Photosynthetic and Evolutionary Determinants of the Response of Selected and (NADP-ME) Grasses to Fire

A thesis submitted in fulfillment of the
requirements for the degree of

MASTER OF SCIENCE

of

RHODES UNIVERSITY

by

TARRYN MARTIN

May 2009

ABSTRACT

species possess characteristics that are considered adapted to burning and these allow them to outcompete species and dominate in fire prone environments. It has therefore been proposed that fire might have played a critical role in the observed expansion of the grasslands, during the late Miocene. The aim of this study was (i) to investigate whether plant response to fire was a result of physiology or (ii) whether it was due to phylogenetic history. This was achieved by doing a pair-wise comparison between Panicoideae (and Panicoideae) and non-Panicoideae (Danthonioideae and Aristidoideae) species. Pre-fire characteristics, that would enhance fire frequency and assist with plant recovery after burning, were compared across phylogenies and photosynthetic type. Post fire plant recovery was then followed in a field and pot comparison which examined the re-growth of the leaf canopy area, leaf mass, above-ground biomass and the cost of this to the below-ground biomass.

The pre-fire characteristics showed both a photosynthetic and phylogenetic response. It was found that the species showed a greater canopy death during winter and had a lower moisture content than the species. These characteristics would potentially contribute towards a larger fuel load in the species. However, the comparison of the dead standing biomass at the end of winter and the below-ground biomass, showed a phylogenetic response with the Panicoideae having a proportionally larger dead standing biomass and below-ground biomass than the non-Panicoideae. These results suggest that not only did the Panicoideae have a larger potential fuel load but that they also shunted carbon below-ground, enabling a fast recovery after being burned.

The post-fire results were more strongly determined by phylogeny than by photosynthetic type. The Panicoideae recovered faster and more completely than the non-Panicoideae grasses, possibly contributing to their success and expansion under conditions of increased fire frequency. Although recovery of the and Panicoideae were similar, frequently burnt grasslands are dominated by the Panicoideae. Hence, this dominance cannot be explained by differences in their fire responses and may be determined by the post-fire environmental conditions that potentially advantage species possessing the photosynthetic pathway.

Panicoideae dominance is limited to mesic environments where fire is the likely driver of grassland expansion while more arid environments are dominated by non-Panicoideae species. Representative species from these non-Panicoid subfamilies showed poor recovery after fire. This suggests that factors other than fire were the likely drivers of these xeric grassland expansions. The ability of these subfamilies, and particularly the species, to cope with drought remains a likely selective mechanism that requires further research.

ACKNOWLEDGEMENTS

- My profound thanks to my supervisor, Brad Ripley, whose advice, guidance, patience, constant enthusiasm and encouragement made this thesis possible.

- To Trevor Abraham and Leigh-Ann de Wet for all their help collecting, growing, burning and separating hundreds of plants. I could not have completed this project without them.

- To Colin Osborne and Samuel Taylor for their patient help and suggestions with the statistical analysis of data.

- To Rob Freckleton and Sarah Radloff for their statistical advice.

- To Michelle Featherstone, Nancy Klaas, Cordelia Leggitt, Kathryn Martin and Scott Martin for the hours they spent helping me carefully separate out plants into their different components.

- To Joe for all his help weeding and watering over 700 potted plants each week.

- To my mother, Carol Martin, for her support and encouragement to return to University to do my Masters degree.

- To the NRF for funding

CONTENTS

LIST OF FIGURES

CHAPTER 1

Figure 1: Relationship between annual precipitation and the percentage of grass species that use the NADP-ME, NAD-ME and PCK biochemical pathways (a) and the relationship between annual rainfall and the percentage of species in the Chloridoideae and Panicoideae subfamilies (b) (Redrawn from Cabido *et al.*, 2007).

CHAPTER 2

Figure 2.1: The new PACCMAD phylogeny showing sub-family relationships. Open circles represent weak support, half shaded circles moderate support and closed circles, strong support. The diagram is based on Bouchenak-Khelladi *et al.,* 2008*.*

Figure 2.2: Mean daily outside and polytunnel temperatures during the pre- and post-fire growth of the grass species▲)(represents the four harvest dates.

Figure: 2.3: The positioning of the plants within the grid prior to burning. One of each species was placed in the centre of each block and soil placed around the plants to prevent the roots and corm from being damaged during burning.

CHAPTER 3

Figure 3.1.1: Average percentage below-ground biomass grouped by photosynthetic type (a) and phylogenetic group (b). (n = 6-7. Vertical bars represent standard errors). Different letters indicate significant differences between means at $P < 0.05$ (Tukey HSD test).

Figure 3.1.2: The percentage below-ground biomass of pot-cultivated and grasses belonging to the indicated subfamilies. (*a*) Responses averaged across species of a particular photosynthetic type within each subfamily. (b) Individual species responses. Abbreviations*: Pa = P. aequinerve, Pe = P. ecklonii, As = A. semialata subsp. eckloniana, Kc = K. curva, Md = M. disticha, Pp = P. pallida, Pc = P. curvifolia, Hc = H. contortus, Hh = H. hirta, Tt = T. triandra, As = A. semialata subsp. semialata, Ac = A. congesta, Aj = A. junciformis, Ad = A. diffusa.* (n = 3-4 for subfamily and n= 5-6 per individual species. Vertical bars represent standard errors). Different letters indicate significant differences between means at P < 0.05 (Tukey HSD test).

Figure 3.2.1: Average percentage of dead leaves grouped by photosynthetic type (a) and phylogenetic group (b). Abbreviations: n.s. = not significant. (n = 6-7. Vertical bars represent standard errors). Different letters indicate significant differences between means at P < 0.05 (Tukey HSD test).

Figure 3.2.2: The percentage of dead leaves of pot-cultivated and grasses at different time intervals between May and July 2008. (*a*) Response averaged across species of a particular photosynthetic type within each subfamily. (b) Individual species responses. Abbreviations*: Pa = P. aequinerve, Pe = P. ecklonii, As = A. semialata subsp. eckloniana, Kc = K. curva, Md = M. disticha, Pp = P. pallida, Pc = P. curvifolia, Hc = H. contortus, Hh = H. hirta, Tt = T. triandra, As = A. semialata subsp. semialata, Ac = A. congesta, Aj = A. junciformis, Ad = A. diffusa.* (n = 3-4 for subfamily and n= 6 per individual species. Vertical bars represent standard errors). Different letters indicate significant differences between means at P < 0.05 (Tukey HSD test).

Figure 3.3.1: Average percentage of dead leaves grouped by photosynthetic type (a) and phylogenetic group (b). Abbreviations: n.s. = not significant. (n = 6-7. Vertical bars represent standard errors). Different

letters indicate significant differences between means at P < 0.05 (Tukey HSD test).

Figure 3.3.2: The potential fuel load of pot-cultivated and grasses belonging to the indicated subfamilies. (*a*) Responses averaged across species of a particular photosynthetic type within each subfamily. (b) Individual species responses. Abbreviations*: Pa = P. aequinerve, Pe = P. ecklonii, As = A. semialata subsp. eckloniana, Kc = K. curva, Md = M. disticha, Pp = P. pallida, Pc = P. curvifolia, Hc = H. contortus, Hh = H. hirta, Tt = T. triandra, As = A. semialata subsp. semialata, Ac = A. congesta, Aj = A. junciformis, Ad = A. diffusa.* (n = 3-4 for subfamily and n= 5-6 per individual species. Vertical bars represent standard errors). Different letters indicate significant differences between means at P < 0.05 (Tukey HSD test).

Figure 3.4.1: Average moisture content of green leaves grouped by photosynthetic type (a) and phylogenetic group (b). Abbreviations: n.s. = not significant. (n = 6-7. Vertical bars represent standard errors). Different letters indicate significant differences between means at P < 0.05 (Tukey HSD test).

Figure 3.4.2: The moisture content of the green leaves of pot-cultivated and grasses belonging to the indicated subfamilies. (*a*) Response averaged across species of a particular photosynthetic type within each subfamily. (b) Individual species responses. Abbreviations*: Pa = P. aequinerve, Pe = P. ecklonii, As = A. semialata subsp. eckloniana, Kc = K. curva, Md = M. disticha, Pp = P. pallida, Pc = P. curvifolia, Hc = H. contortus, Hh = H. hirta, Tt = T. triandra, As = A. semialata subsp. semialata, Ac = A. congesta, Aj = A. junciformis, Ad = A. diffusa.* (n = 3-4 for subfamily and n= 5-6 per individual species. Vertical bars represent standard errors). Different letters indicate significant differences between means at P < 0.05 (Tukey HSD test).

CHAPTER 4

differences between treatments for each subfamily and between means of the control and burnt plants for individual species at P < 0.05 (Tukey HSD test).

Figure 4.2.2.1: Average leaf mass of control and burnt plants grouped by Phylogenetic group and photosynthetic type. The above-ground biomass was completely removed by the fire at 0 and statistical comparisons were made on data from plants harvested at weeks 3, 6 and 9. (n=6-7 and vertical bars represent standard errors). * indicates significant differences between treatments at P < 0.05 (Tukey HSD test).

Figure 4.2.2.2: Leaf mass of pot-cultivated control and burnt and grasses belonging to the indicated subfamilies. The above-ground biomass of the burnt treatment was completely removed by the fire at time 0 and statistical comparisons were made on data from plants harvested at weeks 3, 6 and 9. Panels a, b c and d indicate the responses average across species of a particular photosynthetic type, within each subfamily. Individual species responses are indicated as *Pa = P. aequinerve, Pe = P. ecklonii, As = A. semialata* subsp. *eckloniana*, *Kc = K. curva, Md = M. disticha, Pp = P. pallida, Pc = P. curvifolia, Hc = H. contortus, Hh = H. hirta, Tt = T. triandra, As = A. semialata* subsp. *semialata, Ac = A. congesta, Aj = A. junciformis, Ad = A. diffusa.* (n = 3-4 for subfamily, n= 5-6 per individual species and vertical bars represent standard errors). * indicates significant differences between means of the control and burnt plants at P < 0.05 (Tukey HSD test).

Figure 4.2.3.1: Average specific leaf area (SLA) of control and burnt plants grouped by phylogenetic group and photosynthetic type. The above-ground biomass was completely removed by the fire at 0 and statistical comparisons were made on data from plants harvested at weeks 3, 6 and 9. (n=6-7 and vertical bars represent standard errors). * indicates

CHAPTER 5

Figure 5.1.1: The contribution of photosynthesis (B-D), reallocation (D), fire stimulus (B-C) and fire inhibition (C-B) to the growth of the canopy area grouped by phylogenetic group and photosynthetic type, over three successive harvests. Abbreviations: Control Plants (C), burnt plants (B) and dark adapted plants (D). * indicates whether the subtraction one treatment from the other was valid based on whether differences between means of the treatments were significant P < 0.05 (Tukey HSD test).

Figure 5.1.2: The contribution of photosynthesis (B-D), reallocation (D), fire stimulus (B-C) and fire inhibition (C-B) to the growth of the canopy area of and species grouped according to subfamily. Panels a, b c and d indicate the responses average across species of a particular photosynthetic type, within each subfamily. Individual species responses are indicated as *Pa = P. aequinerve, Pe = P. ecklonii, As = A. semialata* subsp. *eckloniana*, *Kc = K. curva, Md = M. disticha, Pp = P. pallida, Pc = P. curvifolia, Hc = H. contortus, Hh = H. hirta, Tt = T. triandra, As = A. semialata* subsp. *semialata, Ac = A. congesta, Aj = A. junciformis, Ad = A. diffusa.* Abbreviations: Control Plants (C), burnt plants (B) and dark adapted (D). (n= 5-6 per individual species). * indicates whether the subtraction one treatment from the other was valid based on whether differences between means of the treatments were significant P < 0.05 (Tukey HSD test).

Figure 5.2.1: The contribution of photosynthesis (B-D), reallocation (D), fire stimulus (B-C) and fire inhibition (C-B) to the growth of the leaf mass grouped by phylogenetic group and photosynthetic type, over three successive harvests. Abbreviations: Control Plants (C), burnt plants (B) and dark adapted plants (D). * indicates whether the subtraction one treatment from the other was valid based on whether differences between means of the treatments were significant P < 0.05 (Tukey HSD test).

Figure 5.2.2: The contribution of photosynthesis (B-D), reallocation (D), fire stimulus (B-C) and fire inhibition (C-B) to the growth of the leaf mass of and species grouped according to subfamily. Panels a, b c and d indicate the responses average across species of a particular photosynthetic type, within each subfamily. Individual species responses are indicated as *Pa = P. aequinerve, Pe = P. ecklonii, As = A. semialata* subsp. *ecklonian*a, *Kc = K. curva, Md = M. disticha, Pp = P. pallida, Pc = P. curvifolia, Hc = H. contortus, Hh = H. hirta, Tt = T. triandra, As = A. semialata* subsp. *semialata, Ac = A. congesta, Aj = A. junciformis, Ad = A. diffusa.* Abbreviations: Control Plants (C), burnt plants (B) and dark adapted (D). (n= 5-6 per individual species). * indicates whether the subtraction one treatment from the other was valid based on whether differences between means of the treatments were significant P < 0.05 (Tukey HSD test).

Figure 5.3.1: The contribution of photosynthesis (B-D), reallocation (D), fire stimulus (B-C) and fire inhibition (C-B) to the growth of the above-ground biomass grouped by phylogenetic group and photosynthetic type, over three successive harvests. Abbreviations: Control Plants (C), burnt plants (B) and dark adapted plants (D). * indicates whether the subtraction one treatment from the other was valid based on whether differences between means of the treatments were significant P < 0.05 (Tukey HSD test).

Figure 5.3.2: The contribution of photosynthesis (B-D), reallocation (D), fire stimulus (B-C) and fire inhibition (C-B) to the growth of the above-ground biomass of and species grouped according to subfamily. Panels a, b c and d indicate the responses average across species of a particular photosynthetic type, within each subfamily. Individual species responses are indicated as *Pa = P. aequinerve, Pe = P. ecklonii, As = A. semialata* subsp. *eckloniana*, *Kc = K. curva, Md = M. disticha, Pp = P. pallida, Pc = P. curvifolia, Hc = H. contortus, Hh = H. hirta, Tt = T. triandra, As = A. semialata* subsp. *semialata, Ac = A. congesta, Aj = A. junciformis, Ad = A. diffusa.* Abbreviations: Control Plants (C), burnt plants (B) and dark adapted (D). (n= 5-6 per individual species). * indicates whether the subtraction one treatment from the other was valid based on whether differences between means of the treatments were significant P < 0.05 (Tukey HSD test).

Figure 5.4.1: The contribution of reallocation (B-D), inhibited recharge (B-D)-(C-D) and stimulated recharge (C-D)-(B-D) from the below-ground biomass grouped by phylogenetic group and photosynthetic type, over three successive harvests. Abbreviations: Control Plants (C), burnt plants (B) and dark adapted plants (D). * indicates whether the subtraction one treatment from the other was valid based on whether differences between means of the treatments were significant P < 0.05 (Tukey HSD test).

Figure 5.4.2: The contribution of reallocation (B-D), inhibited recharge (B-D)-(C-D) and stimulated recharge (C-D)-(B-D) from the below-ground biomass of and species grouped according to subfamily. Panels a, b c and d indicate the responses average across species of a particular photosynthetic type, within each subfamily. Individual species responses are indicated as *Pa = P. aequinerve, Pe = P. ecklonii, As = A. semialata* subsp. *eckloniana*, *Kc = K. curva, Md = M. disticha, Pp = P. pallida, Pc = P. curvifolia, Hc = H. contortus, Hh = H. hirta, Tt = T. triandra, As = A. semialata* subsp. *semialata, Ac = A. congesta, Aj = A. junciformis, Ad = A. diffusa.* Abbreviations: Control Plants (C), burnt

plants (B) and dark adapted (D). (n= 5-6 per individual species* indicates whether the subtraction one treatment from the other was valid based on whether differences between means of the treatments were significant P < 0.05 (Tukey HSD test).

List of Tables

CHAPTER 1

CHAPTER 3

CHAPTER 4

CHAPTER 5

CHAPTER 1: INTRODUCTION

1.1. OVERVIEW

The majority of land plants utilise a photosynthetic mechanism termed photosynthesis, named for its primary products of the carboxylation reaction that contain three carbons. However, some species have evolved a more efficient, alternative photosynthetic mechanism that produces four-carbon products and is termed photosynthesis. The pathway has evolved independently at least 45 times in 19 families of angiosperms (three monocot families and 16 dicot families) (Ehleringer *et al.*, 1991; Sage, 2004; Sage *et al.,* 1999a) spanning the last 30 million years since the Oligocene (Osborne and Beerling, 2006; Roalson, 2008). Within each family there are also multiple, independent origins with grasses having 18 independent lineages (Ehleringer *et al.,* 1991; Roalson, 2008). This fascinating example of convergent evolution has stimulated much research into factors that may have driven these evolutionary events. The hypothesis has been widely used to explain the evolution of the pathway (reviewed in Sage, 2004) however recent studies show that other factors such as heat, drought, salinity, disturbance, competition and seasonal rainfall patterns, or a combination of these, may have also played a role in the selection for photosynthesis (Roalson, 2008).

Equally fascinating is that grasses showed a global expansion during the late Miocene, twenty five million years after they were thought to have first evolved (Osborne and Beerling, 2006). This raises important questions about what drove this expansion. It is well known that the mechanism confers potentially greater efficiencies of light, nitrogen and water use on species relative to species, conditions associated with recently burnt environments and that and grasses occur in disturbance (fire) prone environments with grasses frequently being dominant. It has therefore recently been proposed that fire might have played a critical role in the observed expansion of the grasslands (Keeley and Rundel, 2003). The aim of this study was to therefore investigate whether species were more adequately adapted to fire driven ecosystems by using a phylogenetically controlled experiment that would account for differences in evolutionary history.

Before proceeding to consider what factors would be of advantage to plants and grassland expansion, it is necessary to review the mechanism, its variants (subtypes) and the particular characteristics they confer.

1.2. CHARACTERISTICS

1.2.1 The and Biochemical pathways

plants use the Photosynthetic Carbon Reduction (PCR) or Calvin-Benson cycle, whereby atmospheric carbon dioxide () and Ribulose-1,5-bisphosphate (RuBP) are catalysed by the enzyme ribulose-1.5-bisphosphate carboxylase oxygenase (Rubisco) to form two 3C phosphoglycerate molecules (PGA) (Calvin and Benson, 1948). PGA is further metabolised to form triose phosphate (TP) which is the starting point for the synthesis of sugars and starch.

The pathway differs from the pathway in that there is a spatial separation between the pathway and the PCR cycle (Hatch and Slack, 1966). In this process, the atmospheric is bound to phosphoenolpyruvate (PEP) in a reaction catalysed by the enzyme phosphoenol pyruvate carboxylase (PEPcase) to form the four carbon compound, oxaloacetic acid. This all occurs in the mesophyll cells in the absence of Rubisco. Oxaloacetic acid is then reduced to malate or converted to aspartate (depending on the subtype) and moved from the mesophyll cells (outer compartment) to the bundle sheath cells (inner compartment) where it is decarboxylated to yield and a three-carbon acid (Hatch, 1987). The concentration rises in the bundle sheath cells to a level that almost saturates Rubisco’s active sites, giving PEPcase and the cycle the name of “ pump” (von Caemmerer, 2000). The enters the Calvin cycle and reacts with Rubisco to form PGA and the other photosynthetic intermediates while the three-carbon acid returns to the mesophyll cells, where it is converted to pyruvate which then reacts with ATP to form more molecules of PEP (Ehleringer and Monson, 1993).

Three biochemical mechanisms are utilised by plants to achieve acid decarboxylation in the bundle sheath cells (reviewed in Kanai and Edwards, 1999). These decarboxylation enzymes are the basis for the naming of the three different subtypes of photosynthesis: the NADP-ME subtype, the NAD-ME sub-type and the PCK sub-type. All three subtypes use the same initial carboxylation reaction whereby is catalysed by PEPcase to yield oxaloacetic acid, however, their reactions differ as follows; the NADP-ME species utilise the NADP-malic enzyme to convert oxaloacetic acid to malate which then diffuses into the bundle sheath cells. Pyrvate is formed during the decarboxylation reaction and returns to the mesophyll cell to be phosphorylated back to PEP. The NAD-ME species use the NAD-malic enzyme to transaminated oxaloacetic acid to aspartate which then diffuses into the bundle sheath cells. Pyruvate is also formed during this reaction except that it is transaminated to alanine which returns to the mesophyll cell where it is converted back to pyruvate and phosphorylated to yield PEP. If PEP carboxykinase is used, PEP is formed and this can be returned directly back to the mesophyll cells for carboxylation by PEPcase.

1.2.2. Evolution of Photosynthesis

The pathway is thought to have evolved in response to declining atmospheric levels (Ehleringer *et. al.*, 1997) although the importance of other factors has been recognised (Roalson, 2008). Rubisco, the enzyme used to catalyse and RuBP to form 3C phosphoglycerate molecules, has a high affinity for oxygen, especially at high temperatures or low concentrations (Ehleringer and Monson, 1993; Sage, 2004). When Rubisco catalyses the oxygenation of RuBP, one PGA molecule and one phosphoglycolate (PG) molecule is formed instead of the 3 PGA molecules which are formed when catalysing and RuBP. Since PG is unable to be used directly to make TP, plants evolved a way to metabolise PG back to PGA. The oxygenation of RuBP and the metabolism of PG are collectively known as photorespiration (Sage, 2004). Photorespiration, however, can decrease net rates of carbon assimilation by as much as 30% since the pathway requires energy and it results in the release of one molecule of for every two molecules of PG processed (Ehleringer *et al.,* 1991, Sage, 2004).

Historically the effects of photorespiration were negligible since the earth had high levels of atmospheric and low (Ehleringer, 1991; Sage, 2004). It was only during the Carboniferous period (280-340 million years ago) and during the last 35 million years that atmospheric levels have dropped to concentrations that have allowed for a significant increase in oxygenation and photorespiration (Sage, 2004). It is therefore believed that the pathway evolved as a means of reducing the loss of carbon and energy associated with photorespiration (Ehleringer *et al.*, 1997).

\- intermediates of *Flaveria* have provided new insights into how this may have occurred. plants evolved a mechanism of enhancing the efficiency of photosynthesis at low concentrations through the scavenging of photorespiratory metabolites, using a precursory pump (Rawsthorne, 1992; Sage, 2004). Glycine decarboxylase, a mitochondrial enzyme associated with the release of , was limited to the bundle sheath cells so that glycine needed to be shunted from the mesophyll cells to the bundle sheath cells where it was decarboxylated to release , which was then used in the PCR cycle. By limiting glycine decarboxylase to the bundle sheath cells a weak pump was created.

In addition to the pump, PEPcase activity in the mesophyll cells increased as a result of the excess leaking back from the bundle sheath cells (Monson, 1999; Rawsthorne, 1992; Sage, 2004). PEPcase is implicated in the transfer of carbon to the mitochondria. As PEPcase activity increased further it began assimilating atmospheric eventually leading to the true pump seen today.

plants are able to reduce the effects of photorespiration through the spatial separation of the biochemical reactions (Ehleringer and Monson, 1993). This is achieved by limiting the Calvin Cycle to the bundle sheath cells and through the creation of a pump which concentrates within the bundle sheath cells. As a result, an atmosphere with a high / ratio is created, reducing the risk of Rubisco binding to instead of (Ehleringer and Monson, 1993).

By eliminating photorespiration and by concentrating within the bundle sheath cells, Rubisco is able to approach its maximum rate of catalysis, allowing species to have greater efficiencies of light, water and nitrogen use relative to species (Long, 1999). Since recently burnt environments are associated with a high light intensity and low nitrogen concentration (Knapp, 1984; Ojima *et al.,* 1994), it is predicted that species are better adapted to fire driven ecosystems than their counterparts.

1.3. THE ROLE OF FIRE

1.3.1 Fire verse Climate

Climate has always been viewed as the main factor affecting global vegetation (Schulze, 1997), however it has recently become clear that fire has been an important evolutionary force and contributing factor to the distribution and abundance of present and past plant communities, in particular grasslands (Bond, 2005; Bond and Keeley, 2005; Bond and Midgley, 1995; Bond *et al.*, 2003a, 2003b; D’Antonio and Vitousek, 1992; Keeley and Rundel, 2003; Keeley and Rundel, 2005). Dynamic global vegetation models (DGVMs) use soil and climate properties as variables to simulate plant growth based on physiological conditions (Bond et al., 2005). These simulated models predict that large areas that are currently wooded and open grasslands, are able to sustain forests based on their climate potential (Bond *et al.,* 2005). This is evident in the most frequently burned ecosystems such as the grassland and savannas in Africa and South America. When modelled using the DGVMs these biomes were found to be furthest from their climate potential. According to the DGVMs these areas are warm and wet enough to support forests if fire was excluded (Bond *et al.,* 2005).

Bond *et al.* (2005) used DGVM’s to assess the effect of fire on vegetation in southern Africa. In a simulation where fire was included as part of the ecosystem processes, the vegetation showed patterns consistent with the actual vegetation seen i.e. grassland with a low tree cover with the exception of the savanna along the eastern coast and the south-west with its evergreen forests. In a simulation where fire was excluded, it was found that trees dominated all the areas in the east with a high rainfall, implying that in the absence of fire this region would be forested (Bond *et al,* 2005). Fire exclusion studies in southern Africa showed a similar pattern to the above simulations (Bond, 2008; Bond *et al.,* 2003a). Regions that experienced >650mm of annual rainfall showed a shift from being dominated by grasslands to being dominated by trees and woody vegetation in the absence of fire, suggesting that fire influenced the distribution of grasslands in these areas. Sites that received <650mm of annual rainfall did not show a change towards fire-intolerant forest or thicket species suggesting that water availability, rather than fire, influenced the development of woody plant cover (Bond, 2008; Bond *et al.,* 2003a). It is clear from the above studies that in some regions fire overrides the effect of climate and plays a significant role in determining the distribution of plant communities (Bond *et al.*, 2005).

1.3.2 The Role of Fire in Plant Communities

Bond and Keeley (2005) compare the effects of fire to those of herbivory pointing out that fire, like herbivores, feeds on and alters organic molecules. Fire, however, is not selective and regularly consumes both dead and living material, irrespective of its nutritional value. Unlike herbivores, fire thrives on the features such as high cellulose and lignin content, characteristics that make plants inedible to herbivores (Bond, 2005). Disturbances, such as herbivory and fire, function to open up canopies by removing the accumulating detritus layer. This increases the amount of available solar energy and changes the temperature regimes within the disturbed areas (Knapp and Seastedt, 1986). The carbon concentrating mechanism flourishes under warm, light saturated conditions such as that brought about by the opening up of the canopy layer (Sage, 2004). An example of this is evident in the invasion of grasses into Hawaii's submontane regions which is discussed in detail further on in this chapter (Hughes *et al.,* 1991).

Communities that are subject to frequent burning are usually replaced by grasslands (D'Antonio and Vitousek, 1992; Hoffman, 1999) and these grasslands will persist provided the burning regime continues (Bond *et al.,* 2003a, 2005*;* D'Antonio and Vitousek, 1992). It has been suggested that fire dependent communities are able to outcompete fire sensitive communities by utilising characteristics that perpetuate fires (Bond *et al.,* 2003a) and that the evolution of flammability would be an import factor in determining fire regimes, with the knock on effect that fire would determine which species are admitted to these communities (Mutch, 1970; Bond and Midgley, 1995). If fire played a role in grassland expansion then it could be hypothesised that grasses show characteristics that perpetuate fires as well as being more fire tolerant than the grass species.

Vitousek and D'Antonio (1992) have identified features in a number of grasses and grass-dominated systems that have enabled these ecosystems to increase their fire frequency. The first feature is that grasslands support large stands of dead biomass which are highly flammable under dry conditions. It could be expected that grasses support higher stands of dead biomass than the grasses. For example, an experiment done on and *Alloteropsis semialata* showed that the species died back in winter, increasing their potential fuel load while the species retained their green leaf area (Ibrahim *et al.*, 2008). The second feature identified by Vitousek and D'Antonio (1992) was that the grass tissues have a high surface area/volume ratio which would result in them drying out quickly and therefore having a low moisture content, increasing their flammability. Thirdly, grasses are able to recover rapidly after a fire compared to woody species enabling them to outcompete and dominate in areas that are subjected to frequent burning. Lastly, the microclimate found in grass canopies supports higher surface temperatures and larger vapour pressure deficits resulting in the tissues drying out more quickly than in forests or woodlands (Knapp and Seastedt, 1986; Vitousek and D'Antonio, 1992).

1.3.3 Ecophysiological Response to Fire

Plant species show varied physiological response to fire suggesting that some species have adapted, and even benefited, from frequent burning while other species are negatively affected by fire (Hartnett, 1991; Knapp, 1985; Reich *et al.*, 1990; Svejcar and Browning, 1988). For example, studies done on the dominant grass, *Andropogon gerardii,* show that burning resulted in an increased photosynthetic rate, leaf conductance, leaf area index, leaf nitrogen content, nitrogen use efficiency (NUE), leaf thickness, tillering and shoot biomass compared to plants that were not burnt (Knapp, 1985; Knapp and Gilliam, 1985; Svejcar and Browning, 1988). When the ecophysiological response of *A. gerardii* was compared to the less dominant *Panicum virgatum*, another perennial grass that occurs in small isolated colonies, it was found that the post burn differences between burnt and unburnt plants of *P. virgatum* were less pronounced than in *A. gerardii* (Knapp, 1985).

Another study was conducted on four tree species in a central Wisconsin oak forest (Reich *et al.,* 1990). As with the *A. gerardii* study, it was found that on average, fire increased the net photosynthetic rate as well as the nutrient content (N, P, K) of leaves on the burnt site for all four species. Reich *et al.* (1990) suggested that the increased stimulation of photosynthesis was a result of increased leaf N concentrations.

Hartnett (1991) compared the response of the perennial forb *Ratiba columnifera* on burned and unburned sites. Unfortunately the net photosynthetic rates were not measured between sites but it was found that contrary to the response observed in *A. gerarrdi, R. columnifera* on unburned sites were larger, produced 50% more stems and had a higher number of flower heads per plant. It was suggested that the different response could be a result of competition between species with the grass species being better adapted at utilising resources in a post fire environment (Hartnett, 1991).

The response to fire can be used to divide grasses into three categories: resprouters (survive fires by sprouting new foliage from heat resistant buds), obligate seeders (which recruit mostly from canopy or soil-stored seed banks) or facultative seeder/resprouters (resprout after mild fires but act as seeders during intense fires) (Pate, 1993; Pate *et al.*, 1990). Studies show that resource partitioning differs between resprouters and seeders with resprouters allocating larger areas of their root tissues to the storage of starch while seeders allocate a larger proportion of their resources into their above-ground biomass, resulting in larger shoot:root ratios than resprouters (Bell *et al.*, 1996; Bowen and Pate, 1993; Hansen *et al.*, 1991; Kruger *et al.* 1997, Pate, 1993 Pate *et al., 1990,* 1991). It has also been recorded that resprouters have slower growth rates. It can be inferred from the above that fire-adapted species would store starch belowground whereas fire-sensitive species might invest their resources elsewhere, such as in above-ground biomass and reproduction.

Below ground starch reserves have been found to assist in the recovery of burnt plants. A study on *Stirlingia latifolia* after fire showed that the depletion of starch in the roots was related to the plants recovery post burn (Bowen and Pate, 1993). During the initial few months of regrowth, after the fire, the plants were found to use 50-75% of their stored starch. The levels of starch in the roots were further depleted by continuously removing the new shoots of the recovering plant, suggesting that root starch reserves may limit the plants ability to produce new shoots during recovery.

Starch reserves in the roots were found to only be replaced once the shoot biomass was similar or equal to that of the pre-fire shoot biomass (Bowen and Pate, 1993). These starch reserves were slowly replaced but did not return to the pre-fire starch levels until the plants had finished flowering. *S. latifolia* took 1.5-2 years to complete reproduction, increase its starch reserves and replace the shoot size to what it was before the fire.

1.4. EXPANSION

1.4.1. When did the pathway evolve?

It is difficult to predict with certainty when exactly the first plants evolved. Based on the atmospheric conditions that predominated during the Carboniferous period (280-340 million years ago) and on sparse isotope signatures, some authors have suggested that they first appeared during this period (reviewed in Sage 2004). There is however no fossil evidence to support this theory and the validity of the isotopic data has been queried (Cerling, 1999).

Strong evidence exists to suggest that the pathway’s earliest origins were found within the Poaceae (Kellogg, 2000). Since the earliest grass fossil records, in the form of pollen, date back to the Palaeocene (55-60 million years ago), the pathway must have evolved sometime afterwards (Osborne and Beerling, 2006). Using the ‘molecular clock’ technique, a method that compares gene sequences to estimate the divergence time, it was estimated that the first grasses occurred in the sub-family Panicoideae at least 20-30 million years ago during the Oligocene (Osborne and Beerling, 2006; Sage, 2004). Recent evidence however points to the first origins of the pathway occurring in the Chloridoideae subfamily 32-25 million years ago (Christin, 2008). This time period is associated with a shift in atmospheric .

There are however 16-17 more recent grass origins, none of which correlate as closely with changes in atmospheric as the Chloridoideae subfamily (reviewed in Roalson, 2008). This suggests that other factors or a combination of factors, such as climate, rainfall seasonality and fire, might have been additional drivers in the evolution of grasses.

1.4.2 When did the grasslands expand?

Twenty five million years after their suggested origin there was an abrupt expansion of plants in terrestrial ecosystems (Cerling *et al.,* 1993*,* Cerling *et* al., 1997; Sage, 2004). This sudden expansion occurred during the Late Miocene (5-8 million years ago) and is well supported by fossil and isotopic records from peat and lake sediments, fossil soils and the tooth enamel of fossil mammals (Cerling, 1999; Cerling *et al.,*1997).

The sudden expansion was a global phenomenon which resulted in a shift from dominated ecosystems to dominated ecosystems, simultaneously, across four continents (Osborne and Beerling, 2006). The question that arises is why the photosynthetic pathway took 25 million years after it first evolved, to expand? Factors other than simply low must have driven the observed expansion of the grasslands.

1.4.3. Current hypotheses explaining grassland expansion

Most authors are in agreement that grasslands expanded during the mid- to late-Miocene (Cerling *et al.,* 1993; 1997; Ehleringer *et al.,* 1991; 1997; Keeley and Rundel, 2005; Quade *et al.,*1989; Sage, 2004). Despite this, various hypotheses have been suggested to explain the sudden, simultaneous expansion of grasslands.

There are two current hypotheses that attempt to explain what caused the sudden expansion of grasslands. The first hypothesis suggests grasslands expanded under conditions of low atmospheric due to the advantage of their concentrating mechanism (Cerling *et al.,* 1993*,* 1997; Ehleringer *et al*., 1991, 1997). As atmospheric concentrations of declined, plants possessing the pathway were able to outcompete plants with the pathway in areas such as the tropics, where the temperatures remained warm (Cerling *et al.,* 1997, 1999). While this is a logical argument, it is unlikely that low atmospheric alone resulted in the expansion of grasslands. New evidence suggests that atmospheric levels did not drop in the late Miocene as previously predicted and can hence not explain the sudden expansion of grasslands (Pagani *et al.*, 1999) Furthermore, disturbance is required for the expansion of grasslands because in its absence wooded areas can outcompete grasses by shading them, even under low levels of atmospheric (Keeley and Rundel, 2003).

A second hypothesis suggests that the grasslands expanded in areas with warm temperatures during the wet growing season and that they are limited by cold temperatures (Chazdon, 1978; Ehleringer, 1978; Ellis *et al.,* 1980; Huang *et al.* 2001; Rundel, 1980; Teeri & Stowe, 1976; Tieszen *et al.,* 1979; Vogel *et al*., 1978;). This hypothesis however does not explain why areas that have a climate potential to be forested are covered in grasslands. For example, Bond *et al.* (2003a) has found that there are areas in South Africa that have not reached their “climate potential” i.e. these areas have the potential to be woody forests but under the current fire regime are presently dominated by grasslands. When fire was excluded from these areas in experimental plots, a shift from the grasslands to woody vegetation was noted (Bond *et al.* 2003a). Keeley and Rundel (2005) suggest that it was the influence of fire rather than climate and atmospheric that played a role in the expansion of grasslands.

1.4.4. Fire as a Contributing Factor to the Expansion of Grasslands

Fire has recently caught the attention of evolutionary biologists and has been proposed to have played a significant role in driving the expansion of C_4 grasslands in the late Miocene (Beerling and Osborne, 2006; Bond *et al.,* 2003a, 2003b, 2005; Keeley and Rundel, 2003; 2005; Osborne and Beerling, 2006). Keeley and Rundel (2003; 2005) proposed that changes in climate during the late Miocene provided the ideal environment for the initiation of fires. Evidence in the oceanic charcoal sediments suggests a significant increase in fire frequency in the late Miocene (reviewed in Keeley and Rundel, 2005). The Siwalik Group sediments in northern Pakistan provide evidence of a marked increase in seasonality in the tropical and temperate regions as well as a strong intensification of Asian Monsoons during the late Miocene (Quade *et al.*, 1989). Asian Monsoons are characterised by a warm growing season, which would promote a high biomass production, followed by a long dry season accompanied by large convection storms and a high frequency of lightning. These conditions would promote sufficient drying out of the biomass to create a highly combustible fuel load which would easily ignite if struck by lightning (reviewed in Keeley and Rundel, 2005).

Present day examples of grass invasions into communities where fire has been excluded might provide evidence as to how fire played a role in the expansion of grasslands during this period. Grass invasions into areas such as the submontane zone of Hawaii, an area characterised by low fire frequencies and fire intolerant species, can initiate a grass/fire cycle (Hughes *et al.,* 1991). This grass/fire cycle occurs when the alien colonizer provides the fine fuel necessary to start and spread fires, increasing the fire frequency and intensity (D'Antonio and Vitousek, 1992). Following these fires, the alien grasses are able to recover more quickly than the indigenous species, escalating the communities' susceptibility to fire and thus altering the species composition. In Hawaii it was found that *Schizachyrium condensatum* alone could initiate a cycle that converted *Metrosideros polymorpha* woodlands into open grasslands dominated by *Melinis minutiflora*, a highly flammable exotic species (Hughes *et al*., 1991).

The simulated effect of deforestation on climate change in the Amazon, showed that with grassland invasions into previously forested areas, the dry season was extended (Lean and Warrilow, 1989). The simulation showed the reduction in precipitation to be larger than the reduction in evapotranspiration in deforested areas, causing an extended dry season not seen in forested areas. An extended dry season has the effect of increasing the frequency and intensity of fires, which would favour grassland expansions (Shukla *et al.*, 1990). Frequent burning would result in these grasslands being maintained.

From the above examples it is clear that frequent burning changes plant communities from forests to grasslands (Bond, 2005). It is predicted that as the forests were opened up by burning, grasses are likely to have outcompeted grasses due to their high productivity under warm conditions and high light intensity (Keeley and Rundel, 2005). The increased productivity of the grasses would have provided a highly combustible fuel load creating a feedback loop that increased fire frequency within their environment, leading to the subsequent expansion and maintenance of grasslands.

1.5. BIOGEOGRAPHIC DISTRIBUTION OF SPECIES

The distribution of species is closely associated with changes in latitude and altitude. species commonly occur at low latitudes, decreasing with an increase in latitude and becoming uncommon above latitudes of 45° and 50° (Sage *et al.*, 1999b; Teeri and Stowe, 1976). Similarly, an altitudinal gradient affects the distribution of and species, with species dominating at low altitudes and species dominating at high altitudes (Chazdon, 1978; Rundel, 1980; Tieszen *et al.*; 1979).

Investigations have found these latitudinal and altitudinal distributions to be influenced by the minimum temperatures during the growing season, seasonality of precipitation and subtype. It was found that species dominated in regions where minimum temperatures during the growing season were 16 to 18°C (Sage *et al.*, 1999b) whereas species were found to dominate in regions where the minimum temperature during the growing season was below 8°C (Teeri and Stowe, 1976; Tieszen *et al.*, 1979). Coupled with this, species predominate in regions where precipitation occurs during the warm, growing season while species are found in winter rainfall regions (Cabido *et al.*, 2007; Ellis *et al.*, 1980; Vogel *et al.*, 1978).

For example, the distribution of and grass species in South Africa showed a geographic separation between the two photosynthetic types (Vogel *et al.,* 1978). The grasses were found to predominate in the winter rainfall region of the Western Cape as well as along the high lying areas of the Eastern Cape and Drakensberg mountains (Ellis, 1977; Vogel *et al.* 1978). The grasses were found to predominate throughout the rest of South Africa on the drier, hotter interior plateau and along the tropical eastern coastal belt.

A strong correlation can be drawn between rainfall patterns, fire frequency and distribution. In general, rainfall patterns in South Africa show an increase from the western half to the eastern half of the country with the exception of the south western cape and eastern coastal belt which also experience a high rainfall (Schulze, 1997). When comparing the rainfall patterns to fire frequencies, there is an obvious overlap with the eastern half of the country, the south western cape and the eastern coastal belt all experiencing a high fire frequency with two exceptions: (1) the forest mosaic pockets that occur directly along the eastern coast and along the Drakensberg escarpment and (2) the Karroid Shrublands that occur over the western interior (Bond, 1997). In the arid areas (<650mm yr^{-1}) fire is limited to years with a high rainfall, when grass biomass is sufficient to provide fuel for a fire (Bond, 1997). In contrast, the mesic grasslands (>650mm yr^{-1}) experience a high frequency of fires and as a result are dominated by grasses illustrating the link between fire frequency and the distribution of grasses.

Since woody species can outcompete graminoids in the absence of ecological factors such as fire and large animal herbivory (Bond *et al*., 2005; Sage *et al.*, 1999b) an important link can be made between fire frequency and distribution i.e. an increase in rainfall increases plant biomass and an increase in plant biomass increases the potential fuel load which in turn can result in an increase the frequency of fires.

The above explanation, however, does not account for the occurrence of species in the western interior, an area characterised by a low rainfall and fire-free vegetation. Ellis *et al.* (1980) examined the distribution of the three biochemical subtypes in Namibia in an attempt to explain the effects of precipitation on grass distributions. It was found the NADP-ME subtype became more abundant with an increase in rainfall while the NAD-ME subtype dominated the arid regions. The PCK subtype occurred in regions of intermediate rainfall.

Further studies using species from the subfamilies Panicoideae and Chloridoideae by Cabido *et al.* (2007) and Taub (2000) found a similar pattern with the NADP-ME subtype dominating the wetter extremes while the NAD-ME species dominated the more arid regions (Fig. 1a). The relationship between the PCK subtype and climatic factors was less evident. When the authors took into account the association of subfamilies with rainfall, a stronger correlation was found than that between decarboxylation type and rainfall (Fig. 1b). The Cloridoideae sub-family is characterised by having species with the NAD-ME sub-type while the majority of NADP-ME grasses are found in the Panicoideae family (Ellis *et al.,* 1980). Since these subfamilies diverged before the origin of photosynthesis, the differences observed in their climatic relationships may be a result of their phylogenetic history rather than a result of their physiology.

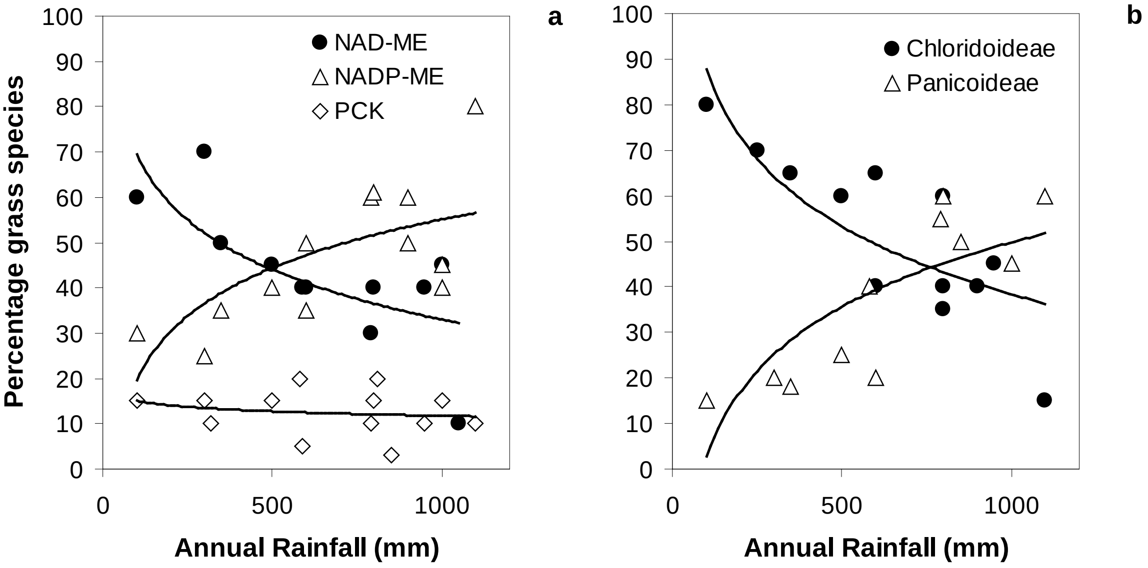

Figure 1: Relationship between annual precipitation and the percentage of C_4 grass species that use the NADP-ME, NAD-ME and PCK biochemical pathways (a) and the relationship between annual rainfall and the percentage of C_4 species in the Chloridoideae and Panicoideae subfamilies (b) (Redrawn from Cabido *et al.*, 2007).

1.6. PHYLOGENY VERSE PHOTOSYNTHETIC TYPE

Only 2.5% of the 300 000 estimated terrestrial species possess the pathway suggesting that this is a relatively new pathway (Roalson, 2008; Sage 2004; Sage *et al.,* 1999b). Over half of these species (4500) are grasses. Despite the low number of species, these grasses cover 35% of the lands surface, contributing about 25% of the world's primary productivity (Sage *et al.*, 1999b).

The pathway has evolved independently and simultaneously at least 45 times in 19 families of angiosperms (three monocot families and 16 dicot families) (Ehleringer *et al.*, 1991; Sage, 2004; Sage *et al.,* 1999a). Phylogenetic evidence indicates that each of these families arose from species providing evidence that photosynthesis evolved independently in each family (Kellogg, 1999). Within each family there are also multiple, independent origins of grasses. An example of this simultaneous yet independent evolution is evident in the *Panicum* genus, where there are three species that exhibit enough biochemical variation in photosynthesis to suggest separate origins (Ehleringer *et al.,* 1991).

The pathway is a remarkable example of convergent evolution. It is therefore important to note that differential responses between the three subtypes (NADP-ME, NAD-ME and PCK) to rainfall and disturbances such as fire and drought may be confounded by phylogenetic history. As mentioned in the previous section, correlations found between the different sub-types and rainfall were solely due to the association of the sub-types with particular grass subfamilies and not a result of their decarboxylation variants, implying that the differences observed in their climatic relationships may be a result of their evolutionary history.

Studies investigating the physiological differences between and species are therefore best done pairwise, comparing a taxon with its sisters (Kellog, 1999). This reduces the confounding effects of genetic background by making comparisons between closely related and species.

1.7. AIMS

The objective of this study was (i) to investigate whether plant response to fire was a result of physiology or (ii) whether it was due to phylogenetic history.

Questions related to Objective 1:

1. Do species recover their above-ground biomass faster than species?
2. What mechanisms do they employ to achieve this?
3. Do species produce a larger, more flammable fuel load at the end of winter, encouraging more frequent fires within their habitat?

Questions related to Objective 2:

4. Do the Panicoideae species recover their above-ground biomass more rapidly than the Aristidoideae and Danthonioideae?
5. What mechanisms do they employ to achieve this?
6. Do the Panicoideae species produce a larger, more flammable fuel load at the end of winter, encouraging more frequent fires within their habitat?

CHAPTER 2: METHODS AND MATERIALS

2.1. SPECIES SELECTION AND DESCRIPTION

Grass species from three different subfamilies, Aristidoideae (NADP-ME), Danthonioideae () and Panicoideae which is split into two different tribes; the Andropogoneae (NADP-ME) and Paniceae (; NADP-ME; NAD-ME; PCK), were selected. The Aristidoideae is sister to the Danthonioideae (Bouchenak-Khelladi *et al.,* 2008) allowing for a pair wise comparison to be made between this group and the Panicoideae, hereafter referred to as the Panicoideae and non-Panicoideae groups, respectively. The purpose of a pair wise comparison was to account for the confounding effects of phylogenetic history, by selecting species from two independent evolutionary lineages of NADP-ME photosynthesis, enabling and species to be effectively compared across phylogenies.

It must be noted that the PACCMAD clade is frequently being revised and as a result, the placement of the subfamilies regularly changes. For example, the position of Aristidoideae within the clade is still uncertain. Sanchez-Ken *et al.* (2007) placed the Aristidoideae clade basal to the other subfamilies while Bouchenak-Khelladi *et al.* (2008) placed it as a sister clade to the Chloridoideae/Danthonioideae group. Both authors however, agree that the placement of this group within the PACCMAD phylogeny is unclear and that further studies need to be done to determine its exact position. Since there is relatively weak support for the positioning of the Chloridoideae, Danthonioideae, Aristidoideae, Micrairoideae and Arundinoideae clades (represented by the open circles in Fig. 2.1.), the relationship between clades can be collapsed in a strict consensus tree, allowing for the assumption to be made that there is an equal distance between each subfamily (Fig. 2.1). For the purpose of this study the most recently revised PACCMAD clade (Bouchenak-Khelladi *et al.,* 2008) was used.

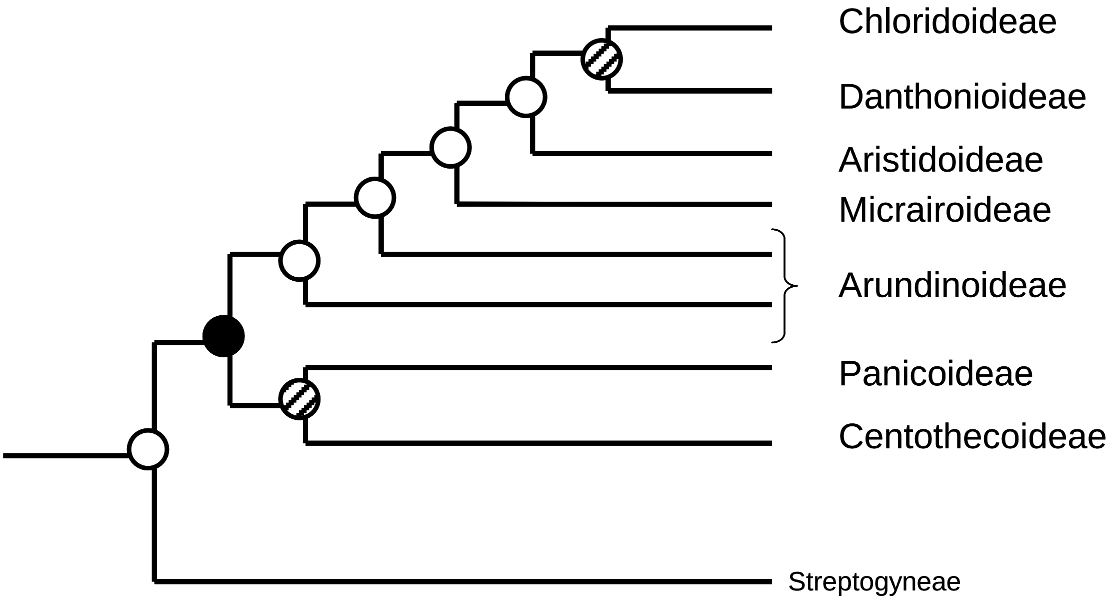

Figure 2.1: The new PACCMAD phylogeny showing sub-family relationships. Open circles represent weak support, half shaded circles moderate support and closed circles, strong support. The diagram is based on Bouchenak-Khelladi *et al.,* 2008*.*

A total of fourteen species from the three different subfamilies were randomly selected for the pot experiment. There were three species within the Panicoideae (*Panicum aequinerve* Nees*, Panicum ecklonii* Nees*, Alloteropsis semialata* (R.Br.) subsp. *eckloniana* (Nees)), four species within the Panicoideae (*Heteropogon contortus* (L.) Roem. & Schult.*, Hyparrhenia hirta* (L.) Staff.*, Themeda triandra* Forssk.*, Alloteropsis semialata* (R.Br.) subsp. *semialata* (Nees)), four species within the Danthonioideae (*Karrochloa curva*

Nees, *Merxmuellera disticha* Nees, *Pentaschistis pallida* Thunb, *Pentaschistis curvifolia* Schrad.) and three Aristidoideae species (*Aristida congesta* Roem. & Schult., *Aristida diffusa* Trin. & Rupr., *Aristida junciformis* Trin.). P. ecklonii have been reported as having both and forms (Gibbs Russell, 1990), however *P. ecklonii* plants collected at Faraway Farm were photosynthetically characterised as plants (Frole, 2008).

Of the fourteen species, six co-occurring species were selected for the field study. These consisted of three species (*A. semialata* subsp. *ecklonian*a, *P. aequinerve* and *P. pallida*) and three NADP-ME species (*A. junciformis, H. hirta* and *T. triandra*).

For easy reference, the species along with the subfamily and photosynthetic type to which they belong have been summarised in Table 1. Additional background information on the growth form, habitat type and biome for each species has been included.

Table 1: Summary of the fourteen study species indicating the photosynthetic type, family, whether the species is an annual or perennial, flowering time, habitat, biome and growth form. (Adapted from Gibbs Russell *et al.*, 1991).

Species	*I*	**Sub-Family**	**Annual/ Perennial**	**Time of Flowering**	**Habitat**	**Biome**	**Growth Form**
Panicum aequinerve Nees		Panicoideae	Short-lived Perennial or annual	September - January	Clay or sand on shallow soils of forest margins or open grasslands, mainly in damp places around boulders.	Grassland and forest	Scrambler
Panicum ecklonii Nees		Panicoideae	Perennial	September - April	Sandy soils and often in moist areas in mountainous regions that are subjected to burning.	Grassland	Shortly rhizomatous and tufted
Alloteropsis semialata (R. Br.) Hitchc. subsp. *eckloniana* (Nees) Gibbs Russell		Panicoideae	Perennial	September – March	Grassland, rocky places and forest margins	Savanna and grassland	Short-rhizomatous and tufted
Karrochloa curva Nees		Danthonioideae	Perennial	October-May	In damp or shady habitats	Nama Karoo, fynbos and grassland	Stoloniferous and tufted
Merxmuellera disticha Nees		Danthonioideae	Perennial	October-May	From coastal regions to high altitude montane bogs	Grassland, fynbos, Nama Karoo and afromontane	Tufted
Pentaschistis pallida Thunb.		Danthonioideae	Perennial	October	Widespread in areas with slight to heavy disturbance	succulent Karoo and fynbos	Tufted
Pentaschistis curvifolia Schrad.		Danthonioideae	Perennial	October - November	Widespread over wide altitude range, usually in Fynbos on sandstone derived soils.	fynbos	tufted

Table 1 continued…

Heteropogon contortus (L.) Roem. & Schult.		Panicoideae	Perennial	October – June	Hillsides and rocky places on well drained soils	Savanna, fynbos, grassland and Nama Karoo	Rhizomatous
Hyparrhenia hirta (L.) Staff		Panicoideae	Perennial	September - June	Stony soils	Savanna, fynbos, grassland, and Nama Karoo	Rhizomatous and tufted
Themeda triandra Forssk		Panicoideae	Perennial	September-June	Undisturbed veld	Savanna, fynbos, grassland and Nama Karoo	Rhizomatous
Alloteropsis semialata (R.Br.) Hitch.subsp. *semialata*		Panicoideae	Perennial	September – March	Grassland and Bushveld	Savanna and grassland	Short-rhizomatous and tufted
Aristida congesta (Roem. & Schult.		Aristidoideae	Perennial (occasionally an annual)	December - May	Hard or stony loam, sandy basalt, black clayey soils, Kalahari sands on stony slopes, open eroded places, old lands, road verges and other disturbed ground.	Savanna and grassland	Densely tufted
Aristida junciformis Trin. & Rupr. subsp. *junciformis*		Aristidoideae	Perennial	November - May	Sandy, clayey, stony soils or shallow soils on stony hillsides, in depressions where water collects and in other damp places, along roadsides and other disturbed ground	Savanna, fynbos and grassland	Stoutly rhizomatous and tufted
Aristida diffusa Trin.		Aristidoideae	Perennial	November - April	Dry, sandy, gravely loam soils on hilly slopes	Savanna and grassland	Densely tufted

2.2. FIELD STUDY

2.2.1. Field Site and growth conditions

Mountain Drive, Grahamstown was selected as the field burn site since six species (three and three NADP-ME) from the fourteen species used in the pot experiment, naturally co-occur here. The area was also known to be frequently burnt by natural fires at the end of winter. The study site was located about 5 km outside of Grahamstown, South Africa (33°19.788'S, 26°31.391'E) and had a predominantly north facing slope with sandy soils. The vegetation type was typically grassy fynbos characterised by an abundance of grasses (Poaceae) and daisies (Asteraceae) (Lubke and van Wijk, 1998).

Grahamstown lies inland and is thus subjected to greater variations in climate than coastal areas (Stone *et al.,* 1998). This area is described as semi-arid and is characterized by hot summers and cold winters (2-10 days of frost per annum) with a mean daily maximum of 27.7°C in February and 4.7°C in July (Mucina and Rutherford, 2006). The region receives on average 681mm of rainfall per annum and is characterised by a bimodal rainfall pattern with the highest amount falling from March to April and again from November to December.

2.2.2. Experimental Design

Ninety six individuals per species were tagged and mapped along a transect. This was to enable their positive identification after the fire. Each individual on the map was allocated a random number to ensure that they were randomly harvested.

The transects were burnt at the end of July 2007 in a natural fire. The first harvest was done eight days after the fire, as the first re-growth of new leaves occurred. Twelve harvests were done in total from August 2007 through until July 2008. Control plants were harvested on the same day as the burnt plants, from an unburned area adjacent to the burnt plants with the same aspect, slope and species composition. Control site selection ensured that all the plants were subjected to the same climatic conditions. The first two harvests were two weeks apart, harvests 3-5 were three weeks apart, harvests 6-8 were four weeks apart and harvests 9-12 were each six weeks apart. Harvest intervals were planned to coincide with the initial high rate of re-growth that diminished over time.

Eight individual plants per species were harvested from the burnt area and control area. To account for differences in plant size, ten tillers from each individual were randomly selected to represent the whole plant. The canopy surface area of each plant was measured by capturing an image of all the leaves that made up the canopy of a single plant and using the computer programme WinDIAS (Delta-T Devices, Cambridge, U.K.) to calculate the total canopy leaf surface area. Each individual was then separated into the following functional components: leaf (defined as the part from the tip of the leaf to the ligule), leaf sheath (defined as the photosynthetic portion from the ligule to the point it arises on the stem), culm (defined as the stem like structure that bears leaves and develops into an inflorescence), inflorescence (defined as the structure found at the tip of the culm and which bears spikelet's) , corm (defined as the swollen, non photosynthetic tissue that the roots and culm were attached to) and dead biomass (defined as the senescent leaves, leaf sheaths, inflorescences and stems) (Abraham, 2007; van Oudtshoorn, 2004). The roots (defined as the underground structures attached to the corm) were not collected as it was not possible to successfully dig up the entire root system from the field.

The plant components were dried in an oven at 70°C until constant weight was obtained and then weighed.

2.3. POT EXPERIMENT

2.3.1. Plant collection

Fifteen individual plants per species were collected between the 12/08/2007 and 30/08/2007. Each plant was divided into four smaller clumps of approximately 15 tillers each, giving a total of 60 individuals per species (840 individuals in total), and potted into 10L pots. Species that were difficult to grow were placed in jars of water in a glass house prior to planting. These were planted out after two weeks, once they showed substantial new root growth. The plants were potted in a natural topsoil, collected from the Waainek study site outside of Grahamstown (33° 19.812' S 26° 31.420E). The soil was similar to that in which the grasses were found to naturally occur.

2.3.2. Pre- and Post-fire growth conditions

The potted plants were grown at the Waainek study site prior to burning (September 2007 - August 2008) and then moved to a polytunnel after being burnt, so that their recovery was not influenced by any late season frosts which would have killed off any new re-growth. The plants were weeded and watered regularly while at the Waainek study site and while in the tunnel.

The pre-fire growth conditions at the Waainek study site are characteristic of Grahamstown's climatic conditions which has a mean daily maximum of 27.7°C in February and 4.7°C in July (Mucina and Rutherford, 2006). A temperature probe (ECH20, Decagon Devices Inc. Pullman, Washington, USA) was used to measure canopy temperature at hourly intervals two months prior to the burn (Fig. 2.2).

The post-fire growth conditions in the polytunnel were measured using a weather station (Vantage Pro Weather Station, Davis Instruments, Hayward, California, USA). The tunnel had an average relative humidity of 62.5% and an average day/night temperature of 15.4 ± 0.4 °C/11.3 ± 0.5°C (Fig.2.2). Daily average temperatures in the tunnel were slightly higher than those experienced outside of the tunnel during the same time period (Fig. 2.2). As a result, the plants were subjected to conditions somewhat representative of the onset of spring without the complication of late season frosts.

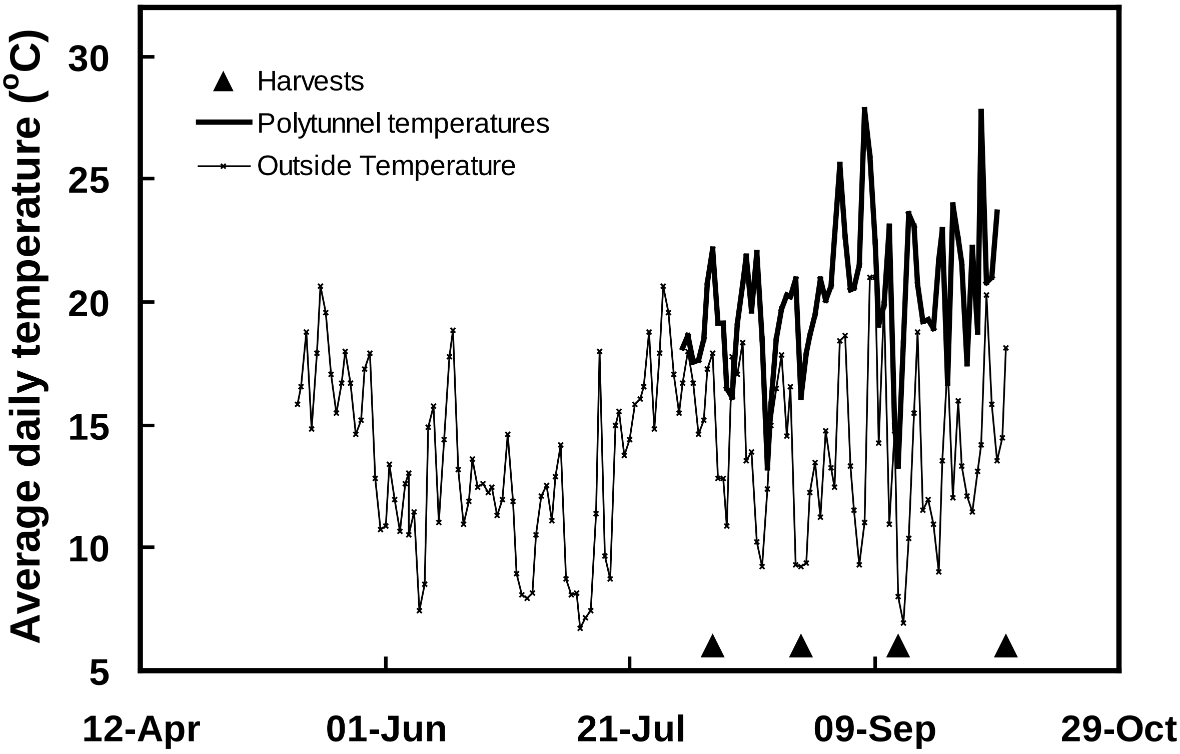

Figure 2.2: Mean daily outside and polytunnel temperatures during the pre- and post-fire growth of the grass species. (▲) represents the four harvest dates.

2.3.3. Experimental Burn

Grasslands generally burn towards the end of the dry season (Sarmiento, 1992). The potted plants were therefore burnt during this period, after the winter frosts had occurred and once the above-ground biomass had died back.

To ensure that all the plants were subjected to the same fire conditions, the plants were laid out in a 6m x 6m block design and all burnt simultaneously on one occasion.

The area was divided into 36 x blocks and one potted plant of each species placed in the centre of each block. To accommodate all fourteen species with the least edge effect, two rows of four individuals were placed down the centre of the block with two rows of three individuals on either side. A border of soil, 12.5cm in width, was placed around the edge of the grouped plants, filling in the remaining square meter (Fig. 2.3).

Figure. 2.3: The positioning of the plants within the grid prior to burning. Plants, one of each species, were grouped in the centre of each block and soil placed around the plants to prevent the roots and corm from being damaged during burning.

The plants were carefully removed from their pots, ensuring minimal root disturbance, and packed closely together according to the block design. Soil was carefully placed in the gaps between individuals to protect their roots from the fire. Where necessary, soil was placed beneath individuals, building them up to ensure that the soil surface of all the plants was at the same height and thus burned evenly.

Once the plants had been burnt, they were carefully returned to their pots and transported, along with the control plants, to a clear, naturally lit polytunnel.

2.3.4. Fire Behaviour

Byram's (1959) equation (*I = hwr)* was used to calculate fire intensity where *I* is the fire intensity (kW m^{-1}), *h* is the heat yield of the fuel (kJ kg^{-}), *w* is the dry weight of the fuel load (kg DM m^{-2}) and *r* is the rate of spread of the fire front (m s^{-1}).

To obtain a fire intensity, characteristic of natural grassland fires (900-1000 kWm^{-1}) a fuel load of 444g per square meter *(w)* was required as well as a wind speed of 2-3 ms^{-1} (Gambiza *et al.*, 2005). Pre-weighed bags of hay were evenly distributed across the burn area (one bag of 444 g of hay per). A two meter wide perimeter of hay was placed around the entire experimental grid to reduce the edge effects. The plants were burnt in a head fire, defined by Trollope (1981) as a fire that burns with the wind, on the 4/08/2008. The plants were burnt once the prevailing wind speed was approximately $2m.s^{-1}$.

The rate of spread of fire *(r)* was calculated by dividing the distance (m) covered by the fire front by the time (s) taken to travel this distance. This was achieved by placing flags at the corners of each block within the grid and recording the time taken for the fire to reach each flag.

A calorific value of 16 890 kJ kg^{-1} for grass was used as a measure of the heat yield (*h)* (Gambiza *et al.*, 2005).

Obtaining a constant wind speed of 2 m.s^{-1} proved more difficult than anticipated. The data obtained from the anemometer (Vantage Pro Weather Station, Davis Instruments, Hayward, California, USA), placed next to the burn site, showed the wind speed to vary from 0.9 m.s^{-1} to 3.1 m.s^{-1} during the burn with an average of 2.3 m.s^{-1}.The fire intensity for the burn was calculated to be 525.5 kW m^{-1}. It is possible that this was an under estimation as the fire did not burn at right angles to the fire front, making it difficult to accurately measure the rate of fire spread, and the wind speed did not remain constant.

2.3.5. Treatments

To effectively measure plant recovery and resource partitioning after burning, two treatments and a set of control plants were used. The control plants were defined as those plants that were not burnt. The burnt plants were divided into two treatments. The plants in treatment one, hereafter referred to as the burnt treatment, recovered under normal conditions. The plants in treatment two, hereafter referred to as the dark-adapted treatment, were placed in the dark during recovery. Dark recovery conditions were constructed by inverting silver painted pots over the burnt plants. The silver paint reduced absorbed radiation, lowering temperature, and the holes in the pots were covered with black material, excluding light but allowing ventilation.

2.3.6. Harvest Protocol

Plants were harvested on four occasions three weeks apart. Six control plants per species were harvested at time 0 on the 5/08/2008. These plants were used to give an indication of below-ground storage reserves and potential fuel load at the end of winter. Subsequently three harvests were done on the 25/08/2008, 14/09/2008 and the 6/10/2008 and on each occasion six individual plants per treatment, per species, were harvested. Treatments included unburnt control plants, burnt plants recovered under normal conditions and burnt plants recovered in the dark.

Immediately after the fire, the above-ground biomass remaining on any burnt plants was measured non-destructively. It was found that all viable photosynthetic tissue had been removed by the fire.

Plants were harvested by removing them from their pots and carefully washing away the soil, ensuring that no below-ground biomass was lost. They were then separated into the following components: leaf, leaf sheath, culm, inflorescence, dead material, corm and roots.

The canopy area of each plant was measured using the computer program WinDIAS (Delta-T Devices, Cambridge, U.K.). The plant components were dried in an oven at 70°C until constant weight was obtained and then weighed.

CHAPTER 3: PRE-FIRE PLANT TRAITS

3.1. INTRODUCTION

It has been proposed that grasslands are dependent on fire to suppress the growth of woody plants, allowing them to dominate in areas that are frequently burnt (Bond, 2005; 2008; Bond and Keeley, 2005; Keeley and Rundel, 2003; 2005). It is therefore predicted that these grasslands exhibit traits that promote fire within their habitat as well as mechanisms that allow them to recover quickly after a fire (Bell *et al.*, 1996; D’Antonio and Vitousek, 1992; Keeley and Rundel, 2005; Sarmiento, 1992).

A combination of traits would promote fire by creating a large, highly flammable fuel load (Keeley and Rundel, 2005). Since grasslands occur in regions with a warm, moist growing season, they are able to support the production of a large biomass. The wet growing season is then followed by a dry season which reduces the moisture content of the leaves (D’Antonio and Vitousek, 1992; Keeley and Rundel, 2005). In addition to the drying out of the leaves it has been observed that the above-ground biomass of perennial grasslands dies back during the dry season (Sarmiento, 1992). A recent study on *Alloteropsis semialata* by Ibrahim *et al.*(2008) showed that the leaves of the and not the subspecies died back during the dry season. This dead biomass then accumulates in the absence of a disturbance such as fire or grazing (Knapp and Seastedt, 1986), creating a fuel load which could easily ignite when subjected to lightning strikes or anthropogenic influences. By this mechanism grasses are thought to promote fire, thereby removing competitors and accessing the subsequent available resources and space

(D'Antonio and Vitousek, 1992; Hughes *et al.,* 1991; Keeley and Rundel, 2005).

The advantage of increasing the frequency of fires within a species' habitat would only be realised if the species were able to recover quickly and thus outcompete its neighbours (Bond and Midgley, 1995). Below ground storage organs play an important role in the recovery of fire-tolerant species after burning (Bell *et al.*, 1996; Bowen and Pate, 1993; Pate *et al.,* 1990). Studies in the Konza Prairie have shown root biomass within grass species to be significantly higher in frequently burnt areas compared to unburned areas (reviewed in Blair *et al.,*1998). This was shown to be attributable to an increase in root production, rather than a decrease in root senescence. Since grasses are strongly associated with frequently burnt areas it might be hypothesised that they would have a larger below-ground biomass and subsequent competitive advantage over species.

The aim of this study was to determine whether grass species confer characteristics that promote fire within their environment as well as to determine whether they have a larger below ground storage that would support their post-fire recovery. To determine this, the proportion of dead biomass on end-of-season grass species and rate of canopy death were compared, as was the moisture content of the live leaves, the flammability between species and the variation between below-ground biomass.

3.2. METHODS AND MATERIALS

3.2.1. Pot Experiment

As discussed in detail in Chapter 2, six individual control plants per species from the pot experiment were harvested at the end of winter, before the burning event. The plants were separated into the below-ground components made up of the roots and corm and above-ground components made up of the culm, leaf sheath, leaf and inflorescence.

Data from this harvest was used to measure variation between species' below-ground biomass, dead biomass and leaf moisture content at the end of the dry season.

Below-ground biomass was calculated as a percentage of total plant biomass. Dead biomass was calculated as the proportion of dead material relative to above-ground biomass and the moisture content of the live leaves was calculated by subtracting the wet weight from the dry weight.

In addition to the above measurements, the proportional death of the leaf canopy of six individual plants per species was measured on the pot cultivated plants, every three weeks throughout winter. The pot-grown grass tussocks were roughly circular in the horizontal dimension and hence the canopy could be easily divided into eighths using a length of wire bent at an angle of 45°. The total number of alive and dead leaves within this fraction of the canopy were counted and then scaled up to represent the entire canopy. The percentage of dead leaves for the entire canopy was calculated and used to measure the change in canopy death as the year proceeded from autumn into winter (27 May – 27 July).

3.2.2. Field Experiment

The rate of mass loss of plant material while being burned is proportional to its flammability (Mutch, 1970). Six to seven replicate above-ground biomass samples were collected from un-burnt similarly grown and Panicoid species (*P. aequinerve, P. ecklonii, A. semialata subsp. eckloniana, H. contortus, H. hirta* and *T. triandra*). Biomass was oven dried at 30°C, as certain compounds can become volatile at higher temperatures, and allowed to equilibriate to an atmospheric temperature of 25°C and relative humidity of 60%. Since wind speed influences flammability, samples were ignited in a fume hood cupboard with a constant vertical wind speed of 0.1 m.s^{-1}. The samples' weight loss over time was continuously recorded, using a balance connected to a computer that automatically logged weight at 10 sec intervals that described a logistic function. Flammability was calculated as the slope of the linear portion of this curve.

Architectural burns, where the plant architecture remained intact, were done by burning individual clumps of between 30-32 g. Non-architectural burns, where plant architecture was removed by cutting clumps of grass into 100 mm sections, were performed by burning 18-20 g of this material.

Data Analysis

A 2-level nested general linear model (GLM) was used to measure variation between species, subfamily, photosynthetic type and phylogenetic group on the data for the arcsine transformed percentage below-ground biomass, the arcsine transformed percentage dead biomass, moisture content and flammability. A nested repeated measures GLM was used to analyse the canopy death over time. In each case, three separate analyses were run to determine whether the major effects could be attributed to photosynthetic type, phylogenetic group or subfamily. The first analysis compared photosynthetic types (verse) with species nested in photosynthetic type, the second compared the Panicoideae and non-Panicoideae groups with species nested in phylogenetic group while the third analysis compared subfamilies (Panicoideae, Danthonioideae, Panicoideae and Aristidoideae) with species nested in subfamily.

A nested GLM was used to account for species belonging only to one subfamily and either one photosynthetic type or one phylogenetic group.
The analysis tested the variance among species within subfamily, photosynthetic type and phylogenetic group (Sokal and Rohlf, 1995). Using this method it is possible to determine whether significant differences exist between photosynthetic types, phylogenetic groups or sub-families even if a significant amount of added variance was found between species.

The raw data was transformed where required and the assumption of sphericity was met by the data for the nested repeated measures GLM. Statistical differences between means were determined by Tukey HSD *post-hoc* tests when the results were significant.

3.3. RESULTS

3.3.1. Below-ground Biomass

The Panicoideae had a significantly larger below-ground biomass (84.4%) compared to the non-Panicoideae grasses (76.3%) (Table 3.1.2 and Fig. 3.1.1b). Although there were differences among the subfamilies (Table 3.1.3), the *post-hoc* tests showed no significant difference between the Panicoideae and Panicoideae and no significant difference between the Danthonioideae and Aristidoideae subfamilies (Fig. 3.1.2a). However, at the level of the individual species, biomass differed within each subfamily (Table 3.1.3 and Fig. 3.1.2b). *K. curva* and *A. congesta*, both of which belong to the non-Panicoideae group, were significantly lower than the other species. These species exerted a marked influence on the results of the non-Panicoideae group and when they were excluded from the analysis the overall Panicoideae/non-Panicoideae differences disappeared.

The Panicoideae's large below-ground biomass suggests that these species will recover faster than the non-Panicoideae after being burnt.

In contrast, overall differences between and photosynthetic types were not significant (Table 3.1.1 and Fig. 3.1.1a).

Table 3.1.1. GLM results for differences in below-ground biomass, dead biomass and moisture content between and photosynthetic types with species nested in photosynthetic type. n.s., not significant; ***, $P < 0.001$.

	Species (Photosynthetic Type)	**Photosynthetic Type**
Below-ground Biomass	*** $_{,70}$= 24.26	*n.s.* $_{,70}$= 1.2
Dead Biomass	*** $_{,70}$= 15.63	*n.s.* $_{,70}$= 0.01
Moisture content	*** $_{,70}$= 13.73	*** $_{,70}$= 139.97

Table 3.1.2. GLM results for differences in below-ground biomass, dead biomass and moisture content between Panicoideae and non-Panicoideae grasses with species nested in phylogenetic group. n.s., not significant; ***, $P < 0.001$.

	Species (Group)	**Group**
Below-ground Biomass	*** $_{,70}$= 21.48	*** $_{,70}$= 34.62
Dead Biomass	*** $_{,70}$= 12.14	*** $_{,70}$= 41.87
Moisture content	*** $_{,70}$= 25.24	n.s. $_{,70}$= 1.90

Table 3.1.3. GLM results for differences in below-ground biomass, dead biomass and moisture content between subfamilies with species nested in subfamily. n.s., not significant; ***, P < 0.001.

	Species (subfamily)	**Subfamily**
Below-ground Biomass	*** $_{,70}$= 25.34	*** $_{,70}$= 12.97
Dead Biomass	*** $_{,70}$= 10.46	*** $_{,70}$= 27.68
Moisture content	*** $_{,70}$= 16.40	*** $_{,70}$= 46.92

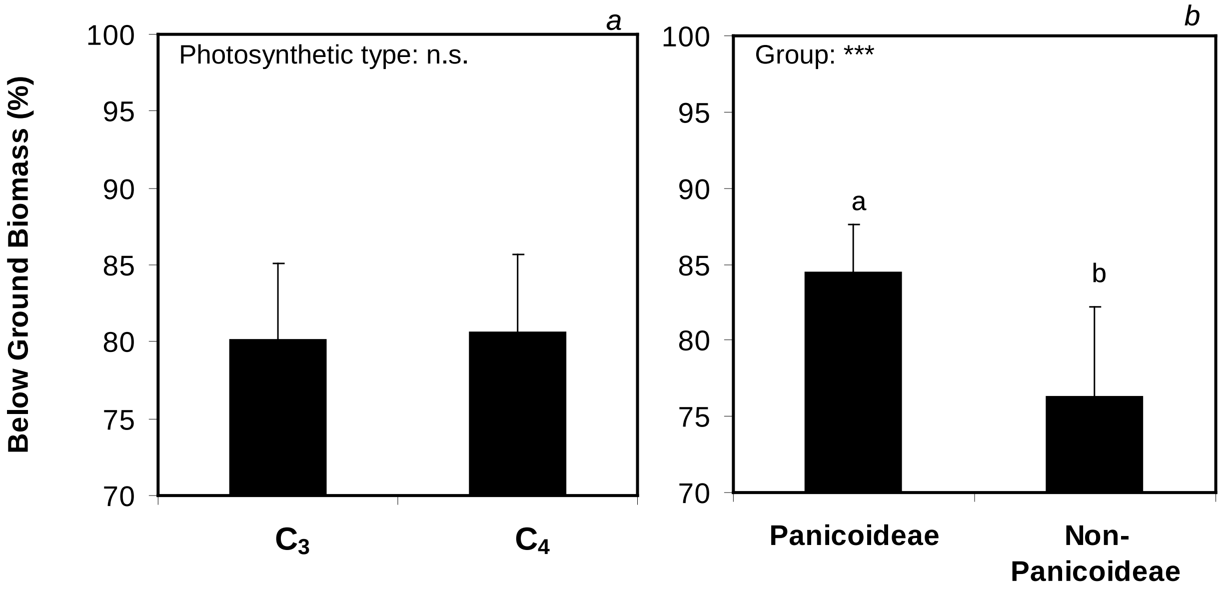

Figure 3.1.1: Average percentage below-ground biomass grouped by photosynthetic type (a) and group (b). (n = 6-7. Vertical bars represent standard errors). Different letters indicate significant differences between means at P < 0.05 (Tukey HSD test).

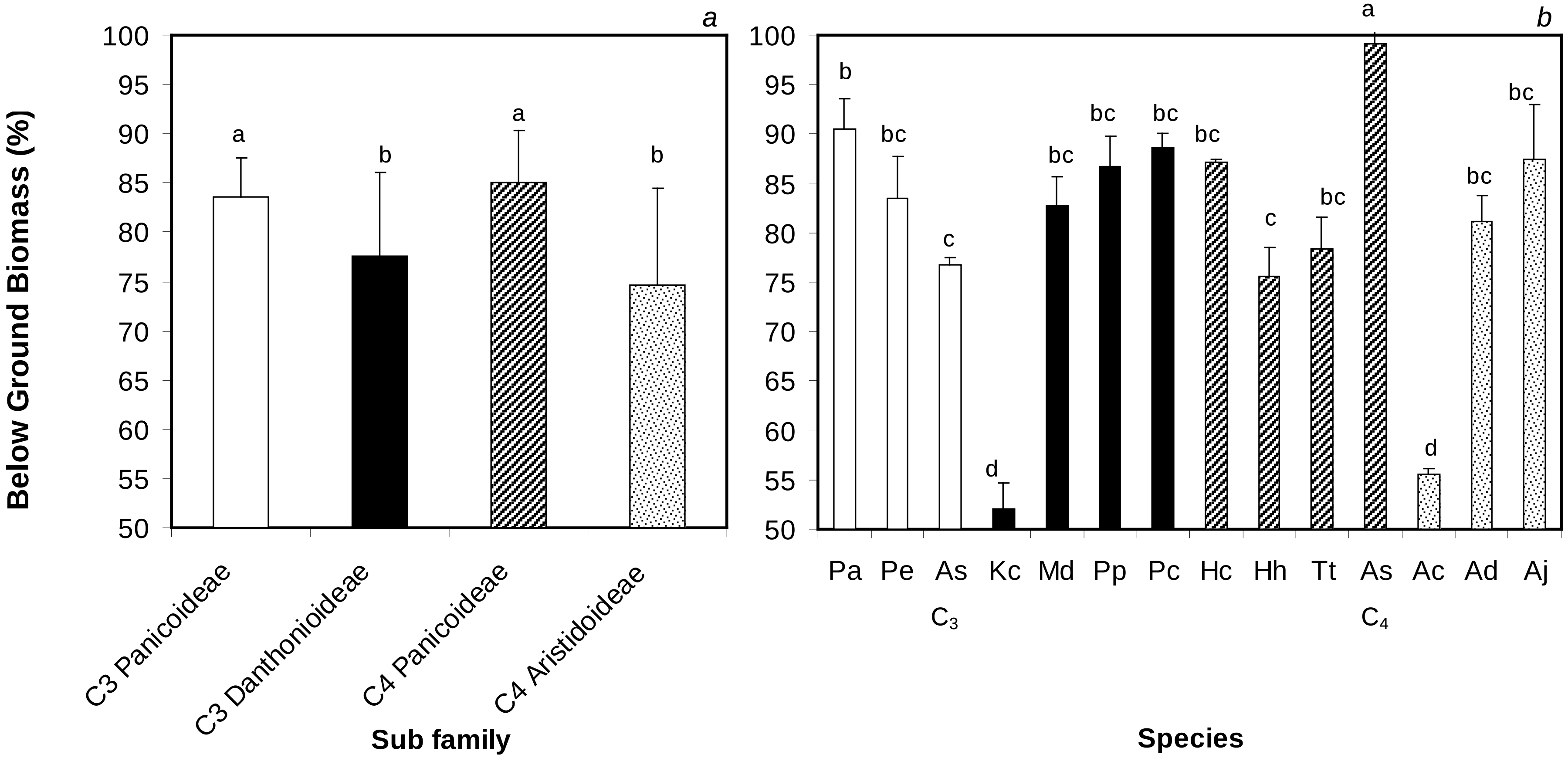

Figure 3.1.2: The percentage below-ground biomass of pot-cultivated C_3 and C_4 grasses belonging to the indicated subfamilies. (*a*) Responses averaged across species of a particular photosynthetic type within each subfamily. (b) Individual species responses. Abbreviations*: Pa = P. aequinerve, Pe = P. ecklonii, As* C_3 *= A. semialata subsp. eckloniana, Kc = K. curva, Md = M. disticha, Pp = P. pallida, Pc = P. curvifolia, Hc = H. contortus, Hh = H. hirta, Tt = T. triandra, As* C_4 *= A. semialata subsp. semialata, Ac = A. congesta, Aj = A. junciformis, Ad = A. diffusa.* (n = 3-4 for subfamily and n= 5-6 per individual species. Vertical bars represent standard errors). Different letters indicate significant differences between means at P < 0.05 (Tukey HSD test).

3.3.2. Canopy Death

The species accumulated a significantly greater proportion of dead leaves over time than the species implying that they had a larger potential fuel load than the species (significant type x time interaction Table 3.2.1 and Fig.3.2.1a). In contrast, overall differences between Panicoideae and non-Panicoideae groups over time were not significant (n.s. group x time interaction Table 3.2.2 and Fig. 3.2.1b). However, a comparison between groups showed that the Panicoideae had a greater proportion of dead leaves irrespective of changes over time.

Within subfamilies, the Panicoideae and Aristidoideae accumulated a significantly greater proportion of dead leaves over time than the Panicoideae and Danthonioideae (Table 3.2.3 and Fig. 3.2.2a), supporting the photosynthetic response. In addition, there were no significant differences in canopy death over time between species within each subfamily (n.s. species x time interaction Table 3.2.3 and Fig. 3.2.2b).

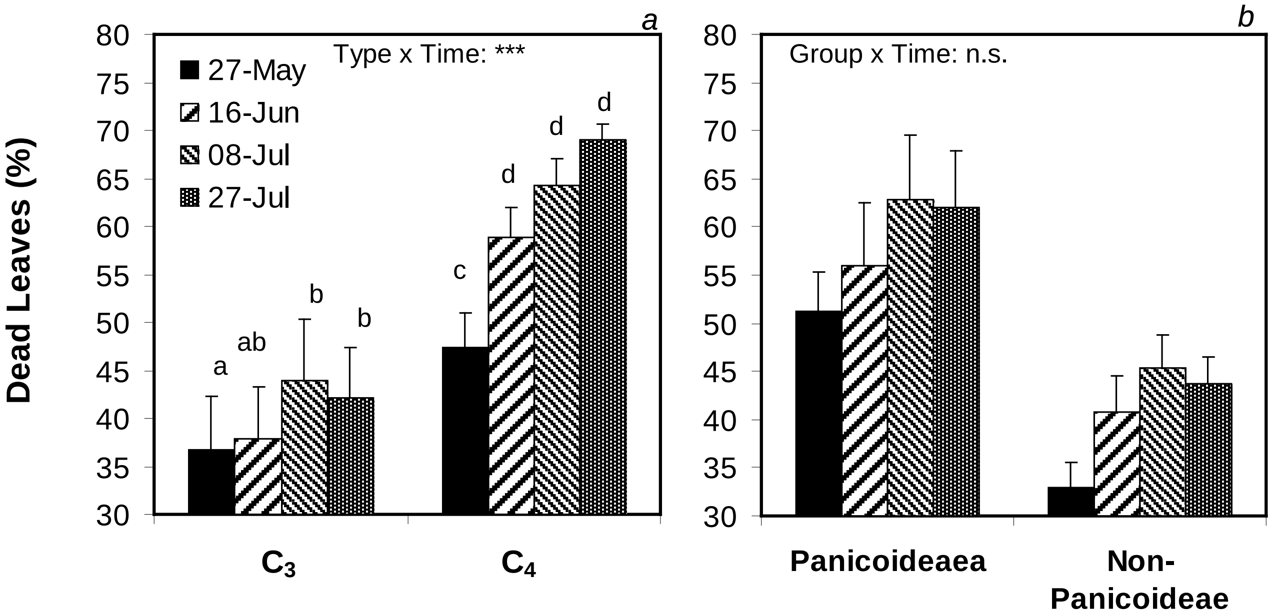

Figure 3.2.1: Average percentage of dead leaves grouped by photosynthetic type (a) and group (b). Abbreviations: n.s. = not significant. (n = 6-7. Vertical bars represent standard errors). Different letters indicate significant differences between means at $P < 0.05$ (Tukey HSD test).

Table 3.2.1 GLM results for differences in canopy death over time between and photosynthetic types with species nested in photosynthetic type with time as the within-subject factor**.** n.s., not significant; ***, $P < 0.001$.

Species (Photosynthetic type)	Photosynthetic Type	Time	Time*Species (Photosynthetic Type)	Time* Photosynthetic Type
*** $_{,70}$= 6.81	*** $_{,70}$= 72.94	*** $_{,210}$= 25.34	n.s. $_{,210}$= 0.94	*** $_{,210}$= 5.44

Table 3.2.2 GLM results for differences in canopy death over time between Panicoideae and non-Panicoideae grasses with species nested in phylogenetic group with time as the within-subject **.** n.s., not significant; ***, $P < 0.001$.

Species (Group)	Group	Time	Time*Species (Group)	Time* Group
*** $_{,70}$= 6.81	*** $_{,70}$= 69.32	*** $_{,210}$= 25.34	n.s. $_{,210}$= 0.94	n.s. $_{,210}$= 0.68

Table 3.2.3 GLM results for differences in canopy death over time between subfamilies with species nested in subfamily with time as the within-subject factor**.** n.s., not significant; ***, $P < 0.001$.

Species (Subfamily)	Subfamily	Time	Time*Species (Subfamily)	Time* subfamily
*** $_{,70}$= 6.81	*** $_{,70}$= 41.81	*** $_{,210}$= 25.34	n.s. $_{,210}$= 0.94	*** $_{,210}$= 3.04

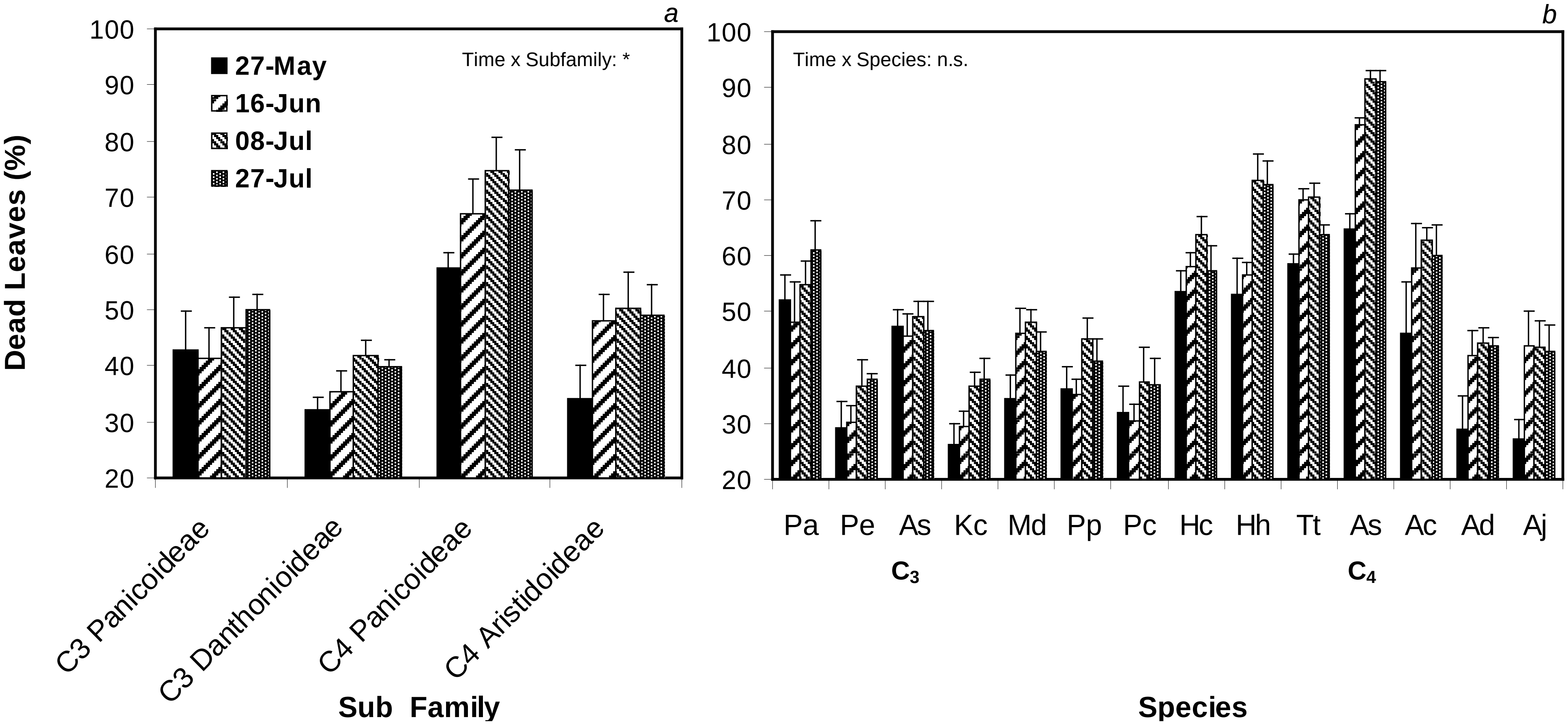

Figure 3.2.2: The percentage of dead leaves of pot-cultivated C_3 and C_4 grasses at different time intervals between May and July 2008. (*a*) Response averaged across species of a particular photosynthetic type within each subfamily. (b) Individual species responses. Abbreviations*: Pa = P. aequinerve, Pe = P. ecklonii, As C_3 = A. semialata subsp. eckloniana, Kc = K. curva, Md = M. disticha, Pp = P. pallida, Pc = P. curvifolia, Hc = H. contortus, Hh = H. hirta, Tt = T. triandra, As C_4 = A. semialata subsp. semialata, Ac = A. congesta, Aj = A. junciformis, Ad = A. diffusa.* (n = 3-4 for subfamily and n= 6 per individual species. Vertical bars represent standard errors). Different letters indicate significant differences between means at $P < 0.05$ (Tukey HSD test).

3.3.3. Dead Biomass

Immediately prior to the burn the Panicoideae had a significantly larger amount of dead biomass (73.8%) compared to the non-Panicoideae (61.7%) (Table 3.1.2 and Fig. 3.3.1b). However, when the differences between subfamilies were compared it was found that the Panicoideae had a significantly larger amount of dead biomass (77.3%) than the other subfamilies, that there was no difference between the Panicoideae (69.2%) and Danthonioideae (69.2%) and that the Aristidoideae had the smallest amount of dead biomass (51.8%) than the other three subfamilies (Table 3.1.3 and Fig. 3.3.2a). At the level of the individual species, dead biomass varied between species only within the Panicoideae with *H. hirta* having the lowest proportional dead biomass compared to the other species within the subfamily and *A. semialata* subsp. *semialata* having the largest proportional dead biomass (Table 3.1.1 and Fig. 3.3.2).

The results suggest that the Panicoideae, in particular the Panicoideae, accumulated dead biomass, potentially increasing their potential fuel load.

In contrast, the amount of dead biomass did not differ between photosynthetic types (Table 3.1.1 and Fig. 3.3.1a).

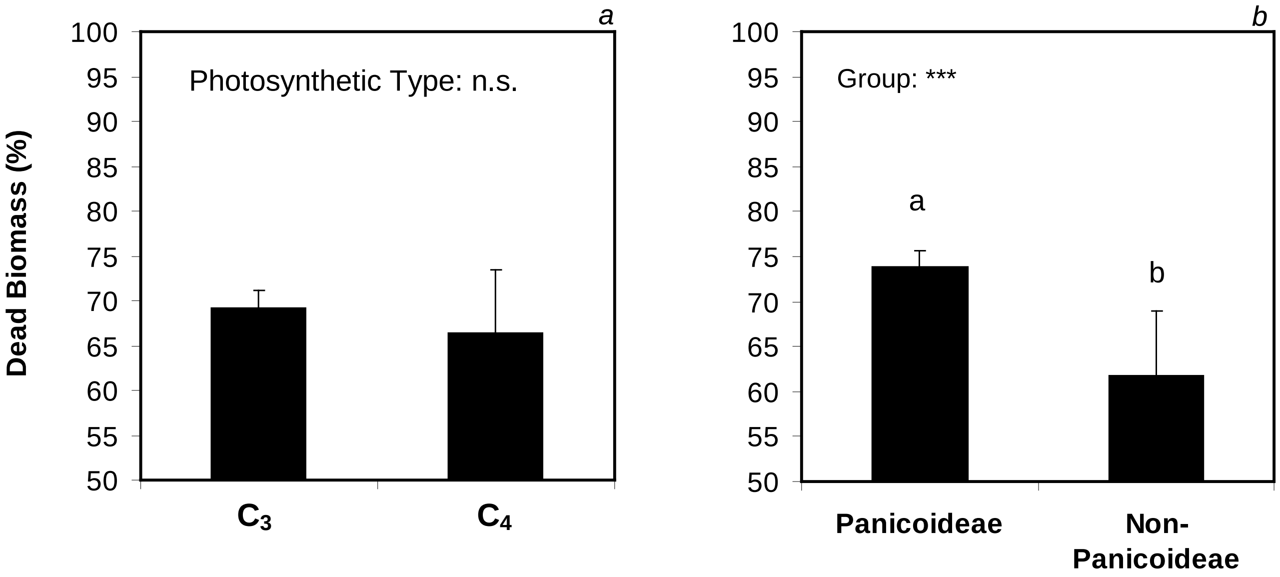

Figure 3.3.1: Average percentage of dead above-ground biomass grouped by photosynthetic type (a) and group (b). Abbreviations: n.s. = not significant. (n = 6-7. Vertical bars represent standard errors). Different letters indicate significant differences between means at $P < 0.05$ (Tukey HSD test).

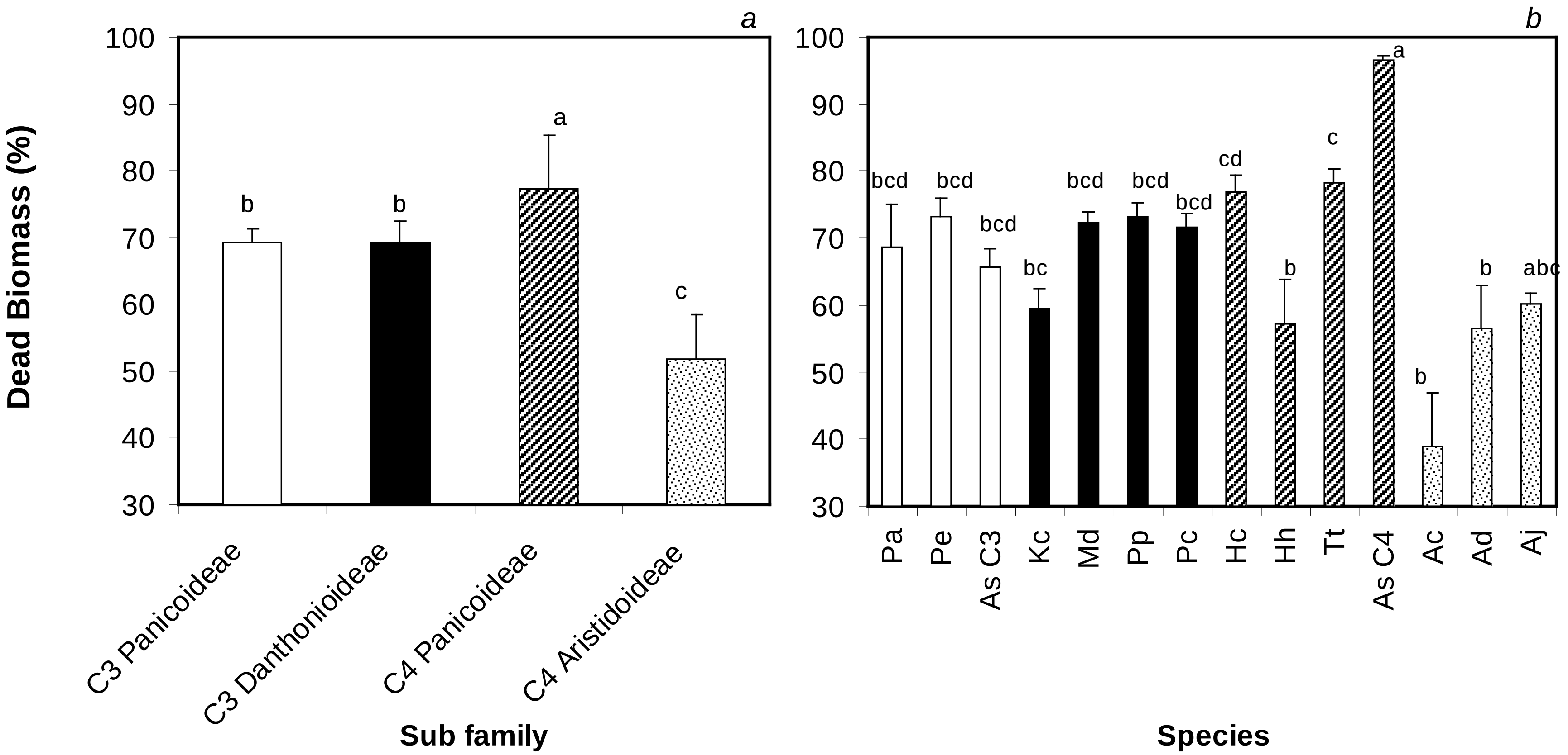

Figure 3.3.2: The dead above-ground biomass of pot-cultivated C_3 and C_4 grasses belonging to the indicated subfamilies. (*a*) Responses averaged across species of a particular photosynthetic type within each subfamily. (b) Individual species responses. Abbreviations*: Pa = P. aequinerve, Pe = P. ecklonii, As* C_3 *= A. semialata subsp. eckloniana, Kc = K. curva, Md = M. disticha, Pp = P. pallida, Pc = P. curvifolia, Hc = H. contortus, Hh = H. hirta, Tt = T. triandra, As* C_4 *= A. semialata subsp. semialata, Ac = A. congesta, Aj = A. junciformis, Ad = A. diffusa.* (n = 3-4 for subfamily and n= 5-6 per individual species. Vertical bars represent standard errors). Different letters indicate significant differences between means at P < 0.05 (Tukey HSD test).

3.3.4. Moisture Content

The species had a significantly lower moisture content (0.04 g.g^{-1}) than the species (0.15 g.g^{-1}) (Table 3.1.1 and Fig. 3.4.1a) and there were no differences among the subfamilies belonging to each of these photosynthetic types implying that the species retarded fire less than the species (Table 3.1.3 and Fig. 3.4.2 *a*).

In contrast, overall differences between Panicoideae and non-Panicoideae groups were not significant (Table 3.1.2 and Fig. 3.4.1b). However, at the level of the individual species, moisture content varied between species with *A. semialata* subsp. *eckloniana* having the highest moisture content (Table 3.1.3 and Fig. 3.4.2 b). When this species was excluded from the analysis, the overall Panicoideae/non-Panicoideae differences were significant.

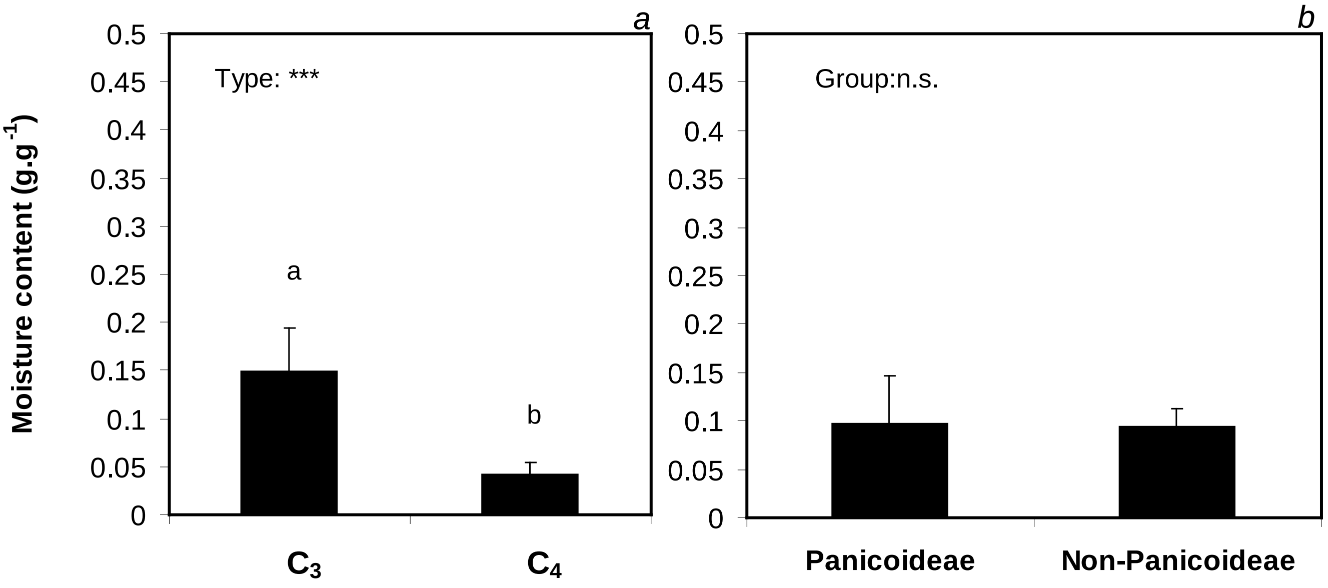

Figure 3.4.1: Average moisture content of green leaves grouped by photosynthetic type (a) and group (b). Abbreviations: n.s. = not significant. (n = 6-7. Vertical bars represent standard errors). Different letters indicate significant differences between means at $P < 0.05$ (Tukey HSD test).

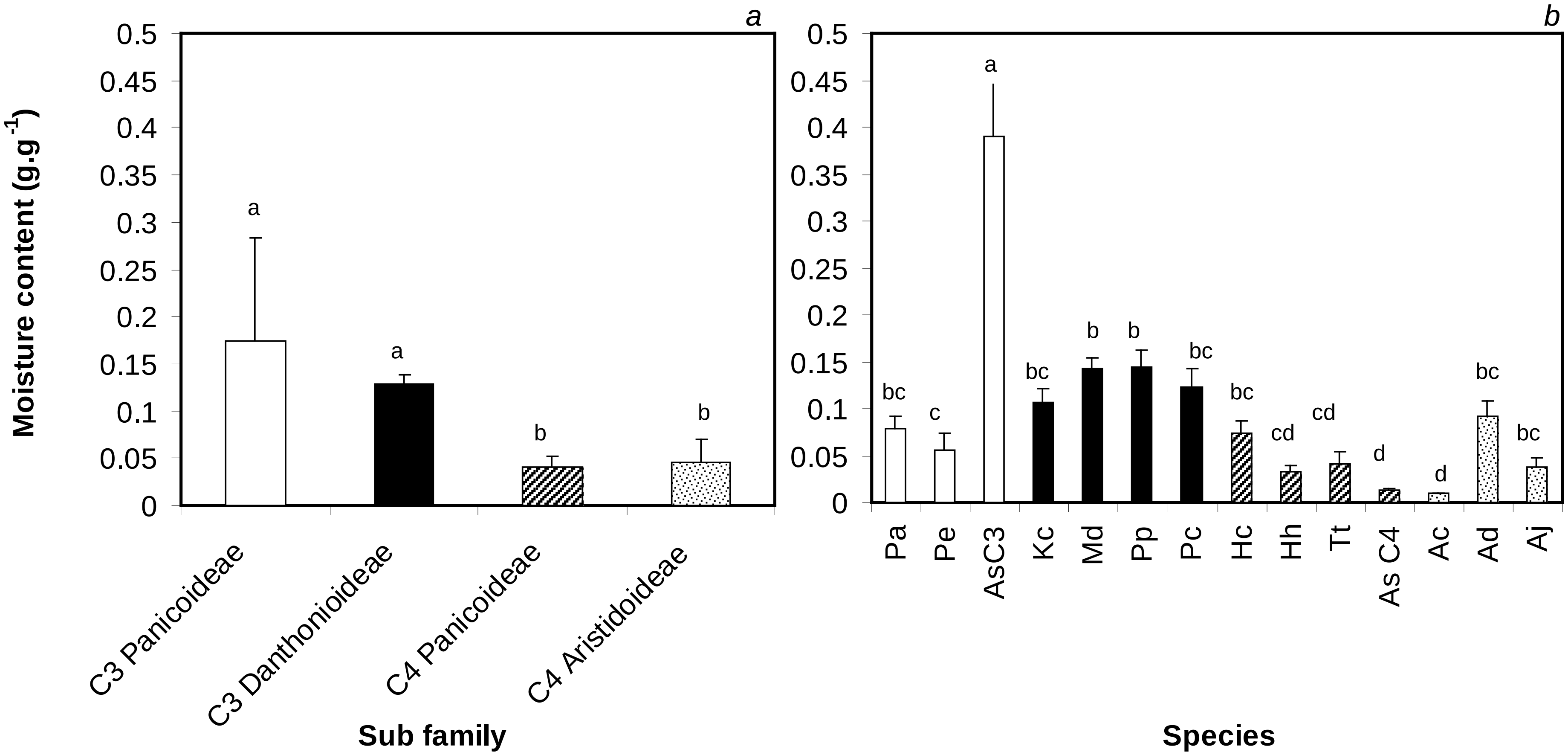

Figure 3.4.2: The moisture content of the green leaves of pot-cultivated C_3 and C_4 grasses belonging to the indicated subfamilies. (*a*) