The effect of altered rainfall seasonality on post-fire recovery of Fynbos and Renosterveld shrublands in the Cape Floristic Region

by

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Thesis presented for the Degree of
DOCTOR OF PHILOSOPHY

in the Department of Biological Sciences
UNIVERSITY OF CAPE TOWN

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Plagiarism Statement

I certify that this thesis is entirely my own work, based on my own experiment, data collection, analyses and interpretation. I have acknowledged all other intellectual material and sources used in the preparation of this document and certify that I have not copied in part or whole or otherwise plagiarised the work of any other persons.

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Acknowledgements

I would like to acknowledge the National Research Foundation, and ACCESS foundations for providing personal and research-related funding. Thank you to the Drie Kuilen Nature Reserve for allowing me to conduct this research on their premises and providing accommodation during the process.

To start, I would like to thank my supervisors, Adam, Timm and Res, for providing a wealth of knowledge and support throughout my research experience. Adam, the enthusiasm and energy you invest into people is second to none and I am lucky to be one of those people. Encouraging me to believe in my own abilities and take pride in my science has been instrumental in my personal and professional development. Timm, your patience and relaxed style of mentorship played a critical role in my research experience and helped me to keep a cool head throughout some of the tougher times. Measuring thousands of plants together is an experience I will not forget any time soon. Res, thank you for your ability to communicate statistics clearly and for your fine attention to detail. Having an enthusiastic connection within the statistical sciences was an invaluable addition to my research experience.

I owe a huge debt of gratitude to everybody on the considerable list of people who helped with field work. This project would not have been possible without you.

To my mom, dad and sister, thank you for your always being there to encourage me and for the long and interesting family discussions. I am truly grateful for a family that encouraged both creativity and critical thinking. Finally, thank you to my wonderful fiancé, Marijke, for her unwavering support throughout my research journey and for never losing patience with my irregular hours and long trips into the field. I am so lucky to have you in my life.

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ABSTRACT:

Shifting climate patterns are a cause for concern for natural ecosystems globally. Of particular concern is the effect of climate change on fire-prone, Mediterranean-type shrublands globally because of the heightened sensitivity of post-fire vegetation to environmental conditions. In this thesis, I focused on investigating the relationships between rainfall seasonality patterns and post-fire vegetation processes in neighbouring Fynbos and Renosterveld shrubland communities within the mega-diverse Cape Floristic Region of South Africa. I investigated vegetation sensitivity to moisture availability at multiple levels of detail including 1) productivity and community structure, 2) growth form responses and 3) physiological performance over three years. Post-fire rainfall patterns were manipulated by artificially increasing summer rainfall and reducing winter rainfall over permanent, field sites, thus reducing annual seasonality and creating soil moisture contrasts between control and treatment plots over warm and cool seasonal periods. At all levels of investigation, post-fire vegetation processes at the Fynbos site were relatively insensitive to variations in moisture availability relative to the Renosterveld site where vegetation processes and community structure were strongly affected. Nutrient limitation and higher soil water potential in coarse, sandstone-derived soils of the Fynbos site could strongly limit the influence of soil moisture patterns on post-fire physiology leading to stable growth, community structure and productivity under a variety of moisture regimes. Soil moisture patterns during the first summer had significant and long-term implications for community structure and productivity patterns in the Renosterveld site, highlighting the sensitivity of vegetation patterns to early post-fire processes. Overall this study demonstrates that post-fire rainfall patterns can have strong effects on vegetation recovery processes but that structurally similar shrublands, which are specialised to differing soil types, could show marked

differences in their response to climate change due to the mediation of climate responses by soils.

CHAPTER 1: General Introduction

1.1. Research rationale

Mediterranean-type, shrubland ecosystems are of great ecological importance because they hold ~20% of the global vascular plant diversity despite only making up ~2% of the Earth's landmass (Cowling et al., 1996). Unfortunately, these complex and diverse ecosystems could be susceptible to anthropogenic climate change and thus attempts to better understand the impact of climate change on these megadiverse ecosystems are of paramount importance.

The factors that influence vegetation structure and function are a primary focus of ecological research globally. Typically, research is focused on key factors including climate patterns, soil types, fire regimes and land-use, which are widely accepted as strong determinants of vegetation patterns (Lavorel *et al.*, 1998; Quillet *et al.*, 2010). However, complex interrelationships and feedback loops exist between the many aspects of these climatic and environmental factors, thus limiting the ability of researchers to understand the relative importance of any single factor. The complexity of natural systems has imposed a significant barrier to predicting vegetation responses to novel environmental and climatic scenarios which is particularly worrying considering the unprecedented, accelerating rates of global temperature increase associated with the Anthropocene (IPCC, 2013; Huntingford *et al.* 2012). Global temperature increases may have variable, complex effects on climate processes at regional scales (Pierce *et al.* 2009). Notably, changing temperatures could alter patterns of rainfall amount and seasonality. This could have potentially negative effects on vegetation patterns, seeing that moisture availability is the primary limiting factors in many vegetation communities. The uncertainty surrounding the vulnerability of vegetation to climate change is thus twofold. Firstly, changes in climate may vary widely from region to region and secondly, we do not have a fully developed mechanistic understanding of ecosystem-climate relationships due to the inherent complexity and regional variation of these relationships.

Therefore, there is an urgent need to develop an integrated understanding of vegetation-climate relationships. Here, focus should be directed toward conceptually linking mechanistic, ecological and evolutionary processes (Rees *et al.*, 2001), and determining how they relate at various spatiotemporal scales.

1.2. Experimentation as a means to investigate natural systems

There is a growing interest in experimental climate studies within Mediterranean-type shrublands globally. A new generation of rainfall manipulation studies have emerged which investigate the effects of the amount and timing of rainfall patterns as well as the interaction between rainfall patterns and other important ecological factors such as fire (e.g. Beier *et al.*, 2012: Parra *et al.*, 2012; West *et al.*, 2012; Esler *et al.*, 2015: Parra & Moreno, 2017, 2018).

The ability to reveal causal relationships between specific climatic or environmental variables and vegetation processes can best be achieved through experiments, particularly those carried out under field conditions (Altwegg *et al.*, 2014). Correlative, observational approaches are generally limited in their ability to reveal causality between response and explanatory variables (Winship & Morgan, 1999). Natural variation in variables may often be insufficient for deriving insights about their effects on measured variables. Therefore, introducing variation into the natural systems through experimental manipulation allows for a greater ability to find the precise functional relationships between variables. Covariation between interlinked variables can be difficult to tease apart in observational studies where experimentation allows for the isolation and manipulation of specific explanatory variables which can help to identify their causal relationships on measured response variables of interest (Resetarits & Bernardo, 2001).

A trade-off between realism and control is an inherent problem for experimentation whereby increased precision and control over variables (often in lab setting) is gained at the expense of realism (Schindler, 1998; Morin, 1998). This is particularly relevant when comparing field and lab experiments (Morin, 1998). Experiments conducted under lab conditions have the advantage of precision and close control of the experimental conditions. Therefore, lab experiments are highly replicable. However, they often do not do well when describing patterns under natural field conditions because the replication of natural conditions in the lab may lack key, unobserved environmental factors which play a critical role in how vegetation functions under normal conditions (Resetaritz and Bernardo, 2001; Finn *et al.*, 2010). Field experiments offer high levels of realism, but because environmental factors are complex and variable in natural ecosystems, confounding site-specific effects on experimental results can be difficult to control and studies can be difficult to compare. Therefore, in field experiments, effort should be made to control for as many confounding factors as possible and to carefully record site-level environmental factors in as much detail as possible so that these effects can be accounted for in the interpretation of the results (e.g. West *et al.*, 2012). Field experiments have the advantage of revealing the actual outcome of a variable being manipulated in natural field conditions (Resetarits & Bernardo, 2001). Logistically, there can often be a trade-off between high replication within a single experimental field site and replication of an experiment over wider spatial scales. This is because setting-up and managing field experiments requires a large investment of time and resources which are often limited. High replication within a single experimental site reduces the potential for confounding environmental variables to influence the experiment because conditions within a single site are more similar than across a range of spatially separated sites. Here, studying fine-scale connections between measured variables at a high level of detail can allow the researcher to derive a strong mechanistic understanding of the relationship between variables that can then be tested beyond the conditions of the experimental site (Resetarits & Bernardo, 2001). In experiments that cover wide spatial scales, less investment into replication within sites may limit the ability to uncover fine-scale mechanistic connections between variables but may be more appropriate for testing whether a hypothesised relationship between variables holds over wider spatial scales.

1.3. Climate change in the Cape Floristic Region

This thesis focuses on the megadiverse, fire-prone Cape Floristic Region (CFR) of South Africa (Bergh *et al.*, 2012) with the aim of developing an integrated understanding of the links between seasonal climate patterns and post-fire vegetation responses. The CFR consists of a mosaic of vegetation types dominated by Fynbos, Renosterveld shrublands. The term, 'Greater Cape Floristic Region' (GCFR), is used when the neighbouring Succulent Karoo biome is also considered. This study does not specifically investigate the Succulent Karoo because it is not fire-prone like Fynbos and Renosterveld. However, the Succulent Karoo is included in the discussions of broad vegetation-climate relationships.

The GCFR boasts around 9000 plant species with ~70% endemism, most of which occur in the winter rainfall zone (Cowling *et al.*, 1996). Fynbos is typically limited to the nutrient poor, sandstone-derived soils while Renosterveld and Succulent Karoo occupy the moderately fertile, clay-rich soils derived from shales (Bergh *et al.* 2014, Bradshaw & Cowling, 2014). These vegetation types all have high species diversity and are floristically distinct from each other (Bergh *et al.* 2014). The climatic associations of different vegetation types are not well understood, although Succulent Karroo tends to dominate hotter, drier climate regions toward the northern reaches of the Western Cape Province and southern Namibian desert. Fynbos and Renosterveld co-occur under milder conditions but are both fire-prone habitats (Bradshaw & Cowling, 2014). Succulent Karoo generally has fuel loads which are too low to sustain fire (Bergh *et al.*, 2014).

Assessing vegetation responses to climate change is a critical area of current ecological research in the GCFR (Midgley *et al.*, 2002;2003; Hannah *et al.*, 2005; West *et al.*, 2012; Esler *et al.*, 2015). However, a clear understanding of how environmental variables affect vegetation processes within these Mediterranean-type shrublands is still lacking. Accordingly, there is a need for experimentation to identify the causal relationships between climate and vegetation process in the GCFR. Despite the importance of experimentation in understanding the causal effects of environmental variables on vegetation patterns, there have been few experimental field studies conducted in the region. One recent study, which experimentally manipulated rainfall patterns in Fynbos, found that amplified summer drought had diverse effects across species and growth forms which could be related to rooting depth (West *et al.*, 2012). My experiment continues along this line of investigation and uses experimental structures to manipulate rainfall seasonality over fixed, field plots. I focus on post-fire vegetation responses to rainfall patterns across two dominant, soil types which are typically associated with different fire-prone shrubland types: Fynbos and Renosterveld.

The intensification of climate extremes and shifts in seasonal patterns have been identified as potential threats to Mediterranean type-ecosystems globally (Hewitson & Crane, 2006; Giorgi & Lionello, 2008; Klausmeyer & Shaw, 2009). Future climate projections for Mediterranean-type ecosystems include hotter, drier extremes, less reliable winter rainfalls and possible increases in summer orographic rainfall events (Tadross et al., 2005; Hewitson & Crane, 2006; IPCC, 2013; Giorgi & Lionello 2008; Altwegg et al., 2014). In Mediterranean-climate regions, climate change projections emphasize the possibility of increased climatic variability, with major droughts or intense rain events becoming more frequent (Cubash et al., 1996). Despite substantial variability and uncertainty in future climate projections, there is some consensus that the patterns of seasonality could change within Mediterranean-type ecosystems globally. It is widely accepted that changes in rainfall seasonality could have profound effects on community structure (Shwinning & Sala, 2004; Chesson *et al.*, 2004).

Changes in the natural seasonal patterns in the GCFR could have wide-scale impacts on the local biodiversity (Midgley *et al.*, 2002; Midgley *et al.*, 2003; Hannah *et al.*, 2005; Altwegg *et al.* 2014). Recent modelling attempts have suggested the potential contraction of species distributions within the Fynbos biome, due climate change, as soon as the year 2050 (Midgley *et al.*, 2003). However, these models are not easily generalisable and do not cover the broad range of species which make up the GCFR. Here, I suggest that developing a broader, biome level approach to understanding vegetation change is required due to the inherent challenges of species-level predictions where diversity is so high. This approach requires the identification and description of generalisable, functional differences between the vegetation types of the region, if any exist.

Vegetation responses to climate are diverse (West *et al.*, 2012; Slingsby *et al.*, 2017) and difficult to investigate because climate is complex and incorporates a multitude of various inter-relating factors. This study focuses on rainfall because moisture availability is of primary importance for plant growth and is predicted to change in the GCFR. Additionally, experimental manipulation of rainfall can be achieved effectively through relatively simple, yet labour intensive, means using exclusion and or water harvesting structures (West *et al.*, 2012; February *et al.*, 2013; Parra *et al.*, 2012; Parra & Moreno, 2018, 2019). In comparison, experiments which manipulate CO_2 (Allen Jr. 1992; Kimbell *et al.*, 1997; Kgope *et al.*, 2010) require much higher expense and infrastructure and those which manipulate temperature are generally limited in the area of coverage of the experimental treatment (Musil *et al.*, 2004).

Seasonal rainfall patterns are a defining feature of the winter rainfall Mediterranean-type regions of the world and have long been associated with high species diversity (Levyns, 1964; Forest *et al.*, 2007; Ackerly *et al.*, 2014;Cowling *et al.*, 2017). Some studies suggest that the relative stability of the Mediterranean climate in the south western range of the Cape throughout the Pleistocene may have contributed to diversity through the increase in speciation and reduction in extinction events (Cowling & Lombard, 2002; Cowling *et al.*, 2017; Colville *et al.*, 2020). This pattern could explain the decline in diversity on the West-East gradient observed as early as the 1930s (Levyns, 1938). However, this focuses on stability of climate and not the role of rainfall seasonality itself. Therefore, there is still very little understanding of the potential mechanistic or evolutionary links between plant function, community composition and seasonal rainfall climate in the region. The corresponding declines in species diversity and winter rainfall seasonality on the west-east gradient in the Cape highlight the need to further investigate the role of rainfall seasonality in determining shrubland community dynamics.

One of the few experimental studies carried out in the GCFR showed that vegetation responses to drought were variable within Fynbos vegetation and influenced by growth form (West *et al.*, 2012). No comparative studies have been carried out in other vegetation types in the region and none have focused on the combination of drought and pulsed rainfall responses, which could form a critical part of vegetation function within the seasonal rainfall zone of the GCFR. Plant responses to moisture pulses have been the focus of arid land ecology which suggest that complex factors may mediate plant responses (Reynolds *et al.*, 2004). I suggest that this focus should be applied to the GCFR in order to study its potential connection with seasonal climate.

1.4. Fire and climate

Of particular concern is the vulnerability of fire-prone shrublands to climate change because of the potentially strong effects of post-fire weather on species emergence patterns (Slingsby *et al.*, 2017) as well as the strong effects of climate on fire-cycles. These effects can have long-term effects on vegetation (Bond & van Wilgen, 1996; Keeley et al. 2005; Batllori et al., 2013; Moreno *et al.*, 2011; Enright et al., 2014; Parra & Moreno 2018). The interaction between fire climate change and disturbance could render fire-prone systems particularly sensitivity to climate change (Slingsby *et al.*,2017). The importance of fire-regimes and their top-down influence on vegetation dynamics has been a central focus of shrubland ecosystem research. Fire is thought to be a major selective influence on vegetation dynamics. Because fires prevent the formation of a climax vegetation state, fire-prone systems are in a state of non-equilibrium and therefore may be particularly sensitive to changes in environmental conditions, especially during stages of post-fire recovery which are most sensitive to environmental conditions. Post-fire populations commonly experience local extinction depending on the particular post-fire conditions (Cowling, 1987). This may be of particular importance because of the potential long-term implications of post-fire species emergence and survival patterns (Bond & van Wilgen, 1996; Keeley *et al.*, 2005; Moreno et al., 2011). Indeed, Fynbos broadly follows the initial floristic composition model (Egler, 1954) which implies that mature vegetation is a subset of the initial species composition that arose after fire and that relatively little secondary recruitment occurs after the first few years after fire (Le Maitre & Midgley, 1992). The strength of the effect of initial conditions remains to be tested. However, it is reasonable to predict that climate change and post-fire weather may have a strong impact on vegetation dynamics which are periodically reset to the recovery stage. The co-occurrence of Fynbos and Renosterveld across most of their range and with similar fuel-loads means that both vegetation types-burn frequently during the regular fire-prone summer drought periods associated with the Mediterranean climate (Kraaij & van Wilgen, 2014). Succulent Karroo generally has a lower fuel load and is thus less prone to fires, despite experiencing warmer temperatures throughout its distribution (Bergh *et al.*, 2014; Bradshaw & Cowling, 2014). Wildfires are typically assumed to be a natural and necessary phenomenon amongst these vegetation types where many plants are adapted to use fire in order to complete their life cycles and to ensure the establishment of new generations.

The potential amplification of vegetation sensitivity to climate in post-fire landscapes means that changes in climate could have more rapid ecological effects in fire-prone vegetation relative to vegetation which is less prone to disturbance. Developing a better understanding of the effects of post-fire weather on key post-fire recovery processes is thus of paramount importance in the megadiverse shrubland ecosystems of the Cape Floristic Region.

1. 5.Thesis layout

Chapter 2: Field manipulation of rainfall seasonality in post-fire Fynbos and Renosterveld study sites

This chapter highlights the details of three-year rainfall manipulation experiment within permanent Fynbos and Renosterveld field study sites. The experiment altered seasonal rainfall patterns through a series of summer irrigations and winter drought treatments, thus reducing annual rainfall seasonality. The experimental design and effects of rainfall manipulation treatments on soil moisture are described in detail. Additionally, field sites are characterised in terms of climate, soils and floristic composition.

Chapter 3: The effects of altered rainfall seasonality on post-fire vegetation productivity and community structure in Fynbos and Renosterveld

This chapter focuses on the patterns of post-fire productivity and community structure in Fynbos and Renosterveld plots, in response to experimentally altered moisture availability. This chapter investigates how structurally similar shrubland communities differ in their responsiveness to post-fire moisture availability at the community level. Productivity represents the broadest level of investigation as it is inclusive of numerous other vegetation processes (e.g. recruitment, establishment, survival, growth). Productivity patterns are investigated in conjunction with measures of community structure (e.g. species abundance and dominance). Patterns are related to soil moisture and nutrient availability patterns described in Chapter 2.

Chapter 4: Growth form responses to altered post-fire rainfall seasonality in Fynbos and Renosterveld

This chapter further interrogates the plot-level patterns of productivity and community structure by investigating dominant growth form responses to altered moisture availability. This is done in order to determine the main drivers of the patterns of productivity and community structure described in Chapter 3. Growth form demographic responses are investigated in terms of volume, counts and growth. Growth form interactions are also investigated to determine the degree to which growth forms interact or compete in the different shrubland communities.

Chapter 5: The effect of altered post-fire rainfall seasonality on the physiological performance of seedlings and resprouts in Fynbos and Renosterveld

This chapter investigates the link between seasonal physiological performance (e.g. leaf hydration, photosynthetic capacity, photosynthetic performance) of key growth forms and their demographic responses to altered rainfall seasonality. Specifically, physiological performance and demographic patterns are contrasted between plants which resprouted after fire and those which emerged from seed. These represent the important life-history stages of the most common regeneration strategies amongst growth forms. The physiological investigation was intended to further validate the link between experimental changes in soil moisture availability and demographic/community responses.

Chapter 6: Synthesis

The synthesis ties together findings from each chapter showing how physiology, demography, community structure and productivity patterns relate to each other and how these processes are affected by climatic and edaphic factors. Furthermore, the importance of rainfall seasonality is discussed from a functional and evolutionary perspective and how changes in rainfall relate to patterns of short-term and long-term sensitivity/vulnerability of shrubland ecosystems.

CHAPTER 2: Field manipulation of rainfall seasonality in post-fire Fynbos and Renosterveld study sites

Summary:

- This chapter describes the concept and design of a three-year rainfall-manipulation experiment in post-fire Fynbos and Renosterveld field sites within the Cape Floristic Region. Additionally, the soil, climate and floristic characteristics of each shrubland site are contrasted.
- Climate patterns indicated winter rainfall dominance with strong, sporadic summer rainfall components due to the inland location of the site. Due to their proximity, both sites experienced the same natural climate patterns.
- Experimental sites were subjected to controlled burns before the onset of rainfall manipulation treatments.
- Experimental summer treatment irrigations and 50% winter rain-removal treatments reduced the yearly seasonality of rainfall over treatment plots relative to control plots by redistributing moisture from the periods of lowest temperature to the periods of highest temperature. This created significant summer and winter moisture soil moisture contrasts which enabled vegetation responses to moisture availability to be assessed across a variety of seasonal conditions.
- Fine textured, shale-derived soils of the Renosterveld site had higher nutrient availability than coarse sandy soils of the Fynbos site. Shale-derived soils also experienced more intense soil drought during the dry summers due to higher soil tension.
- Floristic diversity differed significantly between sites due to strong species turnover across the sharp soil boundary between sites.

2.1. Introduction

This chapter describes the post-fire rainfall manipulation experiment which was conducted at the Drie Kuilen Private Nature Reserve (33.5805° S, 20.0332° E) located in the Western Cape of South Africa **(Fig. 2.1).** In this experiment, seasonal rainfall inputs were manipulated using irrigations and imposed droughts with the objective of investigating the effects of seasonal moisture availability patterns on post-fire vegetation processes.

2.1.1. General description of study sites

The Drie Kuilen Nature Reserve is situated in the mountainous Langeberg region that comprises a mosaic of Matjiesfontein Shale Renosterveld and Langeberg Sandstone Fynbos (Mucina & Rutherford, 2006). The Fynbos site and Renosterveld site were located within a kilometre of each other, either side of a distinct soil ecotone. The Renosterveld site occurred on fine-textured, shale-derived soils in the valley while the Fynbos site occurred on the ridge of the valley (within <200m altitude difference) on coarse sandstone-derived soils **(Fig. 2.2)**. Topsoil depth was <0.5 m at both sites overlying hard, bedrock layers. Succulent Karoo, although also present in patches of the Drie Kuilen Nature Reserve, was not included in this study despite it being a dominant vegetation type within the GCFR **(Chapter 1)**. This is because Succulent Karoo is not a fire-prone system and this experiment was specifically focused on post-fire vegetation processes.

2.1.2. Historical climate

The Drie Kuilen Nature Reserve falls within the transitionary zone between the strongly winter rainfall region toward the south west of South Africa and the eastern, inland regions which have aseasonal rainfall **(Fig. 2.1)** (Schulze, 2007, Bradshaw & Cowling, 2014). Accordingly, climate patterns from 1991-2004 for Drie Kuilen Nature Reserve indicate that most rainfall occurs in winter but with a stronger summer rainfall component than what would be expected toward the extreme south-western reaches of the Western Cape. Mean annual precipitation was <300mm placing this site at the dry end of the aridity spectrum within the Cape Floristic Region (**Table 2.1, Fig. 2.3a)**. The Drie Kuilen historical weather data showed higher mean annual precipitation (MAP) and lower winter rainfall seasonality relative to the nearby districts of Montagu and Touwsrivier. Unfortunately, available datasets for Drie Kuilen and surrounding areas did not cover the exact same periods of time and so seasonality patterns were not compared statistically. Nonetheless, patterns were useful for broadly describing the climate regime for the region. Generally, in Drie Kuilen and surrounding areas, highest temperatures coincided with the lowest rainfall months and the lowest temperatures with the highest rainfall months resulting in a broad asynchrony of moisture and energy resources.

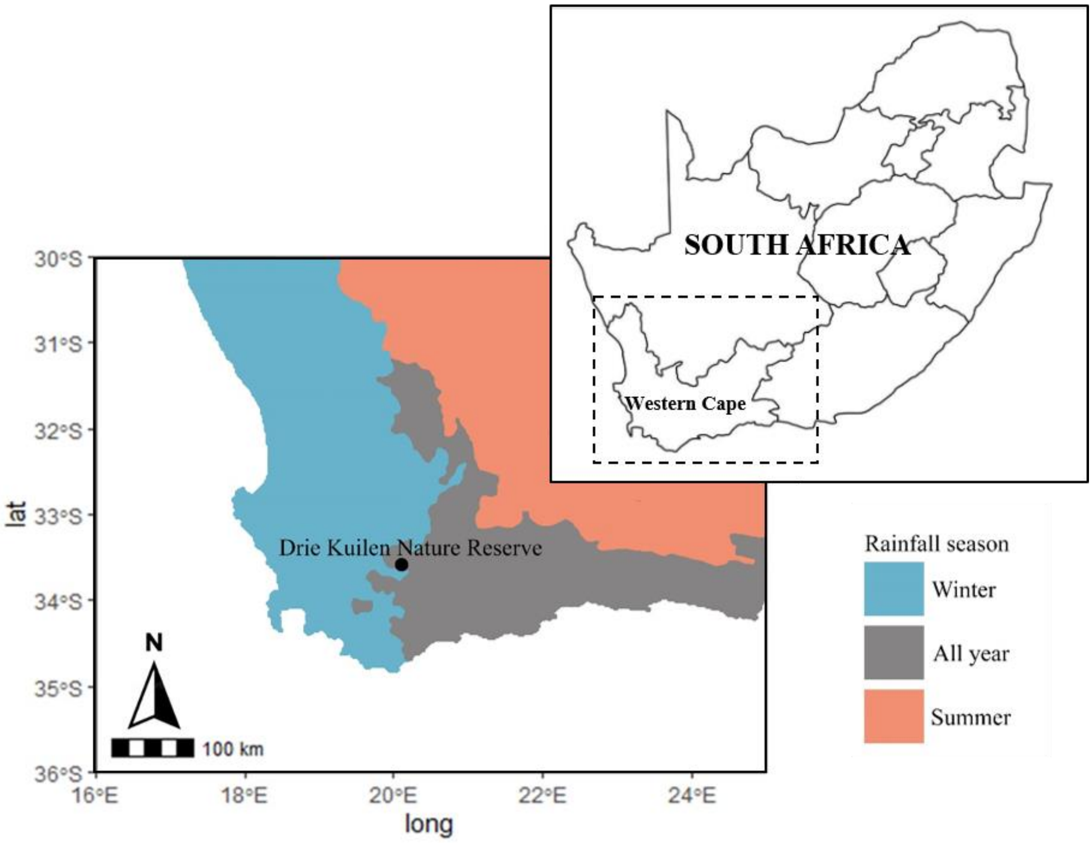

Figure 2.1: Map showing the location of the Drie Kuilen Nature Reserve in the Western Cape of South Africa and the regional rainfall seasonality zones. Rainfall zones adapted from Schulze (2007).

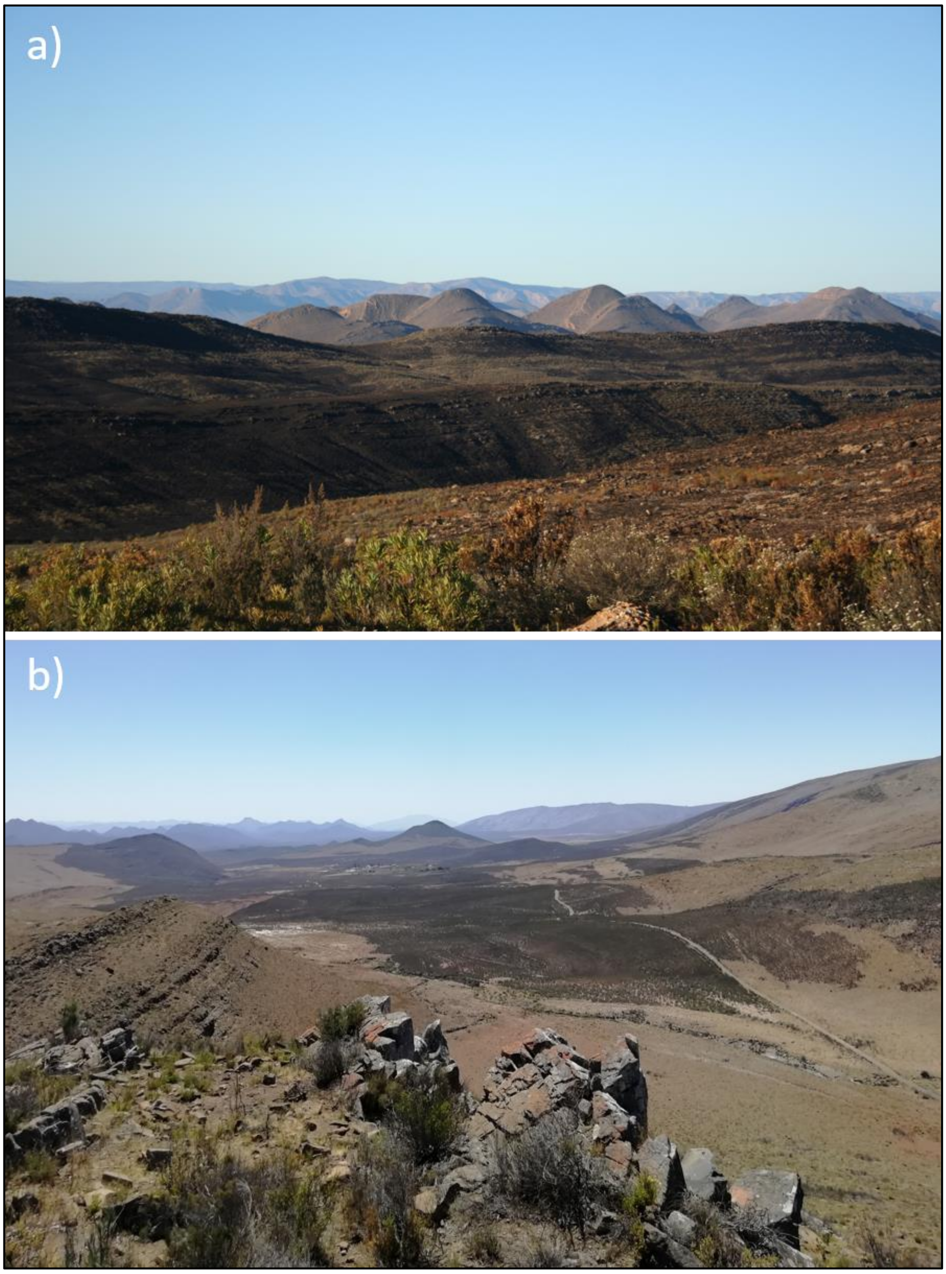

Figure 2.2: Photographs taken within the Drie Kuilen Nature Reserve during the experiment. **a)** View from the Fynbos site showing semi-arid mountainous surrounds. **b)** View of Renosterveld site in valley below the Fynbos site.

Table 2.1: Mean annual precipitation (MAP), summer (November – April) and winter (May – October) rainfall percentages from Drie Kuilen Nature Reserve and surrounding areas using available historical rainfall datasets.

Weather Station	MAP (mm) ± SD	% winter ± SD	% summer ± SD
Montagu (2000 - 2019)	206 ± 82	65 ± 14	35 ± 14
DeDoorns (2000 - 2018)	296 ± 112	70 ± 10	30 ± 10
Drie Kuilen (1991 – 2004)	238 ± 49	59 ± 15	41 ± 15
Pooled Mean	247 ± 147	65 ± 23	35 ± 23

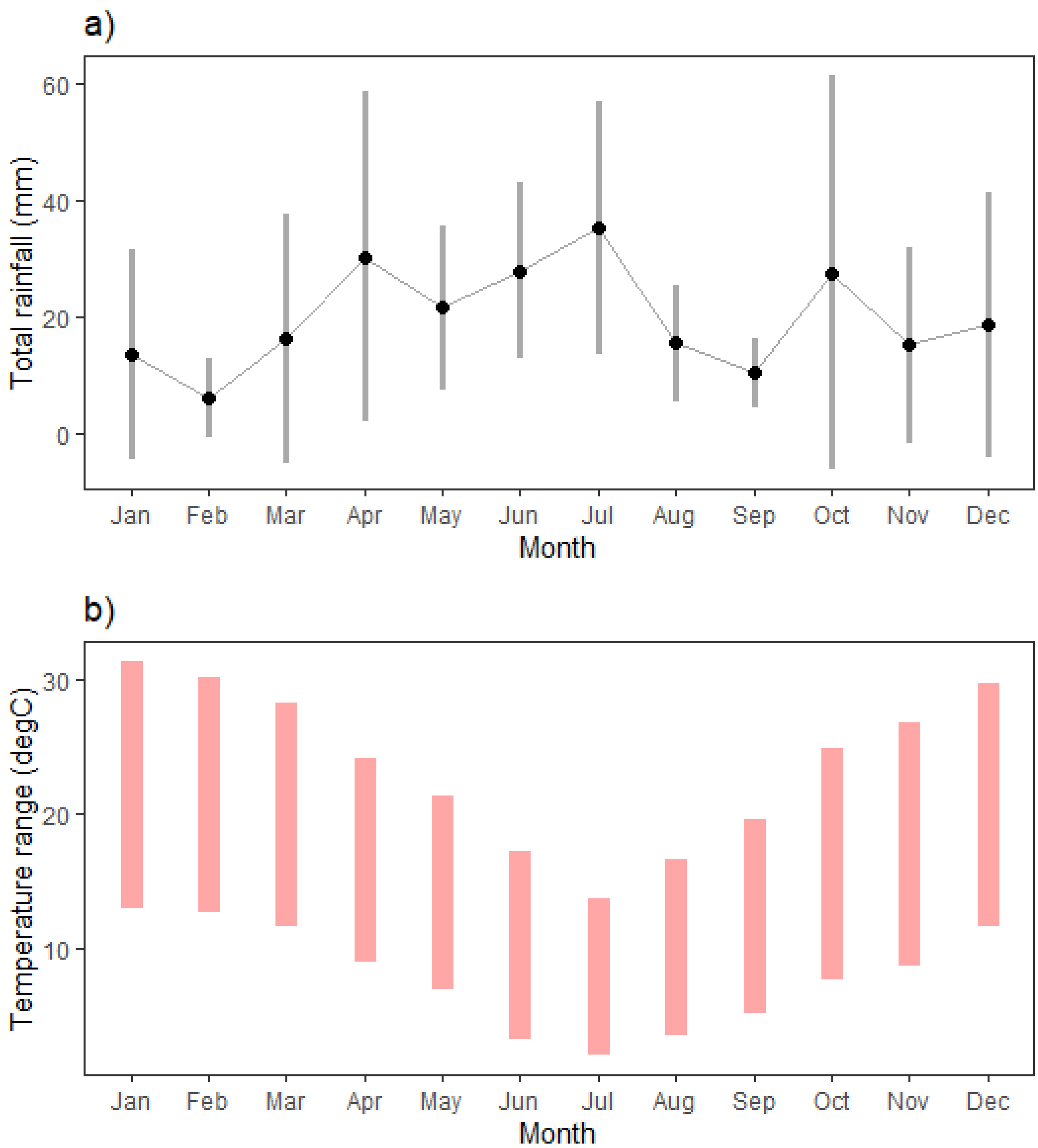

Figure 2.3: a) Historical rainfall from Drie Kuilen Nature Reserve for the period 1991-2004. Points represent monthly means over the years 1991-2004 and grey bars represent standard deviation of monthly means over that period. **b)** Mean daily temperature ranges from Drie Kuilen Nature Reserve for the period 1991-2004. The tops of red bars represent the mean maximum daily temperatures and the bottom of red bars represent the mean minimum daily temperatures for each month over the period 1991-2004.

2.2. Methods

2.2.1 Experimental design and rainfall manipulation

In this experiment I manipulated post-fire rainfall over neighbouring Fynbos and Renosterveld shrubland sites and to monitored vegetation responses. This study adds to recent rainfall manipulation studies in the Greater Cape Floristic Region (West *et al.*, 2012), and globally (Parra *et al.*, 2012; Parra & Moreno, 2017, 2018), with the goal of documenting real vegetation responses to manipulated rainfall patterns. As discussed in Chapter 1, field studies have the advantage of realism, but results can be difficult to generalise due to unseen, site-specific factors. However, the addition of high-resolution environmental measurements (see section 2.2.2) allows for confounding variables to be measured and accounted for in the interpretation of experimental results. Similar experiments carried out in spatially separated regions should clearly document the underlying environmental conditions so that the results can be contrasted in the context of underlying environmental conditions. Additionally, the detailed investigation of vegetation processes at multiple levels of detail (e.g. physiology, demography, productivity) can provide valuable mechanistic insights into the relationships between response and explanatory variables. These mechanistic relationships can then be tested in subsequent experiments over broader spatial scales.

The primary function of my rainfall manipulation experiment was to investigate the influence of rainfall seasonality patterns on a variety of post-fire vegetation processes. Rainfall manipulation was achieved by experimentally reducing the winter component of rainfall and increasing the summer rainfall component over permanent, post-fire treatment plots. This was not intended to simulate predicted changes in rainfall, which are uncertain, but rather to test the responsiveness of vegetation to clearly contrasting seasonal moisture availability patterns. Winter periods were defined as the period between mid-May to mid-October and summer periods were defined as the period between mid-October to mid-May. This was based on previous temperature and rainfall data for the region (**Fig. 2.3a,b**). This rainfall manipulation design allowed for the comparison of the effects of moisture availability on vegetation over both cool and warm seasonal periods between control and treatment plots. The basic concept of the experimental design was to redistribute winter rainfall onto treatment plots during the hottest summer months, thus reducing rainfall seasonality in the treatment plots. Treatment plots were compared against control plots which received natural rainfall.

According to the historical rainfall data (**Fig. 2.3a**), Drie Kuilen received ~160 mm of rainfall in winter (65% of total annual rainfall) and ~86 mm in summer (35% of total annual rainfall). The manipulation of climate was intended to reverse the seasonality of rainfall in a series of treatment plots. Therefore, the winter drought treatment was designed to reduce natural winter rainfall from ~ 65 % to ~ 35 % and the summer irrigation treatment was designed to increase summer rainfall from ~35% to ~65% of the total annual rainfall. These percentages were broad targets because it was not possible to account for the potential noise introduced by natural variability in rainfall during the experiment. Based on pooled historical rainfall means **(Table 2.1)**, it was calculated that adding 90mm of rainfall during summer and halving winter rainfall would roughly achieve the target seasonality changes over experimental plots. 90mm rainfall inputs were separated into three separate 30mm irrigation events to minimize runoff and to more closely mimic large natural rainfall events. Large, infrequent summer irrigations closely resemble the natural occurrences of summer rainfall in the region and were more logistically feasible than numerous small irrigation events. Large irrigation events also ensured greater soil moisture effects due to the potential for evaporative effects to render small irrigations ineffective.

Before the start of the experiment, permanent, galvanised-steel rainfall structures were erected over each of the twelve 4 m × 4 m plots in 2016. This was carried out at one Fynbos and one Renosterveld site. These structures were used as permanent, weather-proof platforms to house roofing and irrigation systems for capturing and adding rainfall to experimental plots. Structures consisted of the 4 upright poles, “uprights”, with two adjustable crossbars. These crossbars supported four rotatable/removable steel frames which supported roofing panels **(Fig. 2.4).** The adjustability of the crossbars and rectangular frames allowed for roofing to easily be removed or adjusted into the required positioning (e.g. orientation and slope of roofing panels – described below).

