increasing fire frequencies result in communities with relatively large, slow-growing species (high LTH, low SLA and high LDMC). Furthermore, biomass ratio increased significantly with a decreasing fire frequency for species A. junciformis and H. contortus. At Ukulinga, warming did not have a significant effect on community nor species-specific traits and no interaction between fire and warming was found. At Brotherton, warming had a significant effect on LDMC and an interactive effect for fire*warming was found for LTH, indicating that LTH increase less in the warming treatment plots compared to ambient plots. CH and NI were not affected significantly by fire nor warming at both study locations. In the following paragraphs the results will be discussed according to the sub-questions.

THE EFFECT OF FIRE

LEAF TABLE HEIGHT

Results of this study show a selection for larger species in the absence of fire at both study locations, which is in accordance with my first hypothesis that frequent fire would result in lower community LTH. Fire return interval significantly increases community LTH at Brotherton, while at Ukulinga only a significant effect was found for ambient data. The latter is most likely caused by the high variation of LTH between the blocks for warming data in annual and triennial burned plots, as a result of absence of all dominant species inside the OCTs that were selected to calculate community weighed trait values. In the annual burned plots, the relatively large species C. validus exlusively dominated the OCT in block three, whereas in the triannual burned plots only the relatively short species H. contortus and T. triandra were present in the OTC in block two. Although the presence of the relatively large species C. Validus at the annual burned plots in block 3 was likely the result of underlaying soil properties, the domination of 1 species in general is more likely to occur in frequently disturbed environments as species richness tends to decrease with increasing disturbance (Forrestel et al., 2014). Looking at ambient data, results of this study indicate a trend in line with multiple studies that investigated grass trait response over a disturbance regime and found that with the absence of disturbances, species are replaced by more shade tolerant, slow growing, and large species (Fynn, Morris, Ward, & Kirkman, 2011; Fynn et al., 2005; Kirkman et al., 2014).

Results of this study show that the increasing community plant height due to fire absence is not only a result of compositional changes, but rather a result of increasing plant height of individual species with the absence of fire. At both study locations fire return interval significantly increases the height of all dominant species, but the degree to which plants grow in height is different per species. Although the increase in height when burning frequencies are low is most likely related to a longer life span, species might also increase in height as a result of a competitive strategy (Moles et al., 2009). Hempson et al. (2019) emphasizes that grasses in fire prone environments outcompete other grasses by allocating the light environment, resulting in upright and tall species. When burning frequencies are low, fuel beds of dead biomass increase in height and obstruct light at ground level to which species need to grow taller to maintain photosynthetic ability. Tall and erect tuft forms require high C:N ratios, indicating high LDMC values, to provide structural support and in turn decompose slower, contributing to dead biomass fuel beds in infrequent disturbed environments (Hempson et al., 2019). Inversely, species that dominate frequently disturbed environments are mostly short species with traits associated with rapid post-fire regeneration (Kirkman et al., 2014). Therefore, accompanying trends that are found in LTH data, it is necessary to look at fire related traits SLA and LDMC, which are discussed in the following paragraphs

SPECIFIC LEAF AREA & LEAF DRY MATTER CONTENT

It was hypothesized that SLA decreases and LDMC increases with the absence of fire. In line with the hypothesis, results of this study show a significant negative effect of fire on community SLA with increasing fire-return interval at both study locations. Although the overall effect shows a decrease in SLA with the absence of fire, species-specific analyses indicate that species respond differently as the SLA of some species decrease with the absence of fire, whereas the SLA of others increase. This implies compositional community trait changes with a selection on species with a lower SLA when burning frequencies decrease. For example, at Ukulinga the species *A. junciformis* has relatively low SLA and high LDMC values (table 5) and increases in relative abundance with an increasing fire-return interval (0% in annual, 7% in biennial and 56% relative abundance in triennial burned plots). Similarly, the community LDMC at Ukulinga increases with increasing fire-return interval but species-specific LDMC show divergent responses over the fire treatments. Compositional changes at both study location imply a community shift towards species with increased toughness of leaves with the absence of disturbances, increasing the ability of leaves to penetrate accumulated litter (Wright et al., 2004).

Following the hypothesis, at Ukulinga, the community SLA and LDMC are inversely related. However, at Brotherton the community LDMC decreases with an increased fire-return interval, showing a similar trend to SLA and thus opposing the hypothesis. Although SLA and LDMC tend to be inversely related, this relation is plant and environment depended (Pérez-Harguindeguy et al., 2013). In general, species tend to be shorter with increased altitude and related increase in weather extremes (Moles et al., 2009). This study confirms this trend as averaged LTH of species at BRT is 53,4 % shorter than averaged LTH of species at Ukulinga (21 cm and 46 cm respectively) (table 5; table 8) and could imply less fuel accumulation with the absence of disturbance as a cause for different LDMC values at both study locations. However, the averaged community LDMC values for both study locations do not differ significantly. Another study done on community LDMC as response to different fire frequencies at two study locations, found diverging results that do not indicate a particular trend in community LDMC (Forrestel et al., 2014). Additionally, differences between study locations are visible when looking at the amount of interactions found significant in the ANOVA analyses. At Ukulinga, the effect of fire and

temperature is significantly dependent on the species for SLA, implying more divergent growth strategies per species compared to Brotherton, where all the dominant species show similar trends. Both results might imply a stronger environmental filter due to the altitude and weather conditions at Brotherton compared to Ukulinga, leading to a lower inter-specific trait variation. Several studies describe how abiotic conditions related to altitudinal gradients can act as filter for SLA and LDMC trait variability in plant communities. De Bello et al. (2013) indicate lower diversity at high elevations in the French Alps, and Pescador et al., (2015) found the opposite for a study done in a Spanish high mountain grassland. Water availability and temperature seem to be limiting factors in these studies. Although Brotherton and Ukulinga have substantial different annual mean precipitation and temperature values (table 2), summer rains temporarily limit precipitation differences between the two study areas. Low annual temperatures at Brotherton could, however, imply an abiotic filter leading to lower trait variability at community and species-specific scales (Moles et al., 2009), also limiting the effects fire might have on trait variability.

Species-specific data analyses also indicate different plant strategies concerning investment in carbon content (LDMC) between the two study locations. Although results of community SLA and LDMC are in line with the hypothesis, at Ukulinga, the SLA of T. leucothrix and E. curvula both significantly increase with lower burning frequencies. At Brotherton, fire did not show significant effects on speciesspecific traits LDMC and SLA at all. These findings are opposing the hypothesis that is based on multiple studies that found low SLA values in grassland communities with the absence of fire disturbance, related to low growth rates (Fynn et al., 2011; Fynn et al., 2005; Kirkman et al., 2014). It is well documented that the SLA is dependent on the successional position of plants, and decreases with age and decline in light intensity (Pérez-Harguindeguy et al., 2013; Poorter et al., 2004). High SLA values are found to be important for early successional plants in highly disturbed areas, whereby the high production of leaves with low carbon content increase the competitive advantage over neighboring plants (Poorter et al., 2004). Increasing SLA values of T. leucothrix with less frequent fires could be related to the underground tillering strategy of T. leucothrix, allowing for more plants in early successional stages with higher SLA and subsequently lower LDMC values in unfrequently burned plots compared to other species (Fynn et al., 2005). Opposing T. leucothrix, T. triandra and H. contortus have aboveground nodes from which tillers develop that are more likely to be vulnerable to fire compared to a below-ground tillering strategy (Everson, Everson, & Tainton, 1988).

BIOMASS RATIO

It was hypothesized that biomass ratios, the ratio of canopy biomass above 10 cm to the biomass below 10, will increase in communities with a high frequency of hot fires. This indicates a plant strategy to aggregate biomass above ground to minimize damage to the main basal meristems (Gao & Schwilk, 2018; Hempson et al., 2019). Opposing the hypothesis, *A. junciformis* and *H. contortus* show a significant increase in biomass ratio with decreasing fire frequency meaning that the biomass ratios are expected to reflect a species architectonical strategies in relation to competition for resources such as light and space (Archibald et al., 2019), the increasing life span of the individuals that grow in the low burn frequency communities might have had a positive influence on biomass at the upper part of the plant, in line with increasing LTH values (Gao & Schwilk, 2018; Hempson et al., 2019). Furthermore, increasing competition for light in dense, non-disturbed, communities, could trigger

erect growth forms, also resulting in increasing biomass ratio values with a decrease in fire-return interval (Hempson et al., 2019).

NUMBER OF INFLORESCENCE & CULM HEIGHT

No clear pattern was found at both study locations as the community NI and CH were not significantly affected by fire nor temperature. This is most likely a result of the interreference between the timing of burning and the phenology of the perennial grasses. In order to measure effects of fire on the phenology of grasses, traits should be measured consistently throughout the season, which was not achievable during this study.

THE EFFECT OF WARMING

It was hypothesized that plant height would increase with warming, in line with multiple studies (Hudson et al., 2011; Zhang et al., 2015). However, no significant effect of temperature on community LTH is found at both locations. This might be the effect of unfavorable ecological effects of the warming chambers, like changes in wind, competition for space and light or altered soil moisture and relative humidity and shading (Marion et al., 1997; Snyman et al., 2013). Furthermore, the high variation between blocks of LTH of the warming data in the annual and triennial burned plots at Ukulinga make it hard to distinguish a clear trend (figure 4; figure 8).

Furthermore, it was hypothesized that SLA would increase and LDMC would decrease with warming. However, no significant effect of warming on community and species-specific SLA was found at both study locations, except for *T. triandra* that shows positive significant relation with SLA and warming. Several studies describe an increase in SLA as a response to warming, using similar experimental designs and warming chambers in the tundra (Hudson et al., 2011; Zhang et al., 2015). A study done in the more temperate region also found that temperature increases are most likely to favor species with a higher specific leaf area (Sandel & Dangremond, 2012).

At Brotherton, warming had a significant negative effect on the community LDMC, in line with the hypothesis and findings of a study done in the tundra biome (Bjorkman et al., 2018). Warming affected the LDMC to a stronger extend in frequently burned plots (annual and biennial), compared to infrequently burned plots (quinquennial and no-burn). In the frequently burned plots, the LDMC values inside the warming chambers were substantially, albeit not significant, lower than in the ambient plots (figure 8). This could imply that warming positively affects the growth species in early successional stages more compared to mature species, leading to high SLA and subsequently low LDMC values. However, a research done on community and species-specific recovery after fire disturbance in a Mediterranean shrubland showed that warming slowed down post-fire succession, indicating the thin line between warming and drought (Prieto et al., 2009). Subsequently, other studies did not find a relation between LDMC and warming without taking into account other environmental variables such as water availability (Hudson et al., 2011; Lamarque, Lavorel, Mouchet, & Quétier, 2014).

It was expected that the NI and CH would be positively affected by warming due to the extended flowering season. No significant warming effect is found on NI and CH at both study locations, although at Ukulinga both trait averages are lower inside the OTCs. This could imply a negative effect of warming but is most likely a result of unwanted effects of the warming chambers, like limited space availability.

INTERACTIVE EFFECT OF WARMING AND FIRE

It was hypothesized that warming increases the biomass production in grasslands (Hudson et al., 2011; Zhang et al., 2015), leading to more intense fires and therefore is was expected that the effect of fire on plants traits would be amplified with warming. However, no interactive effect of fire and warming was found at Ukulinga for the community traits in the ANOVA analysis. Looking at linear regression outputs and opposing the hypothesis, for all community traits the ambient data shows significant trends over the fire treatments, but the warming data does not. This might imply that warming levels out the effect fire can have on communities.

The same is seen for species-specific LTH data at Ukulinga and this could imply that the positive effect of warming on growth levels out the positive effect of decreasing fire frequencies on growth, so that LTH differences between fire treatments become less. At Brotherton, an interactive effect between fire and warming for community LTH shows that the effect of warming on LTH is greater in frequently burned plots (annual and biannual) compared to plots with a long fire-return interval (figure 9), as described before. Furthermore, a significant interaction effect was found for Brotherton community NI, as warming data shows a higher variability of NI values across the fire treatments due to an explicitly high NI number in biennial plots.

From these results, it can be argued that plant communities at a higher altitude and thus a lower average temperature are more likely to show interaction effects between warming and fire. The lower annual temperatures at Brotherton could imply a grass community with elevational specialization leading to reduced thermal tolerance. This could indicate a limited capacity to survive heat waves as a result of climate change, compared to Ukulinga (Laurance et al., 2011).

To investigate the interactive effects of warming and fire, it is important to take into account other environmental variables such as water availability and atmospheric CO_2 concentrations (Jin et al., 2011) as it is well documented that high atmospheric temperatures in combination with water deficits can lead to increased wildfire occurrences (Jolly et al., 2015).

RESEARCH LIMITATIONS

Field-based studies allow for mechanistically testing for plant trait responses to isolated fire and warming treatments that are unachievable using historical observations. The well managed long-term experiments Ukulinga research farm and Brotherton research trail gave opportunity to investigate plant responses to fire and warming treatments simultaneously at two different locations. However, the experimental set-up of this study does inevitably lean on several factors that could have influenced the validity of this research. First of all, an issue of scale may have influenced the results of this study as the sampling design is based on the species composition data of 2018, assuming similar dominant species inside and outside the warming chambers. However, since the warming chambers only occupy a small area, compared to the whole plot, not all dominant species were present in all the warming chambers. This however, only seem to be of influence in the annual burned data for Ukulinga as the species *C. Validus* is calculated to occupy 15% of relative abundance of the annual burned plot in block 3, but in the warming chamber in the same plot this species occupied 100% of the relative abundance. These potential errors were also transferred to the community trait data, as relative abundance per species was calculated using the species composition data. Thus, the community trait data gives a good

overview of how community traits respond to disturbances since the functioning of ecosystems is to a large extent determined by the trait values of the dominant species (Garnier et al., 2001). However, this method is dependent on reliable community data that was harder to calculate for the warming chambers due to the scale issue. A use of weighed trait data infers the potential of balancing out species-specific trends occurring over treatments. By simultaneously looking at species-specific data tackled this problem was tackled and gave insight in whether changes occurred due to compositional changes or trough species-specific trait adaptation. Investigating community and species-specific traits simultaneously also gave the opportunity to study to what extend individual species contribute to community responses.

In a shifting climatic regime, the research of plant responses to warming is very important and could be done by using open top warming chambers. Results indicate that warming did not seem to affect functional traits at both study locations. Although open top warming chambers are a widely used to mimic global warming, the unfavorable alterations of environmental factors inside the OCTs, like changes in wind, space limitation, altered soil moisture and relative humidity could have affected the results (Marion et al., 1997). Additionally, repeated destruction of the OCTs due to weather extremes and vandalism during the research period caused temporarily elimination of the OCTs and this might have influenced plant response. Furthermore, since the OCTs only cover a small area and the treatments are only replicated 3 times, a relatively small area of grassland could be sampled to test the effect of warming. Lastly, the warming chambers at Ukulinga have been operative for only a short time frame (OTCs were placed in 2019 and 2017 at Ukulinga and Brotherton respectively), which could have led to underdeveloped responses of plants.

FURTHER RESEARCH AND MANAGEMENT IMPLICATIONS

Knowledge on the response of grassland species to disturbances like fire and warming are crucial for the implementation of successful conservation strategies. To complement results from this study, suggestions for further research include trait monitoring over a longer time frame, preferably covering a whole growing cycle to eliminate the trait differences paired with successional stages rather than treatment responses. Furthermore, to have a more complete understanding of vegetation responses to fire and warming, environmental variables related to climate change such as soil type and moisture availability should be monitored per plot. Lastly, it is suggested to include intra-specific trait variability when determining the response of several species to fire and warming treatments, which was not possible in this study due to time limitation. Intra-specific trait variation describes the natural trait variation of individuals of the same species and provides one of the key prerequisites for natural selection (Bolnick et al., 2011). Quantification of intra-specific trait variation helps to understand to what extent traits change as a response to disturbances. This study provides insights on the response of particular plant traits to fire and temperature, which enables applicability of specific knowledge to a large range of environments (Fynn et al., 2005).

CONCLUSION

This study gives insight on the effect of different fire frequencies and warming including their interactive effect on plant responses at both community and species-specific level. It can be concluded that mesic grassland communities change by selecting for species with high LTH and low SLA values when fire frequencies decrease. Plant strategies for carbon investment measured in LDMC were different between the study locations and highlight the importance of taking into account other environmental variables when looking at plant responses. Overall, fire has substantial effects on mesic grasslands, altering the distribution of resources and creating niches for a high diversity of species. The responses of plants to fire regimes is of crucial importance in order to implement successful conservation strategies. Furthermore, the community LDMC at Brotherton showed a significant response to warming and the interactive effect of warming and fire. Although in this study the effect of fire was more prominent visible than the effects of warming, the effects of increasing temperatures and the effects hereof on plant communities are crucial to understand in the face of climate change.

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APPENDIX

A. Species composition & selection

A.1 Species composition Brotherton Research Trail

Table 12. Species composition at Brotherton: relative abundance of 3/4 most dominant species per plot and cumulative relative abundance averaged per treatment of 4 most dominant species per treatment.

TREATMENT	REP	PLO T ID	NAME SPECIES AND RE	NAME SPECIES AND RELATIVE ABUNDANCE (%)					CE(%)
ANNUAL	А	3	Koecap(37,75)	Hetcon(20,54)	Traspi(16,59)	Trileu(12,98)	Stibalo(2,48)	87,86	
	В	12	Stibalo(43,89)	Koecap(26,09)	Trileu(21,48)	Thetri(5,91)	Hetcon(2,63)	97,37	
	С	21	Koecap(49,13)	Thetri(32,88)	Harfal(7,35)	Trileu(6,94)	Stibalo(1,85)	96,30	
	D	45	Thetri(31,55)	Koecap(30,46)	Trileu(17,70)	Hetcon(14,97)	Harfal(5,33)	94,67	
	Av. 3 r domin	nost Iant:	Koecap(35,86)	Thetri(19,07)	Trileu(14,78)	Hetcon(8,88)	Stibalo(12,06)		92,42
BIENNIAL	А	2	Thetri(40,81)	Hetcon(20,99)	Stibalo(17,35)	Trileu(9,03)		79,13	
	В	15	Thetri(41,21)	Trileu(11,94)	Stibalo(11,68)	Hetcon(8,74)		73,59	
	С	19	Trileu(32,30)	Thetri(25,79)	Hetcon(11,94)	Stibalo(9,03)		79,07	
	D	31	Thetri(65,16)	Trileu(23,02)	Hetcon(7,45)	Stibalo(1,09)		97,82	
	Av. 3 r domin	nost iant:	Thetri(43,24)	Trileu(19,07)	Hetcon(12,29)	Stibalo (9,80)			84,37
QUIN-	А	6	Thetri(63,35)	Harfal(28,09)	Trileu(4,09)	Stibalo(3,60)	Moncer(0,97)	99,13	
QUENNIAL	В	13	Thetri(45,63)	Trileu(34,13)	Hetcon(16,44)	Allsem(2,35)	Allsem(2,53)	98,73	
	С	17	Trileu(47,47)	Thetri(24,83)	Harfal(20,05)	Stibalo(6,81)	Haplosca (0,85)	99,15	
	D	37	Trileu(41,10)	Koecap(21,61)	Thetri(20,28)	Hetcon(15,35)	Harfal(1,17)	98,33	
	Av. 3 r domin	nost iant	Thetri(38,52)	Trileu(31,70)	Harfal(12,33)	Hetcon(7,95)	Stibalo(2,60)		93,10
NO BURN	А	11	Harfal(72,72)	Stibalo(13,70)	Trileu(13,58)	n.a.		99,50	
	В	24	Harfal(81,88)	Trileu(8,99)	Stibalo(6,68)	Thetri(2,45)		100	
	С	35	Harfal(45,83)	Thetri(28,18)	Trileu(22,80)	Stibalo(3,18)		99,99	
	D	44	Harfal(62,32)	Trileu(29,05)	Thetri(5,26)	Allsem(3,36)		99,99	
	Av. 3 r domin	nost iant:	Harfal(65,69)	Trileu(18,61)	Thetri(8,97)	Stibalo (5,89)			99,87

Table 13. Brotherton: List of most dominant species, sorted from most dominant to least dominant based on cumulative abundance across all averaged treatment plots.

SPECIES ABBREVIATION	SPECIES NAME	CUM. REL. ABUNDANCE	DOMINANT IN TREATMENT
THETRI	Themeda triandra	109,8	all
TRILEU	Trystachya leucothrix	84,16	all
HARFAL	Harpochlia falx	65,69	nb
KOECAP	Koeleria capensis	35,86	an
HETCON	Heteropogon contortus	29,12	an, bi, qq
STIBALO	Stiburus alopecuroides	24,46	all

A.2 Species composition Ukulinga research farm

Table 14. Species composition at Ukulinga: relative abundance of 4/8 most dominant species per plot and cumulative relative abundance averaged per treatment of 4 most dominant species per treatment.

TREATMENT	REP	PLOT ID	NAME SPECIES AND	RELATIVE ABUNDANCE	E(%)			CUM COVERAGE (%)
ANNUAL	А	2.1	Diham(24,85)	Trileu(14,79)	Hetcon(14,79)	Thetri(14,20)	Melnis(5,91)	74,56
	В	2.2	Thetri(40,49)	Trileu(15,49)	Ariju(1,41)	Eracur(0,70)		58,10
	С	2.3	Ariju(60,48)	Eracur(12,31)	Thetri(11,66)	Trileu(11,67)		96,11
	Av most dominant		Thetri(23,79)	Hetcon(22,90)	Cymval(15,95)	Trileu(12,30)	Diham(8,80)	83,74
BIENNIAL	А	4.1	Thetri(35,17)	Trileu(25,23)	Ariju(15,29)	Hetcon(6,88)		82,41
	В	4.2	Trileu(54,91)	Thetri(21,97)	Eracur(4,12)	Ariju(2,52)		83,52
	С	4.3	Eracur(22,72)	Hetcon(17,17)	Trileu(14,13)	Ariju(4,47)	Thetri(4,72)	62,79
	Av most dominant		Trileu(31,43)	Thetri(20,54)	Eracur(12,47)	Ariju(7,43)	Hetcon(8,55)	80,41
TRIENNIAL	А	7.1	Ariju(60,48)	Eracur(12,31)	Trileu(11,66)	Thetri(11,66)		96,11
	В	7.2	Ariju(80,21)	Trileu(3,79)	Hetcon(2,04)	Eracur(1,17)		96,21
	С	7.3	Cymval(46,46)	Eracur(20,96)	Ariju(19,55)	Trileu(4,25)		91,22
	Av most dominant		Ariju(56,41)	Cymval(15,49)	Eracur(11,48)	Trileu(6,57)	Hetcon(0,68)	90,63

Table 15. Ukulinga: list of dominant species, sorted from most dominant to least dominant based on cumulative abundance across all averaged treatment plots.

SPECIES ABBREVIATION	SPECIES NAME	CUM ABUNDANCE	DOMINANT IN TREATMENT
ERACUR	Eragrostis curvula	62,49	bi, tri
THETRI	Themeda triandra	58,17	an, bi
ARIJU	Aristida junciformis	41,57	bi, tri
HETCON	Heteropogon contortus	40,11	all
TRILEU	Trystachya leucothrix	27,32	all

A.3 SELECTION OF SAMPLED SPECIES

Table 16. Ukulinga and Brotherton: 4 most dominant species (based on averaged relative abundance per treatment) for both + cumulative relative abundance (CRA) for each treatment.

STUDY SITE	TREATMENT	4 MOST DOMINAN	T SPECIES & THEIR	RELATIVE ABUNDAN	CE (%)		CRA (%)
UKULINGA	Annual	Thetri(23,79)	Hetcon(22,90)	Cymval(15,95)	Trileu(12,30)	Diham(8,80)	83,74
RESEARCH FARM	Biennial	Trileu(31,43)	Thetri(20,54)	Eracur(12,47)	Ariju(7,43)	Hetcon(8,55)	80,41
	Triennial	Ariju(56,41)	Cymval(15,49)	Eracur(11,48)	Trileu(6,57)	Hetcon(0,68)	90,63
BROTHERTON	Annual	Koecap(35,86)	Thetri(19,07)	Trileu(14,78)	Hetcon(8,88)	Stibalo (12,06)	92,42
RESEARCH TRAIL	Biennial	Thetri(43,24)	Trileu(19,07)	Hetcon(12,29)	Stibalo (9,80)		84,37
	Quinquennial	Thetri(38,52)	Trileu(31,70)	Harfal(12,33)	Hetcon(7,95)	Stibalo(2,60)	93,10
	No burn	Harfal(65,69)	Trileu(18,61)	Thetri(8,97)	Stibalo (5,89)		99,98
					Averaged:		88,87

B: TECHNICAL DESIGN



Figure 17. Schematic overview of the technical design of this study and subsequently shows how data collection will be used to eventually answer the research questions.

C: VISUALIZATION BOXPLOT SPECIES-SPECIFIC DATA

UKULINGA RESEARCH FARM



Figure 18. Boxplots of Ukulinga species-specific trait data over all fire treatments, divided into ambient and warming treatments.





Figure 19. Boxplots of Brotherton species-specific trait data over all fire treatments divided into ambient and warming treatments

D: ANOVA OUTPUT COMMUNITY TRAITS CULM HEIGHT & NUMBER OF INFLORESCENCES

UKULINGA RESEARCH FARM

Table 19. ANOVA output of Ukulinga community trait data for culm height (CH) & number of inflorescences (NI).

		СН			NI
	Df	F	р	F	р
fire	2	0.54	0.619	0.46	0.659
temp	1	1.17	0.393	3.41	0.202
fire* temp	2	0.75	0.529	0.12	0.886
error	4				
total	17				

E: LINEAR REGRESSION TABLES

UKULINGA RESEARCH FARM

Table 20. Output linear regression model with data per species for all the fire treatments and selections of warming treatments.

		WARMING TREATMENT	R ²	F	P VALUE	ERROR	в
LTH	Trileu	am	0.2650	9.0126	0.0060	45.0617	14.9167
		wa	-	-	-	-	-
	Unterer	am & wa	0.0981	4.6788	0.0361	70.5326	15.6214
	Hetcon	wa	-	-	-	-	-
		am & wa	0.3536	21.8792	0.0000	50.9426	21.8792
	Eracur	am	0.2168	6.9202	0.0144	116.5691	24.2500
		wa	0.1704	-	0.0072	170 1100	24 7010
	Thetri	am & wa	0.1794	9 3 2 4 6	0.0072	1/6.1188	24.7819
	incur	wa	-	-	-	-	-
		am & wa	0.1366	5.0630	0.0314	37.9481	19.0784
СН	Trileu	am	0.2208	7.0849	0.0134	154.7913	36.0278
		wa	1.0e+03 *	1.0e+03 ~	1.0e+03 ~	2 4434	24.6310
		am & wa	1.0e+03	1.0e+03	1.0e+03	1.0e+03	31.4690
			0.0000	0.0017	0.0002	1.1824	
	Hetcon	am	-	-	-	-	-
		wa am & wa	-	-	-	-	-
	Eracur	am	-	-	-	-	-
		wa	-	-	-	-	-
		am & wa	1.0e+03*	1.0e+03*	1.0e+03*	1.0e+03*	40.0784
	Thetri	am	0.0001	0.0041	0.0000	1.6006	
	mean	wa	-	-	-	-	-
		am & wa	1.0e+03*	1.0e+03*	1.0e+03*	1.0e+03*	28.7252
	-		0.0000	0.0001	0.0008	1.1645	
NI	Irileu	am	-	-	-	-	-
		am & wa	0.1142	5.5443	0.0232	15.0305	1.5619
	Hetcon	am					
		wa					
	Fracur	am & wa	-		-	-	
	Liacui	wa	-	-	-	-	-
		am & wa	-	-	-	-	-
	Thetri	am	-	-		-	-
		wa am ^e wa	-	-	-	-	-
SLA	Trileu	am	-	-	-	-	-
		wa	0.3856	10.0412	0.0060	636.7742	60.3632
		am & wa	0.1599	8.1835	0.0065	597.8605	54.3085
	Hetcon	am	1.0e+03 *	1.0e+03 *	1.0e+03 *	1.0e+03 *	66.5854
		wa	0.2520	5.3906	0.0338	384.1930	62.6313
		am & wa	0.1507	7.0962	0.0111	935.6920	64.7704
	Eracur	am	-	-	-	-	-
		wa am & wa					
	Thetri	am	-	-		-	-
		wa	-	-	-	-	-
	* 11	am & wa	-	-	-	-	-
LDIVIC	Irileu	am	1.0e+03 *	1.0e+03 ~	1.00+03 ~	1.0e+03 * 5.6483	154.6799
		wa	1.0e+03 *	1.0e+03 *	1.0e+03 *	1.0e+03 *	148.5027
			0.0002	0.0028	0.0001	1.5573	
		am & wa	1.0e+03 *	1.0e+03 *	1.0e+03 *	1.0e+03 *	152.2090
	Hetcon	am	0.0001 1.0e+03.*	0.0035 1.0e+03.*	0.0001 1.0e+03.*	3.9277	189 2299
			0.0001	0.0016	0.0002	2.0656	100:LLJJ
		wa	1.0e+03 *	1.0e+03 *	1.0e+03 *	1.0e+03 *	177.3814
		am 8 wa	0.0000	0.0003	0.0006	2.1579	192 7012
		dill & Wa	0.0000	0.0003	0.0006	2.1073	193./913
	Eracur	am	1.0e+03 *	1.0e+03 *	1.0e+03 *	1.0e+03 *	211.4456
			0.0001	0.0036	0.0001	2.7157	
		wa	-	-	-	-	-
		am & wa	1.0e+03 *	1.0e+03 *	1.0e+03 *	1.0e+03 *	201.6634
	Thetri	am	1.0e+03 *	1.0e+03 *	1.0e+03 *	1.0e+03 *	215.1356
			0.0000	0.0003	0.0006	6.0835	
		wa	-	-	-	-	-
		am & wa	1.0e+03*	1.0e+03*	1.0e+03*	1.0e+03*	230.2301
	1		0.0000	0.0010	0.0003	4.3332	

BROTHERTON RESEARCH TRAIL

Table 21. Output linear regression model with Brotherton data per species for all the fire treaments and selections of warming treatments.

		WARMING TREATMENT	D ²	F	DVALLE	EPPOP	B1	82
ITU	Thotri		0.4925	20 5096	0.0002	10 1220	17 5/17	2 6167
L111	meur	aiii w2	0.4825	20.3030	0.0002	22 7870	1/.541/	4 5000
		200 8 W2	0.5475	19 2022	0.0000	22.7873	0 4125	4.5000
	Trileu	am	0.5026	22 2281	0.0000	19 5744	16 1250	3 8083
	Inneu	am	0.3020	22.2201	0.0001	13.3744	10.1250	3.8083
		wa	0.6576	42,2487	0.0000	27.2222	8,7917	6.1917
		am & wa	0.5687	60.6638	0.0000	24,7264	9.1528	
	Hetcon	am	-	-	-	-	-	-
		wa	-			-		
		am & wa	-	-	-	-	-	
	Stibalo	am	0.5973	34.1199	0.0000	15.6075	8.8929	4.1964
		wa	0.4007	12.7030	0.0021	23.7517	9.5128	3.6795
		am & wa	0.5017	44.2957	0.0000	18.6301	6.9723	
СН	Thetri	am	0.3777	13.3505	0.0014	121,9455	39,500	7.3667
		wa	0.4618	18.8749	0.0003	636.8235	-2.6667	20.0167
		am & wa	0.3551	25.3292	0.0000	444.0603	19.8306	
	Trileu	am	0.2903	8.9980	0.0066	866.0193	83.2083	16.1167
		wa	0.5285	24.6564	0.0001	445.0350	67.6667	-19,1250
		am & wa	0.3450	24.2343	0.0000	768,7281	7.5250	
	Hetcon	am	-	-	-	-	-	-
		wa	-	-	-	-	-	-
		am & wa	-	-	-	-	-	-
	Stibalo	am	-	-		-	-	
		wa	-	-	-	-	-	-
		am & wa	-	-		-	-	
NI	Thetri	am	-	-	-	-	-	
		wa	0.3550	12.1069	0.0021	12,9189	-1.5833	2.2833
		am & wa	0.1986	11.3965	0.0015	12,7850	1.7389	
	Trileu	am	0.3640	12,5905	0.0018	48,9682	17.2500	-4.5333
		wa	-	-	-	-	-	-
		am & wa	0.1082	5.5813	0.0224	182.1833	1.2722	
	Hetcon	am	-	-	-	-	-	
		wa	-	-	-	-	-	
		am & wa	-	-	-	-	-	
	Stibalo	am	-	-	-	-	-	
		wa	-	-	-	-	-	
		am & wa	-	-	-	-	-	
SLA	Thetri	am	1.0e+03 *	1.0e+03 *	1.0e+03 *	1.0e+03 *	194.8976	-9.5658
			0.0001	0.0013	0.0003	2.0492		
		wa						
		am & wa	1.0e+03 *	1.0e+03 *	1.0e+03 *	1.0e+03 *	57.0205	
			0.0000	0.0009	0.0004	2.4474		
	Trileu	am	-	-	-	-	-	-
		wa						
		am & wa	-	-	-	-	-	
	Hetcon	am	-	-	-	-	-	-
		wa						
		am & wa	1.0e+03 *	1.0e+03 *	1.0e+03	1.0e+03	58.8093	
			0.0000	0.0008	*0.0004	*1.1665		
	Stibalo	am	1.0e+03 *	1.0e+03 *	1.0e+03 *	1.0e+03 *	114.8626	3.5250
			0.0000	0.0003	0.0006	1.1827		
		wa						
		am & wa	-	-	-	-	-	
LDMC	Thetri	am	-	-	-	-	-	-
		wa						
		am & wa	1.0e+03 *	1.0e+03 *	1.0e+03 *	1.0e+03 *	479.8261	10.6881
			0.0000	0.0013	0.0003	5.0784		
	Trileu	am	1.0e+03 *	1.0e+03 *	1.0e+03 *	1.0e+03 *	371.6841	15.1487
			0.0002	0.0067	0.0000	1.0202		
		wa						
		am & wa	1.0e+03 *	1.0e+03 *	1.0e+03 *	1.0e+03 *	392.4343	8.2450
			0.0000	0.0017	0.0002	2.4543		
	Hetcon	am	0.4265	11.8979	0.0033	465.5406	385.1724	21.4844
		wa						
		am & wa	1.0e+03 *	1.0e+03 *	1.0e+03 *	1.0e+03 *	399.7537	19.3661
			0.0002	0.0083	0.0000	1.0879		
	Stibalo	am	1.0e+04 *	1.0e+04 *	1.0e+04 *	1.0e+04 *	449.7579	-4.4627
			0.0000	0.0000	0.0001	1.1011		
		wa						
		am & wa	1.Ue+03 *	1.Ue+03 *	1.Ue+03 *	1.Ue+03 '	487.9062	21.2680
	I		0.0001	0.0029	0.0001	8.3575		

F: QQ PLOTS ANOVA ANALYSES

UKULINGA RESEARCH FARM



Figure 20. QQ plots for Ukulinga community traits.



Figure 21. QQ plots for Ukulinga averaged species-specific data.

BROTHERTON RESEARCH TRAI



Figure 22. QQ plots for Brotherton community data.



Figure 23. QQ plots for Brotherton averaged species-specific data.

G: TESTS FOR NORMAL DISTRIBUTION LINEAR REGRESSION MODELS

		LTH	SLA	LDMC
Ukulinga	Trileu	0	0	1
	Thetri	0	0	1
	Hetcon	0	1	0
	Eracur	0	0	0
	Community	1	0	0
Brotherton	Thetri	0	1	1
	Trileu	0	1	1
	Hetcon	1	1	0
	Community	0	0	0

Table 22. Normal distribution data. Null hypothesis= data comes in normal distribution. H =1 means rejection of null-hypothesis with 0.05% confidence level.

Table 23. Normal distribution residuals of regression Null hypothesis= data comes in normal distribution. H =1 means rejection of null hypothesis with 0.05% confidence level.

Ukulinga	Trileu		0	
	Thetri			
	Hetcon		1	
	Eracur			
	Community	1	1	0
Brotherton	Thetri			
	Trileu			
	Hetcon			
	Community	0	1	0



H: VISUALIZATION OF TRAIT VARIABILITY FOR BOTH STUDY LOCATIONS



Figure 24. Species-specific mean trait values averaged over all treatment for Ukulinga (first graph) and Brotherton (second graph).

I: DIGITAL HERBARIUM



Figure 25. Digital herbarium including part of species collected in this research. From top to bottom: *Eragrostis curvula, Themeda triandra, Hetropogon contortus. Harpochlia falx, Stiburus alopecuroides, Trystachya leucothrix.* Photos from own archive, shot during field work in Jan/Feb 2020.