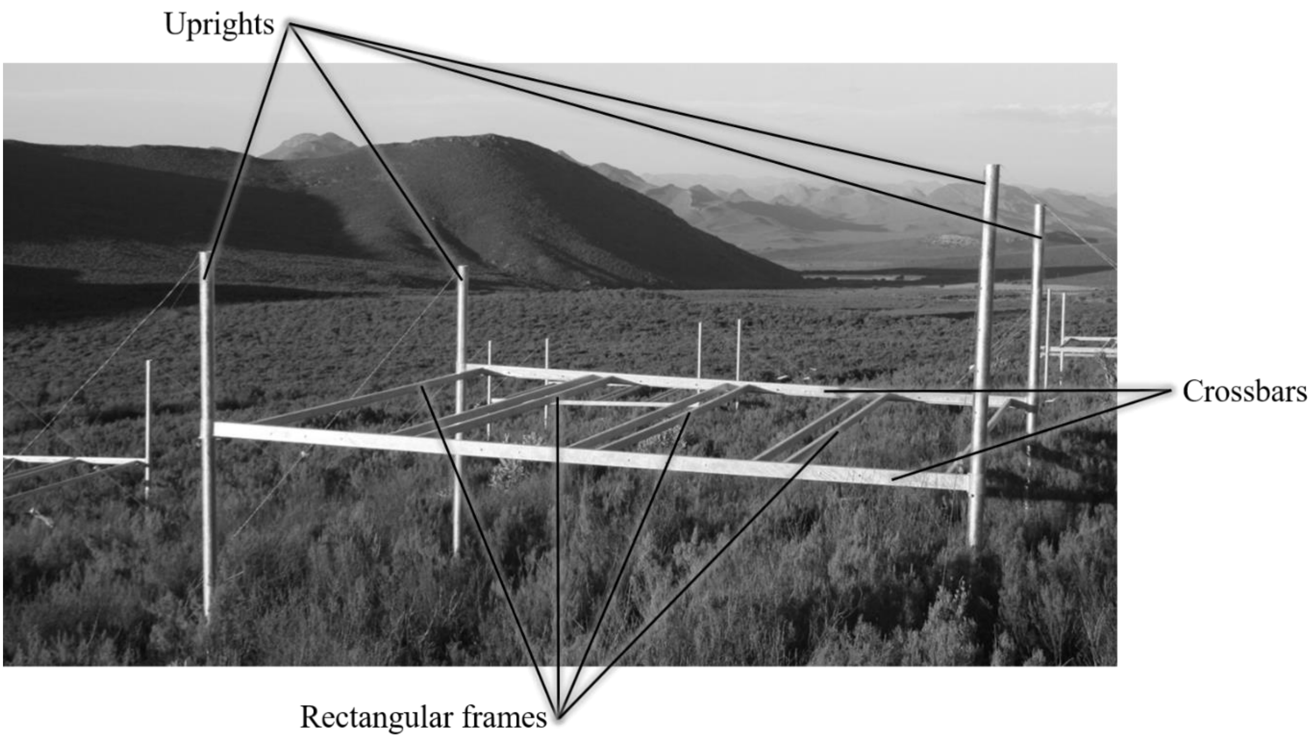

Figure 2.4: Components of the galvanised-steel structures erected at experimental Fynbos and Renosterveld sites within the Drie Kuilen Nature Reserve. These structures provided a strong, weather-proof platform allowing for roofing and irrigation systems to be attached over permanent experimental plots.

Experimental Fynbos and Renosterveld sites, with erected galvanised steel structures were subjected to controlled burns during May 2016 under the supervision of the NCC fire services **(Fig. 2.5).** This was initiated before the onset of rainfall manipulation treatments.

Figure 2.5: Controlled burn at the Fynbos site during May 2016. One of the galvanised-steel structures, which were erected over plots, is visible through the flames. An equivalent burn was carried out at the Renosterveld site (not shown).

After the controlled burn, roofing (to capture rain) and irrigation systems (to add rain) were added to steel structures **(Fig. 2.6 a-d)**. Winter rainfall was captured over plots using U-shaped strips of UV-neutral, clear polycarbonate sheeting secured onto rectangular steel frames ~ 1.5 meters above plots, covering 50% of the area above plots and thus preventing ~50% of natural winter rainfall (**Fig. 2.6c**). The roofing panels were secured to rectangular frames **(Fig. 2.4)** using self-tapping, water-proof screws. PVC gutter systems were connected to the rectangular frame on the downhill boarder of treatment plots **(Fig. 2.6c)**. Gravity-fed irrigation systems consisted of 16mm drip-irrigation tubing (www.agriplas.co.za) with drippers spaced at 15cm apart. This tubing was secured at a level height to the uprights using galvanised steel wire. Drip tubing spanned the length of the experimental plots and were spaced ~30cm apart to provide even coverage irrigation over plots **(Fig. 2.6b, d)**. Structures were fitted with 700 mm tall, fine-mesh, anti-herbivory fences (**Fig. 2.6e**) so that small and large grazing animals would be deterred from entering the plots. Herbivores in the reserve included large grazing antelope and micro-mammals.

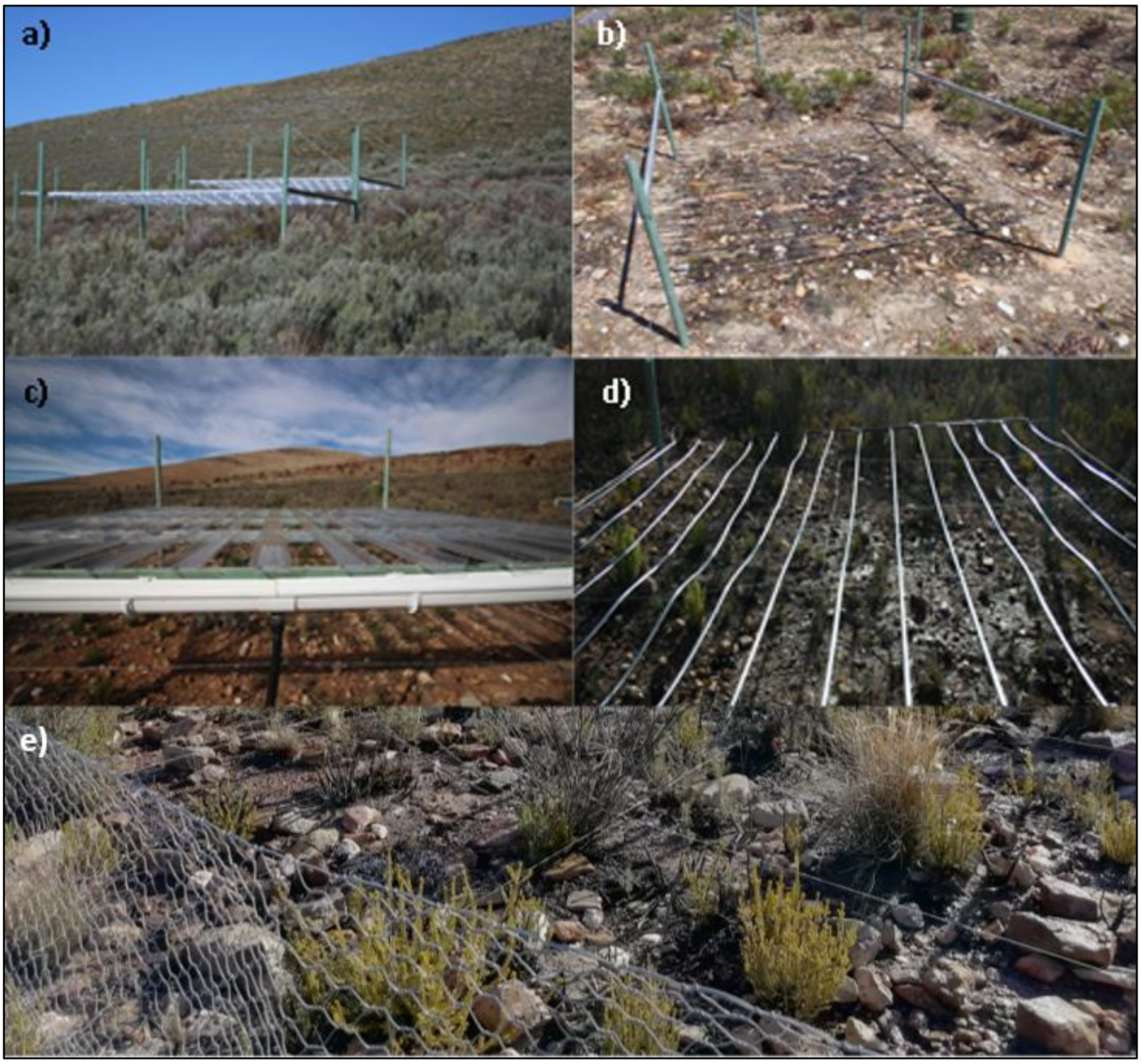

Figure 2.6: a) Control plots with roofing panels oriented to let rainfall run onto plots (no rainfall capture) **b)** Treatment plot after 30 mm irrigation event **c)** Treatment plot with clear plastic roofing panels and gutter for winter rainfall collection **d)** 16 mm irrigation tubing on treatment plot **e)** Anti-herbivory fence and strings marking 1×1m grid blocks within plots.

The two study sites each contained twelve plots (6 treatments and 6 controls) arranged in blocks using a randomised complete block design across the landscape. The control and treatment plots were all located on a north-facing slopes and faced the direction of predominant rainfall and wind conditions (NW). In the treatment plots, winter rainfall (i.e. mid-May to mid-October) flowed from upward-orientated, U-shaped polycarbonate roofing panels into gutter systems **(Fig. 2.6c)**. From the gutters, captured rainfall flowed through broad black tubing into 5000L storage tanks situated downslope of the experimental site (**Fig. 2.7a,c**). Control plots had the same roofing structure as treatments during winter, but U-shaped, polycarbonate roofing panels were orientated into a downward position to allow rain to fall onto the plots rather than be captured and stored. The control plot roofing controlled for any potential lighting effects from the roofing panels over treatment plots but did not affect natural moisture inputs. The orientation of U-shaped polycarbonate sheeting had no effect on lighting. During summer periods, the clear plastic roofing components were removed from all structures by removing rectangular frames from steel structures **(Fig. 2.4)**. During summer stored winter rainfall in 5000L tanks was pumped into smaller, 500L tanks situated uphill of each treatment plot and allowed to flow onto plots through the gravity-fed drip irrigation systems (**Fig. 2.6, 2.7**). From 2017-2019 treatment plots were irrigated once a month for three months during the hottest time of the year (January – March). Each irrigation event was equivalent to 30 mm of rainfall per plot released over ~six hours having a saturating effect on shallow treatment soils **(Fig. 2.7b)**.

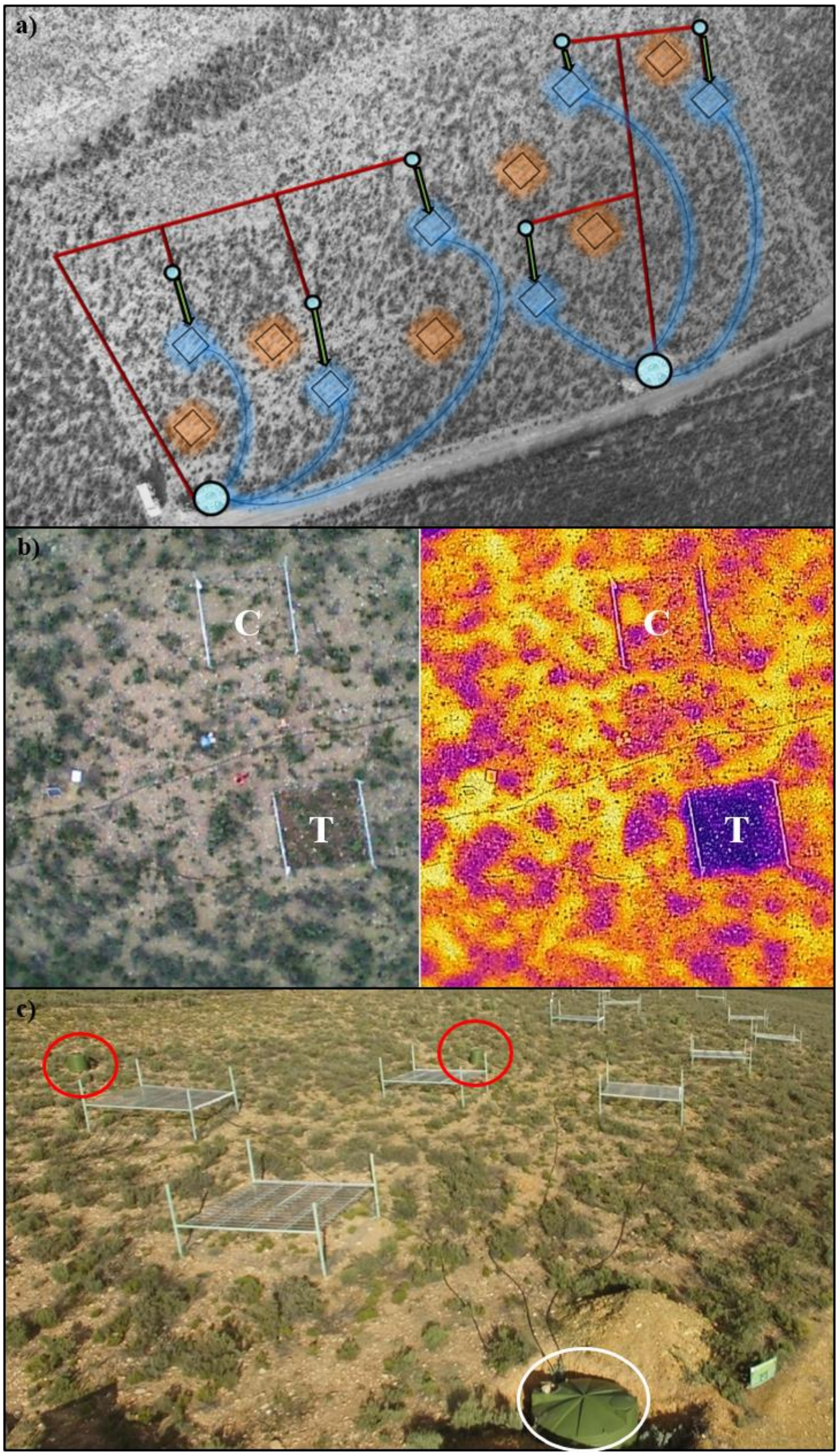

Figure 2.7: Experimental setup of irrigation and drought treatments. **a)** Rainfall capture occurred over treatment plots (blue squares) in winter and captured rainfall was stored downhill in large tanks (large circles) via hosing (blue lines). Stored rainfall in large tanks was redistributed to smaller tanks (small circles) during summer using a pump system (long red lines). Small tanks were used to irrigate treatment plots during summer using a gravity fed system (green arrows). **b)** Aerial and thermal image showing treatment plot (T) irrigation next to control plot (C) directly after a summer irrigation **c)** Aerial image of top and bottom water tanks and experimental plots.

2.2.2. Micro meteorological measurements

At each site a weather station, powered by a solar panel, was erected to record detailed environmental measurements throughout the experiment (**Fig. 2.8)**. Campbell Scientific CR23X Data Loggers were connected to a range of environmental sensors to record rainfall (TE525MM-L tipping bucket rain gauge), relative humidity and air temperature (HC2S3-L Temp & Relative Humidity Probe) at each site. Soil water potential sensors (Decagon MPS-6 Calibrated Water Potential Sensors) were also buried at a depth of 20cm in the soil at each plot as close to the centre of the plot as possible. These sensors recorded soil water potential and soil temperature concurrently for the duration of the experiment. The temperature sensitivity of soil water potential measurements in dry conditions (<500kPa) required water potential readings to be normalised by soil temperature. This was done by multiplying water potential readings with concurrent soil temperature readings and then scaling resultant values back to the normal range of water potential. In one control plot and one treatment plot at each site, volumetric water content sensors (Decagon EC-5) were installed at a depth of 20cm in the soil. While these volumetric water content sensors had limited replication, these sensors allowed for the relationship between soil water potential and volumetric water content to be modelled.

Weather station data were recorded every ten seconds and an average of these readings was logged every thirty minutes. Soil sensors lost power for twenty days during the winter of 2017 during a powerful storm, but power was restored as soon as possible. High higher accuracy of soil water potential data in the wet range (>500kPa) meant that soil water potential measurements were most useful as an indication of moisture availability peaks rather than minimum values. Below -1500KpA is generally considered extremely dry.

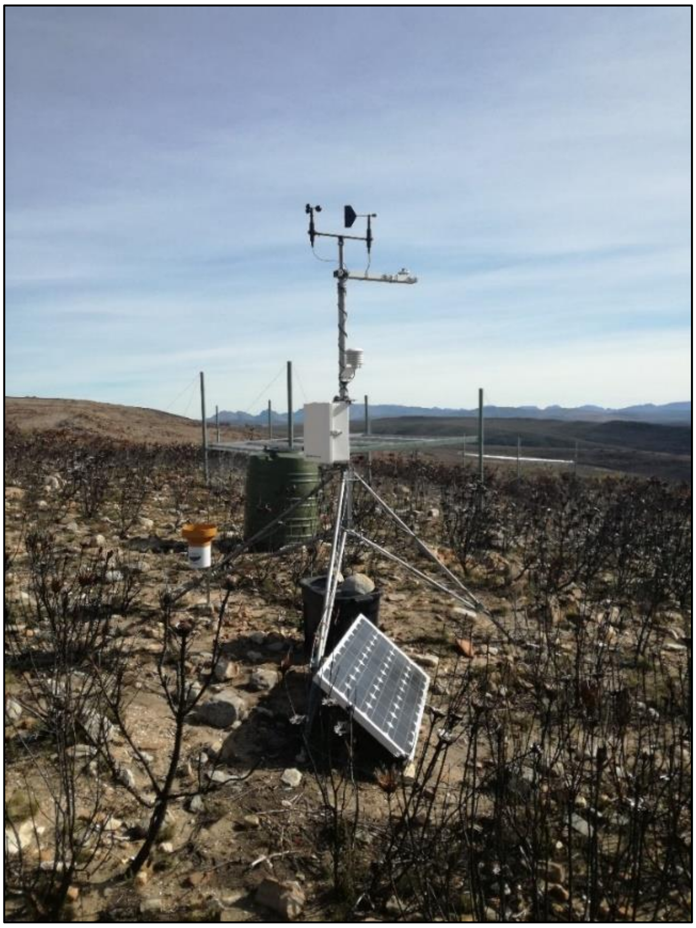

Figure 2.8: Solar-powered weather station at the Fynbos site recording meteorological and soil moisture measurements throughout the experiment. A second station was set up at the Renosterveld site (not shown).

2.2.3. Soil sampling and nutrient concentration analysis

Soil samples (n = 7) were collected randomly across each experimental site (i.e. Fynbos and Renosterveld) during September 2018. These samples were collected from the top 30cm of soils using an auger and were taken from patches of open ground located outside of experimental plots. This was intended to prevent disturbance inside the experimental plots. Soil samples were stored in open plastic bags and air-dried until constant mass. Dried samples were transported to Bemlab, Cape Town (www.bemlab.co.za) where they were analysed for concentrations of P, N, C, B, Zn, Mn, Cu, Fe, Na, Ca, K, Mg (units = mg/kg). This is a SANAS accredited testing laboratory in accordance with ISO17025:2005.

2.2.4. Species identification for site floristic analyses

In this chapter, an initial analysis of floristic composition was used to compare broad site similarities and differences in terms of species composition. Throughout the experiment, every emerging plant was identified (as close to species level as possible) over a series of 6 field surveys over three years. Species identifications were limited to the plants within control and treatment plots (i.e. 12 × 16m^2 plots per site). Demographic surveys are described in detail in Chapter 3. Species were identified using field guides and by comparing photos to a digital database of species occurring on the reserve (available on request). Additionally, samples were collected off-plot to help with identification. Species identification was not always possible as individuals were either too young or not in flower during the survey periods. In such cases, each unidentified species was given a unique working name so that they could be identified as separate species from other unknown species (Table S1).

2.2.5. Statistical analyses

All statistical analyses were conducted in R 3.5.1 (R Development Core Team, 2018).

2.2.5.1. Treatment effects on soil water potential

To compare the soil water potentials (WP) in treatment plots versus control plots, soil water potential data were plotted as effect sizes (ES):

(1) $$\mathrm{ES} = \frac{\mathrm{mean(Soil\ WP\ treatments) - mean(Soil\ WP\ controls)}}{\sqrt{\mathrm{(SE(Soil\ WP\ treatments) + SE(Soil\ WP\ controls))}}}$$

Here, means and standard errors (SE) were calculated for water potential measurements logged at the dataloggers at 30minute intervals (see 2.2.2) resulting in a time-series of effect sizes for each site. Soil water potential means and standard errors were calculated across all control and treatment plots for each site. ES values are in units of SE and thus I considered differences of water potential in treatment plots from levels in control plots to be significant where -2 > ES > 2.

2.2.5.2. Soil moisture release curves

Soil moisture release curves were used to asses how soil tension changed with shifts in volumetric water content. Soil moisture release curves were modelled using non-linear regression on the sigmoidal relationship between soil water potential (kPa) and volumetric water content (cm3/cm3). Values were modelled using concurrent water potential and volumetric water content data logged at 30minute intervals.

2.2.5.3. Principal components analysis

Climate, soil-nutrient and species composition data were contrasted across Fynbos and Renosterveld sites using three separate principal component analyses (PCA). Within each analysis, multiple variables (e.g. climate variables, soil nutrient variables, species) were reduced to two dimensions (i.e. principal components), Dim1 and Dim2. 95% confidence intervals were used to compare differences between Fynbos and Renosterveld sites.

Climate data for each site were grouped by month to account for variation throughout the year. Point clusters in 2D multivariate space thus represented yearly variation in climate data for each site. These data were analysed using key variables including temperature (monthly mean, minimum, maximum), rainfall (total) and relative humidity (monthly mean, minimum, maximum). Values were scaled to have unit variance before analysis. This was done so that all climate variables contributed equally to the analysis despite having different natural variances.

Soil nutrient data included P, N, C, B, Zn, Mn, Cu, Fe, Na, Ca, K, Mg (units = mg/kg). These data were also scaled to have unit variance before analysis. To supplement this analysis, multiple two-sample t-tests were used to compare the concentrations of individual soil nutrients across sites.

Species composition data were composed of species presence absence matrices for each plot and each survey period in the Fynbos and Renosterveld study sites. This analysis was intended to characterise broad floristic differences between sites over the entire experimental period. To supplement this analysis, a summary of plant families, genera and species present at each site throughout different stages of the experiment was tabulated.

2.3. Results

2.3.1. Climate and treatment effects

Over the duration of the experiment, the Fynbos and Renosterveld field sites experienced hot summer and cool winters characteristic of the region (**Fig. 2.3a**) (Bradshaw & Cowling, 2014), as shown by patterns of temperature and atmospheric vapour pressure deficit (VPD) (**Fig. 2.9a,b,c**). Rainfall was generally winter dominated **(Fig. 2.9c, S1)** but a strong summer rainfall component was prevalent in all years. Summer rainfall occurred in large single rainfall events ranging in timing from spring to peak summer. Generally, late summer and early autumn periods were extremely arid and had low rainfall coupled with high temperatures and VPD (**Fig. 2.9c**). Peak winter months (i.e. June - August) had low minimum temperatures (below freezing) and low atmospheric VPD whereas minimum temperatures in peak summer months (January – March) were >10 degrees warmer months. Maximum monthly air temperatures were ~20 degrees warmer than minimum temperatures. The summer of 2019 was especially wet relative to the previous summers due to multiple, large rainfall events.

The treatment irrigations and imposed winter droughts effectively redistributed rainfall from periods of lowest temperature and VPD to periods of highest temperature and VPD (**Fig. 2.9**.c). This created contrasting moisture input pattern under both warm and cool periods allowing for the assessment of the importance of moisture availability in different seasonal periods. The resultant combination of summer and winter treatments transformed the 'rainfall' distribution from ~ 80/20 % winter dominated to ~ 35/65 % summer dominated in year 1 and from ~ 65/35 % winter dominated to ~ 30/70 % summer dominated in year 2. The treatments effectively inverted the seasonal distribution of rainfall over treatment plots relative to controls. These ignore rain angle and runoff, which could have influenced the effectiveness of the drought and irrigation treatments.

The net effect of the irrigation treatments was a reduced winter rainfall seasonality and reduced summer drought relative to the natural winter rainfall climate patterns which were experienced in control plots. It was predicted that the net treatment effect would increase the synchrony of moisture and temperature resources throughout the year by redistributing moisture from cool periods to warm periods.

2.3.2. Effects of rainfall manipulation on soil water potential

Soil moisture was represented by water potential measured in kPa, where water potential values close to zero indicate wet soils and negative values indicate dry soils. Treatment plots had wetter soils during summer irrigations and drier soils than control plots during winter (particularly during rainy periods). This suggests that my experiment affected soil water potentials to a degree that was clearly detectable over the random noise (**Fig. 2.9e**). Generally, the effects of the three 30mm summer irrigations on soil moisture were larger than the effects of the 50% winter rainfall reduction treatments as indicated by larger effect sizes (ES) **(Fig. 2.9e)**. Surface runoff and edge effects may have contributed to a weakening of the effect of winter rainfall removal relative to summer irrigations which were highly controlled.

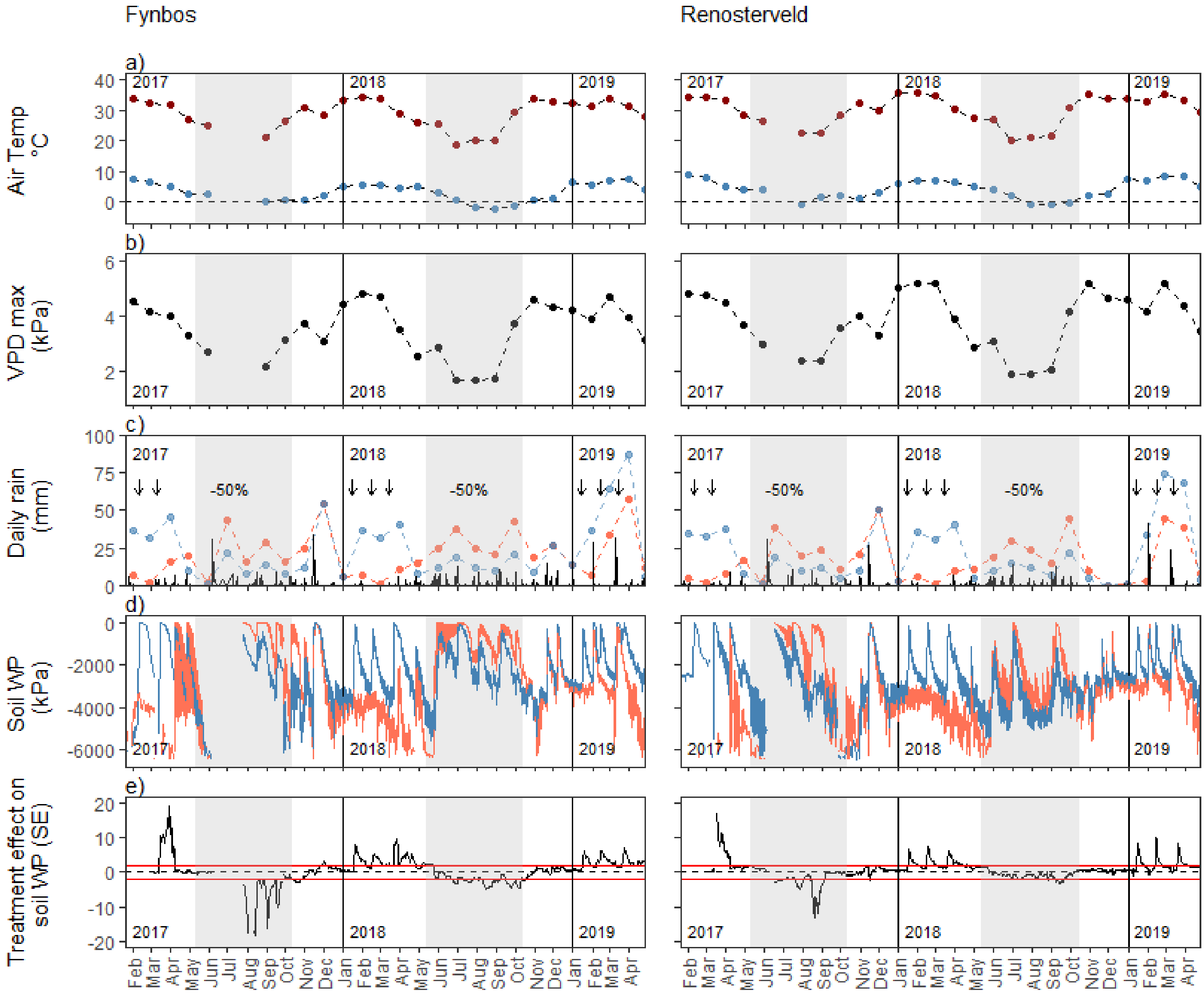

Figure 2.9: Climate and soil moisture patterns in Fynbos and Renosterveld sites at the Drie Kuilen Nature Reserve. **a)** Maximum (red) and minimum (blue) monthly air temperatures (°C) **b)** Maximum monthly atmospheric vapour pressure deficit for each site over the experimental period (kPa) **c)** Summed daily rainfall (mm). Blue dashed line and points = simulated monthly rainfall totals in treatment plots. Red dashed line and points =recorded monthly means in control plots. Summed monthly rainfall (mm) is represented by the solid black line. Black arrows = irrigation events (3*30mm). "-50%" represents the reduction in rainfall during 'winter' treatments **d)** Soil water potential (kPa) (±SE) of control (orange ribbon) and treatment (blue ribbon) plots. **e)** The treatment effect size (SE) on soil water potential relative to the control. Horizontal black dashed line = zero difference from control plots. Horizontal red lines = +2 and -2 standard errors away from zero effect (effect considered to be a significant effect if line exceeds 2 standard errors from zero.) Note, no effect size could be obtained before March 2017 as only a single sensor was present in one control and one treatment plot at each site. Shaded areas = 'winter period'. Unshaded areas = 'summer period'.

2.3.3. Soil moisture release curves and saturation lengths

With equivalent declines in soil volumetric water content (%) from saturation (drying soils), shale-derived soils (Renosterveld site) approached much more highly negative water potential values (dry soils) relative to sandstone-derived soils (Fynbos site). This pattern was detected with all soil volumetric water content and water potential throughout the experiment (**Fig. 2.10a**). Such responses are in line with what is expected for fine versus coarse textured soils (Minasny & McBratney, 2007). Over the course of the experiment shale-derived soils experienced more highly negative water potentials more often than sandstone-derived soils despite maintaining higher levels of volumetric water content (**Fig. 2.10b,c**).

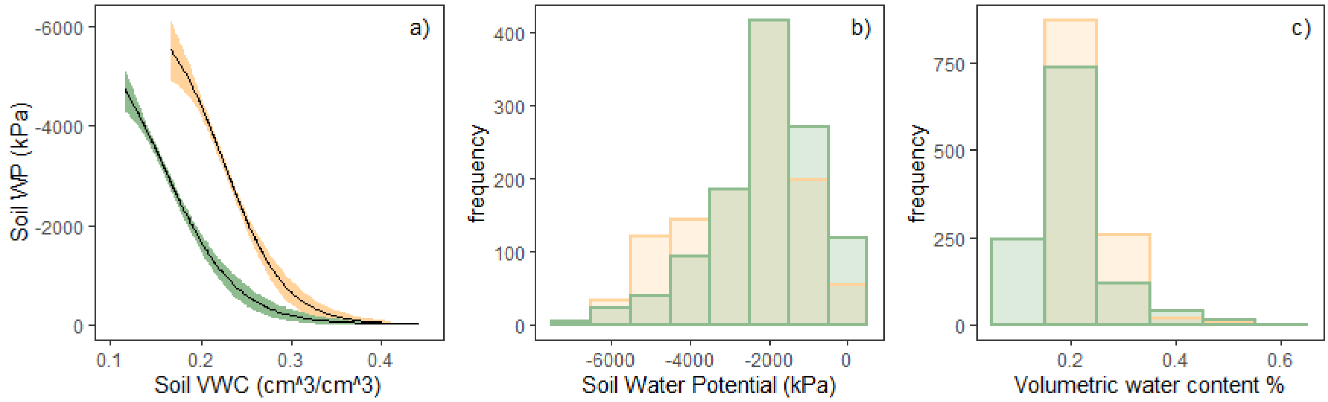

Figure 2.10: Soil moisture properties of sandstone Fynbos (green) and shale Renosterveld (yellow) soils at the Drie Kuilen Nature Reserve. **a)** Moisture release curves showing the modelled relationship between soil water potential and volumetric water content. Mean modelled relationships are represented by soil black lines and 95% confidence intervals are shaded in the respective colours defined for each soil type. **b)** Histogram showing the distribution of soil water potential for each soil type throughout the duration of the experiment. **c)** Histogram showing the distribution of soil volumetric water content for each soil type throughout the duration of the experiment.

2.3.3 PCA – Climate, soils & floristic composition

Sites were not strongly separated on either principal component axis (Dim1 & Dim2) with respect to key temperature, rainfall and humidity variables (**Fig. 2.11a**). This suggests that sites experienced highly comparable climate conditions throughout the experiment which is not surprising due to their proximity. The direction of the slight offset of the Renosterveld site cluster from the Fynbos site cluster in multivariate space was positively correlated with max and mean temperature, and negatively with rainfall, suggesting that the Renosterveld site could have experienced periods of higher aridity relative to the Fynbos site.

Fynbos and Renosterveld sites were well separated in multivariate space in terms of their soil nutrient status (**Fig. 2.11b**). Most nutrients increased in concentration (mg/kg) toward the Renosterveld cluster of points. No nutrients increased in concentration toward the Fynbos cluster of points (**Fig. 2.11b**). Interestingly, Nitrogen did not vary in the direction of either site, but Phosphorus was much more highly concentrated in shale-derived soils of the Renosterveld site. As additional statistical support for describing soil nutrient differences between sites, t-tests were used to contrast the concentration of each nutrient between Fynbos and Renosterveld site soil samples (**Table 2.2**). Taken together these results showed that the Renosterveld site soils had higher concentrations of numerous key nutrients relative to the Fynbos site soils.

Site floristic composition (**Fig. 2.11c**) separated strongly along the first principal component axis (Dim1), indicating that sites were floristically distinct over the course of the experiment. However, the axes, Dim1 and Dim2, explained relative low variation (e.g. 38.5% & 5.3%, respectively) within the data suggesting that floristic differences between sites were highly complex. Species drivers of site differences (i.e. loadings) were not included because high species numbers made visual interpretation impractical.

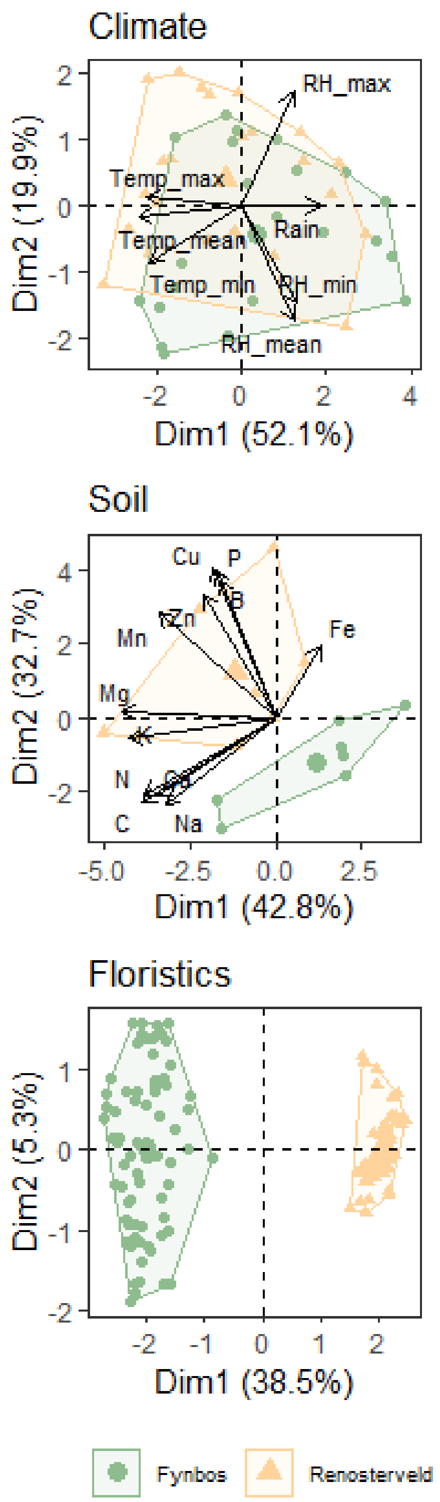

Figure 2.11: Climate, soil and floristic differences between Fynbos and Renosterveld sites at the Drie Kuilen Nature Reserve. Principal component analyses with two dimensions (Dim1 & Dim2). **Climate)** Variation in key climate variables throughout the experiment (including each month as a separate point). **Soil)** Biplot showing variation in key soil nutrients. **Floristics)** Variation in species composition using a matrix of species presence/absence per plot in each survey (control and treatment plots included).

Table 2.2: Soil nutrient properties of Fynbos and Renosterveld sites at the Drie Kuilen Nature Reserve.

Nutrients	Fynbos Sand Mean (SE)	Renosterveld Shale Mean (SE)	P-value (t-test)
Nitrogen (%)	0.11 (0.02)	0.12 (0.01)	0.642
Carbon (%)	1.45 (0.21)	1.51 (0.12)	0.798
Phosphorus (mg/kg)	**9.57 (0.69)**	**18.14 (1.87)**	**0.001**
Boron (mg/kg)	0.38 (0.02)	0.53 (0.07)	0.071
Zinc (mg/kg)	**0.44 (0.04)**	**1.72 (0.57)**	**0.04**
Manganese (mg/kg)	**10.3 (2.18)**	**72.4 (12.59)**	**<0.001**
Copper (mg/kg)	**0.24 (0.02)**	**0.67 (0.07)**	**<0.001**
Iron (mg/kg)	99.86 (5.45)	88.86 (11.14)	0.392
Potassium (cmol(+)/kg)	0.28 (0.03)	0.39 (0.05)	0.09
Calcium (cmol(+)/kg)	2.48 (0.46)	2.51 (0.19)	0.959
Magnesium (cmol(+)/kg)	**1.08 (0.12)**	**1.61 (0.12)**	**0.008**
Sodium (cmol(+)/kg)	0.08 (0.01)	0.07 (0.02)	0.012

Floristic differences between sites were present soon after burning and were maintained from the baseline survey to the end of the experiment (**Table 2.3**). Floristic differences were driven primarily by high species turn-over between sites with more than 70% of species at the Fynbos site, and more than 60% of species at the Renosterveld site being unique to that site. There was also turnover at the genus and family levels but to a much lesser extent (**Table 2.3**). Both sites were host to a variety of geophytic, herbaceous, shrubby and graminoid growth forms. A full list of species, genera and families recorded at each site are presented in **Table S1**.

Table 2.3: Numbers of families, genera and species identified within Fynbos and Renosterveld experimental plots at the Drie Kuilen Nature Reserve. Percentage of counts which are unique to Fynbos or Renosterveld sites (not shared) are displayed in brackets.

Survey Baseline	Fynbos	Renosterveld	Shared
# Families	24 (38%)	17 (12%)	15
# Genera	31 (48%)	24 (33%)	16
# Species	57 (75%)	39 (64%)	14
All Surveys combined			
# Families	30 (27%)	24 (8%)	22
# Genera	40 (33%)	36 (25%)	27
# Species	97 (74%)	68 (63%)	25

2.4. Discussion

The experiment was designed to manipulate rainfall patterns over neighbouring Fynbos and Renosterveld shrubland sites and to closely record vegetation responses. The Fynbos and Renosterveld sites occurred under the same climatic conditions due to their proximity but differed strongly in soil texture and fertility and post-fire floristic species composition (**Fig. 2.11**). The field manipulations of rainfall successfully reduced soil moisture in treatment plots during winter periods and increased soil moisture in treatment plots during summer periods, relative to control plots which experienced winter-dominant rainfall and periods of summer drought (**Fig. 2.9d,e**).

Closely recording many aspects of abiotic site elements is essential for making meaningful inferences about vegetation changes which are influenced by these abiotic elements. The results of this chapter, therefore, form the abiotic reference point to help explain vegetation patterns in Fynbos and Renosterveld sites in the upcoming chapters.

Due to Fynbos and Renosterveld sites having highly comparable climates, the experiment was able to contrast the effect of changing rainfall patterns across sites from equal baseline rainfall conditions. Therefore, imposed droughts or irrigations lead to equivalent changes in rainfall seasonality across sites. The treatment effects created a contrast of moist versus dry soils between control and treatment plots in both summer and winter periods, and lead to an overall change in rainfall seasonality. In Mediterranean-type, winter rainfall climates the predominant winter rainfall coincides with low light and temperatures while favourable light and growing temperatures coincide with severe summer drought (Specht, 1988). Therefore, the addition of rainfall in summer and the removal of rainfall in winter lead to a redistribution of seasonal rainfall within treatment plots, presumably increasing the overall synchrony of resource availability annually. In addition to a higher synchrony of resources, redistributed rainfall reduced the intensity of summer drought. While drought was increased during winter in treatment plots, winter drought is presumably less intense for vegetation than summer drought because low atmospheric VPD in winter reduces evapotranspiration and thus reduces the rate of moisture loss from vegetation and soils.

Soils at the sites were representative of the typical vegetation-soil relationships in the GCFR at the regional scale. Fynbos typically occurs on nutrient poor, sandstone-derived soils and Renosterveld typically occurs on moderately fertile shale-derived soils (Bergh *et al.*, 2014; Bradshaw & Cowling, 2014). The floristic data are consistent with the paradigm that Fynbos and Renosterveld are unique vegetation types with strong edaphic associations (Bergh *et al.*, 2014). The strong differences in species composition from <1 year after fire is consistent with the idea that species were likely to be edaphically specialised to their specific soil types potentially as early as the germination phase, although the effects of dispersal barriers and different pre-fire vegetation structure between sites cannot be ruled out.

Taken together, this experiment successfully altered post-fire soil moisture availability patterns in two floristically and edaphically distinct shrubland sites (Fynbos and Renosterveld) which co-occurred under the same natural climate regime. The results described in this chapter will be referred to in Chapter 3, 4, 5 and 6.

CHAPTER 3: The effect of altered rainfall seasonality on post-fire vegetation productivity and community structure in Fynbos and Renosterveld

Summary

- In this chapter I tested the sensitivity of post-fire productivity patterns and community structure to experimentally altered rainfall seasonality in neighbouring shrubland communities: Fynbos and Renosterveld.
- Productivity patterns were relatively insensitive to altered rainfall patterns at the Fynbos site whereas productivity in Renosterveld was responsive to both summer irrigations and winter drought treatments throughout the experiment indicating sensitivity to moisture availability irrespective of season. Consistent with these differences in moisture sensitivity, Renosterveld site also showed higher natural seasonality in productivity under the control plot, winter rainfall conditions whereas Fynbos productivity was continuous and not strongly seasonal.
- At the Renosterveld site, shifts in community compositions during the sensitive, first summer period had lasting implications for community structure, consistent with 'event-dependence hypothesis'.
- Despite low overall vegetation responsiveness to moisture availability patterns at the Fynbos site, plots with higher summer productivity under summer treatment irrigations lead to strong consequences for productivity under the following droughted winter period suggesting that soil nutrient impoverishment in the sandstone-derived soils of the Fynbos site may limit productivity. These negative feedbacks were not seen in Renosterveld despite significantly larger productivity responses.
- These results suggest that post-fire productivity patterns Fynbos and Renosterveld shrublands may be unequally coupled to rainfall patterns and that soil nutrients and texture could mediate the interactions between vegetation productivity and climate.

3.1. Introduction

Projected climate shifts for the Cape Floristic Region (Tadross et al., 2005; Hewitson & Crane, 2006; IPCC, 2013; Giorgi & Lionello, 2008; Klausmeyer & Shaw, 2009: Altwegg et al., 2014) could have dramatic effects on vegetation patterns. Future climate projections for Mediterranean-type, winter-rainfall ecosystems include hotter, drier extremes, less reliable winter rainfall and possible increases in summer orographic rainfall events (Tadross et al., 2005; Hewitson & Crane, 2006; IPCC, 2013; Giorgi & Lionello 2008; Altwegg et al., 2014). Global models project increased climatic variability for Mediterranean-type winter rainfall regions, with major droughts or intense rain events becoming more frequent (Cubash et al.,1996). Despite substantial variability and uncertainty in future climate projections, there is some consensus that the patterns of seasonality could change within Mediterranean-type, winter rainfall ecosystems globally. Therefore, regardless of the exact changes in rainfall which may occur, it is important to test vegetation responses to a broad range of rainfall seasonality patterns (see West et al., 2012, Parra et al., 2012; Esler et al., 2015). This study tested post-fire vegetation responses to manipulated rainfall patterns using an experimental field study in the Cape Floristic Region of South Africa.

The potential impact of shifting rainfall patterns could be particularly worrying for fire-prone ecosystems, such as the shrublands of the Cape Floristic Region of South Africa. This is because early recovery periods in post-fire shrublands could be particularly sensitive to environmental resource availability patterns (Quintana et al. 2004; Moreno et al. 2011). Newly recruiting plants in the post-fire environment generally have limited access to deeper soil moisture layers and surviving, resprouting plants are in a state of recovery from severe loss of above-ground biomass (Slingsby et al., 2017). Water is a primary limiting factor for germination, growth, survival and reproduction (Johnson et al., 1993; Zhang et al., 2005, Heelemann et al., 2008; Mustart et al., 2012) and therefore the amount and timing of rainfall can have a strong influence on early post-fire vegetation recovery patterns, with potentially long-lasting implications for mature vegetation composition and diversity patterns. Long-term ramifications associated with specific events surrounding a fire (e.g. fire intensity, fire season and/or post-fire weather) has been termed the "event-dependent hypothesis" (Bond & van Wilgen, 1996; Moreno et al., 2011).

Predicting post-fire vegetation responses to shifting rainfall patterns is complex because some stages of plant development (e.g. germination, growth, reproduction) may be dependent on specific temperature and moisture combinations in certain species and growth forms (Kuiper, 1964; Brits et al., 1993; Johnson et al., 1993: Heelemann et al., 2008; Thomas et al., 2010; Mackenzie et al., 2016), which may limit such processes to very specific (and often short) periods of time throughout the year under the winter rainfall climate. Winter rainfall climates are typically thought to have a broad asynchrony of resources seeing as periods of highest moisture availability (e.g. winter) occur during unfavourable light and temperature conditions (Specht, 1988). Therefore, shifts in rainfall seasonality patterns could either increase or decrease the duration of favourable growth conditions, depending on the specific seasonal changes that occur. Species may also have a range of internally regulated phenological rhythms which may vary in their responses to changing environmental conditions (Pierce, 1984). The plasticity of plant developmental processes amongst species in the Cape Floristic Region has received little attention until recently (Power et al., 2019).

Vegetation responses to rainfall may also differ across the structurally similar, yet floristically distinct, shrubland types of the Cape Floristic Region (e.g. Fynbos and Renosterveld), which typically occur on different soils (Bergh et al., 2014, Bradshaw & Cowling, 2014). Edaphic factors such as soil fertility and texture can influence the relationship between rainfall inputs and vegetation activity. For example, nutrient impoverishment can limit growth responsiveness to moisture at the level of the individual plant (Chapin, 1980; DeWitt & Wilson, 1998; Valladares et al., 2002; Valladares et al., 2007; Murren et al., 2015) or community (Chapin et al., 1986) and soil texture can reduce or enhance the intensity of soil drought (Minasny & McBratney, 2007). As shown in Chapter 2, soil nutrient availability was much lower in sandstone-derived soils at the Fynbos site relative to the moderate fertility of shale-derived soils at the Renosterveld site (Table 2 – Chapter 2). The coarse texture of the sandstone-derived soils at the Fynbos site also had lower soil drought intensity relative to the fine textured, shale-derived soils of the Renosterveld site (Fig. 9,10- Chapter 2). Broadly speaking, these soil features should influence Fynbos and Renosterveld climate responses at the regional scale because of their strong soil-vegetation associations (Bergh et al., 2014, Bradshaw & Cowling, 2014). Adaptation of different shrubland communities in different soils could lead to edaphic specialisation which could alter the functionality of vegetation in response to shifts in key climatic variables (Pratt et al., 2012; Esler et al., 2015).

The degree to which rainfall patterns affect the patterns of vegetation recovery in the Cape Floristic region remains to be tested experimentally under field conditions. This chapter investigates the sensitivity of Fynbos and Renosterveld communities to experimentally altered rainfall seasonality patterns. Vegetation responses to altered moisture availability were recorded in terms of 1) post-fire productivity patterns and 2) community structure. This chapter thus is focused around vegetation responses at the community-level which is the broadest scale of investigation in Chapters 3-5. Experimental rainfall manipulations in treatment plots (see Chapter 2) were used to alter soil moisture availability in both summer and winter periods thus allowing for an assessment of the relative importance of summer versus winter moisture availability for post-fire vegetation processes. The study makes use of a unique and highly detailed dataset including an analysis of the changes in volume of every plant (> 50 000 individual measurements) and species growing on control and treatment plots after fire over three years. This dataset was collected concurrently with the environmental field site measurements reported in Chapter 2 and thus the vegetation patterns are discussed in the context of previously discussed environmental patterns and treatment effects on soil moisture.

3.2. Materials and Methods

3.2.1. Field surveys

Detailed demographic surveys were conducted twice a year for three years (2016 – 2019) starting in November 2016 **(Table 3.1)**. This followed from an initial, pre-treatment winter period (Winter 1) after the fire where no treatment effects were applied, allowing re-emergence and germination to occur under normal moisture conditions. The November 2016 survey was the baseline survey which recorded differences in plant coverage across plots before any treatment effects were applied **(Table 3.1)**. Relative changes from an assumed post-fire baseline of zero plant coverage (immediately post-fire) would have introduced bias because natural variations in the distributions of resprouters, seeds and geophytes across plots would not be accounted for. Therefore, the initial winter period allowed for these natural differences to be recorded as baselines for each plot.

Each survey consisted of identifying all plant species in all plots (6 control and 6 treatment plots in each of the sites), counting the number of individuals present and measuring the height and breadth of each. To make surveys repeatable and to aid in not double-counting plants during surveys, permanent sampling grids were set up dividing each plot into 16, 1 m × 1 m sub-plots. Grids demarcating these sub-plots were made using durable, wax coated string, which was secured onto the anti-herbivory fence surrounding the plots (**Fig. 2.6e**). Although individual plants were not tagged due to impracticality and potential disturbance, for each new survey, the field data sheet reflected the recorded history of each 1 m^2 sub-plot, including species name and dimensions of each individual plant ever measured in the sub-plot. Most species recruited once over a brief, discrete period of time (most immediately after fire), meaning that plant numbers could be easily monitored over time. Generally, cadavers were also visible after mortality. This enabled accurately tracking of the demographic changes in each of the sub-plots over time. At the Fynbos site, graminoids (grasses, sedges, restios) were not individually measured due to this growth form being too numerous and difficult to separate. Instead, their cover was estimated as a percentage of each 1 m × 1 m block. This percentage was later converted to volume using the estimated heights of these graminoids noted over time. The resultant dataset is comprised of over fifty thousand individual measurements made over three years.

Surveys were undertaken in the austral autumn (April) and late spring (October) each year, roughly partitioning the year into two contrasting periods of moisture availability and temperature (**Table 3.1**). The April surveys were intended to capture changes in vegetation over the hot, dry summer season as well as some transitionary spring and autumn periods. The October surveys were intended to capture the effects of the cool, wet winter season as well as some transitionary spring and autumn periods. Each survey was conducted over a week-long period. The “winter” and “summer” periods were chosen to contrast the influence of hot/dry and cold/wet seasonal extremes. These periods were chosen before the start of the experiment. Although an effort was made to keep the survey dates as equidistant as possible, due to logistical constraints and weather interference, there was some variation in the time between surveys.

Table 3.1: Demographic survey structure throughout experiment at the Drie Kuilen Nature Reserve.

Survey	Description	Date	Defined Seasonal Periods
-	Fire	11 May 2016	
a	Pre-treatment (Baseline)	21 - 24 November 2016	(W1) Winter 1 (Fire:a) 194 days
b	Post Summer 1	9 - 12 April 2017	(S1) Summer 1 (a:b) 139 days
c	Post winter 2	16 - 19 October	(W2) Winter 2 (b:c) 191 days
d	Post summer 2	8 - 12 April 2018	(S2) Summer 2 (c:d) 175 days
e	Post winter 3	22 - 26 October 2018	(W3) Winter 3 (d:e) 197 days
f	Post summer 3	8 - 12 April 2019	(S3) Summer 3 (e:f) 168 days

3.2.2. Measured and derived demographic variables

Raw volume measurements made on plants in cm^3 were calculated using the formula:

(2) $$Plant\ volume = Plant\ height * Plant\ breadth^2$$

Plant volumes were summed per plot for measures of total accumulated plot volume. Relative productivity was calculated according to the formula:

(3) $$Relative\ productivity = (\log(Plant\ Volume_{t,j+1}) - \log(Plant\ Volume_{t,j}))/Days$$

Where t = plot number, j = survey number (a=1,b=2,c=3,d=4,e=5,f=6), and *Days* = the number of days between survey j and j+1 **(Table 3.1)**.

Converting volume into a relative change from survey t and $t+1$ accounted for the feature of plants where larger plants display larger absolute volume gains/losses compared to smaller plants. This equates to the relative effort put into growth by plants rather than the absolute changes. Furthermore, due to baseline differences in plant coverage, it was necessary to quantify relative changes rather than absolute changes. Normalising the measurement by the number of days between measurements corrected for potential errors associated with longer periods having more time for plants to grow than shorter periods.

3.2.3. Data Analysis

3.2.3.1. Productivity analysis

All statistical analyses were conducted in R 3.5.1 (R Development Core Team, 2018). Relative changes in productivity were used to quantify ‘natural’ productivity patterns in control plots as well as to understand the effect of the treatment on productivity patterns. Mixed effects models were used to analyse the effects of rainfall on relative plot productivity with a focus on comparing treatment and control groups within and across Fynbos and

Renosterveld sites. Plot numbers were included as random effects in order to account to for random differences in plot cover, terrain, aspect, and plot history.

To evaluate plot-level productivity patterns I used pooled plant volume measurements from all measured plants, across all growth forms, and looked at relative changes in the pooled volume over time (i.e. *relative productivity,* see 3.2.2). The results from this analysis therefore accounted for, but did not quantify, unseen and untested community organisation factors such as competition and facilitation between and within species. Modelling species-specific responses and the inter-relationships with each other is extremely complex and is likely to be highly dependent on the specific community of plants. The pooled analysis strategy was based on the assumption that plant communities collectively make demands on resources available in the environment and so net community responses or sensitivity to climate should be considered as a unit. The sensitivity or responsiveness of a single species to environmental change can only be understood in the context of the surrounding plant community because species do not occur in isolation and the activity of one species can influence others.

Analysis of the overall seasonal patterns of relative productivity within and across groups was conducted using mean summer and winter relative productivity values which were averaged over the three-year experiment. Subsequently, I ran season specific analyses of the treatment effects on relative productivity using subsets of the data for each specific seasonal period (S1,W2,S2,W3,S3).

The interconnectivity of relative productivity rates over time was analysed using linear models and linear regression. Positive productivity-feedbacks would suggest that increases in relative productivity disproportionately increase the ability of a plant to access resources, increasing its subsequent productivity performance exponentially. This could be the case if resources are discontinuous in the soil and need to be discovered and tapped into before they can be utilised. Alternatively, positive productivity-feedbacks could be the result of increased overall efficiency of plants with size where larger vegetative structures are able to acquire exponentially more resources. Negative productivity feedbacks would suggest that growth could lead to the temporary or permanent depletion of resources in the environment or in stored nutrient reserves, thus negatively affecting subsequent relative productivity. This could occur of resources are scarce and easily depleted or if the maintenance or metabolic costs of growth are unsustainable with the natural rates of resource rejuvenation. Neutral growth feedbacks would suggest there is no effect of growth or plant size on resource acquisition or depletion and thus growth during one period would have little effect on subsequent relative productivity.

3.2.3.2. PCA analysis of species abundance and dominance

Principal components analysis (PCA) was used to identify shifts in community composition and structure of Fynbos and Renosterveld sites over time. In the first PCA, species abundance (i.e. count) matrices were used to investigate shifts in multi-species recruitment and establishment patterns in terms of numbers of plants in the post-fire environment.

The second PCA used volume weighted species matrices (e.g. accumulated above ground volume for each species in each plot) were taken to represent species dominance patterns in terms of plot biomass.

Separate PCAs compared the composition of control and treatment plots for each survey period (e.g. Nov2016, Apri2017…) within each site. Analyses were run separately for species abundance and volume-weighted species matrices.

3.3. Results

3.3.1. Net plot volume accumulation patterns

Broad differences in patterns of net plant volume accumulation were apparent between Fynbos and Renosterveld sites by the end of the experiment (i.e. at survey f, April 2019) **(Fig. 3.1a)**. Here, the patterns of plant volume accumulation differed more between control and treatment plots at the Renosterveld site compared to Fynbos site **(Fig. 3.1a)**. Net volume accumulation at survey f did not differ significantly across Fynbos treatment and control plots; $t(20) = -0.037$, $p = 0.971$, but differed strongly between Renosterveld control and treatment plots; $t(20) = -5.57$, $p = <0.001$. Renosterveld control plots accumulated more net plant volume than both Fynbos control and treatment plots; $t(20) = -2.51$, $p = 0.021$; $t(20) = -2.47$, $p = 0.023$. Not only were net accumulation patterns different between sites, seasonal patterns of change in net plot volume over the sequence of surveys (a-f) were also clearly unique in Renosterveld control plots compared to other groups. Renosterveld control plots showed a distinct stepped pattern in volume accumulation which indicated that plot volume was primarily accumulated during winter seasons. This pattern was clear on the absolute (**Fig. 3.1a**) and log scales (**Fig. 3.1b**).

Due to the potential cumulative effects of baseline plot differences (i.e. during baseline survey a), relating net plot volume accumulation patterns to control and treatment effects could be misleading. Therefore, follow-up analyses were run on relative or proportional changes in productivity using the log scale (i.e. *relative productivity* -see methods 3.2.2).

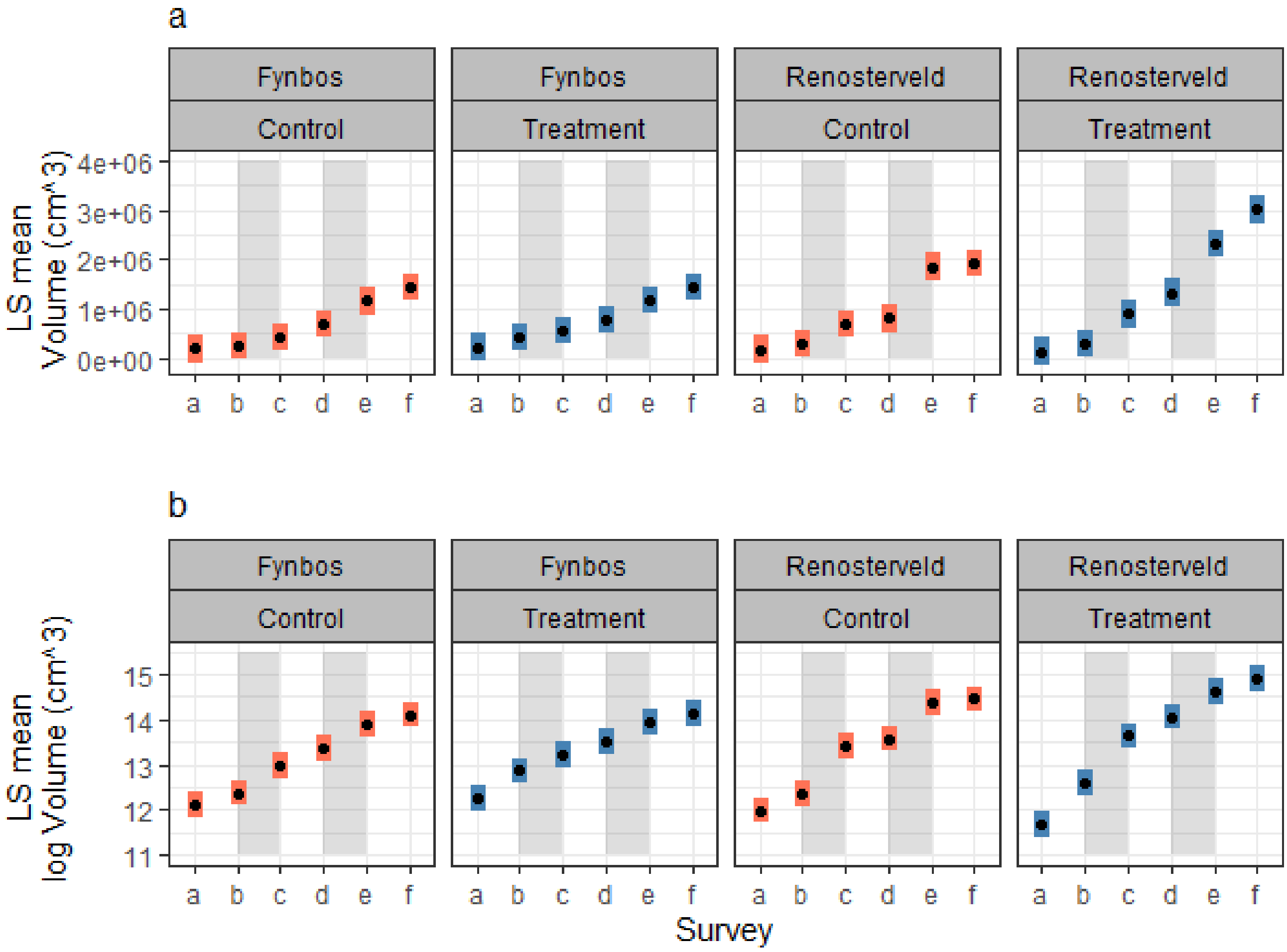

Figure 3.1: a) Summed plot volume for each survey (a-f). b) Log of the summed plot volume for each survey (a-f). Points represent least square means drawn from the full mixed effects model and coloured bands represent 95% confidence intervals. Grey bands represent winter periods.

3.3.2. Relative plot productivity

The full mixed effects model highlighted the effects of season (summer/winter) and site (Fynbos/Renosterveld) as important factors influencing relative productivity over the experimental period. There were also significant interactions between season and site as well as season and the treatment effects. This suggests that the magnitude of the effect of season on relative productivity was different in Fynbos and Renosterveld and in control and treatment groups (CT) **(Table 3.2)**. The three-way interaction was not significant implying that the model was not able to detect any overall difference on the impact of the treatment on productivity seasonality between Fynbos and Renosterveld sites.

Control plot relative productivity was higher in winter periods compared to summer periods. However, this natural growth seasonality was much more pronounced in Renosterveld where differences in the summer versus winter relative productivity values were larger than in Fynbos (**Table 3.3**). The treatment effect reduced the seasonality of relative productivity (seen in control plots) at both sites as relative productivity differences between summer and winter were not statistically significant in treatment plots (**Table 3.3**). Renosterveld control and treatment plots both achieved periods of higher maximum relative productivity than Fynbos (**Fig. 3.2a**).

Table 3.2: Mixed effects model results showing the effects of ”Season”, “Site” and “Control/Treatment” on relative productivity.

Factor	Df	denDF	F value	Pr (>F)
Season (Summer/Winter)	1	92	12.70778	**<0.001**
CT (Control/Treatment)	1	20	2.71205	0.115
Site (Fynbos/Renosterveld)	1	20	9.42679	**0.006**
Season*CT	1	92	10.86541	**0.001**
Season*Site	1	92	6.67216	**0.011**
CT*Site	1	20	2.61524	0.122
Season*CT*Site	1	92	1.03654	0.313

Table 3.3: Site-specific contrasts showing the differences in relative productivity between summer and winter and between control and treatment plots.

Group	Factor	Estimate	SE	df	t-ratio	p.value
Relative productivity seasonality in control versus treatment plots						
Renosterveld controls	Summer – Winter	-0.00348	0.00067	92	-5.231	**<0.001**
Renosterveld treatment	Summer – Winter	-0.00061	0.00067	92	-0.917	0.362
Fynbos controls	Summer – Winter	-0.00108	0.00067	92	-1.630	0.107
Fynbos treatments	Summer – Winter	0.00043	0.00067	92	0.648	0.519
Treatment effects on summer & winter relative productivity						
Fynbos summer	Control - Treatment	-0.00062	0.00060	20	-1.035	0.313
Fynbos winter	Control - Treatment	0.00090	0.00073	20	1.235	0.231
Renosterveld summer	Control - Treatment	-0.00221	0.00060	20	-3.717	**0.001**
Renosterveld winter	Control - Treatment	0.00066	0.00073	20	0.903	0.377

3.3.3. Treatment effects on relative plot productivity

Season specific treatment effects showed that the sensitivity of vegetation was highest to the treatment effects in the first summer after fire at both Fynbos and Renosterveld sites which both showed a positive relative productivity response to the first summer irrigations. However, the responsiveness of Renosterveld productivity to treatment effects was sustained throughout the experiment whereas no effects were detected in Fynbos after the first summer (**Fig. 3.2b**). In Renosterveld, where effects were statistically significant, summer irrigation treatments always had a positive effect on relative productivity relative to control plots and winter drought treatments always had a negative effect on relative productivity relative to controls. The seasonal treatment effects in Renosterveld appear to have reduced the natural seasonality of productivity (**Fig. 3.1, 3.2a**) by altering the timing and amount of productivity throughout the experiment (**Fig. 3.2b)**. Additionally, the treatment effects also increased overall relative productivity in Renosterveld between survey a – f, relative to control plots **(Fig. 3.2b, Table 3.4).** No significant overall treatment effect on relative productivity was detected in Fynbos **(Fig. 3.2b)**.

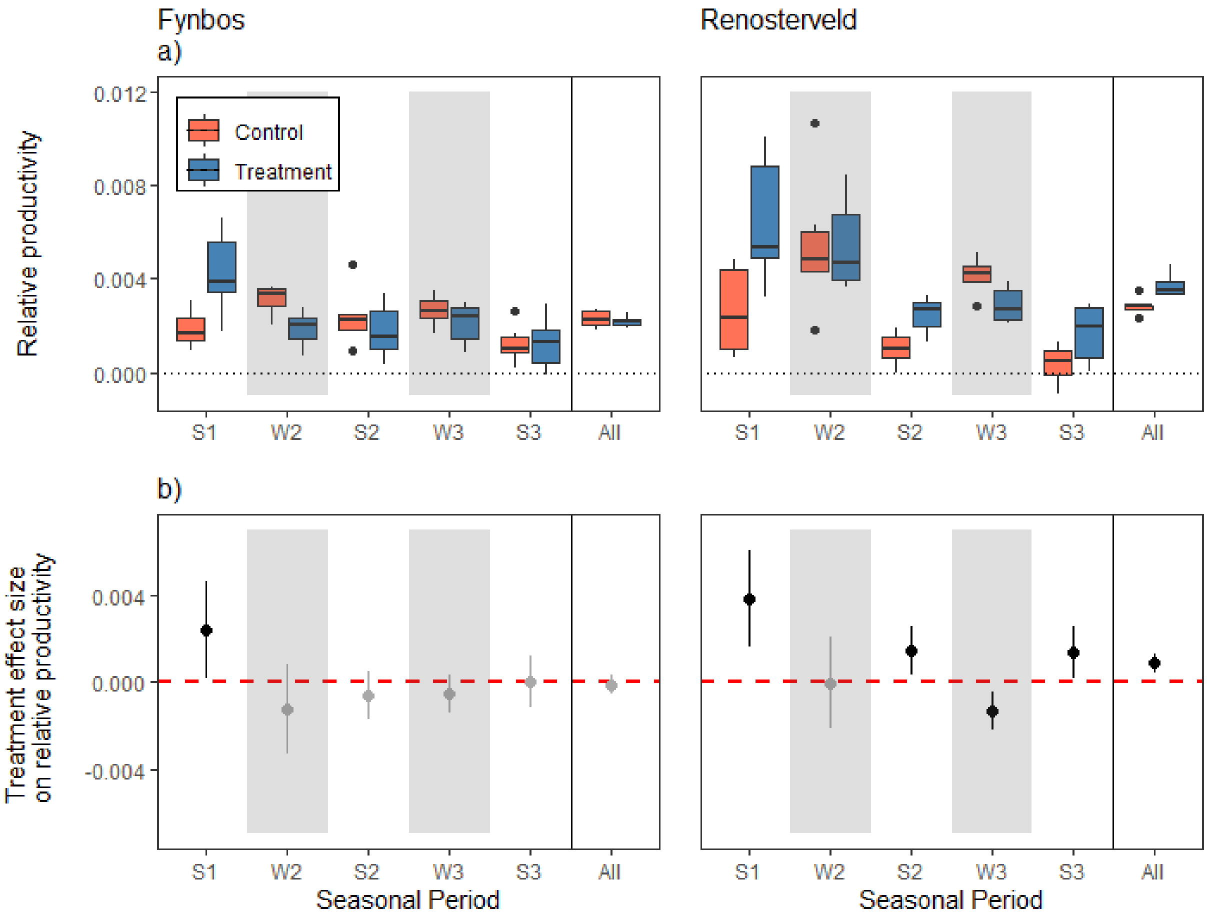

Figure 3.2: a) Box and whisker plot of season-specific relative productivity in control and treatment plots for Fynbos and Renosterveld sites. Box and whiskers display median, UQR,LQR, min, max and outliers. b) Season-specific treatment effects (defined as treatment means minus control means) on relative productivity. Black symbols represent treatment effect sizes different from zero (dashed line) at the 95 % confidence level while grey symbols represent non-significant differences. Vertical bars represent the 95% confidence intervals surrounding treatment effect size estimates. 95%. First summer (S1) = survey a to b, Second winter (W2) = survey b to c, Second summer (S2) = survey c to d, Third winter (W3) = survey d to e, Third summer (S3) = survey e to f, All = survey a to f. Grey bands represent winter periods.

Table 3.4: The differences in season-specific treatment effect sizes between Fynbos and Renosterveld sites drawn from mixed effects models. These values supplement the visual differences displayed by overlapping/non-overlapping CI of treatment effects in Fig. 3.2b.

Period	Estimate	SE	df	T	p
S1	0.00141	0.00162	20	0.86881	0.395
W2	0.00122	0.00151	20	0.81259	0.426
S2	0.00207	0.00082	20	2.53290	**0.020**
W3	-0.0007	0.00064	20	-1.16627	0.257
S3	0.00131	0.00085	20	1.54221	0.139
All	0.00099	0.00031	20	3.19064	**0.005**

3.3.4. Productivity feedback effects

Strong evidence for negative productivity feedbacks (see Methods section 3.2.3.1) were detected in Fynbos plots (**Fig. 3.3**) even though overall relative productivity seasonality and responsiveness were generally insignificant in Fynbos (**Fig. 3.2b**). During two distinct summer-winter contrasts, Fynbos treatment plots with larger relative productivity under summer irrigation periods had smaller relative productivity during the following winter drought treatment, leading to strong negative correlations between relative productivity in S1 and W2 as well as S2 and W3 (**Fig. 3.3**). Conversely, there was no feedback detected in Renosterveld suggesting that Renosterveld could increase its relative productivity without any clear effect on subsequent performance (**Fig. 3.3**). The productivity trade-offs in Fynbos treatment plots were not driven by the same plots in the two summer/winter contrasts (S1 vs W2 & S2 vs W3) as indicated by the difference in order of the coloured points in the S1/W2 and S2/W3 contrasts **(Fig. 3.3)**. This suggests that the strong negative relationships were not influenced by natural, chance differences in plot community structure where some plots could have had naturally higher summer or winter productivity, unrelated to the treatment effect. It also suggests that specific plot compositions can have varied responses over time which are not the same each year. Regardless of the plot-specifics, large responses to the summer irrigations consistently lead to reduced performance in the subsequent winter drought. While these relationships were clear in Fynbos treatment plots, the large S2 relative productivity rate of a single plot in the Fynbos control plots was not associated with reduced subsequent performance (**Fig. 3.3**). This could imply that productivity responses may not be as detrimental if not followed by a drought period. This specific plot also had a relatively high proportion of resprouting plants which could be less reliant on shallow moisture availability.

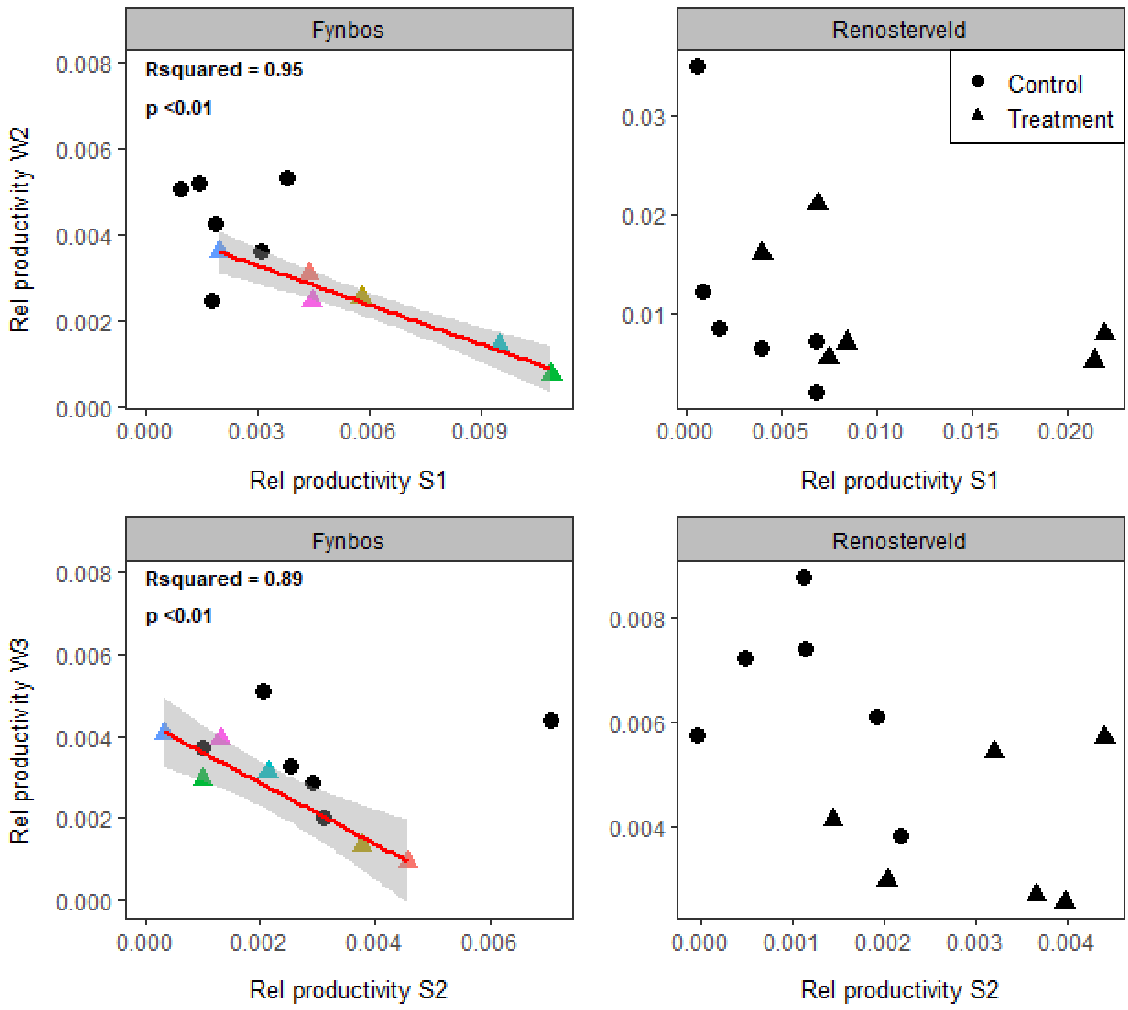

Figure 3.3: Interconnectivity between seasonal relative productivity during two consecutive summer/winter periods. First summer (S1) = survey a to b, Second winter (W2) = survey b to c, Second summer (S2) = survey c to d, Third winter (W3) = survey d to e. Significant linear relationships are displayed using red lines with confidence bands represented by grey shading. For significant relationships, data points are coloured by plot so that plot-specific responses can be compared between the two summer/winter contrasts. p and R-squared values are displayed for the significant relationships.

3.3.5. Treatment effects on community composition

Principal components analysis (PCA) demonstrated greater compositional responses to altered rainfall patterns in the Renosterveld site, in terms of both species abundance patterns (i.e. counts) **(Fig. 3.4**), and species dominance (i.e. volumes) **(Fig. 3.5)**. The Fynbos site showed little compositional change in relation to the treatment effects.

Shifts in species abundance patterns (i.e. counts) were only detected at Renosterveld in response to the first summer irrigations, indicated by a strong separation of control and treatment means and 95% confidence ellipses in multivariate space from Nov2016 (Baseline) to Apr2017 **(Fig. 3.4)**. However, after the first summer, control and treatment species abundance patterns remained highly stable in multivariate space. This indicated that species abundance patterns in Renosterveld were most sensitive to rainfall during the first summer and that changes in species abundance patterns during the first summer had lasting effects on community composition (**Fig. 3.4**). There was no clear relationship between shifts in species abundance patterns (as detected by PCA) and seasonal productivity responses **(Fig. 3.2)** over the course of the experiment. Fynbos productivity responses in the first summer **(Fig. 3.2)** were not matched by compositional shifts **(Fig. 3.4).** Additionally, continued productivity responses in Renosterveld after the first summer **(Fig. 3.2)** which were not matched by concurrent compositional shifts **(Fig. 3.4)**. This indicates that seasonal productivity responses to altered moisture availability were primarily the result of high growth plasticity within the established species, although some combination of growth and shifts in abundance could influence productivity patterns in the first summer at the Renosterveld site.

The volume-weighted PCA **(Fig. 3.5)** also indicated a stronger shift in community structure/species dominance at the Renosterveld site and relatively low sensitivity at the Fynbos site. However, this separation of control and treatment plots in multivariate space for the Renosterveld site only began to materialise in the later stages of the experiment. The slow accumulation of differences in species dominance patterns between control and treatment plots in Renosterveld is strongly representative of the raw volume patterns which show increasingly large differences forming between control and treatment plots over time **(Fig. 3.1)**. This indicates that the combination of early compositional shifts and sustained growth responses to treatment effects can lead to strong differences in patterns of species dominance and overall productivity patterns which accumulate over time.

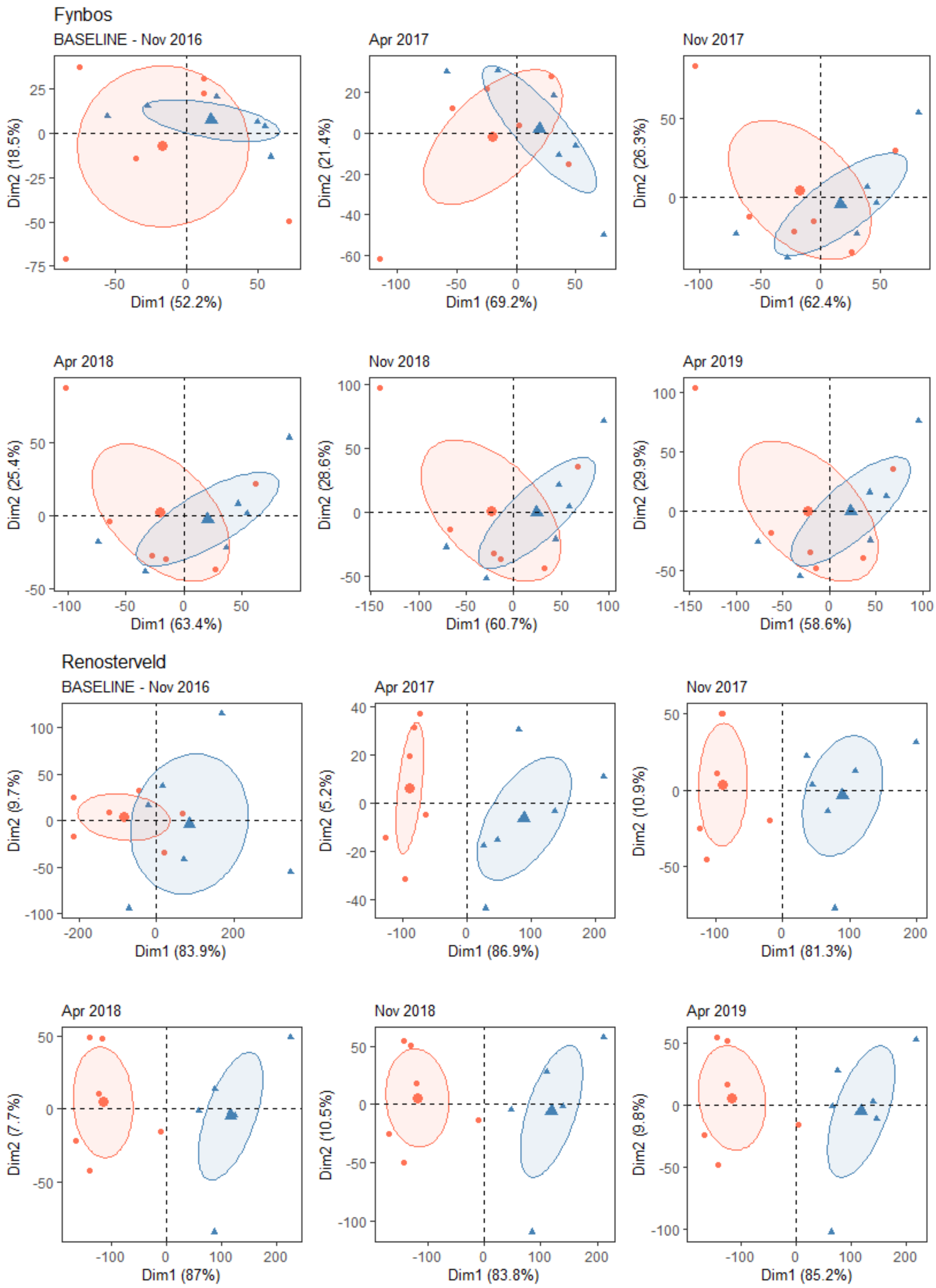

Figure. 3.4: Fynbos and Renosterveld site species compositional differences between control and treatment plots over time as determined by PCA analysis using species abundances (i.e. counts). Small orange circles represent control plot compositions and large orange circles represent the mean of control plot clusters. Small, blue triangles represent treatment plot compositions and large, blue triangles represent the mean of treatment plot clusters. 95% confidence ellipses are shaded for control and treatment groups.Dim1 and Dim2 is the first and second dimension of the PCA, respectively. The percentage of variation explained by each dimension is indicated in brackets.

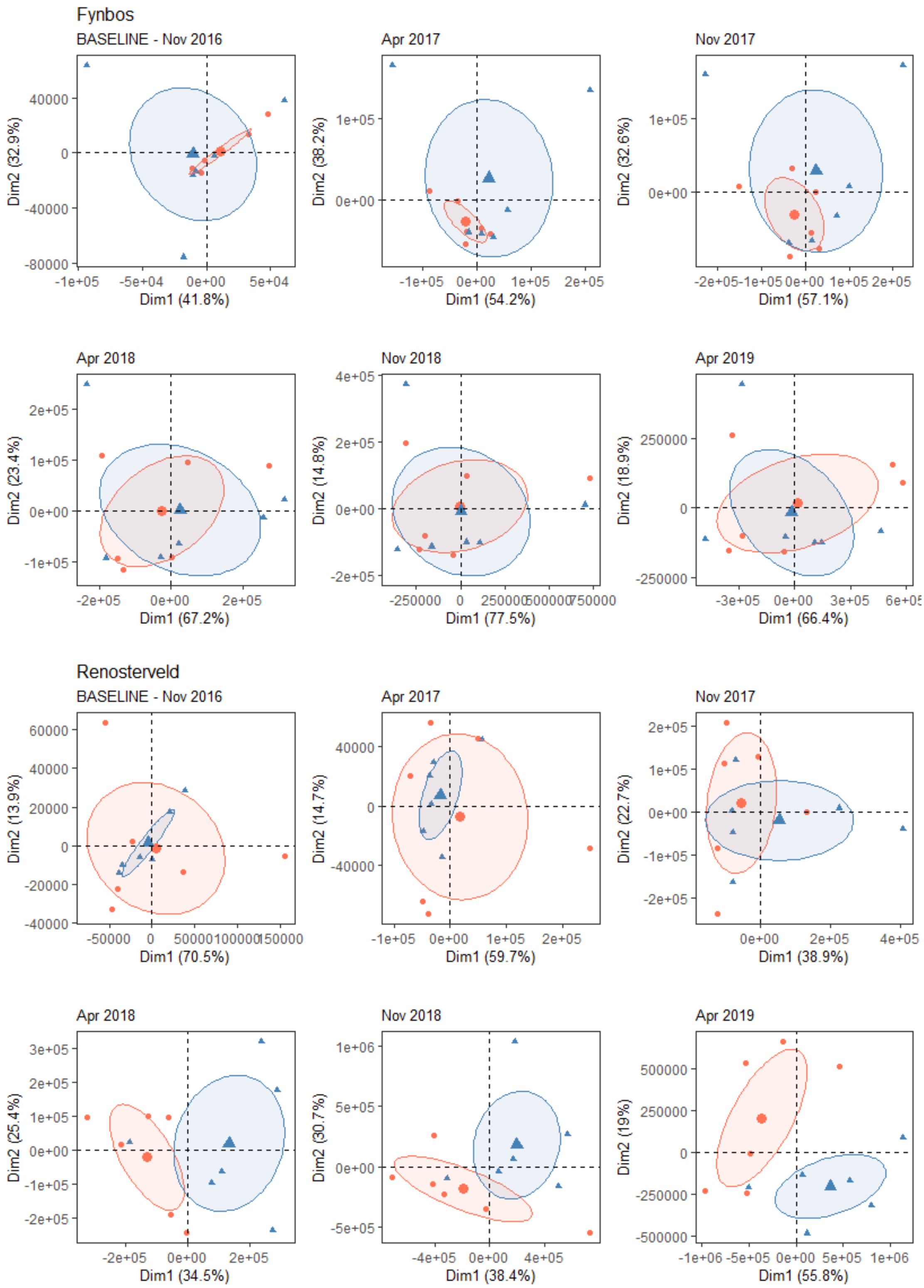

Figure. 3.5: Species compositional differences between control and treatment plots over time as determined by PCA analysis using species volumes. Small orange circles represent control plot compositions and large orange circles represent the mean of control plot clusters. Small, blue triangles represent treatment plot compositions and large, blue triangles represent the mean of treatment plot clusters. 95% confidence ellipses are shaded for control and treatment groups.Dim1 and Dim2 is the first and second dimension of the PCA, respectively. The percentage of variation explained by each dimension is indicated in brackets.

3.4. Discussion:

Neighbouring, post-fire Fynbos and Renosterveld communities differed in terms of their responsiveness to altered moisture availability. Productivity patterns at the Renosterveld site were more highly responsive to summer irrigation treatments and winter drought treatments compared to the Fynbos site which was relatively insensitive to moisture patterns after the first summer. Along with higher sensitivity of productivity, the Renosterveld site also showed larger shifts in community structure in response to altered rainfall whereas Fynbos community structure was not affected. These patterns highlight the potential for soil properties to mediate shrubland community responses to moisture availability patterns. Furthermore, the study demonstrated amplified sensitivity of vegetation processes during the first summer after fire with potential long-term implications on community structure and patterns of diversity (i.e. event-dependant succession).

Patterns of plot productivity detected in the experimental Fynbos and Renosterveld sites **(Fig. 3.2)** were consistent with regional scale data presented in (Cramer and Hoffman, 2005 supplementary material) which showed a lower mean inter and intra annual variability in the Normalised Difference Vegetation Index (NDVI) values of Fynbos relative to Renosterveld. NDVI and productivity are closely linked (Casadesús *et al.*, 2000; Hope *et al.*, 2012) and therefore the low NDVI variability in Fynbos at the regional scale is consistent with low sensitivity to rainfall found in this experiment. Due to the broad separation of Fynbos and Renosterveld across soil-types in the Cape Floristic Region (Bergh *et al.*, 2014; Bradshaw & Cowling, 2014), it is likely that edaphic effects play a role in regional-scale shrubland responses to rainfall. Edaphic effects have been shown to affect species distributions and plant function within the region (Esler & Cowling, 1993; Esler *et al.*, 2015).

Experimental sites were considered as representative of regional scale vegetation-soil relationships in the Cape Floristic Region. Strong soil fertility (**Table 2.2**) and textural differences between the soils at Fynbos and Renosterveld sites, appeared to mediate the relationship between productivity and post-fire moisture availability. Low nutrient availability at the Fynbos site could have directly limited the productivity responses to moisture because plants require both moisture and nutrients to grow (Chapin 1980, Chapin *et al.*, 1986). A given rate of vegetation productivity has an inherently greater chance of depleting nutrient reserves on infertile soils than on fertile soils due to a decrease in supply relative to demand (Chapin 1980). This study provided evidence of nutrient depletion/limitation within the vegetation of the nutrient-impoverished, sandy Fynbos soils (**Table 2.2**) during early post-fire succession. Here treatment plot productivity during irrigated summers had slight, but clear negative consequences on subsequent productivity during imposed winter droughts **(Fig. 3.3)**. This, along with generally low moisture sensitivity (naturally and to treatment effects), suggests that vegetation productivity in Fynbos was primarily constrained by chronic nutrient scarcity rather than moisture availability. Conversely, larger productivity responses to irrigations in the moderately fertile (Renosterveld) soils were not associated with any negative feedback effects suggesting that nutrient availability was not limiting over the course of the experiment and that vegetation activity was strongly moisture driven.

While direct nutrient limitation is likely to play a direct limiting role on productivity responses in Fynbos, the extent to which Fynbos communities have also developed internally canalised, conservative growth traits requires further investigation (Chapin, 1991; Power *et al.*, 2019). The evolution of conservative growth traits are often favoured in nutrient limited environments (Chapin, 1991; Diaz *et al.*, 2004; Wright *et al.*, 2004; Chapin, 1980; Valladares *et al.*, 2007; Murren *et al.*, 2015; Power *et al.*, 2019) and would ensure that resource use and uptake patterns during temporarily favourable conditions do not lead to unsustainable metabolic and maintenance costs in the long-term (Cheung *et al*, 2013; Murren *et al.*, 2015). This could be tested using reciprocal transplant, or nutrient addition experiments to test whether Fynbos plants would respond to moisture under conditions of higher nutrient availability. There is some evidence to suggest strong edaphic specialisation in Fynbos communities may inhibit their performance under higher nutrient conditions (Esler *et al.*, 2015) .This has important implications for determining whether Fynbos responses to climate are constrained directly by nutrient availability constraints or the adaptation/specialisation of canalised, conservative traits (Chapin, 1991; Power *et al.*, 2019).

The Fynbos and Renosterveld productivity responses to moisture presented in this chapter are consistent with the apparent trade-off between conservative, specialised growth and rapid acquisitive type growth, respectively, as seen amongst many plant lineages globally (Diaz *et al* 2014; Wright *et al.*, 2004). This trade-off could mediate community level responses to climate across structurally similar Fynbos and Renosterveld sites where rapid moisture responses and growth plasticity could be favoured in more fertile soils relative to nutrient impoverished soils which should select for conservative traits (Power *et al.*, 2019). Soil texture can also mediate productivity responses to rainfall because different soil textures affect moisture retention within soils between rainfall events (Minasny & Mcbratney, 2007). The fine-textured shale-derived soils in Renosterveld had lower and more rapidly declining soil water potential values during drought compared to Fynbos (**Fig. 2.10**). This amplified drought for plants in terms of plant-available moisture meaning that Renosterveld plots experienced higher variability in moisture availability (i.e. harsher droughts) while Fynbos dry/wet cycles were buffered to some extent by the maintenance of higher soil water potentials through drought. The buffering against natural drought in Fynbos soils would consequently dilute the treatment irrigation and drought effects. The combination of relaxed drought intensity and nutrient limitation in sandy soils provide an intuitive explanation for relative stability of productivity in Fynbos which appeared to be buffered against inter- and intra-annual climate variability relative to Renosterveld.

In addition to site productivity responses to moisture availability patterns, community compositional shifts were also most evident at the Renosterveld site suggesting that productivity and community sensitivity was broadly linked, but not closely correlated in time. The findings in the Renosterveld site are consistent with the 'event-dependant hypothesis' (Bond and van Wilgen, 1996; Moreno *et al.*, 2011) which predicts that the sensitivity of vegetation to early-post fire climate conditions could have lasting implications on mature vegetation trajectories and community composition. This highlights the potentially rapid impacts of climate change on fire-prone vegetation over successive fire events. However, the results also demonstrated that Fynbos communities may be somewhat buffered from the effects of early post-fire weather thus decoupling vegetation trajectories from post-fire weather. These ideas are explored further in Chapter 4.

Community stability under varying moisture regimes could help to explain the distribution of Fynbos over wide rainfall gradients (Bergh *et al.*, 2014; Bradshaw & Cowling, 2014). Conversely, instability in Renosterveld could help to explain the rapid climate-driven transitions between mountain Renosterveld and Succulent Karoo communities (Levyns 1964; Bergh *et al.*, 2014; van der Merwe & van Rooyen, 2011a,b). The extent to which major shrubland communities differ in terms of their response to rainfall patterns should be tested experimentally across a range of precipitation and temperature extremes (see West *et al.*, 2012, Parra *et al.*, 2012; Esler *et al.*, 2015).

This chapter demonstrated that patterns of productivity and community structure are differentially coupled to rainfall seasonality across Fynbos and Renosterveld shrublands. In the next chapter, the underlying growth form and demographic drivers of community level responses are investigated further.

CHAPTER 4: Growth form responses to altered post-fire rainfall seasonality in Fynbos and Renosterveld

Summary:

- In this chapter I investigated post-fire responses of perennial plant growth forms (e.g. perennial seedlings, woody resprouts, prostrate resprouts, graminoids) to experimentally altered rainfall patterns in Fynbos and Renosterveld sites. This investigation highlighted which growth forms were responsible for plot level productivity responses and shifts in community structure reported in Chapter 3.
- Growth forms showed varied growth and survival responses to altered rainfall seasonality over the three-year experiment but were generally positively impacted by summer irrigations and negatively impacted by imposed winter droughts. Within growth forms, opposing growth responses to summer irrigations and winter droughts lead to low net effects of altered rainfall seasonality on growth and rates in all growth forms. However, the sensitivity of seedling emergence and survival patterns to rainfall during the first summer lead to compounding effects on seedling volumes over the three-year experiment. Post-fire rainfall patterns thus have important implications for long-term patterns of community structure, consistent with the event-dependence hypothesis.
- Growth forms at the Renosterveld site generally displayed stronger seasonal growth and productivity responses to the treatment effects relative to growth forms at the Fynbos site site explaining the stronger seasonal productivity responses at the plot level (Chapter 3).
- In Renosterveld, seasonal productivity patterns of growth forms were unified under the winter rainfall regime but diverged amongst growth forms under reduced rainfall seasonality. This indicated the potential for altered rainfall seasonality to cause shifts in patterns of resource use and competition amongst growth forms. Complex, unrelated phenological patterns amongst Fynbos growth forms and weak treatment responses reflected a greater need for growth forms to partition, conserve and compete for limited nutrients.

4.1. Introduction:

Chapter 3 demonstrated that post-fire recovery patterns of Fynbos and Renosterveld shrubland sites were differentially sensitive to natural and altered rainfall seasonality (e.g. summer irrigations and winter droughts). Overall, seasonal plot productivity patterns in Fynbos were relatively insensitive to natural winter rainfall seasonality and lacked sensitivity to altered rainfall patterns. Conversely, Renosterveld plot productivity peaks were strongly seasonal under the winter rainfall climate but shifted to match moisture availability patterns under altered rainfall seasonality. The lack of moisture sensitivity in Fynbos was discussed in terms of nutrient limitation and soil texture in sandstone-derived soils. In addition to greater rainfall sensitivity at Renosterveld site, stronger divergence in community composition (i.e. species abundance patterns) was recorded between control and treatment plots over the first summer as well as a stronger progressive shift in above ground species dominance (i.e. net species volumes) over the three-year experiment. Therefore, rainfall patterns in the early, post-fire environment could have long-term ramifications for patterns of vegetation productivity and community structure (i.e. species abundances and dominance), consistent with the idea of "event-dependent succession" (Bond & van Wilgen, 1996; Moreno et al., 2011). In this chapter, I investigated which growth forms were the main drivers of observed patterns in plot level productivity and community structure reported in Chapter 3. This was achieved by separately analysing the demographic responses of key dominant perennial growth forms to experimentally altered rainfall seasonality patterns.

Within post-fire communities, the sensitivity of demographic patterns to seasonal moisture availability could be specific to different growth forms due to inherent differences in numerous aspects of their life-history, form and functionality (Kruger, 1977; West *et al.*, 2012). Numerous plant traits have been proposed as important determinants of plant responses to environmental change, including relative growth rates, leaf, stem and root morphology, physiology and leaf chemistry (Lavorel *et al.*, 1997; Eviner & Chapin, 2003: West *et al.*, 2012). Differences in post-fire regeneration strategy within perennial growth forms could also strongly affect their sensitivity to rainfall and drought. For example, in resprouting perennials (fire survivors), the persistence of deeper underground rooting structures after fire may buffer these growth forms from shallow soil moisture fluctuations (Shwinning & Sala, 2004). Notably, perennial growth forms in the seedling-state could be most sensitive to the timing in moisture availability because their developing rooting structures lack storage potential and access to deeper moisture sources (Parra & Moreno, 2017,2018). While growth form responses to climate change may be complex, characterising functional groups with similar or unique responses to climate can be a practical approach to studying such responses (Chapin *et al.*, 1996).

Not only may growth forms differ in their intrinsic sensitivities to moisture availability, their responses to moisture availability could also be affected by complex relationships with other growth forms (Holzapfel & Mahall, 1999; Bloom & Mallik, 2006). Competitive interactions between growth forms for both moisture and nutrients have been identified as an important feature of Mediterranean-type ecosystems which undergo periods of stress and disturbance (Vila & Sardans, 1999). The strength of competition between growth forms depends on the degree of overlap in resource use and the relative competitive abilities of the growth forms present. From a resource balance perspective, it is expected that the degree of competition should increase with decreasing resource availability. Therefore, it might be expected that differences in nutrient and moisture availability between Fynbos and Renosterveld sites could affect the competitive interactions amongst growth forms. However, to combat the overlap in resource use, and thus reduce competition, growth forms may partition resources through time and in space to varying degrees (Westoby, 1979; Reynolds *et al.*, 2004; Turner, 2008). For example, seasonal phenological and developmental patterns could differ across growth forms leading to non-overlapping seasonal resource use. Growth forms could also separate resources in space with some accessing deeper or shallower moisture sources and different forms of nutrients (Reynolds *et al.*, 2004; Ogle & Reynolds, 2004). Conservative growth traits could also aid in community resource conservation by reducing resource use at the individual plant level (Chapin, 1980; Murren *et al.*, 2015). Taken together, it is not altogether clear how complex differences in growth form functionality interact at the community level and how changes in rainfall patterns could affect growth form function and community dynamics.

In this chapter I explore the diversity of growth form responses to experimental summer irrigations and imposed winter droughts during the first three years after fire. This investigation is intended to identify which growth forms were responsible for the main differences in plot level productivity patterns and changes in community structure, reported in Chapter 3. Growth form responses to altered moisture were investigated in terms of relative changes in volume, count and plant sizes. Additionally, seasonal productivity patterns were correlated amongst growth forms within control and treatment plots to explore patterns of community phenology under differing rainfall regimes. As in Chapter 3, growth form responses were contrasted between Fynbos and Renosterveld sites, which differed in their soil moisture and nutrient properties. Stronger moisture deficits in Renosterveld and stronger nutrient limitation in Fynbos provided a unique opportunity to assess the relative influence of moisture and nutrient factors on individual growth form and community responses.

4.2. Methods

4.2.1. Vegetation measurement surveys

In this chapter I focused analysing the rainfall responses of key perennial growth forms which were present at both shrubland sites **(Fig. 4.1)**. I used subsets (e.g. growth form groupings) of the full demographic dataset described in Chapter 3 (3.2.1). Analyses contrasted relative changes in growth form volumes, counts and plant sizes between control and treatment plots. Analyses were based on the post-fire demographic dataset collected over 6 surveys (a = Nov 2016, b = Apr 2017, c = Oct 2017, d = Apr 2018, e = Oct 2018, f = Apr 2019) from 2016-2019. These surveys allowed for relative changes and treatment effects to be assessed over distinct seasonal periods: 1st Summer (S1), 2nd Winter (W2), 2nd Summer (S2), 3rd Winter (W3) and 3rd Summer (S3) (**see Table 3.1 for details on surveys and seasonal periods).**

4.2.2. Rainfall treatments

Treatment effects were designed to manipulate seasonal moisture availability patterns in the post-fire Fynbos and Renosterveld sites. Control plots received natural rainfall, resulting in cool, wet winters and periods of drought during warm summers, typical of a winter rainfall climate. Treatment plots received winter drought treatments and summer irrigations resulting in reduced seasonality in soil moisture availability. The redistribution of winter rainfall onto plots during summer treatment irrigations alleviated drought during the hottest summer months, and reduced moisture availability under cold seasonal periods which are presumably unfavourable for growth. Therefore, the treatment effects increased the overall synchrony of moisture and energy resources throughout the year. Treatment effects were analysed over distinct seasonal periods S1,W2,S2,W3,S3 (**see Chapter 2 for full description of treatment effects**).

4.2.3. Growth form groupings

Dominant perennial growth forms were grouped into 4 groups based on above-ground growth habit, and below ground structures (**Fig. 4.1**). Grouping of species into growth forms was necessary for the analysis since species diversity was high and describing the responses for all species was impractical. Growth form categories were based on evidence from off-plot excavations as well as research into the habits of species where excavations were not possible.

Perennial seedlings (PS) (**Fig. 4.1a**) included newly germinated seedlings predominantly from shrubby, obligate reseeder species with a lifespan >2 years (i.e. perennial) and with no surviving adults present in the post-fire landscape. PS displayed substantial investment in rooting structures and were generally woody to some degree from early stages of development. Most dominant at the Drie Kuilen study sites were members of the Scrophulariaceae family; *Selago corymbosa* (Fynbos) and *Microdon polygaloides* (Renosterveld). Note that brackets indicate the site to which species were associated.

Woody resprouts (WR) (**Fig. 4.1b**) included long-lived species which had resprouted from large, woody underground root structures and which had an upright, shrubby form. These resprouts represented surviving individuals from obligate resprouter species as there were no seedlings of these recovering shrubs detected post-fire, e.g. *Agathosma capensis* (Fynbos), *Clutia rubricaulis* (Fynbos & Renosterveld), *Passerina obtusifolua* (Renosterveld) and *Elytropappus rhinocerotis* (Renosterveld).

Prostrate resprouts (PR) (**Fig. 4.1c**) included long-lived woody, resprouts from resprouter species which had a prostrate or creeping growth habit. E.g. *Roepera fulva (*Fynbos & Renosterveld), *Hermannia aspera* (Fynbos & Renosterveld), *Indigofera heterophylla* (Renosterveld) and *Aspalathus spinescens* (Fynbos). These were separated from woody resprouts primarily on the basis of height because plant vertical structure could imply adaptation to different light and moisture niches (Linder & Campbell, 1979) and a creeping growth habit could lead to plants being closely affected by the temperature of the soil. Additionally, prostrate resprout rooting structures were generally not as extensive and deep as the woody resprouts.

Graminoids (G) (**Fig. 14, d**) included species from the Poaceae and Cyperaceae families. E.g. *Ehrharta villosa* (Fynbos and Renosterveld), *Isolepis spp.* (Fynbos and Renosterveld). The majority of Poaceae and Cyperaceae included in this group maintained above ground biomass year-round and arose from persisting underground structures after fire.

While there was substantial variation between species within the chosen growth form groupings, they represent the simplest way to broadly separate species based on above-ground and below-ground habit. This classification is loosely adapted from that used by Cowling *et al.*, 1994 and Agenbag, 2006.

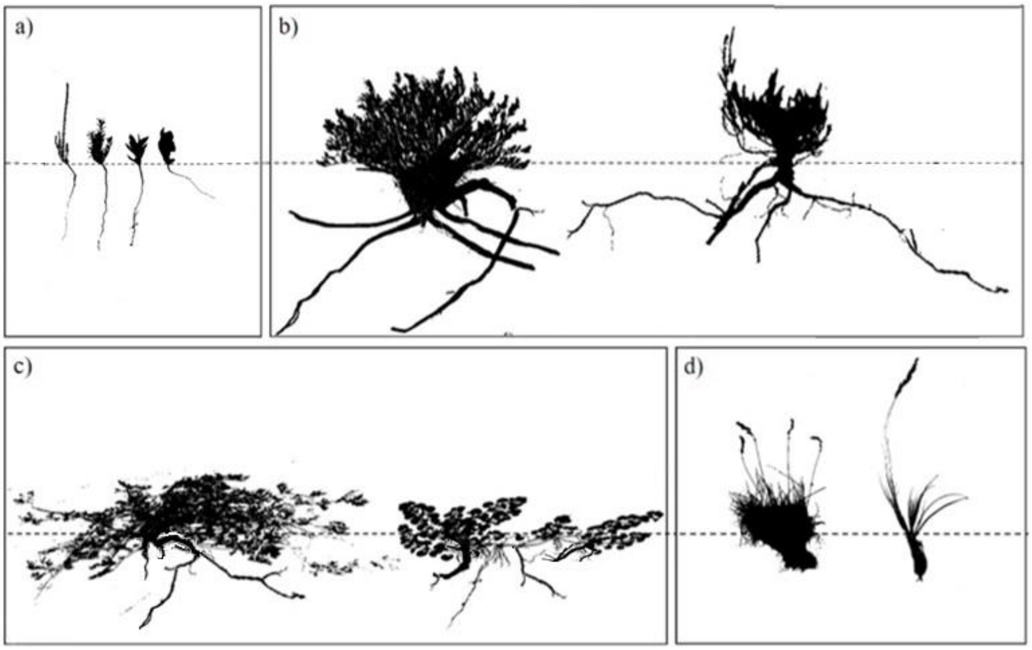

Figure 4.1: Growth form in the post-fire environment. a) Perennial seedlings (PS), b) Woody resprouts(WR), c Prostrate resprouts (PR), d) Graminoids (G). Dotted horizontal line represents the ground soil surface. Growth forms are depicted roughly to scale in terms of how their sizes relate to each other within the first year after fire.

4.2.4. Data analysis

4.2.4.1. Quantification of the effects of manipulated rainfall patterns on growth forms within Fynbos and Renosterveld sites.

I analysed the effects of the summer irrigations and winter droughts on the relative changes in growth form volumes, counts and plant sizes. Analysing relative changes accounted for any baseline differences in growth form abundance and size which had emerged before the first summer irrigation (i.e. over the first winter) as well as differences in the length of time between surveys **(Table. 3.1).** Relative changes thus allowed for a fair comparison of sensitivity amongst growth forms and across sites.

For each growth form I investigated relative changes (Δ) in:

1) net volume/plot $= {'}\Delta\mathrm{Vol}{'}$,

2) plant counts/plot $= {'}\Delta\mathrm{Count}{'}$,

3) mean plant size/plot $= {'}\Delta\mathrm{Size}{'}$.

Relative change (i.e. ΔVol , ΔCount and ΔSize) was analysed separately over distinct seasonal periods: S1 (Nov 2016 – April 2017), W2 (April 2017 – Oct 2017), S2 (Oct 2017 – April 2018), W3 (April 2018 – Oct 2018), S3 (Oct 2018 – April 2019) and over the whole experiment, Net (i.e. Nov 2016 to April 2019). See Chapter 3, **Table 3.1** for full description of survey and seasonal periods.

ΔVol , ΔCount and ΔSize for periods S1, W2, S2, W3, S3, Net were calculated using Equation 4, 5 and 6.

(4) $\Delta\text{Vol} =$ Relative change in net plant volume =

$$(\log(Volume_{\,t,i,j+1}) - \log(Volume_{\,t,i,j}))/Days$$

(5) $\Delta\text{Size} =$ Relative change in plant size =

$$(\log(Size_{\,t,i,j+1}) - \log(Size_{\,t,i,j}))/Days$$

(6) $\Delta\text{Count} =$ Relative change in plant counts =

$$(\log(Count_{\,t,i,j+1}) - \log(Count_{\,t,i,j}))/Days$$

Where t = plot number, i = growth form, j = survey number (a=1,b=2,c=3,d=4,e=5,f=6), and *Days* = the number of days between survey j and j+1 **(Table 3.1)**.

I considered ΔVol to be representative of changes in the productivity of growth forms because net volume patterns resulted from patterns of individual plant growth and changes in plant counts. ΔSize was representative of relative growth rates as it represented the change in mean size of plants. ΔCount represented both changes in mortality and recruitment. Relative changes in total accumulated volume, size and count of growth forms on plots are from here referred to as ΔVol, ΔSize, ΔCount, respectively.

At the Fynbos site, graminoids (grasses, sedges, restios) were not individually measured due to this growth form being too numerous and difficult to separate. Instead, their cover was estimated as a percentage of each 1 m × 1 m block. This percentage was later converted to volume using the estimated heights of these graminoids noted over time. Due to this limitation, ΔCount and ΔSize values were not available for Fynbos graminoids.

Mixed effects models were used to quantify the effects of summer irrigations and winter droughts on relative changes in growth form volumes, sizes and counts (ΔVol, ΔSize and ΔCount). I tested the effects of the explanatory variables; “Treatment/Control” and “Fynbos/Renosterveld”, as well as their interaction on patterns of ΔVol, ΔSize and ΔCount in each growth form. This was tested separately for discrete seasonal time intervals (S1,W2,S2,W3,S3, Net). Plots were added as random effects to account for variability in vegetation emergence patterns at the beginning of the experiment as well as unmeasured differences in topography, rockiness, and plot-history. Analyses were run separately for all three measured variables; ΔVol, ΔSize and ΔCount, and for each growth form. Using model parameters, multiple pairwise contrasts between control and treatment groups provided treatment effect sizes. Treatment effect sizes represented the estimated difference in the ΔVol, ΔSize and ΔCount between treatment and control groups (i.e. more or less change over time caused by treatment effects).

For comparisons of effect sizes across Fynbos and Renosterveld sites, the coefficient of the interaction between Site and Treatment in the model were used instead of contrasts. This interaction coefficients were equivalent to the difference in the treatment responses in Renosterveld plots and Fynbos plots for each seasonal period. In site contrasts, Renosterveld plots were used as the point of reference.

This multi-step analytical approach is summarised in **Figure 4.2.**

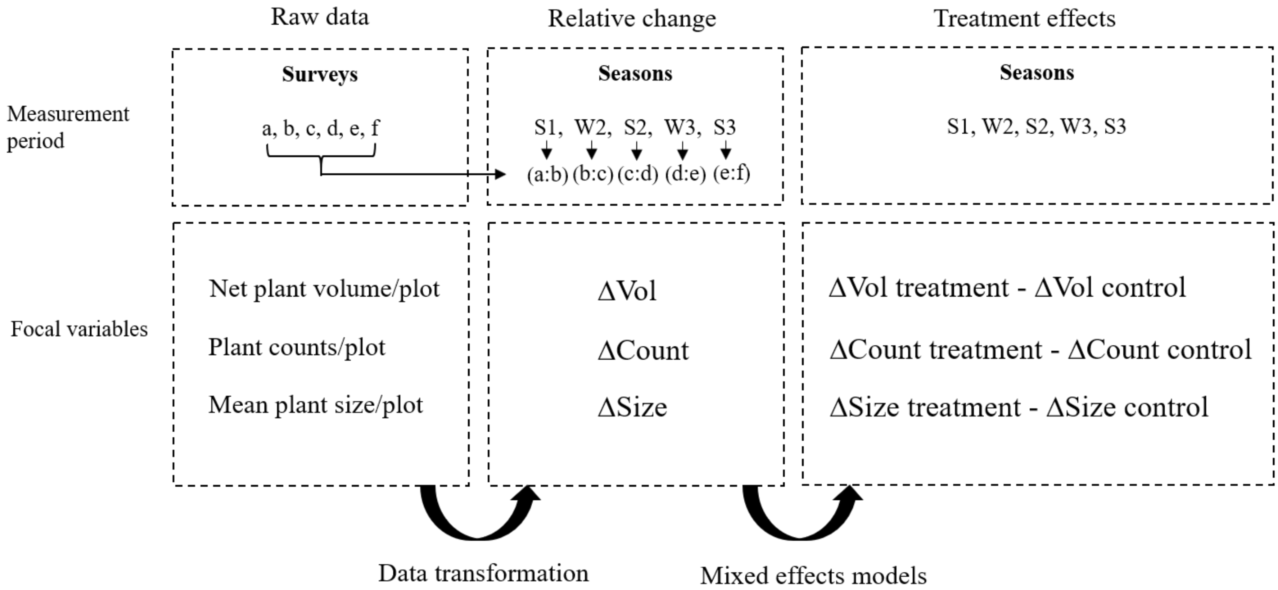

Figure 4.2: Conceptual summary of the multi-step analytical approach used to detect complex treatment responses to manipulated rainfall patterns. This was applied separately for different growth forms or groups of interest. Abbreviations for "Surveys" and "Seasons" are explained in Table 3.1.

4.2.4.2. Relationships between growth form productivity within Fynbos and Renosterveld sites

Correlations matrices were used to assess the relationships between growth form productivity (represented by ΔVol) patterns under natural (i.e. winter rainfall) field conditions. Here the control plot ΔVol values in S1, W2, S2, W3, S3 for each growth form were correlated with corresponding control plot ΔVol values for each other growth form. Neutral, positive and negative correlations would indicate unrelated, similar or opposing natural productivity patterns amongst growth forms, respectively. However, these data could not distinguish definitively between competitive effects and naturally opposing productivity patterns, or between facilitative effects and naturally similar productivity patterns. Nonetheless, correlations were still useful when discussing the potential effects of nutrient availability on community dynamics. Positive and negative correlations were identified as those with significant positive or negative correlation coefficients ($p < 0.05$).

4.3. Results:

4.3.1. General demographic observations amongst growth forms in post-fire Fynbos and Renosterveld sites.

Here I highlight general observations in the ΔVol, ΔCount, and ΔSize (**Fig. 4.3-4.5**) data to broadly characterise the post-fire demographic patterns in growth forms at both Fynbos and Renosterveld sites. The observations highlighted in this section are generalisations that can be made across both control and treatment plots and across sites. This is included to familiarise the reader with the most general demographic differences between growth forms before the outcomes of the statistical analyses are described. This is necessary because treatment effects represented the differences in the relative changes (i.e. ΔVol, ΔCount, and ΔSize) between control and treatment plots which can be difficult to interpret without first contextualising what these relative changes represent. The treatment effects on ΔVol, ΔCount, and ΔSize are described and contrasted between Fynbos and Renosterveld sites in section 4.3.2.

Woody resprouts numbers were stable throughout the experiment, indicated by low ΔCount values **(Fig. 4.4)**. They also sustained low, positive, productivity and relative growth rates at both sites, represented by small, positive ΔVol **(Fig. 4.3)** and ΔSize **(Fig. 4.5)** values, respectively. Prostrate resprouts and graminoids showed high seasonal variation in ΔVol **(Fig. 4.3),** ΔCount **(Fig. 4.4)** and (ΔSize) **(Fig. 4.5),** with negative values (i.e. seasonal declines) in some summer periods. Negative ΔVol, ΔCount or ΔSize in summer periods, followed by positive winter values was indicative of seasonal leaf fall/die-back and recovery within these growth forms. This was concluded on the personal observation that many prostrate resprouters and graminoids re-appeared in the same locations after the temporary absence of living, above-ground structures during summer periods. Perennial seedlings suffered high mortality in the first summer period, indicated by negative ΔCount values, but their numbers remained relatively stable throughout the rest of the experiment **(Fig. 4.4)**. This sensitive first summer period coincided with high relative growth rates in perennial seedlings, as indicated by their large positive ΔSize values during the first summer and second winter **(Fig. 4.5)**. These growth rates diminished over time **(Fig. 4.5)** suggesting that high relative growth rates could be important for early seedling establishment but were not maintained into later stages of development.

Figures S2-S4 represent growth form volume, count and size measurements for each survey before being converted into relative changes (i.e. ΔVol, ΔCount, and ΔSize).

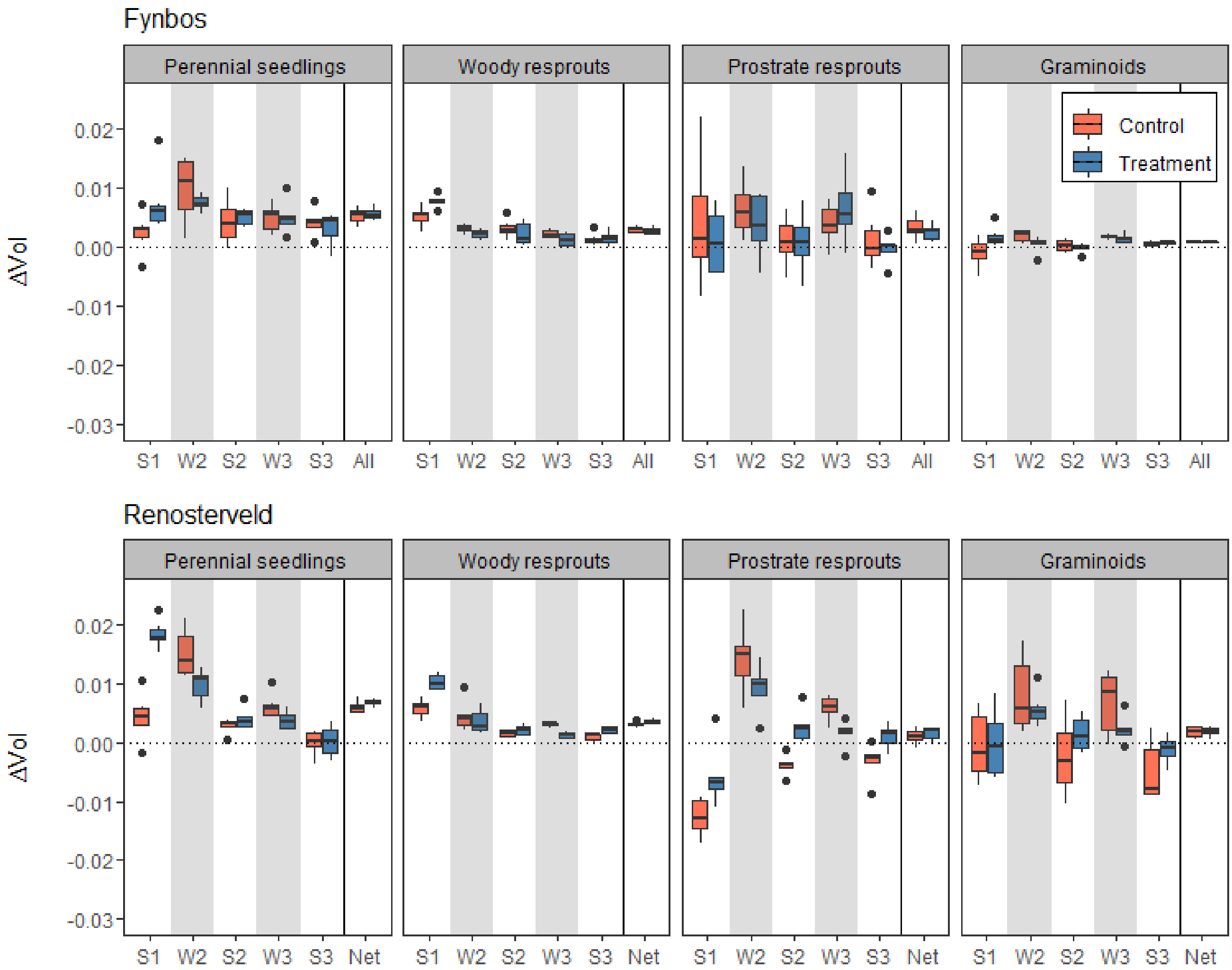

Figure 4.3: Relative changes in accumulated growth form volumes (ΔVol*)* over seasonal periods S1,W2,S2,W3,S3 and over the full duration of the experiment (Net) for growth forms in Fynbos and Renosterveld sites. Positive and negative values indicate increases and decreases in volume, respectively.

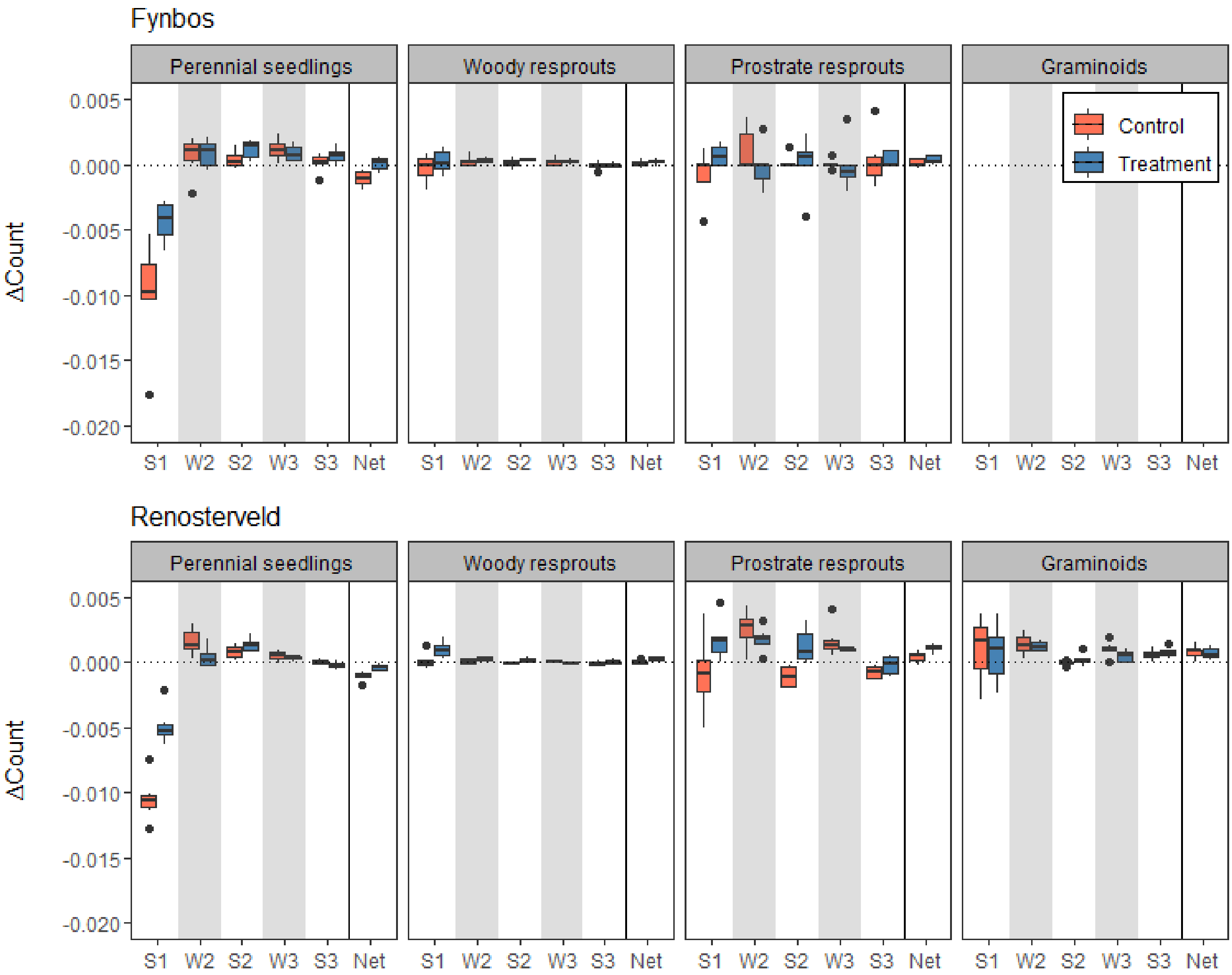

Figure 4.4: Relative changes in plant counts (ΔCount) over seasonal periods S1,W2,S2,W3,S3 and over the full duration of the experiment (Net) for growth forms in Fynbos and Renosterveld sites. Graminoid ΔCount data was not available for the Fynbos site – see section 4.2.4.1. Positive and negative values indicate increases and decreases in plant counts, respectively.

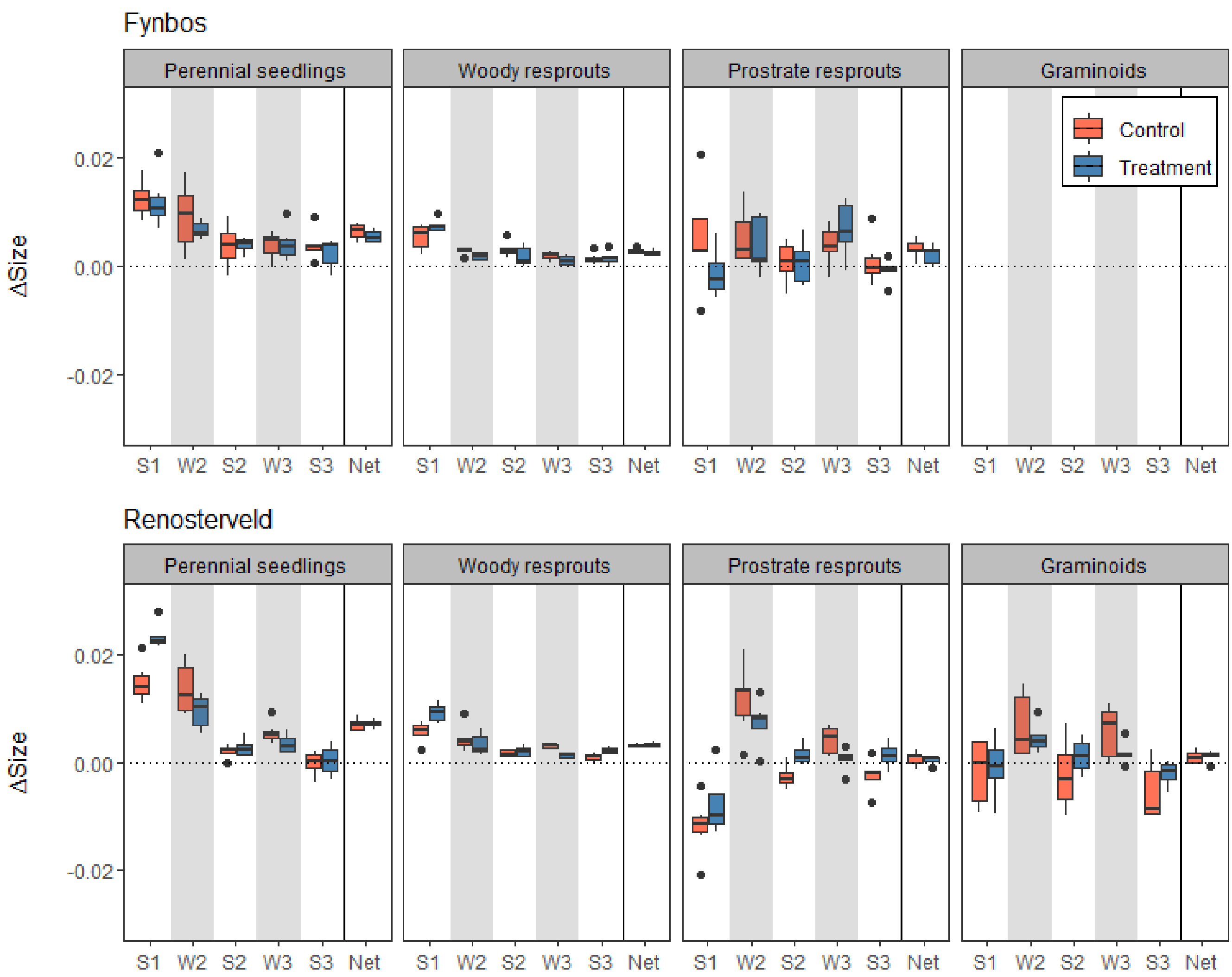

Figure 4.5: Relative growth rate (ΔSize) over seasonal periods S1,W2,S2,W3,S3 and over the full duration of the experiment (Net) for growth forms in Fynbos and Renosterveld sites. Graminoid ΔSize data was not available for the Fynbos site – see section 4.2.4.1. Positive and negative values indicate increases and decreases in mean plant size, respectively.

4.3.2. Diverse responses to altered moisture availability amongst growth forms at Fynbos and Renosterveld sites.

Mixed effects models detected a diverse range of ΔVol, ΔCount and ΔSize responses to experimentally altered moisture availability amongst growth forms at the Fynbos and Renosterveld sites **(Fig. 4.6, 4.7)**. Here, treatment responses represented the estimated differences in ΔVol, ΔCount and ΔSize between control and treatment plots over summers (‘S1’,’S2’,’S3’), winters (‘W2’, ‘W3’) and over the duration of the experiment (‘Net’).

Where significant ΔVol, ΔCount and ΔSize responses to the treatment effects were detected amongst growth forms, all responses to summer irrigations were positive and all responses to winter droughts were negative **(Fig. 4.6, 4.7)**. This included responses in perennial seedlings, woody resprouts, prostrate resprouts and graminoids at both sites **(Fig. 4.6, 4.7)**. However, significant responses to treatment effects were not detected in all growth forms for each seasonal period (‘S1’,’W2’,’S2’,’W3’,’S3’). Responses to treatment effects were thus partitioned amongst perennial growth forms over the seasonal treatment periods **(Fig. 4.6, 4.7)** indicating that the potential for growth forms to respond to moisture availability patterns were affected by other factors (e.g. internal development, community competition). Although responses were not uniform across growth forms, at least one growth form response was detected in all seasonal treatment periods (S1, W2, S2, W3, S3) at the Renosterveld site **(Fig. 4.7)** indicating a sustained community level responsiveness throughout the experiment.

Higher moisture availability generally had a positive effect on ΔVol, ΔCount and ΔSize in all perennial growth forms during some stage of their development. This was true for higher moisture availability in summer (i.e. treatment plots) and winter (i.e. control plots). Most notably, large positive effects of the first summer irrigations were detected on ΔCount of perennial seedlings **(Fig. 4.6e, 4.7e).** These positive responses indicated large reductions in the natural mortality rates observed in control plots **(Fig. 4.4, Fig. S3)**. Percentage mortality for perennial seedlings in the first summer was 71.7 % (± 13.4) for Fynbos control plots, 44.1 % (±11.1) for Fynbos treatment plots, 76.0 % (± 6.2) for Renosterveld control plots and 48.3 % (±11.5) for Renosterveld treatment plots. This demonstrated that the survival patterns of perennial plants emerging from seed (i.e. perennial seedlings) were most closely coupled to post-fire rainfall patterns in the first summer.

The mixed effects models also detected differences in the treatment responses of growth forms between Fynbos and Renosterveld sites. Here Renosterveld growth forms were used as the point of reference between sites **(Fig. 4.8)**. Therefore, where Renosterveld responses to summer irrigations were larger than concurrent responses in Fynbos, modelled differences in ΔVol, ΔCount and ΔSize responses between sites **(Fig. 4.8)** resulted in positive summer values (S1,S2,S3). Similarly, negative values during winter periods (W2 & W3) resulted from larger negative responses to winter droughts in Renosterveld **(Fig. 4.8).**

The first summer irrigations resulted in equivalent reductions of mortality in perennial seedlings across Fynbos and Renosterveld sites, indicated by the lack of difference in ΔCount responses between Fynbos and Renosterveld in the first summer (S1) **(Fig. 4.8e)**. However, only the perennial seedlings at the Renosterveld site displayed large, positive ΔSize responses to the first summer irrigation **(Fig. 4.7i)**, indicating increased relative growth rates in addition to increased survival **(Fig. 4.7e)**. This resulted in larger ΔSize **(Fig. 4.8i)** and ΔVol **(Fig. 4.8a)** responses to the first summer irrigations at the Renosterveld site. Prostrate resprouts at the Renosterveld site showed larger positive ΔVol, ΔSize and ΔCount responses to summer irrigations and larger negative ΔVol, ΔSize and ΔCount responses to winter droughts compared to prostrate resprouts at the Fynbos site **(Fig. 4.8c, g, k).** This indicated that their emergence, productivity, growth rate & survivorship patterns was more closely coupled to moisture availability at the Renosterveld site. As discussed in section 3.2.1, prostrate resprouts showed varying degrees of natural seasonal dieback. This could affect the values of ΔVol, ΔSize and ΔCount variables. The larger responses in prostrate resprouts at the Renosterveld site thus indicate stronger seasonal dieback or seasonal leaf shedding. Differences in the treatment responses of both woody resprouts and graminoids between sites were not significant but also tended towards larger positive ΔVol and/or ΔSize responses in summer and larger negative responses in winter **(Fig. 4.8b, j, d)**. Overall, responses to summer irrigations and winter droughts were most apparent at the Renosterveld site **(Fig. 4.7).**

While significant responses to summer irrigations were detected in some growth forms at both sites, neutral and/or opposing responses to treatment effects in subsequent seasons lead to the dilution/counteracting of initial effects over time. Thus, there were no net effects of the treatments on ΔVol in any growth form in Fynbos **(Fig. 4.6)** or Renosterveld **(Fig. 4.7).** Net treatment responses over time were thus affected by combination of all seasonal treatment effects (e.g. three irrigations and two droughts) as well as changes in responsiveness of growth forms over time. Notably, large positive ΔCount responses in perennial seedlings during the first summer ('S1') had lasting effects on counts as indicated by the net positive effect by the end of the experiment **(Fig. 4.6e, 4.7e)**

Taken together, these responses indicated that many growth forms were sensitive to moisture availability patterns in both summer and winter periods but long-term effects (three year) of growth form responses were the result of these complex, multi-seasonal responses. Notably, seedling establishment patterns were highly affected by post-fire rainfall patterns with long-term effects on seedling numbers. Overall, growth forms at the Renosterveld site showed stronger demographic responses to altered moisture patterns relative to those in Fynbos **(Fig. 4.6 – 4.8).** This was consistent with stronger responses in relative plot productivity at the Renosterveld site **(Fig. 3.2)**. The combination of seasonal growth form responses to treatment effects determined plot level responses. However, larger growth forms had a disproportionately large effect on plot level productivity responses **(Fig. 3.2)** because plot level productivity responses were calculated using pooled growth form data. This is clearly evident when growth form treatment responses are scaled by the proportional plot volume and counts of each growth form (**Fig. S5)**

Logged volume, count and size data were included in the supplementary material **(Fig. S2,S3,S4)**.

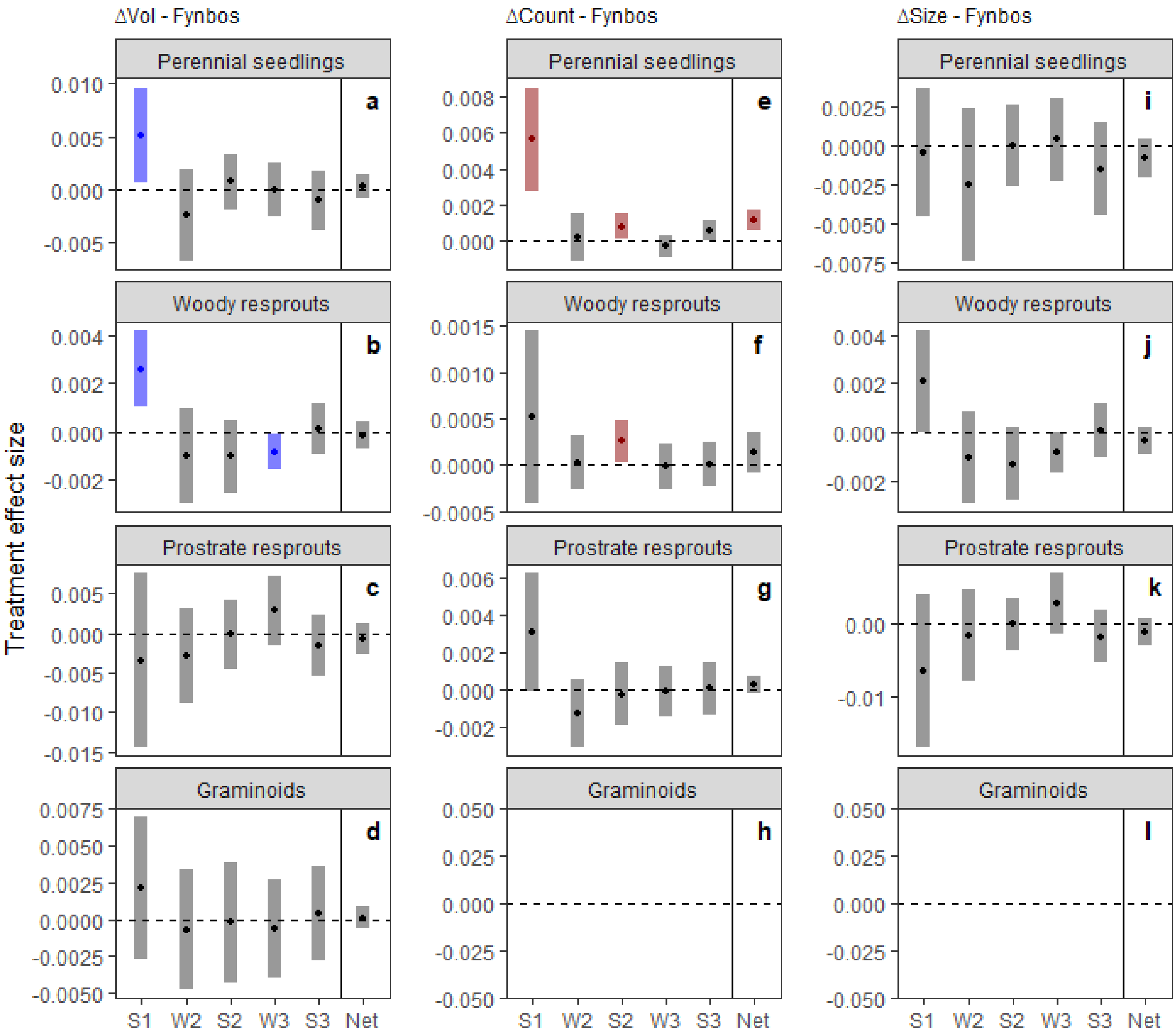

Figure 4.6: Season-specific treatment effects on ΔVol, ΔCount and ΔSize for all Fynbos growth forms seasonally and over the course of the experiment. Summers 1 – 3 are represented by the abbreviations 'S1', 'S2', 'S3, winters 2 and 3 are represented by the abbreviations 'W2', 'W3', and the full duration of the experiment is represented by "Net". Points represent effect size means (relative to control plot trends) and bars represent 95% confidence intervals. Effect sizes represented the modelled differences in ΔVol, ΔCount and ΔSize between control and treatment plots. Positive values indicated higher ΔVol, ΔCount and ΔSize in treatment plots and negative values indicated lower ΔVol, ΔCount and ΔSize in treatment pots. Significant effects ($p < 0.05$) are indicated in colour. Dashed horizontal lines represent a zero effect. Graminoid ΔCount and ΔSize data was not available for the Fynbos site – see section 4.2.4.1. Letters a-l are included for reference to the text.

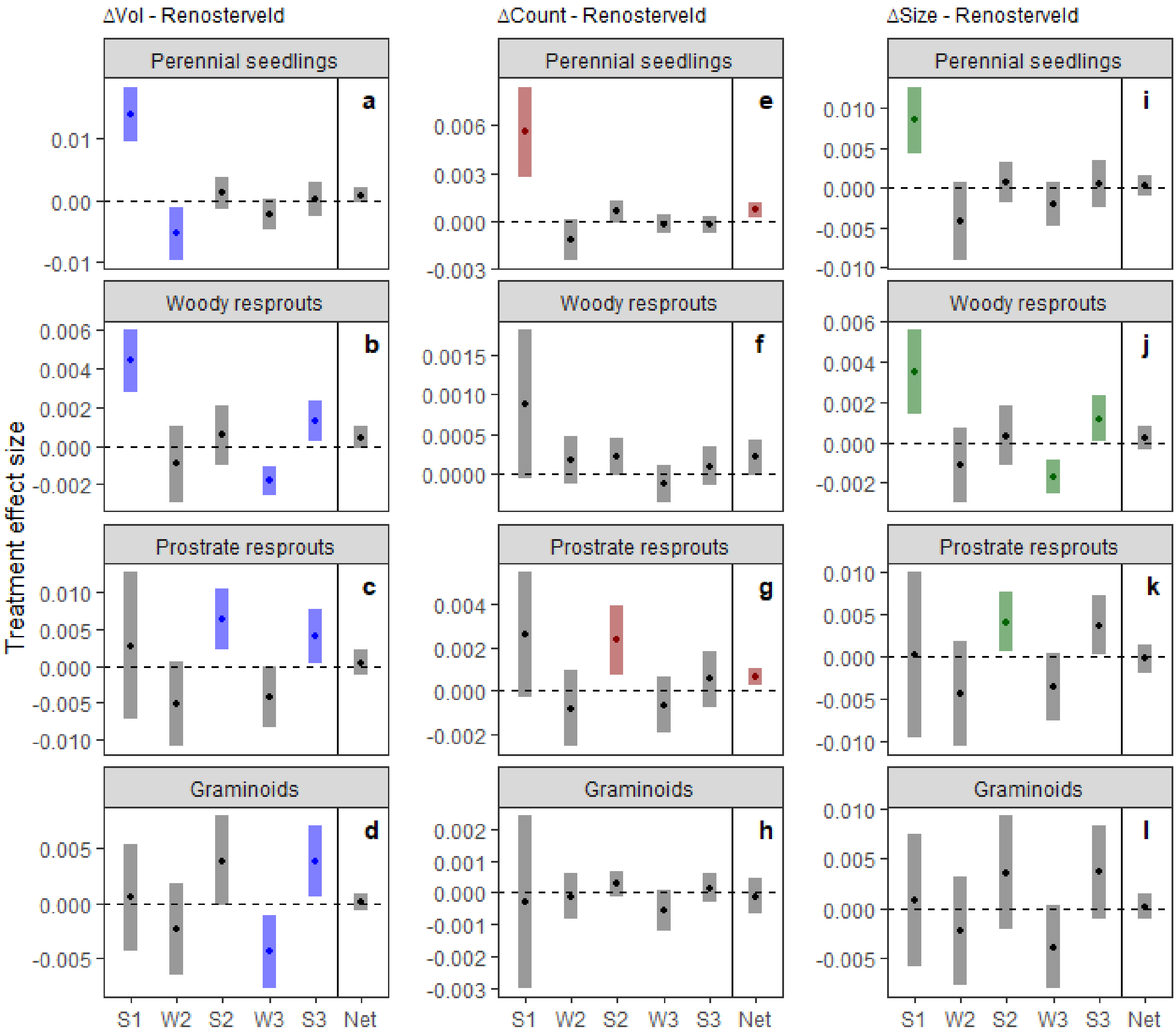

Figure 4.7: Season-specific treatment effects on ΔVol, ΔCount and ΔSize for all Renosterveld growth forms seasonally and over the course of the experiment. Summers 1 – 3 are represented by the abbreviations 'S1', 'S2', 'S3, winters 2 and 3 are represented by the abbreviations 'W2', 'W3', and the full duration of the experiment is represented by "Net". Points represent effect size means (relative to control plot trends) and bars represent 95% confidence intervals. Effect sizes represented the modelled differences in ΔVol, ΔCount and ΔSize between control and treatment plots. Positive values indicated higher ΔVol, ΔCount and ΔSize in treatment plots and negative values indicated lower ΔVol, ΔCount and ΔSize in treatment pots. Significant effects ($p < 0.05$) are indicated in colour. Dashed horizontal lines represent a zero effect. Graminoid ΔCount and ΔSize data was not available for the Fynbos site – see section 4.2.4.1. Letters a-l are included for reference to the text.

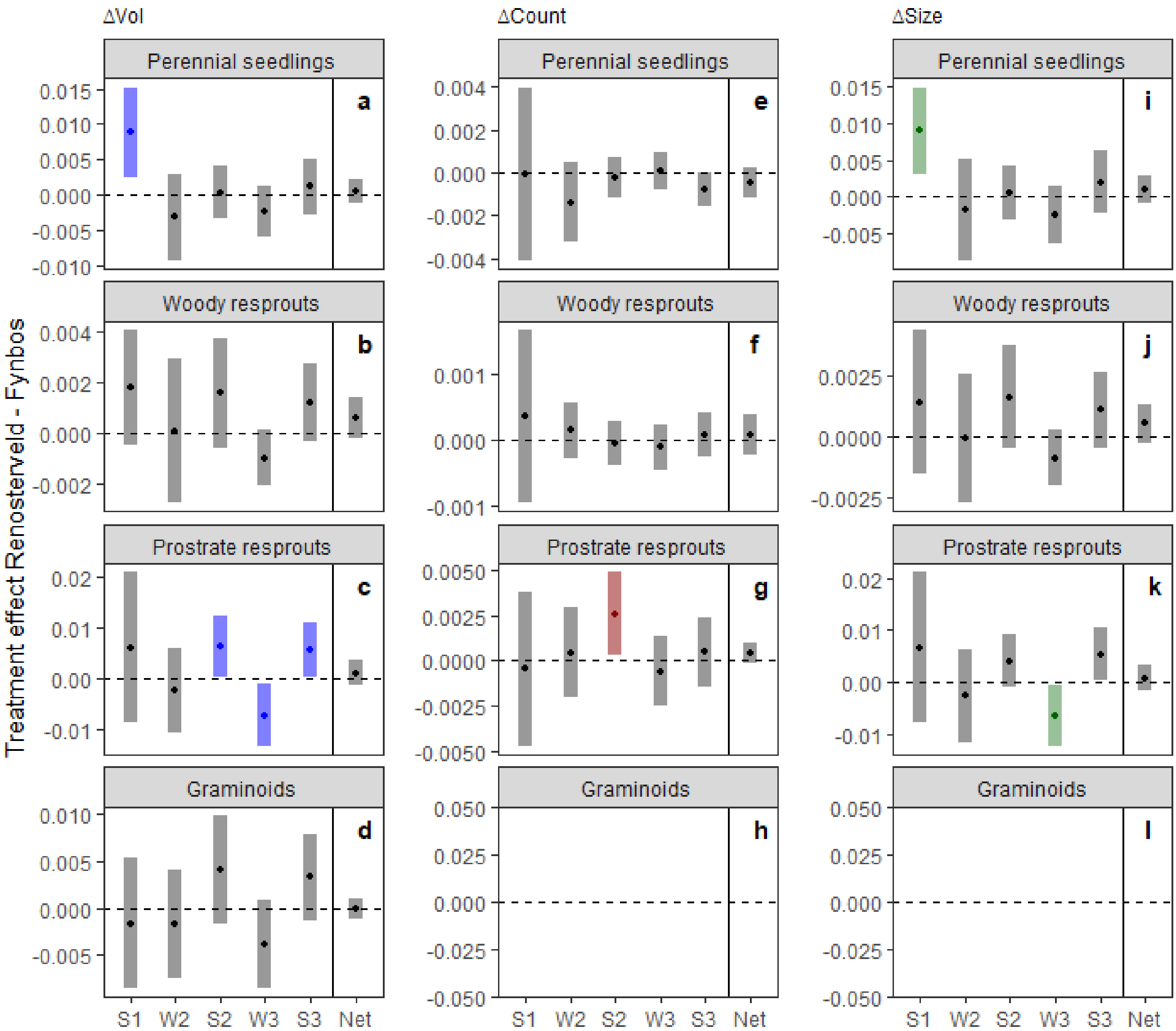

Figure 4.8: Differences in treatment effects on ΔCount, ΔVolume and ΔSize across Fynbos and Renosterveld sites. Summers 1 – 3 are represented by the abbreviations 'S1', 'S2', 'S3, winters 2 and 3 are represented by the abbreviations 'W2', 'W3', and the full duration of the experiment is represented by "Net". Points represent effect size means (relative to control plot trends) and bars represent 95% confidence intervals. Effect sizes represent the modelled difference in treatment response size between Fynbos and Renosterveld sites. Positive summer (S1, S2, S3) values indicated larger positive responses to summer irrigations at the Renosterveld site while negative winter (W2, W3) values indicated larger negative responses to winter droughts. Significant effects ($p < 0.05$) are indicated in colour. Dashed horizontal lines represent a zero effect. Graminoid ΔCount and ΔSize data was not available for the Fynbos site – see section 4.2.4.1.

4.3.3. The influence of growth forms on plot level community structure

Growth form dominance, in terms of absolute plot volumes **(Fig. 4.9),** showed high overall similarity across control and treatment plots at the Fynbos site whereas patterns of growth form dominance showed substantial divergence between Renosterveld control and treatment plots over time. Here, Renosterveld seedlings displayed progressive increases in dominance over the three-year experiment. This pattern was not matched by ∆Volume, ∆Count or ∆Size treatment responses in seedlings during the later stages of the experiment indicating that shifts in seedling dominance were driven primarily by compounding effects of early changes in seedling establishment patterns. While seedling survival was equally sensitive to first summer drought and irrigations across Fynbos and Renosterveld sites, stronger initial seedling growth ∆Size and ∆Vol responses **(Fig. 4.8a, i)** and higher initial seedling counts **(Table S2)** at the Renosterveld site resulted in a larger cumulative effect of these early establishment patterns over time. This is consistent with recorded shifts in species abundance patterns over the first summer and progressive shifts of species dominance over time reported in Chapter 3 **(Fig. 3.4, 3.5)** and highlights the seedlings are strong drivers of community patterns**.** In other growth forms, the opposing effects of summer irrigations and winter droughts lead to shifts in seasonal growth and productivity patterns (**Fig. 4.6 – 4.8)** but a low net effect on dominance patterns over time **(Fig. 4.9).**

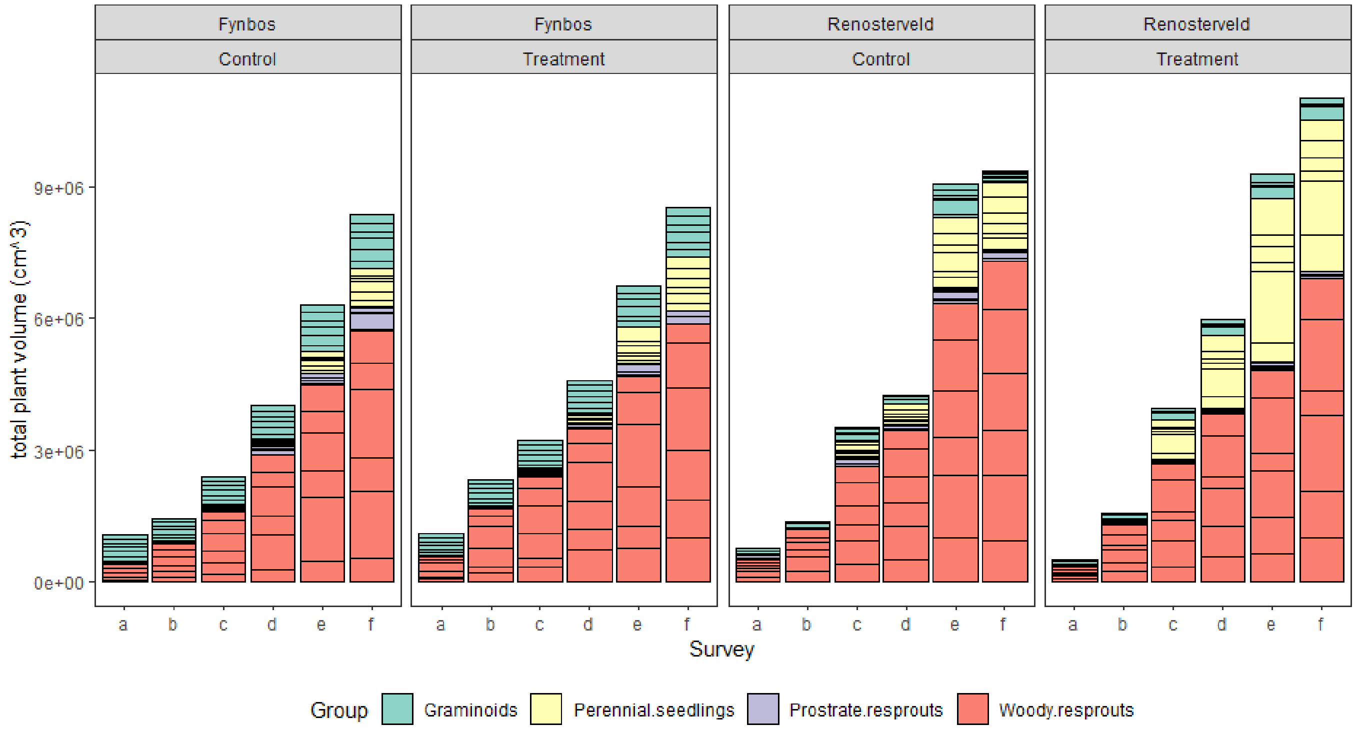

Figure 4.9: Stacked total plot volumes of dominant perennial growth forms on control and treatment plots in Renosterveld and Fynbos sites over surveys a to f. Blocks indicate different plots.

4.3.4. Relationships between seasonal growth form productivity (ΔVol) patterns:

Correlations of seasonal (i.e. S1,W2,S2,W3,S3) ΔVol values amongst growth forms were used to indicate similar, opposing or unrelated patterns of productivity under control and treatment plot conditions **(Fig. 4.10, 4.11)**. Positive correlations were indicative of similar patterns of seasonal productivity, neutral correlations were indicative of unrelated patterns of productivity and negative correlations were indicative of opposing patterns of seasonal productivity.

In the Fynbos control plots, a mixture of negative and neutral ΔVol correlations existed amongst growth forms **(Fig. 4.10a)** while in Fynbos treatment plots growth form productivity patterns were generally not correlated **(Fig. 4.10b)**. In the Renosterveld control plots, seasonal ΔVol values were positively correlated amongst all perennial growth forms except for woody resprouts and prostrate resprouts. This indicated a high degree of congruence amongst growth form productivity patterns at the Renosterveld control plots under natural seasonal rainfall conditions **(Fig. 4.11a)**. In the Renosterveld treatment plots, seasonal ΔVol values were varied indicating a divergence in productivity patterns between growth forms under the less seasonal rainfall availability **(Fig. 4.11a)**.

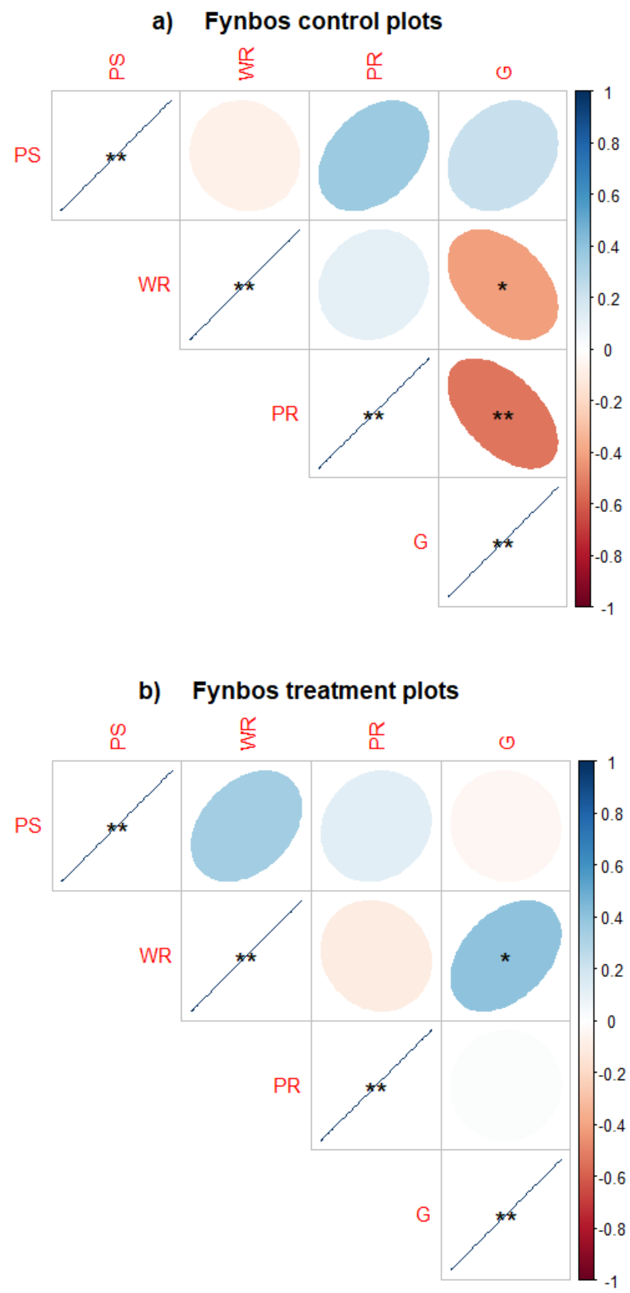

Figure. 4.10: Correlations between concurrent ΔVol measurements made amongst Fynbos growth forms in **a)** control plots and **b)** treatment plots, over all seasonal periods (S1,W2,S2,W3,S3). Shaded ellipses represent the strength and direction of the correlation between any two groups. Statistical significance of relationships were denoted by * = 95%, ** = 99%, significance levels. Colours are used to aid in distinguishing between positive (blue), neutral (white) and negative (red) relationships. PS = “Perennial seedlings”, WR = “Woody resprouts”, PR = “Prostrate resprouts”, G = “Graminoids”

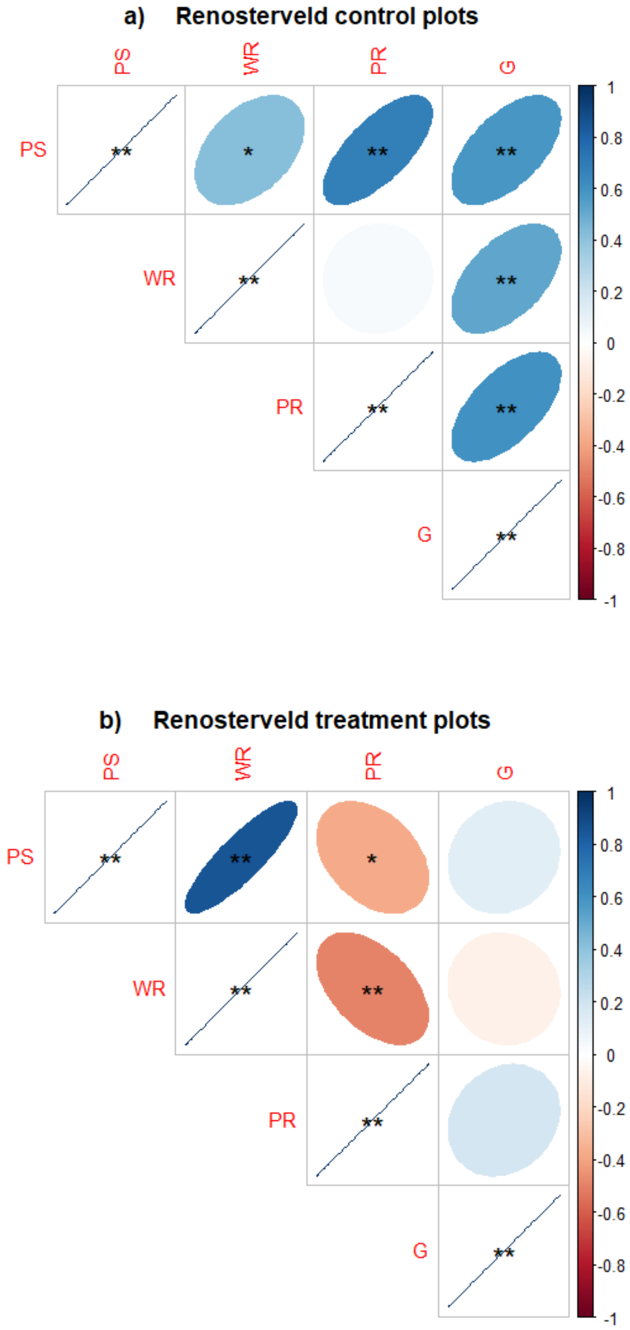

Figure 4.11: Correlations between concurrent ΔVol measurements made amongst Renosterveld growth forms in **a)** control plots and **b)** treatment plots, over all seasonal periods (S1,W2,S2,W3,S3). Shaded ellipses represent the strength and direction of the correlation between any two groups. Statistical significance of relationships were denoted by * = 95%, ** = 99%, significance levels. Colours are used to aid in distinguishing between positive (blue), neutral (white) and negative (red) relationships. PS = "Perennial seedlings", WR = "Woody resprouts", PR = "Prostrate resprouts", G = "Graminoids"

4.4. Discussion

Growth forms showed varying responses to summer irrigations and winter droughts over the three-year experiment. Generally, summer irrigations had positive impacts on productivity, growth and survival while winter droughts had negative impacts. Shifts in rainfall patterns over the first summer period after fire had particularly strong effects on patterns of seedling establishment with compounding effects on community structure over time. Shifts in rainfall seasonality also demonstrated the potential to alter seasonal productivity patterns amongst growth forms thus changing their annual patterns of resource use and competition. Overall, growth (ΔSize) and productivity (ΔVol) was more sensitive to treatment effects **(Fig. 4.6 - 4.8)** amongst growth forms at the Renosterveld site, consistent with larger productivity responses at the plot level **(Fig. 3.2)**.

At both sites the survival of perennial seedlings was closely dependant on rainfall over the first summer after fire **(Fig. 4.6e, 4.7e)**, whereas the survival of resprouting growth forms was less affected by rainfall. At the Renosterveld site, increased seedling survival and high initial seedling counts explained strong shifts in community composition (**Fig. 3.4**) with compounding effects on above-ground seedling volumes over time since fire **(Fig. 3.5, 4.10)**. While early seedling establishment in Fynbos was also sensitive to altered moisture, lower initial seedling numbers and the lack of growth responses lead to a lower overall influence of seedling dynamics on community structure **(Fig. 4.10)**. These patterns were consistent with 'event-dependent' succession (Bond & van Wilgen, 1996; Moreno *et al.*, 2011) and highlight the influence of post-fire rainfall patterns on long-term community patterns (Keeley *et al.*, 2005). While the experiment monitored the effects of moisture availability from the first summer onward, rainfall and other environmental filters during the first winter after fire (i.e. pre-baseline survey) could also have resulted in changes in seedling survival. The potential for post-fire weather to cause dramatic changes in seedling survival suggests that post-fire community composition and structure could be strongly affected by shifts in rainfall seasonality.

Presumably, the survival of shallow rooted perennial seedlings were initially (i.e. during first summer) more sensitive to rainfall patterns due to greater initial reliance on shallow soil moisture availability (Moreno & Oechel, 1992; Parra and Moreno, 2018; Penuelas *et al.*, 2001; Quintana *et al.*, 2004; Luis *et al.*, 2008). This coincided with their highest growth rates suggesting that initial post-fire post fire seedling development was centred around rapid, moisture-dependent establishment with high vulnerability to drought related mortality **(Fig. 4.4)**.

Conversely, woody resprouts showed little drought-driven mortality **(Fig. 4.4)** and slower relative growth rates **(Fig. 4.5)**, characteristic of the 'persistence-niche' (Bond & Midgley, 2001; Keith *et al.*, 2007). Survival of adult resprouters is important for the persistence and stability of resprouting populations because shifts in adult survival can have a disproportionately large effect on population size (Pfister, 1998). Accordingly, changes in post fire rainfall patterns lead to small changes in relative growth rate of woody resprouts with little effect on survival **(Fig. 4.6, 4.7)**. Prostrate resprouts and resprouting graminoids displayed a different persistence strategy where plants generally survived summer drought but demonstrated seasonal leaf shedding and above-ground die-off during droughted summer periods. Leaf shedding has been documented as a strategy of drought avoidance (Palacio *et al.*, 2007; Pineda-García *et al.*, 2013) allowing plants to avoid the physiological risks associated with maintaining leaf function under droughted conditions (West *et al.*, 2012). This strategy may have been necessary in the prostrate resprouts which had shallower roots than the woody resprouts **(Fig. 4.1)** and were thus still sensitive to shallow soil moisture availability**.** However, these resprouting growth forms maintained the capacity to store below-ground resources and re-emerge after droughts. Taken together, these patterns suggest that tolerance of moisture heterogeneity in resprouting growth forms could be achieved through deep rootedness or highly seasonal leaf phenology which is timed with favourable moisture periods.

My findings suggest that different regeneration life history strategies could be affected by climate changes at different temporal scales. For example, the sensitivity of seedlings to post-fire weather could lead to strong reseeder population fluctuations between successive fire events, depending on the prevailing conditions. Although resprouters were resistant to post-fire rainfall treatments, resprouting populations could respond to shifts in environmental factors over longer time scales (Slingsby *et al.*, 2017). These ideas are explored in more detail in Chapter 5.

An emergent theme in my study is the difference in responsiveness between Fynbos and Renosterveld sites. As, discussed in Chapter 3, nutrient limitation and lower soil moisture tension in (i.e. higher aridity) in the Fynbos soils **(Table 2.2, Fig. 2.10)** could limit growth form responses to altered rainfall patterns. Conversely, moderate soil fertility and high soil moisture tension during drought could favour strong growth responses to moisture in Renosterveld. Consistent with these expectations, I detected higher sensitivity of growth rates to altered rainfall patterns amongst Renosterveld growth forms **(Fig. 4.8 i-l)**. The inability for Fynbos seedlings to increase growth rates under summer irrigations suggested that nutrient limitation could be a particularly strong limiting factor on growth potential. Additionally, stronger seasonal leaf shedding/dieback in Renosterveld prostrate resprouts **(Fig. 4.4, 4.5)** could be linked to a combination of more arid soil conditions and lower requirement for foliar nutrient conservation due to higher soil fertility. Conversely, nutrient poor environments could select against seasonal leaf fall because the costs of rebuilding lost vegetative structures are greater than maintaining them (Cowling & Campbell, 1983).

The relationships between growth form productivity patterns differed across sites and under different rainfall regimes. Similarity of growth form productivity patterns at the Fynbos site was low under both control and treatment plot rainfall regimes. I suggest that complex phenological patterns amongst Fynbos growth forms as well as low overall responsiveness to treatment effects could reflect the lower nutrient availability at the site **(Table 2.2)** which increases the requirement for growth forms to compete for, partition (Westoby, 1979; Reynolds *et al.*, 2004; Ogle & Reynolds, 2004; Turner, 2008) and conserve (Murren *et al.*, 2015) available nutrients. Each of these factors could explain why Fynbos growth forms displayed opposing and unrelated patterns of productivity. In highly diverse, nutrient-impoverished shrubland communities, such as Fynbos, coexistence of multiple, functionally different growth forms is likely to be fostered by factors that promote the separation of resource use in time (Walter & Stadelmann 1974; Cody 1986; Smith & Nobel 1986; Ehleringer *et al.* 1991; Lin *et al.* 1996). In Renosterveld control plots, high congruence in seasonal productivity patterns amongst growth form was reflective of a seasonally pulsed shrubland community which is less nutrient limited. However, reduced rainfall seasonality in Renosterveld lead to lower similarity between growth form productivity patterns. This suggested that decreased rainfall seasonality could lead to a divergence in growth form productivity patterns. Reduced rainfall seasonality could allow for growth forms to explore a wider annual climatic window for growth. Shifting phenological patterns amongst growth forms could affect the competitive dynamics between growth forms.

This chapter linked post-fire demographic changes to shifts in moisture availability. However, the degree to which the described demographic patterns can be explained directly by plant physiological performance under different soil moisture conditions requires further investigation. If shifting moisture patterns differentially affect growth forms and communities on different soils, this should be reflected in the differential physiological performance of these growth forms. The next chapter investigates physiological responses to altered rainfall patterns in seedlings and resprouts which showed different growth and survivability during the first summer after fire in Fynbos and Renosterveld sites.

CHAPTER 5: The effect of altered post-fire rainfall seasonality on the physiological performance of seedlings and resprouts in Fynbos and Renosterveld

Summary:

- In this Chapter I investigated the physiological sensitivity of seedlings and resprouts to post-fire, summer rainfall patterns in Fynbos and Renosterveld sites. Summer droughts were highlighted previously as important bottlenecks for seedling emergence patterns whereas resprouts were less sensitive during this time (Chapter 4).
- Plant physiological responses (hydration, maximum quantum yield, photosynthesis) to altered summer moisture availability were contrasted between seedlings and resprouts across neighbouring Fynbos and Renosterveld sites. This was tested over three successive summer periods after fire.
- Natural summer droughts led to strong declines in physiological performance, especially for seedlings during the first summer after fire. This was consistent with high seedling mortality during this time. Summer irrigations strongly reduced levels of plant stress, consistent with the large reduction in seedling mortality in treatment plots. Resprouts at both sites were generally less physiologically sensitive to summer droughts and summer irrigations.
- The physiological sensitivity of plants to drought and irrigations was higher in fine-textured shale soils of the Renosterveld site. Fynbos seedlings and resprouts showed remarkable physiological insensitivity to summer drought after the first summer with lower treatment responses. These patterns were consistent with previous findings showing lower growth and productivity responses to altered rainfall at the Fynbos site. Differences in site sensitivity across sites reflected differences in soil drought intensity between sandstone and shale soils (Chapter 2).
- This chapter confirmed the link between seasonal plant physiological performance and emergent demographic patterns in post-fire growth forms under altered seasonal moisture availability patterns (Chapter 3 & 4). Linking physiological performance of growth forms with their demographic patterns highlighted the functional connection between fine-scale biochemical processes and vegetation community dynamics thus supporting a bottom-up explanation of emergent vegetation patterns.

5.1. Introduction

As shown in Chapter 4, seasonal patterns of soil moisture availability differentially affected shrubs with different post-fire regenerative strategies (e.g. reseeding and resprouting strategies). There was a particularly strong soil moisture effect on patterns of seedling establishment in reseeding shrub species (i.e. perennial seedling), with ramifications for community structure. The resprouts of resprouting species (i.e. woody resprouts and prostrate resprouts) were less affected by moisture availability. Furthermore, sensitivity of growth responses was highest at the Renosterveld site which reflected higher soil nutrient availability. This chapter investigates to what degree these patterns can be explained by underlying physiological performance of these growth forms under different seasonal soil moisture conditions. This will demonstrate the ability to predict demographic and community level changes using a bottom up approach. Specifically, I investigate plant physiological responses of seedlings and resprouts to variations in moisture availability during hot summer periods (December – January) due to their strong differences in drought sensitivity reported in Chapter 4. This investigation assessed how alleviating summer drought (e.g. through treatment irrigations) could affect physiological performance during these stressful summer periods and how differences in post-fire regeneration strategy and soil type can affect plant responses to moisture availability. The three-year rainfall manipulation experiment, described in Chapter 2, allowed for the causal relationships between plant physiology and soil moisture availability to be determined in both Fynbos and Renosterveld field sites.

For decades, research has focused on reseeding and resprouting regenerative strategies in fire-prone shrublands and how circumstances surrounding fires (e.g. weather, frequency, severity) could affect the relative success of these contrasting strategies (Keeley & Keeley, 1981; Lamont, 1985; Enright & Lamont, 1989; Bond & Midgley 1995; Vesk & Westoby, 2004; Enright *et al.*, 2014, Slingsby *et al.*, 2017; Pausas *et al.*, 2016; Parra & Moreno, 2018).

Reseeding is classified as a strategy of regenerating from seed in the post-fire environment. Plants which exclusively use this strategy have been referred to as 'non-sprouters' (Bond & Midgley 1995; Schutte et al. 1995; Pratt et al. 2012), or 'obligate seeders' (Pausas et al. 2004; Keeley et al. 2012). Resprouting is classified as the strategy which allows plants to survive fire and resprout from surviving rooting structures in the post-fire environment. Plants which use this strategy exclusively to persist through fire have been referred to as 'obligate resprouters' (Pausas & Keely, 2014). Some plants have the ability to resprout after fire and produce seedlings between fire events and have been referred to as 'facultative sprouters' (Pratt et al. 2012) or 'facultative seeders' (Keeley et al. 2012).

A potential life-history trade-off exists between seedling fitness and adult persistence within reseeders and resprouters. Here, it has been found that reseeders may allocate more resources to seedling fitness whereas, resprouters partition more resources towards adult plant persistence (Thomas & Davis, 1989; Vilagrosa *et al.*, 2014; Pausas *et al.*, 2016; Parra & Moreno, 2017; Altwegg *et al.*, 2015). Therefore, population dynamics could be most sensitive to seedling survival in reseeding species and adult survival in resprouting species (Pfister, 1998). Therefore, there is a need for experiments to contrast seedling (from reseeders) and resprout (from resprouters) performance in the post-fire environment (Parra & Moreno, 2017, 2018) where both growth forms (e.g. emerging seedlings, surviving resprouts) are in their most sensitive state (Pausas *et al.*, 2016). This experiment focuses on the physiological sensitivity of these focal life history stages, namely seedlings from reseeders (hereafter called seedlings) and resprouts from resprouters (hereafter called resprouts), and examines their responses to shifts in seasonal moisture availability patterns.

Both seedlings and resprouts under a Mediterranean-type climate typically encounter hot dry summers within a year of post-fire emergence/recovery, yet their physiological sensitivity to these conditions could differ dramatically due to different levels of development (i.e. age) and access to moisture resources (i.e. rooting depth) in the immediate post-fire environment (Parra & Moreno, 2017, 2018). Deep roots and stored reserves in resprouting plants could aid in persistence through drought periods, potentially allowing these growth forms to avoid severe dehydration by decoupling them from shallow surface soil drought. This is consistent with the findings in Chapter 4 (e.g. low resprout mortality) as well as recent rainfall manipulation studies showing that deep rooted plants or surviving resprouts generally succumbed less to drought than shallow-rooted plants or seedlings (West *et al.*, 2012; Parra & Moreno, 2018). However, resprouting plants often have lower xylem cavitation resistance, potentially rendering them susceptible to extended drought periods (Pausas *et al.*, 2016). Resprouters have been also found to be sensitive to long term changes in climate in a recent study (Slingsby *et al.*, 2017). In seedlings, the initial lack of developed root structures and/or underground storage organs could reduce their potential to avoid shallow soil drought after post-fire germination (Pausas *et al.*, 2016). Newly emerging seedlings may even temporarily be limited to the utilisation of moisture in the upper few centimetres of soil which are prone to large fluctuations in seasonal moisture availability. This emphasises the need for seedlings to establish rooting systems rapidly after emergence or to have high drought tolerance of above ground structures (Pausas *et al.*, 2016).

There has long been assumed that there is a trade-off between the physiological tolerance of water shortage and growth rate under favourable conditions (Orians & Solbrig, 1977; Chapin, 1980; Smith & Huston, 1989). Therefore, seedlings could face a fundamental trade-off between growing rapidly to access deeper, ‘stable’ soil moisture resources, and surviving drought should their efforts to access stable moisture sources be insufficient. Seemingly, rapid seedling growth rates and high mortality in early post-fire development observed in Chapter 4 could indicate that rapid growth rates were favoured over drought resistance during early post-fire establishment although this was not tested empirically. The “asset protection principal" in animal ecology suggests that young organisms are inherently more vulnerable to mortality and have less to lose and more to gain through "risky" or rapid developmental patterns (Clark *et al.*,1994).

This chapter quantified and contrasted the physiological sensitivity of seedlings and resprouts to a post-fire summer drought as well as their responsiveness to summer irrigations. Physiological sensitivity and responsiveness were assessed using measures of leaf hydration, leaf fluorescence and photosynthetic performance. Due to differences in survivability of seedling and resprouts highlighted in Chapter 4, it was expected that physiological sensitivity to summer moisture availability should be highest in seedlings, particularly during the first summer after fire. Additionally, sensitivities and responses across growth forms were contrasted across Fynbos and Renosterveld sites due to the potential effects of soils on drought intensity and nutrient availability. It was expected that the responsiveness of growth forms in terms of growth and survivability (Chapter 4) should be reflected in physiological performance which is highly sensitive to moisture availability.

5.2. Methods

5.2.1. Field measurements and sample collection strategy

Physiological performance was measured in seedlings and resprouts in peak summer periods (December – March) in droughted control plots and irrigated treatment plots from 2017-2019. Where possible, physiological measurements and sample collections were carried out in both treatment and control plots before and after the drought/irrigation period, thus making it possible to causally examine the relationship between rainfall and physiology. Measurements were conducted on the most commonly occurring species at each site and (**Table 5.1**). In each of six control and six treatment plots at both sites, a single plant was measured from each of the key species. This was repeated for each survey period **(Table 5.2).** Due to logistical reasons, the bulk of the physiological measurements were made during the summer period December 2017 – March 2018 (Summer 2 of the experiment) and the summer period December 2018 – March 2019. However, a preliminary dataset was also collected during the first summer (Feb 2017, March 2017) (Table 7). All measurements made and all samples collected over the duration of the study **(Table 5.2)** were taken from the same individual plants. The physiological measurements were made non-destructively as far as possible so as not to disturb the natural vegetation processes. Therefore, due to measurement limitations and the risk of damaging plants, measurements during the first summer drought (Summer 1) were highly limited as plants were small. See **Table 5.2 and Table 5.3** for a full break-down of measurements made over the three summer periods. Measurements and samples were taken from the healthiest looking branches carrying the most recently produced leaves.

The specific methods associated with different physiological measurements and sample collections are described below in sections **5.2.2 – 5.2.5.**

Table 5.1: Seedling and resprout species measured.

Site	Family	Key study species	Growth form	Regeneration strategy
Fynbos	Rutaceae	*Agathosma capensis*	Resprout	Resprouter
	Peraceae	*Clutia rubricaulis*	Resprout	Resprouter
	Scrophulariaceae	*Selago glabrata*	Seedling	Reseeder
	Fabaceae	*Aspalathus shawii shawii*	Seedling	Reseeder
Renosterveld	Asteraceae	*Elytropappus rhinocerotis*	Resprout	Resprouter
	Thymelaeaceae	*Passerina obtusifolua*	Resprout	Resprouter
	Peraceae	*Clutia rubricaulis*	Resprout	Resprouter
	Asteraceae	*Othonna paviflora*	Seedling	Reseeder
	Scrophulariaceae	*Microdon polygaloides*	Seedling	Reseeder

Table 5.2: Timeline showing the dates and seasonal categories of physiological measurements.

Survey Date	**Category**	**Physiological measurements made**
Feb 2017	Summer 1 (Preliminary data)	Fv/Fm
Mar 2017		Fv/Fm
Dec 2017	Summer 2	Fv/Fm, RWC, A, g
Jan 2018		RWC, A, g
Mar 2018		Fv/Fm, RWC, A, g
Aug 2018	Winter	RWC
Dec 2018	Summer 3	Fv/Fm, RWC, A, g
Jan 2019		RWC, A, g
Mar 2019		Fv/Fm, RWC, A, g

Table 5.3: Symbols and definitions for physiological indices

Measurement Abbreviation	**Measurement Type**	**Units**
RWC	Relative water content	%
Fv/Fm	Maximum quantum efficiency of photosystem II photochemistry	Unitless
g	Stomatal conductance	mol H_2O m^{-2} s^{-1}
A	Photosynthetic CO_2 assimilation rate	µmol CO_2 $m^{-2}s^{-1}$

5.2.2. Leaf Gas Exchange

The li-cor 6400 infrared gas analyser (IRGA) with a 2*3 LED chamber was used to measure photosynthetic gas exchange on target plants. From these measurements I derived photosynthetic carbon assimilation rates (A) and stomatal conductance to H^2O (g). During measurements, relative humidity in the 2*3 LED chamber was held near ambient 35-45% as far as possible. CO_2 was set to 410ppm. Temperature was not controlled. Light was set at 1500 (µmol $m^{-2}s^{-1}$). Leaves were sealed in the chamber for approximately two minutes each, allowing for readings to stabilise before logging measurements. Blank readings were taken before each plot to track ambient changes and the IRGAs were matched before clamping onto each plant for increased accuracy of measurement. The leaf area of the material measured in the chamber was photographed onto the background of the 2*3 chamber head stuck to a white back board. Leaf area was then measured using ImageJ (**ImageJ**: Rasband, W.S., **ImageJ**, U. S. National Institutes of Health, Bethesda, Maryland, USA, https://**imagej**.nih.gov/ij/, 1997-2018. Schneider, C.A., Rasband, W.S., Eliceiri, K.W.). Assimilation rates and stomatal conductance were corrected for leaf area measured. During surveys, control/treatment plots were measured in alternating order so any changes in ambient conditions over the duration of the measurement survey would be incorporated equally into the control and treatment plot surveys.

5.2.3. Dark adapted maximum quantum yield of photosystem II as an indication of damage to the leaf photosynthetic apparatus

The PAM-100 was used to conduct leaf chlorophyll fluorescence measurements. Dark adapted maximum quantum yield Fv/Fm was used as a proxy for drought stress as the measured value is reflective of damage to the photosynthetic apparatus (Maxwell & Johnson, 2000). I conducted fluorescence measurements at night so that whole plants were dark-adapted rather than shading individual leaves/branches during the day. This was done in order to prevent potential whole-plant effects during measurements. I used UV-strings to tag target plants during the day, which aided in finding them more easily at night using a UV torch. The UV torch was prevented from shining directly on target plants for any length of time. Head torches, which were used during the night-time surveys, were set to low-intensity and were prevented from shining directly on target plants. A weak fibreoptic measuring light was used to measure the initial fluorescence in the dark-adapted state (F_o), when all PSII centres are open, followed by a saturating light pulse and a subsequent measurement of the maximal fluorescence (F_V), when all PSII centres are closed. A distance clip was used to hold the measuring beam at a constant distance and 60 degree angle from the leaf measuring surface. Due to many of the plants having very small leaf size, clumps of leaves were held between the distance clip and a neutral (non-fluorescing) surface. Fv/Fm values were therefore representative of multiple leaves.

5.2.4. Leaf relative water content (RWC)

Directly after the morning gas exchange surveys, a single cutting was taken from each of the focal plants in each plot. Cuttings were taken from the same plants which were measured in gas-exchange survey. Samples were immediately stored in 2 ml plastic centrifuge tubes and sealed with parafilm to prevent dehydration. Before sealing tubes, a small ball of damp cottonwool was inserted into each tube to maintain high relative humidity within the tube. The leaf material and cottonwool were prevented from touching using a small ball of clingfilm. Tubes from a single plot were wrapped in tinfoil and stored in a cooler box during the survey and transported back to the lab. The fresh weight of cuttings was measured immediately after collection using a Metler Tolledo balance. The fresh weight was determined within an hour of collection in all cases. After determining the fresh weight, samples were rehydrated for 5.5 hours by submerging them in deionised water. A preliminary test confirmed that this was sufficient time for full rehydration for the suite of test species. Turgid weight was measured after removing samples from water, patting them dry with Kimberly Clarke wipes and weighing. Samples were left to dry at 60 degrees Celsius until constant mass. Dry weight was measured. Relative water content (RWC) was calculated using the formula:

7) $$RWC = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}}$$

5.2.5. Analytical approach

All statistical analyses were conducted in R 3.5.1 (R Development Core Team, 2018). The analyses were aimed at quantifying the effects of key factors (e.g. site, treatment, growth form) on measures physiological variables during discrete seasonal periods. In the primary analysis, measured physiological variables (e.g. RWC, Fv/Fm, A & g) were analysed using mixed effects models with the interacting explanatory factors: 1) 'survey' (Dec 2017/2018, Jan 2018/2019, Mar 2018/2019), 2) 'site' (Fynbos/Renosterveld), 3) 'treatment' (control/treatment) and 4) 'growth form' (seedling/resprout). E.g. RWC ~ survey * site * treatment*growth form. Plot and growth form were added as nested random effects to account for the hierarchical structure of the data. Pairwise, post-hoc t tests were used to quantify the effect sizes of treatment irrigations on discrete measurement dates (e.g. difference in RWC between control and treatment plots in Dec 2018). This was carried out for all site*growth form group combinations with a sample size of n=6 for control and treatment groups at each measurement date.

5.3. Results

The results compared the physiological sensitivity of seedlings and resprouts to contrasting moisture availability patterns over the first three summers after fire in neighbouring Renosterveld and Fynbos sites. Control plots experienced natural Mediterranean-climate summer drought while treatment plots had reduced summer drought due to 3*30mm summer irrigations. Sensitivity of physiological performance to post-fire soil drought was indicated by natural declines in physiological performance in droughted control plots while positive responsiveness to moisture availability was indicated by positive treatment irrigation effects. The combination of natural declines and treatment responses was used to indicate general differences in sensitivity between growth forms and sites. Soil water potential differences between control and treatment plots are outlined in **5.3.1**. Key measured physiological variables included leaf relative water content (RWC) **(5.3.2)**, dark adapted maximum quantum yield of photosystem II (Fv/Fm) **(5.3.3)**, photosynthetic CO_2 assimilation (A) and stomatal conductance (g) **(5.3.4)**.

5.3.1. Soil water potential responses to summer drought and treatment irrigations

As previously described in Chapter 2, treatment irrigations in January, February and March lead to distinct saturation pulses in soil water potential indicated by 'spikes' in water potential reaching values close to zero **(Fig. 5.1)**. This pattern was contrasted against control plots where water potential values generally remained low (i.e. more negative) throughout summer periods. These patterns show that treatment irrigations lead to strong increases in summer soil moisture availability, thus reducing the impact of summer droughts which are characteristic of the region. Notably, higher natural rainfall in summer 3 lead to two uncontrolled periods of soil saturation in both control and treatment plots. This could be expected to dilute strength of treatment, control soil moisture contrasts during the third summer. Control plots had lower soil water potentials than treatment plots on all measurement dates over the duration of the experiment **(Fig. 5.1)**. Measurements coincided with peak saturation periods in treatment plots thus maximising the differences between soil moisture between control and treatment plots at the time of measurement. For a full description of soil moisture fluctuations throughout the experimental period, see Chapter 2.

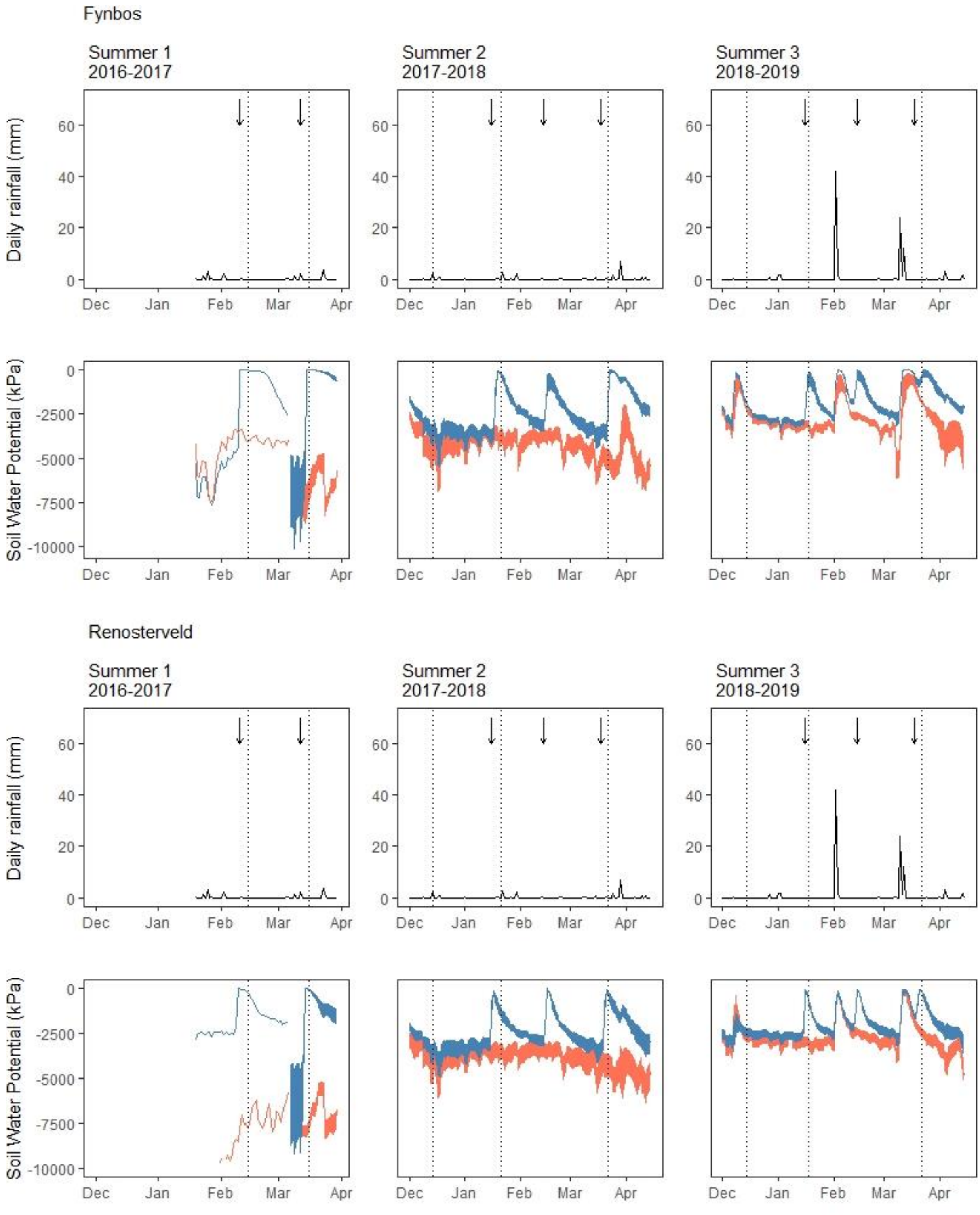

Figure 5.1: Natural daily rainfall patterns (mm) and soil water potential (kPa) in control (orange) and treatment (blue) plots over summer periods in Drie Kuilen Nature Reserve between 2016-2019. Dotted vertical lines represent the dates of the various physiological measurement surveys made over the course of the experiment. December surveys were considered as "pre-drought" baseline values preceding the period of treatment irrigations. Black arrows represent irrigation dates. * Adapted from Figure 7d, Chapter 2.

5.3.2 Leaf RWC responses to soil moisture availability

Leaf relative water content (RWC) was sensitive to patterns of post-fire soil moisture availability in seedlings and resprouts at Fynbos and Renosterveld sites. However, the relationship between soil moisture availability and leaf RWC differed between growth forms and sites, and varied over time since fire **(Fig. 5.2, Table S3)**. Leaf RWC was only measured over the second and third summer after fire.

In control plots, leaf RWC declined progressively over the second summer (Dec2017 – Mar2018) in all growth forms **(Fig. 5.2a)**. These declines were most evident at the Renosterveld site where mean leaf RWC reached ~50% in both seedlings and resprouts by the end of summer (Mar2018). In control plots, late summer (Mar2018) leaf RWC in Renosterveld seedlings was significantly lower than the leaf RWC of seedlings at the Fynbos site; $t(20) = -3.01$, $p = 0.007$ and began declining from earlier in the summer (i.e. Jan2018) **(Fig. 5.2a)**. Taken together, these results showed that Renosterveld seedlings were especially sensitive to leaf dehydration in control plots over the second summer. Conversely, Fynbos seedlings showed remarkable dehydration resistance through summer compared to the other growth forms **(Fig. 5.2a)**.

By the end of the second summer (i.e. Mar2018), the effects of treatment irrigations resulted in significantly higher leaf RWC in Renosterveld seedlings; $t(20) = 6.40$, $p = <0.001$, Fynbos seedlings; $t(20) = 4.69$, $p = <0.001$, Renosterveld resprouts; $t(20) = 7.24$, $p =<0.001$, and Fynbos resprouts; $t(20) = 4.20$, $p = <0.001$ **(Fig. 5.2a, b).** This indicated that summer irrigations helped both seedlings and resprouts to maintain higher leaf RWC values through drought, especially in the Renosterveld site where natural declines were stronger.

All growth forms increased their leaf RWC the winter period (Aug2018), irrespective of winter drought effects (See Chapter 2 for details of the winter droughts). This indicated that cool winter periods could have allowed for recovery from substantial leaf dehydration and also demonstrated a reduced influence of moisture availability on leaf dehydration during this time.

In the third summer after fire (Dec2018 – Mar2019), only the Renosterveld seedlings showed strong declines in leaf RWC **(Fig. 5.2a)** and corresponding positive effects of the summer irrigations; $t(20) = 3.92$, $p = <0.001$ **(Fig. 5.2a, b**). Here, persisting effects of dehydration damage from the previous year's drought were indicated by rapidly declining leaf RWC in control plots as early as Dec2018. Higher leaf RWC in treatment plots was evident in Dec2018 before the onset of the seasonal irrigations; $t(20) = 2.37$, $p = 0.028$. However, seedlings in Renosterveld treatment plots also showed increasing variability in leaf RWC over the third summer. This was not explained by harsher droughts because the third summer had high rainfall **(Fig. 5.1)**. Strong variability in Renosterveld seedlings in the third summer indicated internal changes within seedling physiology or an emergent effect of interactions with other plants. Overall, these results indicated the strong and sustained sensitivity to moisture availability of Renosterveld seedlings. Conversely, resprouts from both sites and Fynbos seedlings all maintained higher leaf RWC over this third summer period **(Fig. 5.2a)** with no corresponding treatment effects **(Fig. 5.2b)**. This implied a reduction in the impact of shallow soil moisture availability on leaf RWC over time for some growth forms.

The full ANOVA model output is displayed in **Table S3**.

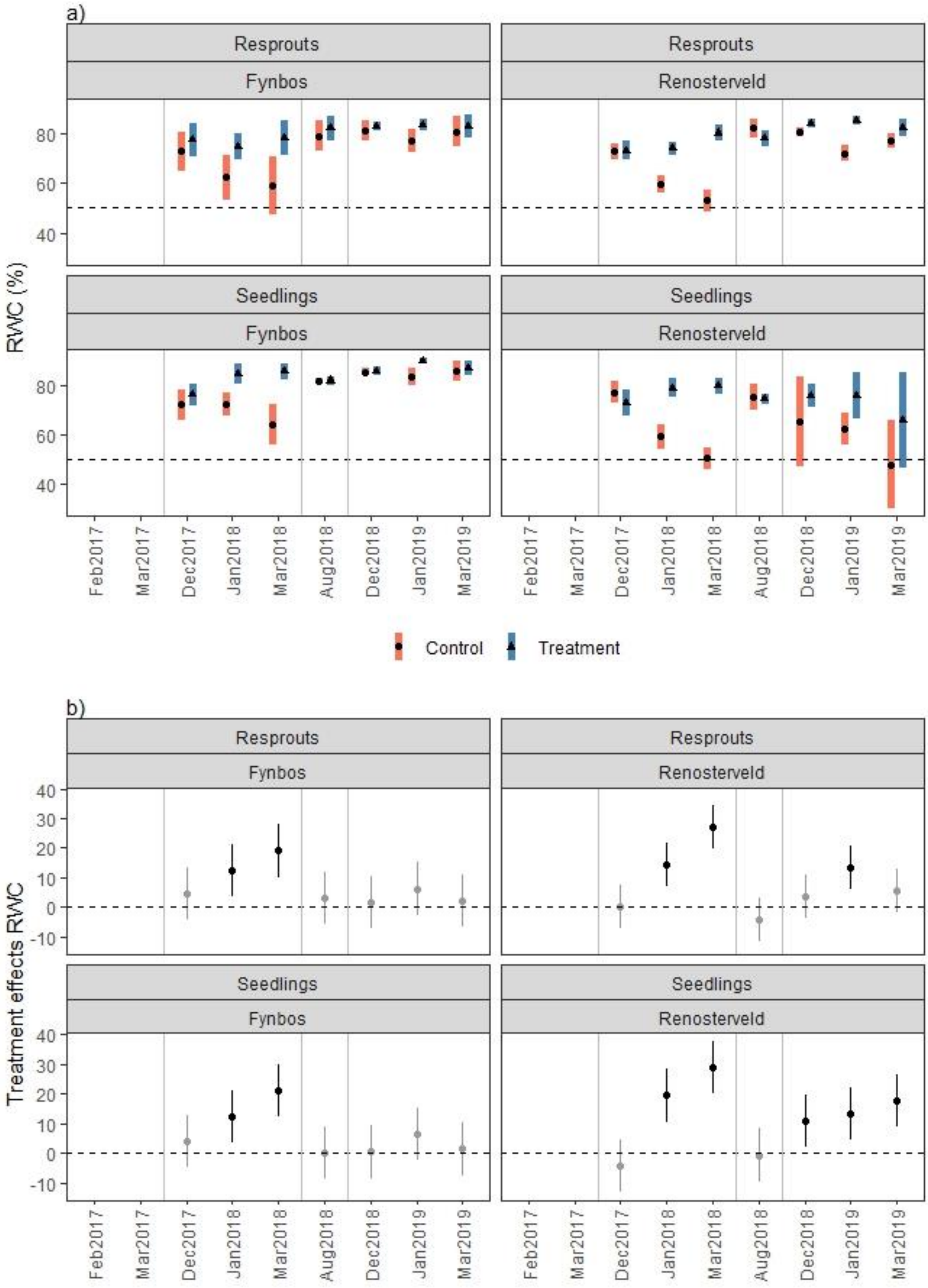

Figure 5.2: a) Leaf relative water content (RWC) % of seedlings and resprouts over summer periods 2016-2019 in treatment and control plots. **b)** Monthly treatment effects on leaf RWC. Significant treatment effects (0.05 % significance level) are indicated in black while insignificant effects are indicated in grey. 50% RWC is indicated by the horizontal dashed line to assist in visual group comparisons.

5.3.3. Dark adapted maximum quantum yield of photosystem II (Fv/Fm) as a measure of damage to leaf photosynthetic apparatus.

Dark adapted maximum quantum yield (Fv/Fm) of photosystem II (Maxwell & Johnson, 2000) was a useful measure of leaf stress under conditions of different soil moisture availability as it was able to indicate damage to photosystem II (Guadagno et al., 2017; Trueba *et al.*, 2019). Fv/Fm values were sensitive to moisture availability patterns but varied strongly between growth forms, across sites and over time **(Fig. 5.3a, Table S4)**. Fv/Fm was measured over all three summers.

Fv/Fm declined over summer drought periods in control plots in all growth forms to varying degrees **(Fig. 5.3a).** This was most apparent in seedlings at both sites, especially during the first summer where late summer Fv/Fm values were extremely low **(Fig. 5.3a).** In all summers, declines in seedling Fv/Fm were larger in the Renosterveld control plots compared to Fynbos control plots. This was indicated by significantly lower late summer (March) Fv/Fm values in Renosterveld control seedlings in the first summer; $t(20) = -4.98$, $p = <0.001$, second summer; $t(20) = -2.66$, $p = 0.015$, and third summer; $t(20) = -6.76$, $p = <0.001$. Late summer Fv/Fm values in control plot resprouts did not differ across sites in any year **(Fig. 5.3a)**. Taken together, these results suggest that seedlings were especially sensitive to drought-related photosynthetic damage, especially in the Renosterveld site.

By the end of the first summer (Mar2017), treatment irrigations resulted significantly higher Fv/Fm values in Renosterveld seedlings; $t(20) = -12.59$, $p = <0.001$, Fynbos seedlings; $t(20) = -6.2$, $p = <0.001$ Renosterveld resprouts; $t(20) = -4.71$, $p = <0.001$ and Fynbos resprouts; $t(20) = -4.37$, $p = <0.001$. By the end of the second summer (Mar2018), treatment irrigations lead to significantly higher Fv/Fm values in Renosterveld seedlings; $t(20) = 5.13$, $p = <0.001$; Fynbos seedlings; $t(20) = 2.6$, $p = 0.017$ and Renosterveld resprouts; $t(20) = 4.18$, $p = <0.001$.

By the end of the third summer (Mar2019), treatment irrigations lead to significantly higher Fv/Fm values only in the Renosterveld seedlings; $t(20) = 2.98$, $p = 0.007$. These results suggest that summer irrigations allowed for growth forms to avoid photosynthetic damage over summer periods, especially in seedlings which experienced the largest natural declines. See supplementary material for full ANOVA output **(Table S4).**

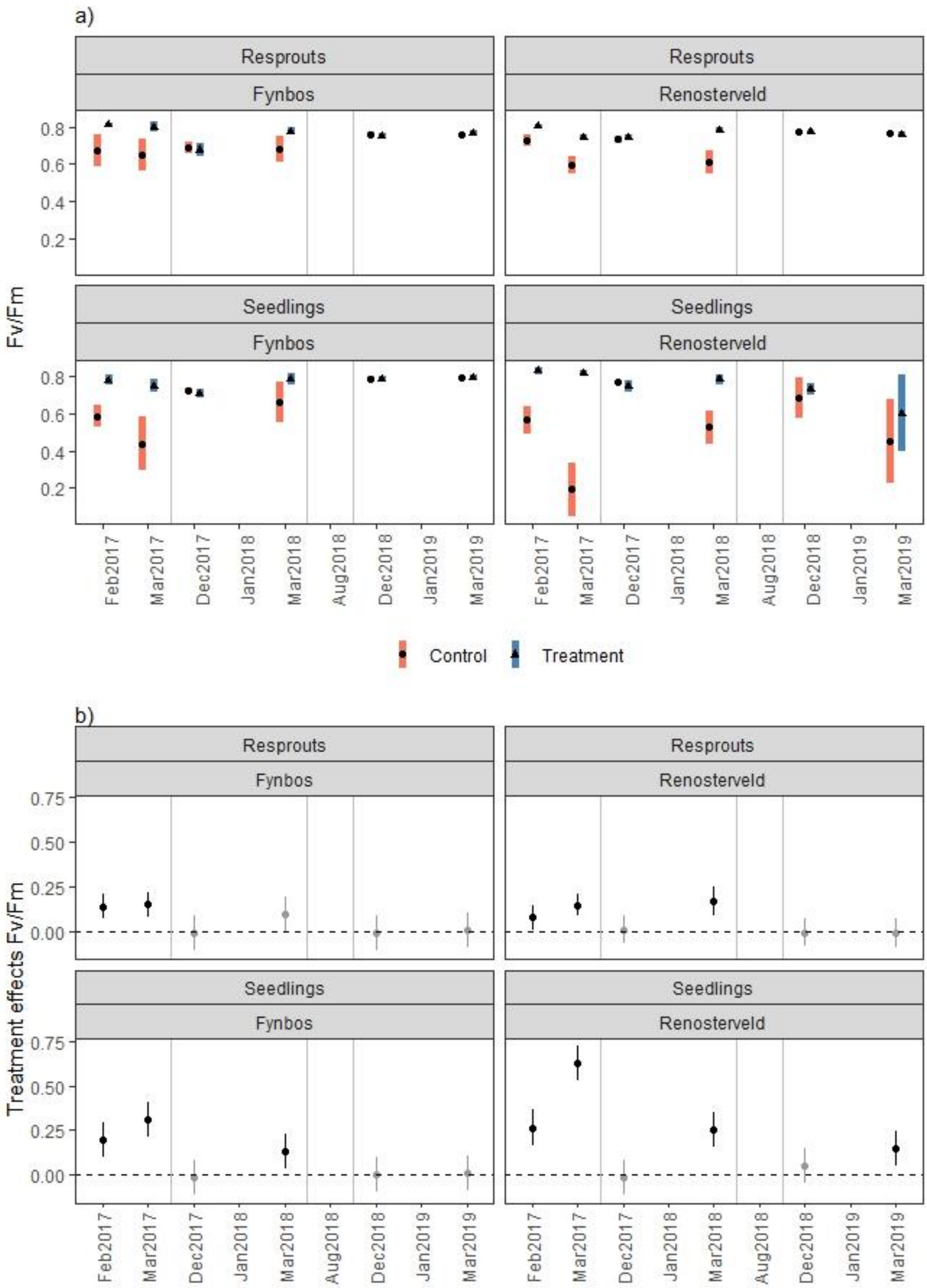

Figure 5.3: a) Dark adapted max quantum yield (Fv/Fm) of leaf tissue in seedlings and resprouts over summer periods 2016-2019 in treatment and control plots. **b)** Monthly treatment effects on dark adapted max quantum yield (Fv/Fm). Significant treatment effects (0.05 % significance level, within group n = 6) are indicated in black while insignificant effects are indicated in grey.

5.3.4. Linking maximum quantum yield and leaf relative water content as a measure of leaf dehydration tolerance

The combination of measurements of leaf RWC and Fv/Fm was found to be a useful indicator of the degrees of leaf dehydration which resulted in potentially permanent or severe leaf damage (Trueba *et al*., 2019). For example, I considered corresponding declines in leaf RWC and Fv/Fm to indicate photosynthetic damage resulting from dehydration while declines in leaf RWC without matching Fv/Fm declines were considered as tolerable changes in hydration which probably did not result in long-term leaf damage. The combination of these measurements **(Fig. 5.2, 5.3)** indicated that leaf dehydration in seedlings **(Fig. 5.2a)** was prone to causing damage to photosynthetic apparatus. In resprouts, leaves appeared to be relatively more resilient to dehydration as leaf RWC declines were not matched by declines in Fv/Fm.

The limited Fv/Fm data collected during the first summer (Feb, Mar 2017) after fire suggests that declines in physiological leaf performance and risk of permanent leaf damage were most severe following summer drought immediately after emergence (i.e. first summer), especially for newly establishing seedlings **(Fig. 5.3)**. This is consistent with the high first summer mortality rates in control plot seedlings at both sites **(Fig. 4.4, 4.6, 4.7)**. The inherent ability to resist dehydration-related stress was thus low in newly developing seedlings within the experiment. This indicates the importance of the timing of moisture availability during early post-fire seedlings emergence. While treatment effects lead to the maintenance of high Fv/Fm values (i.e. healthy leaves) in treatment plot seedlings (**Fig. 5.3b)** during the first summer, the evident mortality (albeit reduced) in treatment plots **(Fig. 4.4)** implies that even well-watered, non-stressed plants were at some risk of rapid dehydration and mortality.

5.3.5. Photosynthetic responses to soil moisture availability patterns

Photosynthetic performance was sensitive to soil moisture availability patterns during summer periods although data were limited to the second summer (Dec2017 – Mar2018).

Rates of stomatal conductance (g) and assimilation (A) declined progressively in control plots over the second summer in both seedlings and resprouts at both sites **(Fig. 5.4, 5.5)**. By the end of the second summer (Mar2018) Renosterveld seedlings in control plots had significantly lower stomatal conductance $t(20) = -2.34$, $p = 0.030$ and assimilation; $t(20) = -3.2$, $p = <0.001$ compared to Fynbos seedlings in control plots. Mean assimilation rates in seedlings and resprouts in Renosterveld control plots had reached zero at the end of the second summer (Mar2018) with some indication of photorespiration (e.g. negative A values) **(Fig. 5.4, 5.5)**. This indicated generally low ability to maintain photosynthetic rates over the second summer in Renosterveld. Both Renosterveld seedlings and resprouts also displayed more rapid declines in carbon assimilation rate (A) coinciding with the early stages of Summer drought (January 2018) **(Fig. 5.4, 5.5)**. Consistent with leaf RWC and Fv/Fm results, these patterns indicate heightened drought sensitivity of growth forms at the Renosterveld site relative to those in the Fynbos site, especially in seedlings. Overall, Fynbos seedlings and resprouts showed higher year-round, photosynthetic function relative to Renosterveld, and generally maintained positive stomatal conductance and assimilation throughout summer droughts (**Fig. 5.4, 5.5).** Fynbos resprouts displayed particularly weak/non-significant treatment effects (**Fig. 5.4b, 5.5b)**. See supplementary material for full ANOVA outputs **(Tables S5, S6).**

Summer irrigations lead to the maintenance of higher stomatal conductance in all growth forms. This was indicated by significantly higher stomatal conductance in treatment plots by the end of summer (Mar2018) in Renosterveld seedlings; $t(20) = 3.94$, $p = <0.001$, Fynbos seedlings; $t(20) = 5.22$, $p = <0.001$ and Renosterveld resprouts; $t(20) = 4.29$, $p = <0.001$. The same positive effects of the treatment irrigations were detected on CO_2 assimilation in Renosterveld seedlings; $t(20) = 6.09$, $p = <0.001$, Fynbos seedlings; $t(20) = 5.2$, $p = <0.001$, Renosterveld resprouts; $t(20) = 6.9$, $p = <0.001$ and Fynbos resprouts; $t(20) = 2.37$, $p = 0.016$.

Photosynthetic responses to treatment irrigations, in terms of stomatal conductance (g) and CO_2 assimilation (A), broadly corresponded with the responses of RWC **(Fig. 5.2)** and Fv/Fm **(Fig. 5.3)**. For example, during the second summer (Dec2017-Mar2018), Fv/Fm responses to moisture availability patterns, or lack thereof **(Fig. 5.3a,b)**, were matched by equivalent responses/non-responses in A and g **(Fig. 5.4a,b, 5.5a,b)**, implying that photosynthetic rates were linked partly to the degree of damage incurred within leaf photosynthetic apparatus amongst growth forms. However, during the third summer (Dec2018-Mar2019).

Changes in photosynthetic performance were found to vary across control and treatment plots independently of changes in leaf RWC (e.g. Fynbos resprouts Dec2017 – Mar2018, e.g. Fynbos resprouts and seedlings and Renosterveld resprouts Dec2018-Mar2019) indicating that photosynthetic rates can vary independently of leaf hydration within certain ranges of leaf hydration. This was generally found to be the case when leaf RWC was high.

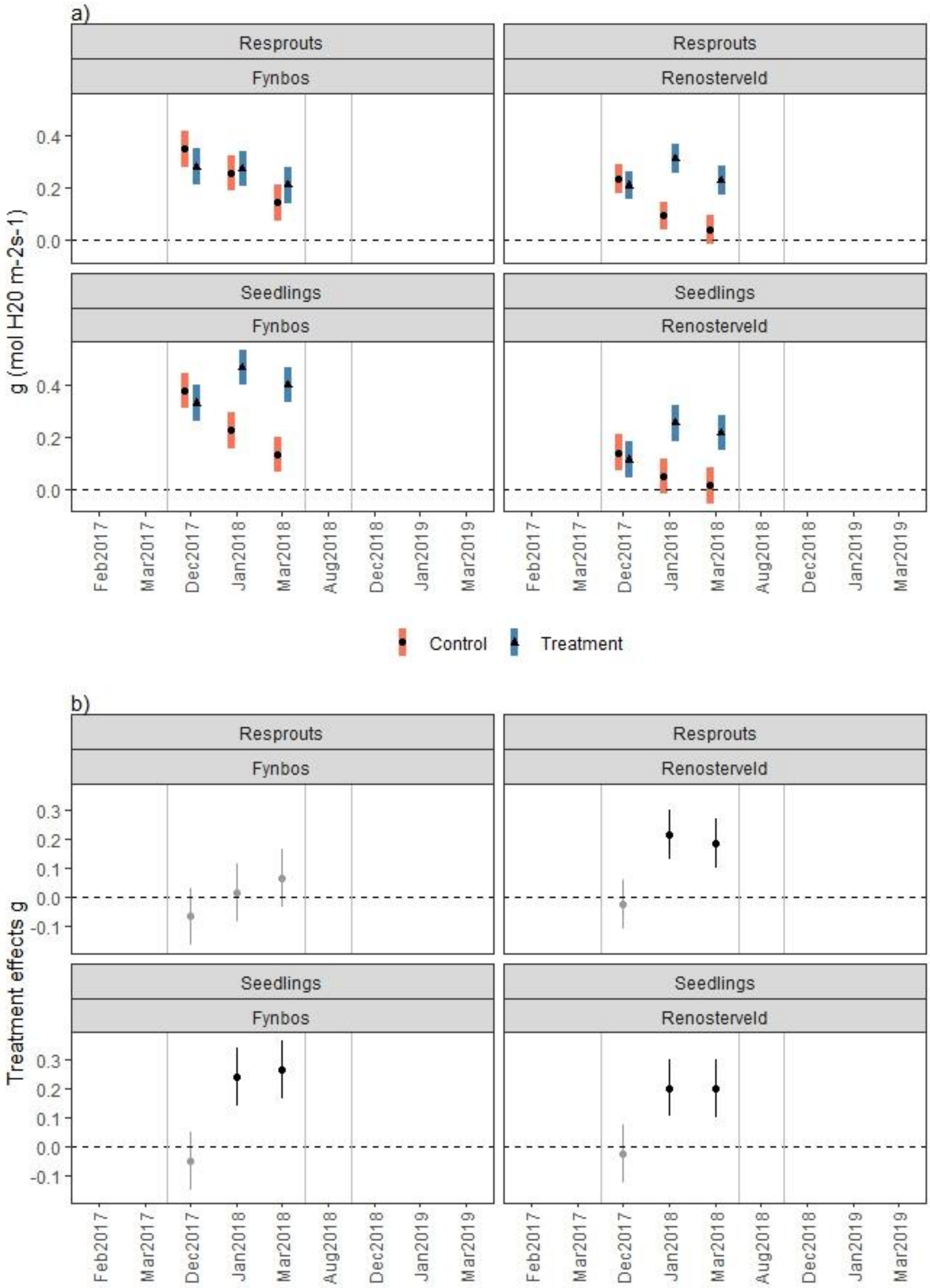

Figure 5.4: **a)** Leaf stomatal conductance (g) of leaf tissue in seedlings and resprouts over summer periods 2016-2019 in treatment and control plots. **b)** Monthly treatment effects on leaf stomatal conductance (g). Significant treatment effects (0.05 % significance level, within group n = 6) are indicated in black while insignificant effects are indicated in grey.

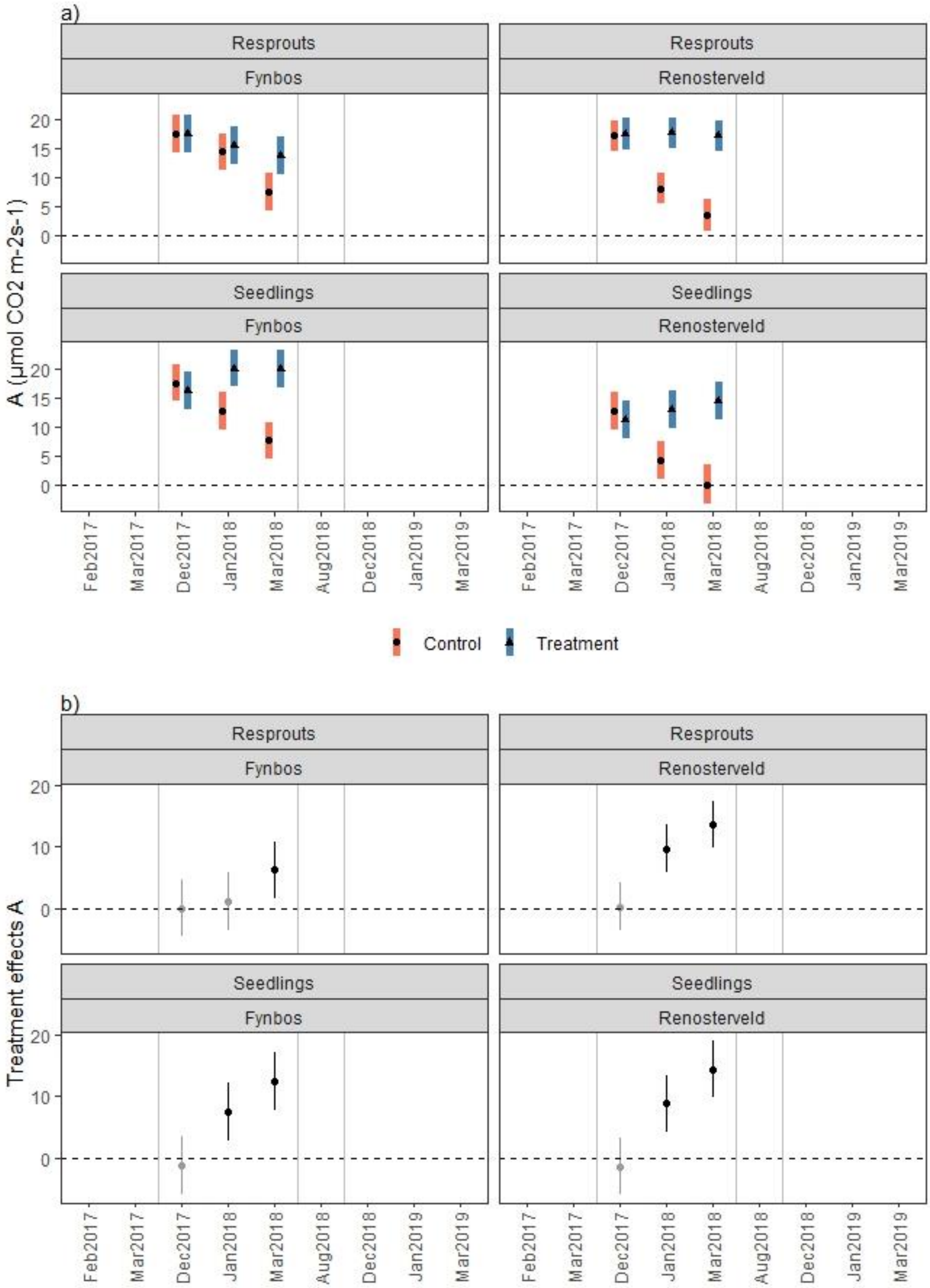

Figure 5.5: **a)** Photosynthetic carbon assimilation rate (A) of leaf tissue in seedlings and resprouts over summer periods 2016-2019 in treatment and control plots. **b)** Monthly treatment effects on photosynthetic carbon assimilation rate (A). Significant treatment effects (0.05 % significance level, within group n =6) are indicated in black while insignificant effects are indicated in grey.

5.4. Discussion

The experiment demonstrated differences in plant physiological sensitivity to post-fire rainfall amongst seedlings and resprouts in Fynbos and Renosterveld sites. The physiological performances of both seedlings and resprouts were negatively impacted by natural summer drought in the post-fire environment but summer treatment irrigations substantially reduced physiological drought stress by maintaining shallow soil moisture availability through periods of low summer rainfall. The physiological sensitivity of all growth forms to moisture availability was highest in the immediate post-fire environment (i.e. first summer). This was most evident in newly developing seedlings where drought stress and mortality were high in the first summer **(Fig. 4.4)**. During this time, seedlings showed strong positive responses to summer irrigations. Plants at the Renosterveld site, especially seedlings, were also found to have higher physiological sensitivity to summer drought compared to those at the Fynbos site, consistent with higher soil moisture tension in shale soils **(Fig. 2.10).** Overall, the differences in physiological sensitivity between seedlings and resprouts across Fynbos and Renosterveld sites was broadly consistent with patterns of demographic sensitivity recorded in Chapter 4. The results demonstrated the importance of the timing of moisture availability for post-fire performance and demographic patterns and highlights the potentially strong limiting effects of Mediterranean-climate summer drought on seedling emergence patterns.

The key findings related to seedlings are resprouts are conceptualised as a sensitivity matrix **(Fig. 5.6)** which ranks the sensitivity of growth forms to successive post-fire summer periods (S1, S2, S3) under a range of summer moisture conditions. This was based on varying levels of observed mortality (Chapter 3) and physiological decline. The sensitivity matrix highlights the increased potential for drought related plant stress and mortality during the first summer period, with decreasing stress potential over successive summers. This decreasing stress gradient after fire is further influenced by growth form, soil-type and amount of summer rainfall. Deep rooted resprouts were less sensitive to drought and showed no mortality whereas newly emerging seedlings were highly susceptible to drought-induced mortality. Potential stress levels were also increased on the fine-particle shale-derived soils consistent with higher soil tension (increased drought intensity) **(Fig. 2.10)**. Increasing summer rainfall reduced stress levels for both seedlings and resprouts on both soil types. This effect was likely to be most important over the first summer period. Rankings of sensitivity from 1-5 were based on the observed demographic (Chapter 4) and physiological responses to natural summer droughts and treatment irrigations.

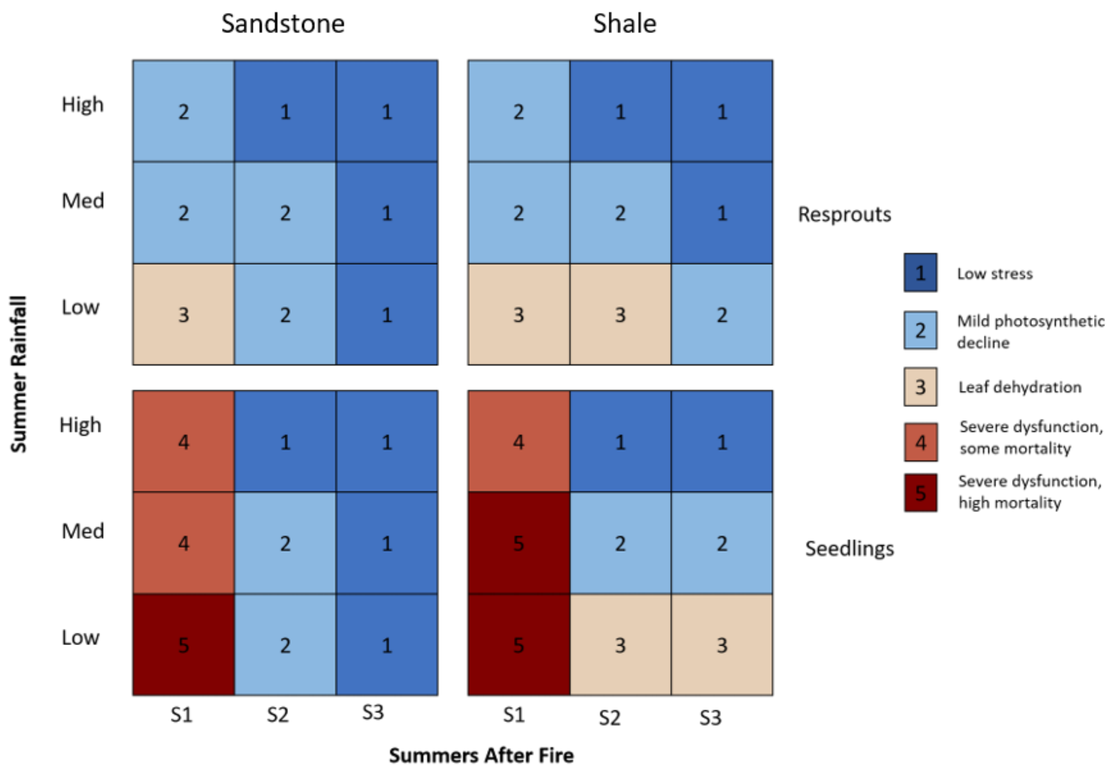

Figure 5.6: Sensitivity matrix showing the estimated degree of post-fire plant dysfunction over three summer periods (S1,S2,S3) after fire in relation to the amount of summer rainfall. Sensitivity ranked 1 – 5 is broadly summarises physiological and demographic sensitivity patterns recorded in chapter 4 and 5.

Reseeding and resprouting species demonstrated characteristics which are consistent with the apparent life-history trade-off between plant persistence and reproductive potential (Chapin *et* *al.*, 1990; Iwasa & Kubo, 1997; Bellingham & Sparrow, 2000). Here, high survival, physiological stability and deeper roots in resprouts was consistent with high allocation to mature plant persistence (Altwegg *et al.*, 2015) allowing resprouts to survive both fire and subsequent drought. Accordingly, these resprouting species demonstrated low reproductive potential in the post-fire environment with few seedlings observed within the first two years after fire (personal observation). Conversely, reseeders showed no persistence through fire with no surviving mature individuals in the post-fire environment but showed high potential post-fire recruitment potential through high seedling production within the first year after fire. This is consistent with the obligate reseeder strategy (Pausas et al. 2004; Keeley et al. 2012). However, the survival of emerging seedlings through subsequent summer drought was closely coupled with summer rainfall, indicating that the ability to realise high reproductive potential in reseeders was strongly influenced by post-fire environmental variability/resource availability (i.e. rainfall). Here, concentrations of reseeding populations are likely to show relatively high instability and mobility (i.e. "boom or bust") between fire events, depending on post fire weather, while resprouters may maintain site occupancy through fires but struggle to populate new sites (Merwin *et al.*, 2012).

The different strategies of adult persistence and reproductive potential should select for different functional and developmental strategies between seedlings and resprouts in the post-fire environment (Altwegg *et al.*, 2015). For example, high physiological sensitivity, fast growth rates and high mortality collectively suggest that post-fire seedlings undergo a rapid, but 'risky' period of development after germination. This is consistent with the ideas of the "asset protection principal" in animal ecology which suggests that young organisms are inherently more vulnerable to mortality and have less to lose and more to gain through "risky" developmental patterns (Clark *et al.*,1994). Seedling development in seasonality arid shrublands should require high growth rates and high responsiveness to moisture in order to rapidly establish rooting structures in deeper soil layers (i.e. deeper than the top few cm) before succumbing to shallow soil drought over the extended summer drought period. The degree of successful seedling recruitment during this time is closely related to the amount of summer rainfall. This finding is mirrored by recent field studies undertaken in other Mediterranean-type regions (e.g. Parra & Moreno, 2018). While high seedling growth rates might be necessary successful establishment and drought avoidance, high growth rates could contribute to the increased vulnerability of newly developing seedlings due to the apparent trade-off between and growth rate under favourable conditions and the physiological tolerance of water shortage (Orians & Solbrig, 1977; Chapin, 1980; Smith & Huston, 1989) . Thus, seedlings which fail to establish sufficient rooting systems could be particularly sensitive to dehydration of above ground structures. However, once seedlings had established themselves, growth rates, mortality rates and physiological sensitivity to moisture availability generally declined indicating the ability to shift physiological activity to different requirements at different stages of development.

Lower physiological sensitivity, slow relative growth rates and low mortality in resprouts are traits characteristic of the 'persistence-niche' (Bond & Midgley, 2001; Keith *et al.*, 2007) and is consistent with studies that have found little impact of drought on mature resprouting plants (Parra & Moreno, 2018). Survival of adult resprouters is critical for resprouting populations as they could be disproportionately sensitive to changes in adult survival (Pfister, 1998).The traits which characterise resprouting are likely to allow persistence trough a range of different disturbances including drought and fire (Zeppel *et al.*, 2015). Of course, initial growth rates of resprouts were much higher from immediately post-burn to the baseline survey after the first winter as they accumulated much larger above ground volumes up to the baseline demographic survey **(Table S2)**. The initial regeneration of resprouters from stored carbohydrates may initially decouple them from immediate environmental resource availability (Bowen and Pate, 1993; Bell & Ojeda, 1999; Clemente *et al.*, 2005; Schutz *et al.*, 2009), thus aiding their rapid initial re-establishment, irrespective of variations in shallow soil moisture availability and enabling them to more easily achieve physiological stability by the time of arrival of the first summer drought. These patterns suggest that seedlings and resprouts both require a period of establishment or reestablishment after fire but that physiological and thus demographic stability is achieved at a faster rate in resprouts. These ideas are consistent with the idea that resprouts should be more well suited to surviving environmental heterogeneity and disturbance because they have invested so much into the persistence strategy rather than high seedling recruitment potential (Altwegg *et al.*, 2015). The superiority of the resprouting regeneration strategy is likely to be balanced by poor seedling performance (Thomas & Davis, 1989; Pratt *et al.*, 2008) or reduced seedling output (Pratt *et al.*, 2014).

Physiological sensitivity to drought and rainfall of growth forms broadly differed between Fynbos and Renosterveld sites. Higher physiological sensitivity was higher in both seedlings and resprouts at the Renosterveld site. Overall, lower seasonal physiological sensitivity of seedlings and resprouts in Fynbos appeared to be consistent with lower sensitivity of productivity and community level responses in Chapter 3 and 4. This implies that differences in physiological processes scale up and influence higher level processes within biomes. Furthermore, this suggests that edaphic differences between Fynbos and Renosterveld shrubland types could have profound, bottom-up effects on shrubland climate responses at the regional scale (Bradshaw & Cowling, 2014). As I have shown previously **(Fig. 2.10)**, fine-textured soils of the Renosterveld site were associated with more rapid reductions in soil water potential during drought compared to the coarse-textured sandy soils of the Fynbos site. Accordingly, during summer drought periods, the highest levels of dehydration, leaf damage and photosynthetic decline appeared to be associated with the fine-textured soils of the

Renosterveld site. Fynbos growth forms, including seedlings in control plots experienced remarkably low sensitivity to low rainfall after the first summer. This result is consistent with previous findings in the Fynbos (Miller *et al.*, 1983). Drought tolerance could be enhanced by the lower transpiration and maintenance costs associated with conservative growth rates (Murren *et al.*, 2015) as well as lower drought intensity of course, sandy soils **(Fig. 2.10)**. Overall, this suggests that plant physiological performance is strongly influenced by soil drought intensity nutrient availability.

Understanding the relationship between summer moisture availability, plant function and life-history characteristics in the post-fire environment is of paramount importance in Mediterranean-type ecosystems because droughts in the post-fire environment are likely to be a strong driver of vegetation function within these diverse regions (West *et al.*, 2012). This study broadly predicts that increased summer rainfall could increase the relative evolutionary advantage of reseeding due to strongly increased post-fire seedling recruitment success. While both seedlings and resprouts are likely to benefit from summer moisture in terms of physiological function, the fitness benefit should be disproportionately larger for reseeders due to high allocation into recruitment potential, which can be realised to a greater potential under favourable conditions. In resprouters, allocation into adult plant persistence through underground storage is likely to have greater fitness under conditions which require temporary decoupling from natural resource availability whereas under non stressful, favourable growth conditions, these traits may become physiologically inefficient (Bellingham & Sparrow, 2000). However, other factors which may be affected by rainfall patterns (e.g. fire frequency, severity) could lead to strong effects on reseeder and resprouter populations which are not covered in this study (Kraaij & van Wilgen, 2014).

Conclusion

This study demonstrated that physiological sensitivity of growth forms to post fire climate is an important driver of demographic patterns as well as higher-level processes such as primary productivity. Post-fire moisture availability in summer appear to be critical factor for recovery patterns of shrublands within the Mediterranean-type climate zone. Notably, the impact of post-fire moisture availability could have disproportionate effects on resprouting and reseeding species due to the inherent differences in their dependence on shallow soil moisture availability. Additionally, edaphic factors could modify the relationship between plant physiology and rainfall due to the effects of soil texture on plant available soil moisture.

Chapter 6: Synthesis

6.1. Overview: Complex relationships between climate and vegetation.

The three-year experimental field study detected effects of altered rainfall seasonality at all levels of the investigation, including soil moisture dynamics (Chapter 2), leaf-level plant physiology (Chapter 5), community demographics (Chapter 4), composition and net primary productivity (Chapter 3). Across Fynbos and Renosterveld field sites, the sensitivity of higher-level vegetation processes (e.g. primary productivity, species composition) to rainfall seasonality appeared to mirror the sensitivity of fine-scale processes (e.g. leaf level physiology) suggesting a degree of interdependence between processes occurring across these scales.

Rainfall pulses in the post-fire environment could thus be described as having a “bottom-up” effect on vegetation structure, whereby complex fine-scale plant physiological and growth responses to patterns moisture availability have cascading effects which determine community level processes and community structure (Ogle & Reynolds, 2004; Reynolds *et al.*, 2004; Báez.*et al.*, 2006). In the post-fire context, changes in fine scale plant functioning and responsiveness to moisture availability could mean the difference between survival or mortality of a large number of newly emerging plants. Thus bottom-up effects in the early post-fire environment could lead to particularly dramatic changes in long-term trends of community structure and the nature of community interactions within mature vegetation stands, consistent with the “event-dependence” hypothesis (Bond & van Wilgen, 1996; Moreno *et al.*, 2011). Community factors such as such as competition, facilitation, fuel load and vegetation structure are emergent phenomena which can, in turn, have top-down effects or form complex form of feedback loops with bottom-up factors.

The investigations into complex climate-soil-vegetation relationship covered within this thesis are summarised conceptually in a network of potential interrelationships between vegetation processes and environmental factors at multiple scales of investigation **(Fig. 6.1).** Complex patterns of seasonality essentially determine the base patterns of potential resource availability for vegetation function. However, soils are highlighted as an important interface between climate factors (i.e. rainfall seasonality) and vegetation processes. Nutrient and textural soil properties thus have the potential to mediate the relationship between vegetation activity and rainfall availability, thus acting as edaphic filters to climate effects. The potential for edaphic effects to influence vegetation function have been highlighted previously in Mediterranean-type shrubland vegetation (Esler & Cowling 1993; Esler *et al.*, 2015; Power *et al.*, 2019). This could lead to regional level edaphic control of vegetation-climate relationships, since Fynbos and Renosterveld communities are generally separated strongly across soil types (Bergh *et al.*, 2014; Bradshaw & Cowling, 2014). Edaphic control of vegetation responses to climate are discussed further in section 6.3. Additionally, the study highlights the potential for fine-scale physiological processes to determine and/or interact with higher level community processes such as community structure and productivity **(Fig. 6.1)**. This was indicated by the congruence of site level patterns across all levels of investigation (e.g. Chapter 3-5). This is indicative of a potentially strong bottom-up control of vegetation structure within megadiverse shrubland ecosystems. However, adaptations and different life history strategies within vegetation communities may further determine the strength of interactions between different vegetation elements and climate processes. For example, factors like canalised, conservative growth traits, physiological drought adaptation and life-history mode could strong influence how climate and vegetation interact.

Taken together, this study contributes to a greater understanding of climate sensitivity within complex post-fire communities of the Cape Floristic Region, highlighting the need to experimentally quantify the sensitivity of vegetation processes to shifts in key climatic variables. Experiments which investigate the responses of complex vegetation systems to climate change should measure various, interacting aspects of vegetation dynamics because this increases the ability to understand and validate patterns observed at any single level of investigation.

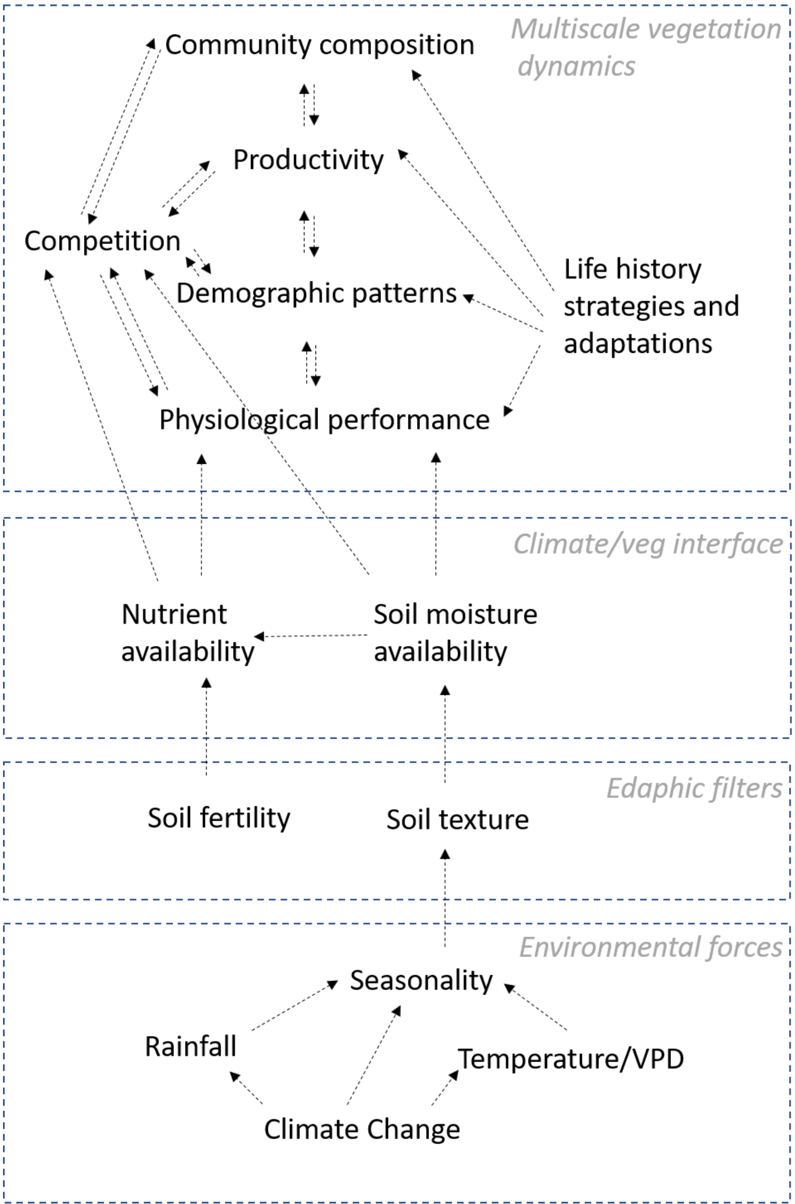

Figure 6.1: Complex, multiscale interconnectivity between measured environmental and vegetation variables. Dashed arrows represent putative causal links between factors. Dotted boxes separate different categories of measured variables. This network is not an exhaustive list of connections and is based primarily on measured variables during the three-year field rainfall manipulation experiment.

6.2. The influence of rainfall seasonality patterns

The timing of rainfall determines the degree of soil moisture and nutrient availability, since nutrient mobility increases with soil moisture (Barber, 1962; Singh & Singh, 2004). This means that seasonal rainfall patterns essentially determine the timing of resource pulses for semi-arid shrubland ecosystems (Schwinning & Sala, 2004; Ogle & Reynolds, 2004; Reynolds *et al.*, 2004: Báez.*et al.*, 2006). Under winter rainfall climates, a general asynchrony of moisture/nutrient resources and favourable growth temperatures/photoperiods exists due to the majority of rainfall occurring during cool months (Specht, 1988). However, rainfall is not restricted to these cool months, with sporadic events occurring during warmer periods. Nonetheless, favourable environmental periods for establishment, growth and reproduction of plants may be limited to relatively brief windows of opportunity where moisture and nutrient resource availability aligns with favourable growth conditions (Specht, 1988). Shifting rainfall seasonality patterns could lead to changes in these windows of opportunity, which is likely to be of high ecological significance during the post-fire period (Parra & Moreno, 2018), where vegetation establishment patterns are most vulnerable to the environmental conditions (Moreno & Oechel, 1992; Parra and Moreno, 2018; Penuelas *et al.*, 2001; Quintana *et al.*, 2004; Luis *et al.*, 2008). In addition to determining these windows of opportunity, rainfall seasonality patterns also determine the periods of potential drought and highest stress (Parra & Moreno, 2018). Under the winter-rainfall climate, the importance of summer moisture availability was apparent for reducing plant stress during these warm periods.

This experiment showed that the importance of rainfall seasonality patterns could differ between different growth forms and post-fire life history strategies. For example, reseeding species populations may have a stronger reliance on post-fire rainfall patterns in determining population sizes because of the high allocation to post-fire recruitment and the inherent sensitivity of seedlings in the post-fire environment (Parra & Moreno, 2018). Conversely, resprouting species populations are more likely to be determined by factors which affect the mature, surviving individuals over longer time scales because population persistence is primarily reliant on these surviving individuals. These responses may include fire frequency (Cowling, 1987), severity, competition, long-term climate changes (Slingsby *et al.*, 2017). Taken together, the temporal scales of climate sensitivity may differ broadly across resprouter and reseeder species. Additionally, the importance of rainfall seasonality differed across edaphic boundaries. For example, the Fynbos site showed little sensitivity to post-fire rainfall patterns despite being located at the arid end of the Fynbos distribution (<300mm) (Bradshaw & Cowling, 2014). This is indicative of extreme resilience to climate in the Fynbos site, presumably lessening the importance of rainfall seasonality. Conversely, the Renosterveld site showed high sensitivity and responsiveness to the same seasonality patterns. Therefore, edaphic factors may mediate the 'importance' of rainfall patterns on vegetation function.

While the shrublands of the Cape Floristic Region may have experienced considerable evolutionary specialisation under a stable, seasonal Mediterranean climate for millennia (Cowling *et al.*, 2004), vegetation at both sites was able to tolerate changes in rainfall seasonality through a degree of insensitivity to rainfall or adjust to altered rainfall seasonality through developmental and demographic plasticity (Bradshaw, 1965). This suggests that shifts in rainfall seasonality within the Cape Floristic Region could be tolerated by vegetation to some degree, perhaps explaining the wide range of seasonal patterns tolerated by shrublands of the Cape Floristic Region across their current distribution (Bergh *et al.*, 2014; Bradshaw & Cowling, 2014). Of course, ecological and selective effects which fell beyond the scope of this experiment could have larger implications for vegetation processes which are compounded over multiple fire-events.

6.3. Edaphic effects could mediate rainfall responses in Fynbos and Renosterveld

Edaphic effects have been shown to affect species distributions and plant function in the Greater Cape Floristic Region (Esler & Cowling, 1993; Esler *et al.*, 2015). This study showed that structurally similar shrubland sites, Fynbos and Renosterveld, were not equally sensitive to rainfall patterns in terms of their physiology (Chapter 5), demography (Chapter 4) and productivity (Chapter 3) in the post-fire environment. The clear difference in sensitivity to rainfall between Fynbos and Renosterveld shrublands adds to growing evidence that these shrublands could have unique relationships with rainfall. For example, these findings are supported by a recent study which compared inter and intra annual variability in NDVI (a useful measure of productivity) to rainfall at the regional scale and showed that Fynbos had lower variability in NDVI than Renosterveld (Cramer and Hoffman, 2005). However, there are still few studies which have compared concurrent rainfall responses across these shrubland types (Pierce & Cowling, 1984; Esler *et al.*, 2015).

These site-specific differences in vegetation sensitivity to rainfall point toward soil properties being significant mediators of vegetation-rainfall relationships due to their effects on nutrient and moisture availability within soils (Bradshaw & Cowling, 2014; Minasny & McBratney 2007). The existence of soil-mediated differences in vegetation-rainfall relationships is an important finding supporting the separation of Fynbos and Renosterveld as highly distinct vegetation types within the Cape Floristic Region even though they are structurally very similar. Until recently, these vegetation types have been categorised under a single biome "Fynbos" despite differences in floristic composition and strong edaphic associations (Bergh *et al.*, 2014; Bradshaw & Cowling, 2014). This study shows that floristic and edaphic differences are also associated with potential differences in plant and community functionality. Taken together, these patterns suggest that Fynbos and Renosterveld not only have distinct evolutionary histories (Verboom *et al.*, 2014, Tolley *et al.*, 2014) but that these histories are likely to have strongly selected for species assemblages which are specialised to specific edaphic conditions, even though they occur under the same climate regime.

Generally, this study showed that sandstone-derived (Fynbos) soils appeared to be associated with constrained productivity and growth responses to rainfall while shale-derived (Renosterveld) soils were associated with stronger responses to rainfall. In the sandstone soils of the Fynbos site, the combination of higher stability in plant-available soil moisture and consistently low nutrient availability, ensured that resource availability was consistently limited by at least one resource (e.g. nutrients) but that this limited supply was relatively stable under both seasonal and aseasonal rainfall patterns. This could drive the slow and steady, conservative growth and productivity patterns in Fynbos which were relatively insensitive to rainfall patterns. Conversely, the strongly (amplified) seasonal plant available soil moisture availability and high mineral nutrient content of shale-derived soils ensured that resource availability was strongly linked to rainfall patterns. This could drive the stronger growth and productivity responses to rainfall seen in Renosterveld.

The selection for plasticity (i.e. variability of a trait in response to environmental variation) in plant responses (e.g. growth) has often been related to heterogeneity in resource supply (Pintado *et al*., 1997; Valladares *et al*., 2000; Gianoli & González-Teuber, 2005; Zunzunegui, 2011) and has been demonstrated to trade off with resource conservatism along soil nutrient availability gradients (Power *et al*., 2019). In Fynbos, while nutrient limitation could be chronically limiting from early post-fire successional stages, there could be some degree of endogenous or genetic limitation to growth at the individual plant level which is driven by selection for conservatism over plasticity. For example, plastic growth responses to temporarily favourable conditions could be unsustainable (Murren *et al*., 2015) and therefore, utilising all available nutrients rapidly after fire during favourable moisture conditions could be maladaptive in Fynbos. The adaptive benefit of plasticity is therefore a balance between cost-benefit ratio associated with a plastic response (Schwinning & Sala, 2004). The extent to which Fynbos is limited endogenously could be tested in future experiments which alter nutrient availability. Some studies have showed that Fynbos plants may be highly specialised to low nutrient soils and could suffer negative consequences under increased nutrient availability (Witowski, 1988; Witowski, 1991; Esler *et al.*, 2015).

6.4. Short and long-term responses to climate change

The experiment demonstrated that the shrublands within the CFR could have potentially different vegetation responses and/or vulnerabilities to future climate change in the short and long term. Short-term sensitivity to rainfall was clearly higher in Renosterveld than in Fynbos in the study. In the context of long-term vegetation stability, it is difficult to predict which shrubland type is most sensitive to potential changes in climate. For example, short-term sensitivity to rainfall patterns, as seen in Renosterveld, could lead to high variability in vegetation processes which could be somewhat erratic due to the inherent variability of post-fire climate, but could also allow for the necessary demographic shifts to track a rapidly changing climate (Aerts, 1999; Callaway *et al.*, 2003; Berg & Ellers, 2010; Burns & Strauss, 2012; Bradshaw 1965; Nicotra *et al.*, 2010; Nicotra & Davidson, 2010). Short-term stability or insensitivity to rainfall patterns, as seen in Fynbos, could buffer vegetation productivity patterns against climate variability within a certain threshold of tolerance but could lead to a lack of flexibility and potential collapse of function should climate shifts range outside of normal variability thresholds. Indeed, it has been shown that specialised communities be particularly vulnerable to collapse and loss of diversity with little chance of being able to adapt to unprecedented environmental situations (Futuyma & Moreno, 1988; Wiens *et al.*, 2005).

These insights could contribute to spatio-temporal distribution patterns of Fynbos and Renosterveld shrublands within the GCFR. For example, the contemporary range of Fynbos occurs over wide environmental gradients (e.g. Mean annual precipitation ±300mm to >3000mm) (Bradshaw & Cowling 2014) and is generally thought to have shown remarkable stability over time scales from centuries to millennia (Tolley *et al.*, 2014; MacPherson *et al.*, 2018, 2019; Gillson *et al.*, 2020). Much of this stability could be explained by the maintenance of fire in Fynbos under a variety of climatic conditions, thus preventing the encroachment of other, non-fire prone vegetation types (Gillson *et al.*, 2020). However, this study makes the case that physical resilience to changes in climate could also play a role in the stability of Fynbos. Higher phenotypic, community and ecological stability at short-time scales and implies that a wide tolerance of climatic gradients could exist in Fynbos due to the low demands made by a conservative growth strategy. Renosterveld (especially Mountain Renosterveld) has strong floristic affinities with the Succulent Karoo (Bergh *et al.*, 2014) and both vegetation types emerged more recently than Fynbos in the biogeographical record (Tolley *et al.*, 2014). Mountain Renosterveld has frequently been described as transitional with Succulent Karoo over fine-scale gradients within the landscape (Levyns 1964; Bergh *et al.*, 2014; van der Merwe & van Rooyen, 2011a,b). This could be indicative of the nutrient-richer soil vegetation being more highly prone to compositional and structural shifts along environmental gradients **(Fig. 6.2)**, while Fynbos occupies a wide environmental range in sandstone soils. Succession between Fynbos to Forest systems has been demonstrated in fire-protected sites with high moisture availability (Power *et al.*, 2019). This study demonstrates the strong edaphic separation between shrubland types which lead to differences in vegetation functionality. Strong edaphic differences between shrublands in sandstone versus shale soils could lead to strong divergence in functionality between shrublands occurring on different substrates over extended periods of time (millennia), thus potentially reinforcing biome boundaries. Climate driven biome shifts are predicted to me more likely in shale soils due to vegetation processes showing a closer coupling with moisture availability patterns in the Renosterveld site (see Chapters, 2-5). Climate driven biome changes may occur gradually relative to sharp changes in community composition between soil ecotones. Climatic thresholds for the different vegetation types are not well understood but it is likely that temperature and moisture extremes may play a role in determining the distribution of these shrublands (Midgley *et al.*, 2002; Bradshaw & Cowling, 2014).

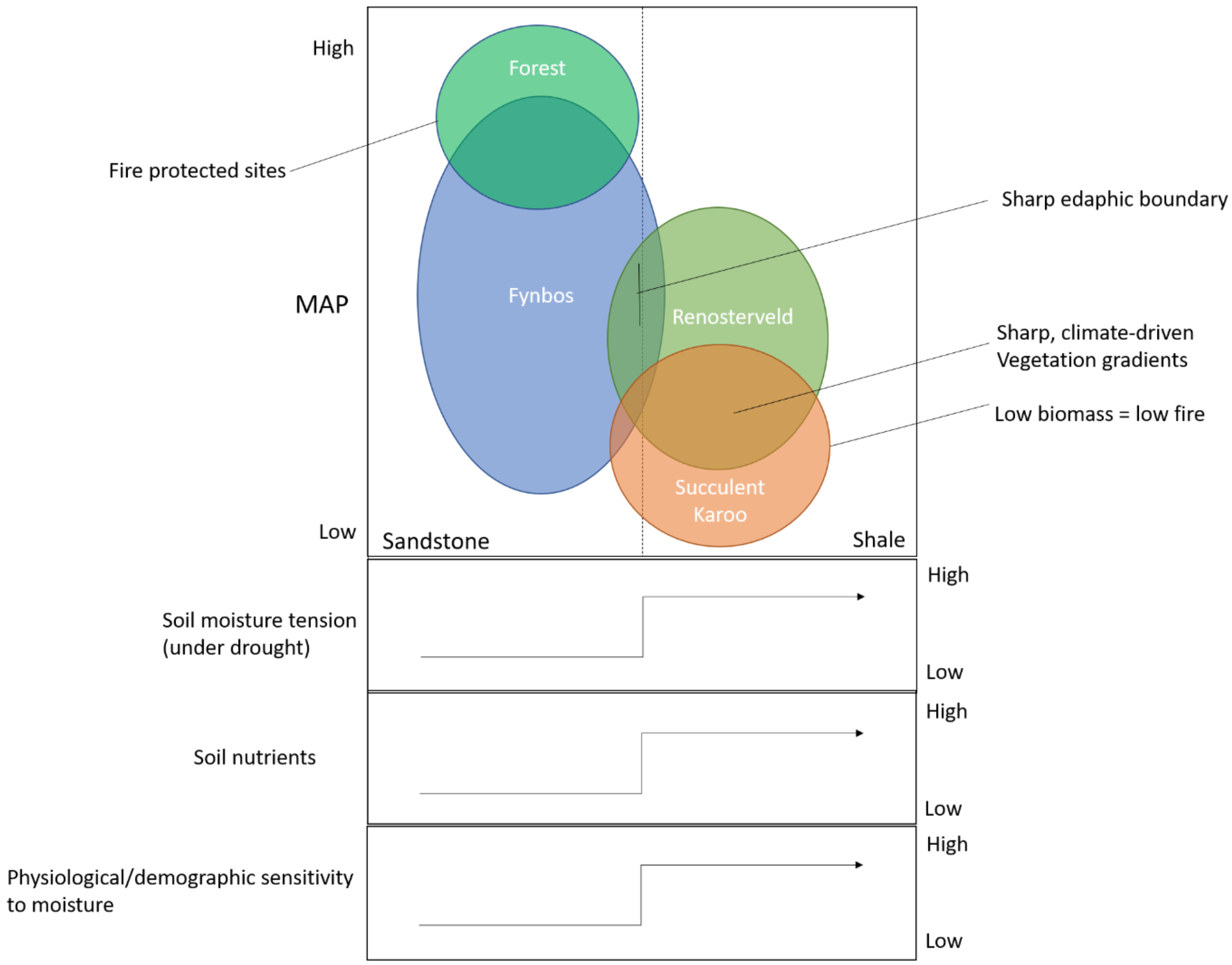

Figure 6.2: Conceptual diagram showing the potential for biomes to occupy specific rainfall and soil niches. Biomes are placed along a vertical axis of mean annual precipitation (MAP), from high (± 3000 mm) to low ((± 0 mm) and separated by soil type on the horizontal axis (sandstone vs shale). Key observed differences in soil tension, nutrients (Chapter 2) and plant performance (Chapter 3,4,5) across soils are indicated by means of black arrows.

My study demonstrated that a fine-scale, mechanistic understanding of vegetation-climate interactions is useful for understanding vegetation patterns at a broader scale. Future research should continue to experimentally test the sensitivity of vegetation to a broad range of possible climate scenarios.

Appendix:

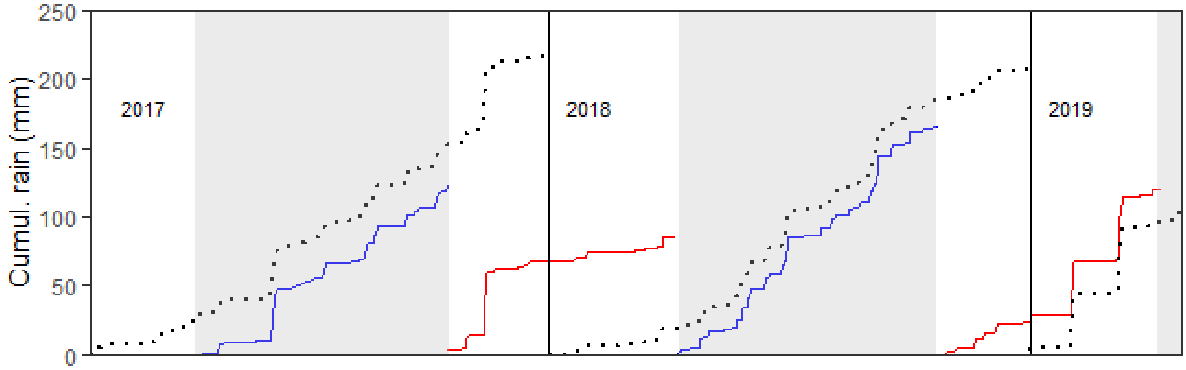

Figure S1: Cumulative yearly rainfall (black dotted line) and cumulative seasonal rainfall at the Drie Kuilen Nature Reserve. Winter = blue lines. Summer = Red lines.

Table S1: Species list recorded at Fynbos and Renosterveld sites during the experiment at Drie Kuilen Nature Reserve. Site preference represents the site which species were more prevalent/dominant while “present at” represents which sites the species were recorded at least once. Each new line represents a unique species.

Family	**Genus**	**Species**	**Site preference**	**Present at**
Agavaceae	*Chlorophytum*	-	Renosterveld	Renosterveld
Aizoaceae	*Galenia*	*africana* (L.)	Renosterveld	Renosterveld
Aizoaceae	*Galenia*	-	Renosterveld	Renosterveld
Aizoaceae	*Ruschia*	-	Renosterveld	Renosterveld
Aizoaceae	*Ruschia*	-	Fynbos	Fynbos
Aizoaceae	*Mesembryanthemum*	-	Fynbos	Fynbos
Asparagaceae	*Lachenalia*	-	Renosterveld	Renosterveld
Asparagaceae	*Asparagus*	-	Fynbos	Fynbos
Asphodelaceae	-	-	Fynbos	Fynbos
Asteraceae	*Elytropappus*	*rhinocerotis* (L.f.)	Renosterveld	Both
Asteraceae	*Othonna*	*paviflora* (P.J.Bergius.)	Renosterveld	Both
Asteraceae	*Metalasia*	*muricate* (D.Don.)	Fynbos	Fynbos
Asteraceae	*Metalasia*	-	Renosterveld	Renosterveld
Asteraceae	*Metalasia*	-	Fynbos	Fynbos
Asteraceae	*Oedera*	*imbricata* (Lam.)	Fynbos	Fynbos
Asteraceae	*Oedera*	*squarosa* (L.)	Renosterveld	Renosterveld
Asteraceae	*Eriocephalus*	*africanus* (L.)	Renosterveld	Renosterveld
Asteraceae	*Anisothrix*	-	Renosterveld	Renosterveld
Asteraceae	*Euryops*	-	Renosterveld	Renosterveld
Asteraceae	*Euryops*	-	Fynbos	Fynbos
Asteraceae	*Helichrysum*	-	Renosterveld	Renosterveld
Asteraceae	*Syncarpha*	-	Fynbos	Fynbos
Asteraceae	-	-	Renosterveld	Renosterveld
Asteraceae	-	-	Fynbos	Fynbos
Asteraceae	*Iridaceae*	-	Fynbos	Fynbos
Borginaceae	*Lobostemon*	*decorus* (Levyns.)	Fynbos	Both
Brassicaceae	*Heliophila*	*cornuta* (Sond.) *var. squamata* (Schltr.)	None	Both
Brassicaceae	*Heliophila*	-	Renosterveld	Renosterveld
Brassicaceae	*Heliophila*	-	Fynbos	Fynbos
Campanulaceae	*Wahlenbergia*	*nodosa* (H.Buek.)	None	Both
Campanulaceae	*Lobelia*	-	Fynbos	Fynbos
Caryophyllaceae	*Dianthus*	-	Renosterveld	Renosterveld
Caryophyllaceae	*Dianthus*	-	Fynbos	Fynbos
Colchicaceae	*Colchicum*	-	Fynbos	Fynbos
Crassulaceae	*Crassula*	*muscosa* (L.)	Rensoterveld	Renosterveld
Cyperaceae	*Isolepsis*	-	Renosterveld	Renosterveld
Cyperaceae	*Isolepsis*	-	Fynbos	Fynbos
Euphorbiaceae	*Clutia*	*rubricaulis* (Eckl.)	None	Both
Euphorbiaceae	*Euphorbia*	*genistoides* (P.J.Bergius.)	Fynbos	Fynbos
Euphorbiaceae	*Euphorbia*	-	Renosterveld	Renosterveld

Fabaceae	*Aspalathus*	*shawii* (L. Bolus.) *subsp. shawii*	Fynbos	Both
Fabaceae	*Aspalathus*	*salteri* (L. Bolus.)	Fynbos	Fynbos
Fabaceae	*Aspalathus*	*spinescens* (Thunb.)	Fynbos	Fynbos
Fabaceae	*Indigofera*	*heterophylla* (Thunb.)	None	Both
Fabaceae	*Rafnia*	*capensis* (L.)	Fynbos	Fynbos
Geraneaceae	*Pelargonium*	*crispum* (Ait.)	Renosterveld	Renosterveld
Geraneaceae	*Pelargonium*	-	Renosterveld	Renosterveld
Geraneaceae	*Pelargonium*	-	Fynbos	Fynbos
Haemoderaceae	-	-	Fynbos	Fynbos
Hyacinthaceae	*Albuca*	*sauveolens* (Jacq.)	None	Both
Iridaceae	*Babiana*	*sambucina* (Jacq.)	Fynbos	Fynbos
Iridaceae	*Moraea*	-	Renosterveld	Renosterveld
Iridaceae	*Moraea*	-	Fynbos	Fynbos
Iridaceae	-	-	Renosterveld	Renosterveld
Iridaceae	*Watsonia*	-	Fynbos	Fynbos
Malvaceae	*Hermannia*	*aspera* (J.C.Wendl.)	None	Both
Malvaceae	*Hermannia*	-	Fynbos	Fynbos
Oxalidaceae	*Oxalis*	-	Renosterveld	Renosterveld
Oxalidaceae	*Oxalis*	-	Fynbos	Fynbos
Poaceae	*Ehrharta*	*villosa* (J.H.Schult.)	None	Both
Poaceae	*Ehrharta*	-	Renosterveld	Renosterveld
Poaceae	-	-	Renosterveld	Renosterveld
Poaceae	-	-	Fynbos	Fynbos
Polygalaceae	*Polygala*	-	Fynbos	Fynbos
Proteaceae	*Protea*	*laurifolia* (Thunb.)	Fynbos	Fynbos
Proteaceae	*Protea*	*repens* (L.)	Fynbos	Fynbos
Restionaceae	*Restio*	-	Fynbos	Fynbos
Restionaceae	-	-	Fynbos	Fynbos
Rhamnaceae	*Phylica*	*lanata* (Pillans.)	Fynbos	Fynbos
Rhamnaceae	*Phylica*	*stipularis* (L.)	Renosterveld	Renosterveld
Rhamnaceae	*Phylica*	-	Renosterveld	Renosterveld
Rhamnaceae	*Phylica*	-	Fynbos	Fynbos
Rosaceae	*Cliffortia*	*ruscifolia* (L.)	Fynbos	Fynbos
Rubiaceae	*Anthospermum*	*galiodes* (Rchb.f.)	None	Both
Rutaceae	*Agathosma*	*capensis* (L.)	Fynbos	Fynbos
Santalaceae	*Thesium*	*strictum* (P.J.Bergius.)	None	Both
Scrophalariaceae	*Selago*	*corymbose* (L.)	Fynbos	Fynbos
Scrophalariaceae	*Selago*	-	Renosterveld	Renosterveld
Scrophalariaceae	*Microdon*	*polygaloides* (L.f)	Renosterveld	Renosterveld
Scrophalariaceae	*Chaenostoma*	*comptonii* (Hilliard)	Renosterveld	Renosterveld
Thymelaeaceae	*Passerina*	*obtusifolua* (Thoday.)	Renosterveld	Both
Zygophyllaceae	*Roepera*	*fulva* (L.)	None	Both
Zygophyllaceae	-	-	Fynbos	Fynbos

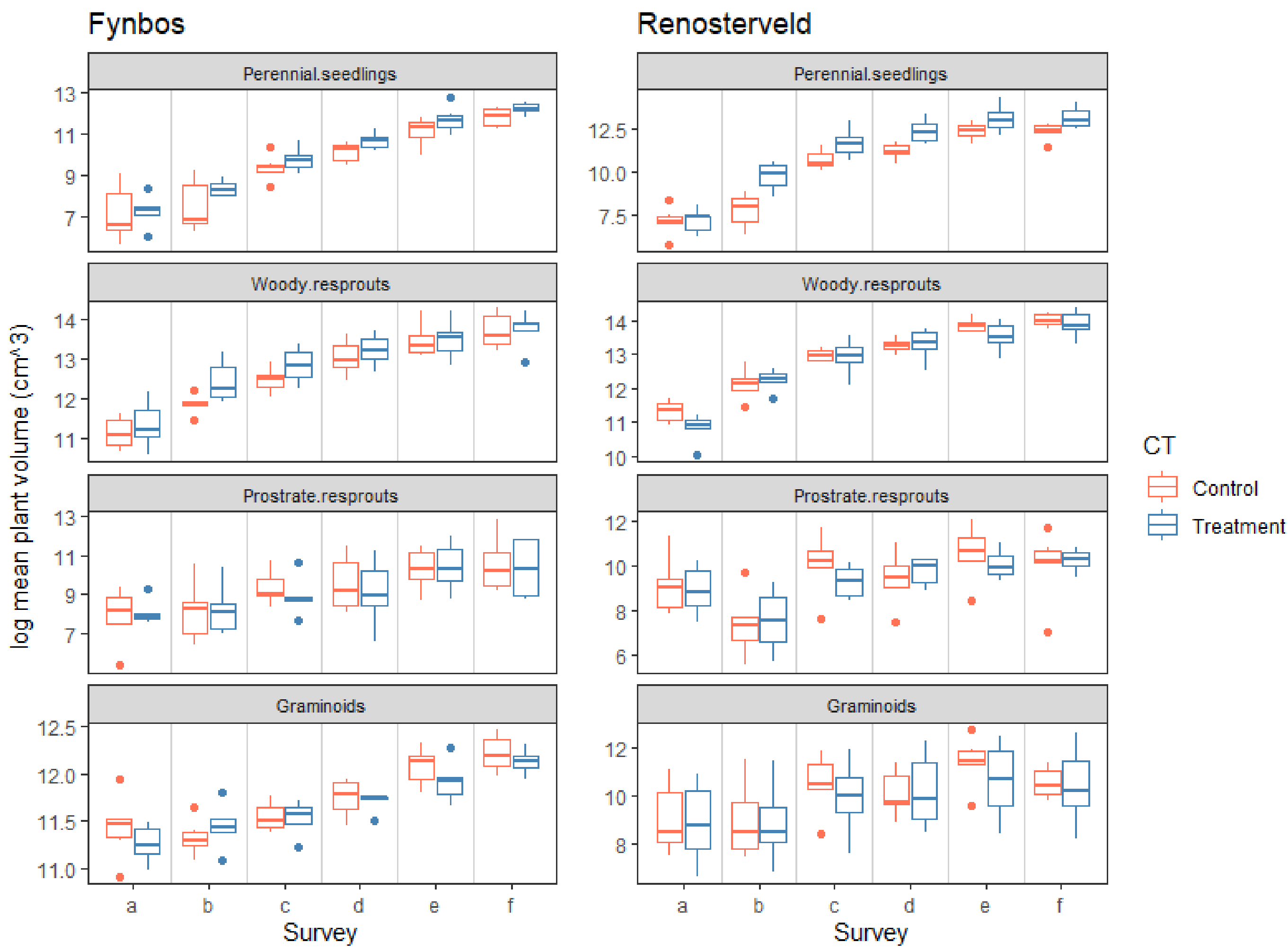

Figure S2: Log growth form volume per plot over surveys (a-f) in control and treatment plots in Renosterveld and Fynbos sites.

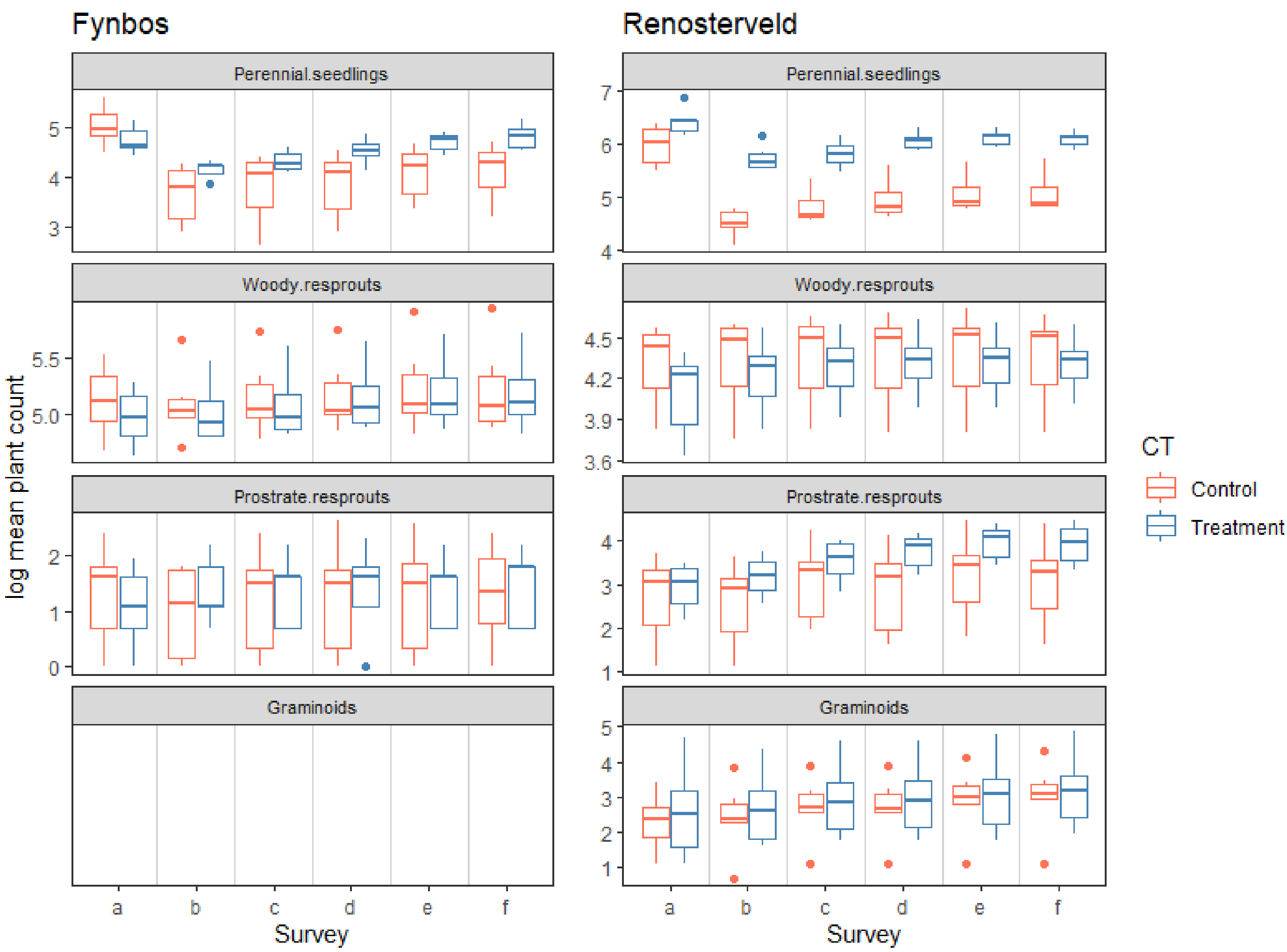

Figure S3: Log growth form count per plot over surveys (a-f) in control and treatment plots in Renosterveld and Fynbos sites.

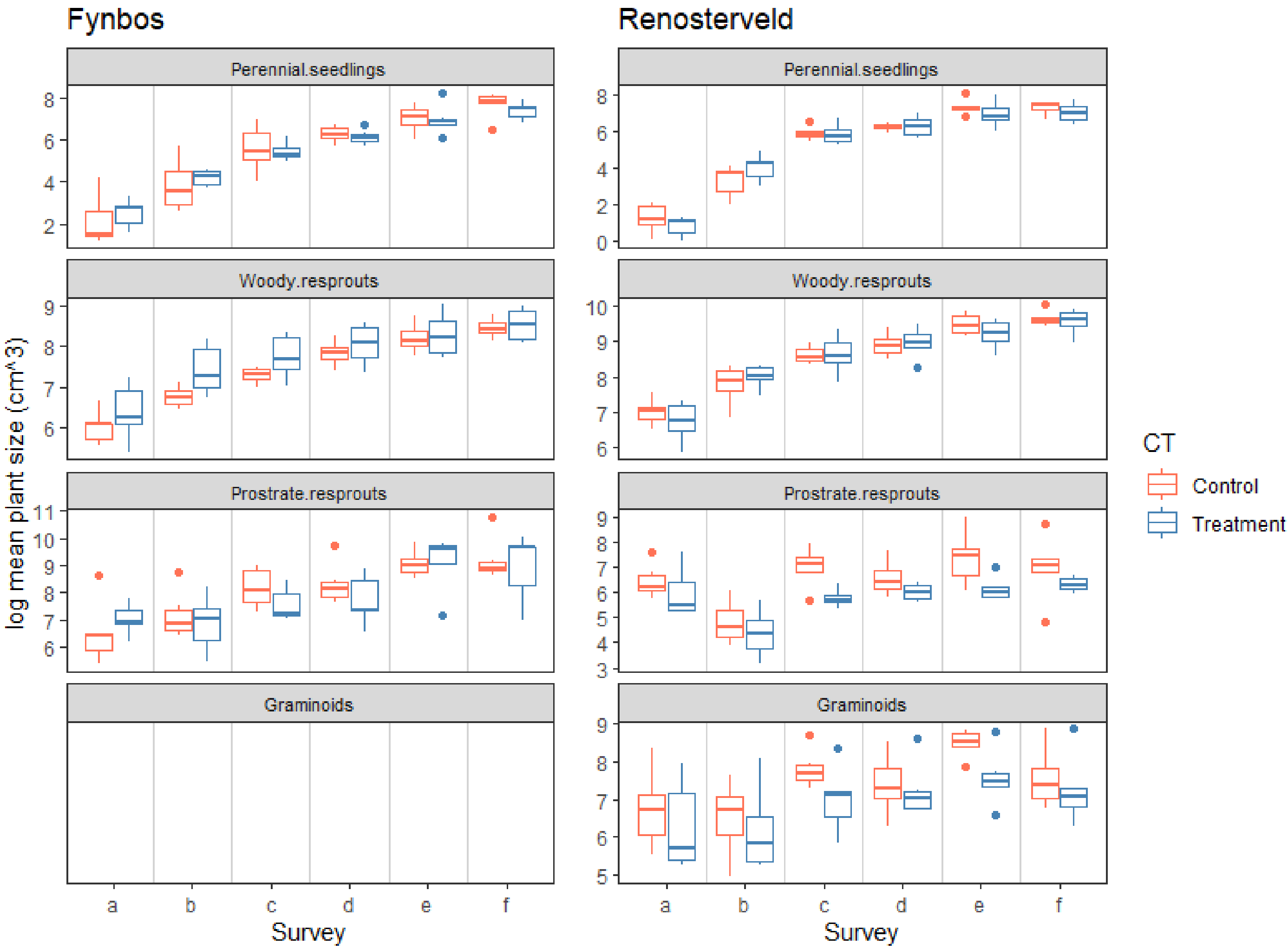

Figure S4: Log mean growth form size per plot over surveys (a-f) in control and treatment plots in Renosterveld and Fynbos sites.

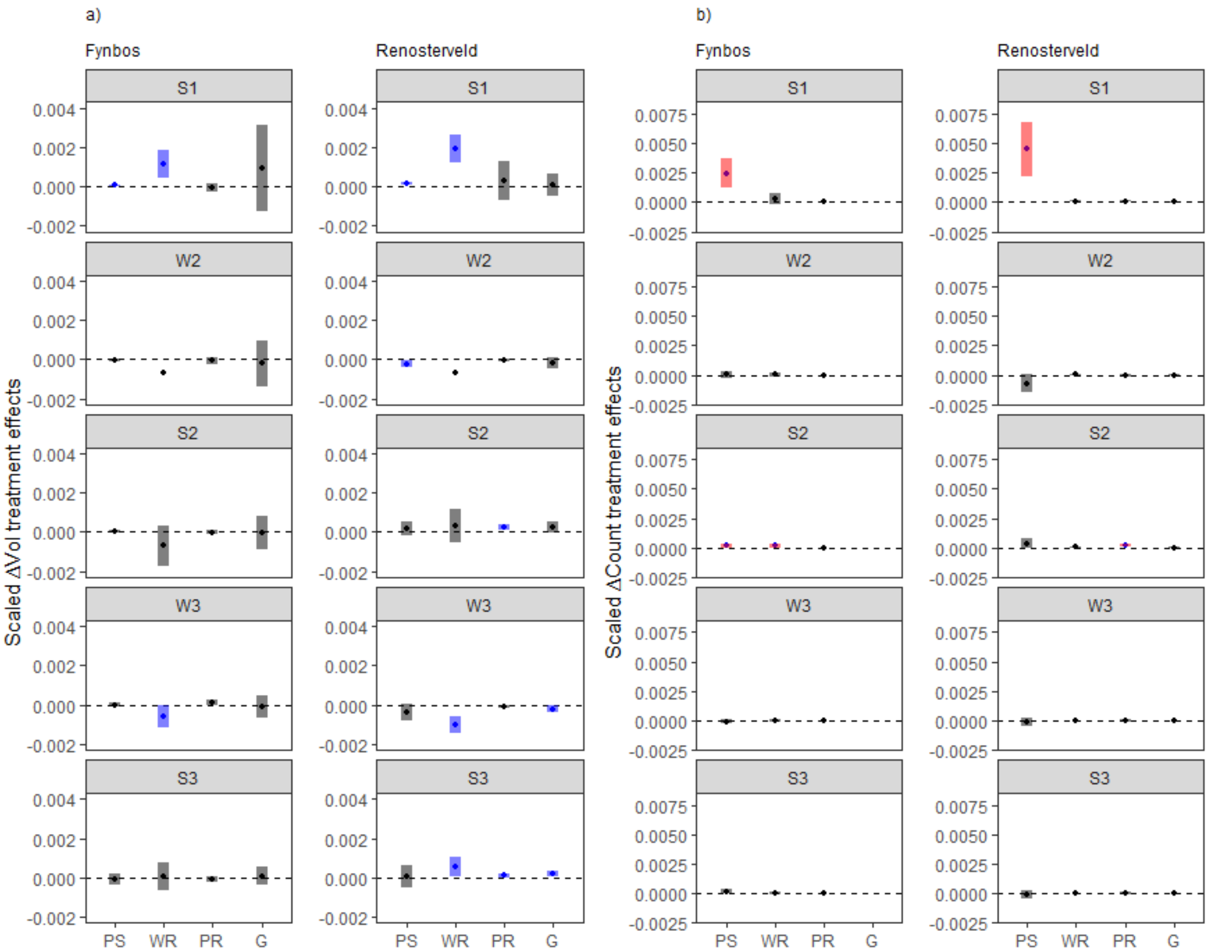

Figure: S5: Scaled growth form ΔVol and ΔCount treatment responses in Fynbos and Renosterveld sites. Adapted from Figure 4.6 & 4,7.

Table S2: a) Initial growth-form counts at the baseline survey (a). **b)** Initial growth-form mean sizes at the baseline survey (a). **c)** Initial growth-form accumulated volumes at the baseline survey (a). Means across plots are displayed with standard errors in brackets. P-values show the statistical significance from multiple two-sided t-tests comparing control and treatment means.

a)	Growth Form	Control count (±SE)	Treatment count (±SE)	P value
Fynbos	Perennial.seedlings	165.0 (±27.5)	119.7 (±14.2)	0.183
	Woody.resprouts	175.0 (±22.3)	148.2 (±14.7)	0.343
	Prostrate.resprouts	4.2 (±1.7)	3.0 (±1.1)	0.570
	Geophytes.Bulbs	14.7 (±3.6)	16.8 (±6.4)	0.775
	Annuals.Biennials.Herbs	5.0 (±1.0)	4.0 (±1.4)	0.578
	Root.parasites	0.2 (±0.2)	0 (±0)	0.363
Renosterveld	Perennial.seedlings	413.3 (±59.8)	628.0 (±73.0)	0.047
	Woody.resprouts	76.8 (±8.4)	61.8 (±7.1)	0.202
	Prostrate.resprouts	20.3 (±5.9)	21.3 (±4.2)	0.893
	Geophytes.Bulbs	0.5 (±0.5)	0.3 (±0.5)	0.788
	Annuals.Biennials.Herbs	2.8 (±1.1)	8.5 (±2.3)	0.060
	Root.parasites	6.0 (±2.2)	9.7 (±3.0)	0.353
b)	Growth Form	Control mean size (cm^3) (±SE)	Treatment mean size (cm^3) (±SE)	P value
Fynbos	Perennial.seedlings	16.9 (±10.1)	14.7 (±3.4)	0.841
	Woody.resprouts	433.2 (±76.5)	704.6 (±193.5)	0.236
	Prostrate.resprouts	1514.1 (±1048.8)	1296.7 (±325.0)	0.851
	Geophytes.Bulbs	411.9 (±149.1)	1199.4 (±1063.3)	0.495
	Annuals.Biennials.Herbs	95.1 (±37.2)	1526.6 (±989.0)	0.257
	Root.parasites	1331 (-)	-	-
Renosterveld	Perennial.seedlings	4.3 (±1.2)	2.5 (±0.4)	0.191
	Woody.resprouts	1160.7 (±174.7)	945.0 (±186.9)	0.419
	Prostrate.resprouts	781.4 (±265.5)	623.3 (±300.5)	0.702
	Geophytes.Bulbs	2412 (-)	770 (-)	-
	Annuals.Biennials.Herbs	70.8 (±36.5)	614.8 (±351.5)	0.197
	Root.parasites	5297.9 (±2057.6)	5086.9 (±1695.6)	0.939
c)	Growth Form	Control volume (cm^3) (SD)	Treatment volume (cm^3) (SD)	P value
Fynbos	Perennial.seedlings	2715.8 (±1400.6)	1800.5 (±531.5)	0.562
	Woody.resprouts	72481.2 (±11971.2)	95865.2 (±22847.1)	0.393
	Prostrate.resprouts	4908.0 (±2012.0)	4208.2 (±1735.7)	0.799
	Geophytes.Bulbs	7000.7 (±2694.6)	20404.7 (±16862.0)	0.466
	Annuals.Biennials.Herbs	460.7 (±170.8)	6606.8 (±3945.1)	0.228
	Root.parasites	1311 (-)	-	-
	Graminoids	97166.7 (±13295.2)	79000 (±6153.6)	0.255
Renosterveld	Perennial.seedlings	1763.2 (±607.4)	1600.2 (±391.1)	0.827
	Woody.resprouters	85081.8 (±10852.5)	53319.8 (±7119.8)	0.038
	Prostrate.resprouters	20028.7 (±12869.8)	11794.5 (±4597.527)	0.568
	Geophytes.Bulbs	7236 (-)	-	-
	Annuals.Biennials.Herbs	346.2 (±201.6)	7708.6 (±4961.3)	0.212
	Root.parasites	49410 (±30045.2)	44678.2 (±14091.0)	0.891
	Graminoids	19727.3 (±10641.2)	17774.8 (±9000.7)	0.891

Table S3: Mixed effects model results showing the individual and interaction effects of ”Season”, “Site”, “Control/Treatment” and “Growth form” on leaf relative water content (RWC).

Response: Leaf RWC	**numDF**	**denDF**	**F-value**	**p-value**
(Intercept)	1	656	21967.79	<0.001
Site (Fynbos, Renosterveld)	1	20	39.65	<0.001
Survey (Feb2017, … , Mar2019)	6	656	19.59	<0.001
CT (Control, Treatment)	1	20	70.60	<0.001
Growth form (Seedling, resprout)	1	20	2.86	0.106
Site:Survey	6	656	3.57	0.002
Site:CT	1	20	2.45	0.133
Survey:CT	6	656	17.79	<0.001
Site: Growth form	1	20	37.56	<0.001
Survey: Growth form	6	656	5.95	<0.001
CT: Growth form	1	20	0.94	0.343
Site:Survey:CT	6	656	2.03	0.060
Site:Survey: Growth form	6	656	5.046	<0.001
Site:CT: Growth form	1	20	1.292	0.269
Survey:CT: Growth form	6	656	0.493	0.814
Site:Survey:CT: Growth form	6	656	0.495	0.813

Table S4: Mixed effects model results showing the individual and interaction effects of ”Season”, “Site”, “Control/Treatment” and “Growth form” on dark adapted maximum quantum yield (Fv/Fm).

Response: Leaf Fv/Fm	**numDF**	**denDF**	**F-value**	**p-value**
(Intercept)	1	646	24988.59	<0.001
Site (Fynbos, Renosterveld)	1	20	8.52	0.009
Survey (Feb2017, … , Mar2019)	5	646	12.43	<0.001
CT (Control, Treatment)	1	20	149.74	<0.001
Growth form (Seedling, resprout)	1	20	43.94	<0.001
Site:Survey	5	646	6.43	<0.001
Site:CT	1	20	3.63	0.071
Survey:CT	5	646	22.43	<0.001
Site: Growth form	1	20	13.66	0.001
Survey: Growth form	5	646	8.29	<0.001
CT: Growth form	1	20	35.09	<0.001
Site:Survey:CT	5	646	1.073	0.374
Site:Survey: Growth form	5	646	4.29	<0.001
Site:CT: Growth form	1	20	9.84	0.005
Survey:CT: Growth form	5	646	7.60	<0.001
Site:Survey:CT: Growth form	5	646	1.85	0.102

Table S5: Mixed effects model results showing the individual and interaction effects of ”Season”, “Site” and “Control/Treatment” and “Growth form” on stomatal conductance (g).

Response: Leaf g	**numDF**	**denDF**	**F-value**	**p-value**
(Intercept)	1	260	635.55	<0.001
Site (Fynbos, Renosterveld)	1	20	51.82	<0.001
Survey (Feb2017, … , Mar2019)	2	260	16.17	<0.001
CT (Control, Treatment)	1	20	36.18	<0.001
Growth form (Seedling, resprout)	1	20	0.094	0.762
Site:Survey	2	260	1.52	0.220
Site:CT	1	20	1.85	0.189
Survey:CT	2	260	33.15	<0.001
Site: Growth form	1	20	15.07	<0.001
Survey: Growth form	2	260	2.14	0.119
CT: Growth form	1	20	4.55	0.046
Site:Survey:CT	2	260	0.51	0.602
Site:Survey: Growth form	2	260	0.10	0.906
Site:CT: Growth form	1	20	5.35	0.032
Survey:CT: Growth form	2	260	1.38	0.252
Site:Survey:CT: Growth form	2	260	1.61	0.202

Table S6: Mixed effects model results showing the individual and interaction effects of ”Season”, “Site” and “Control/Treatment” and “Growth form” on photosynthetic carbon assimilation rates (A).

Response: Leaf A	**numDF**	**denDF**	**F-value**	**p-value**
(Intercept)	1	260	1187.97	<0.001
Site (Fynbos, Renosterveld)	1	20	17.54	<0.001
Survey (Feb2017, … , Mar2019)	2	260	27.39	<0.001
CT (Control, Treatment)	1	20	62.35	<0.001
Growth form (Seedling, resprout)	1	20	7.77	0.011
Site:Survey	2	260	1.25	0.287
Site:CT	1	20	4.55	0.046
Survey:CT	2	260	33.68	<0.001
Site: Growth form	1	20	19.41	<0.001
Survey: Growth form	2	260	1.82	0.165
CT: Growth form	1	20	1.17	0.292
Site:Survey:CT	2	260	1.51	0.222
Site:Survey: Growth form	2	260	0.11	0.893
Site:CT: Growth form	1	20	2.91	0.104
Survey:CT: Growth form	2	260	1.37	0.257
Site:Survey:CT: Growth form	2	260	0.66	0.520

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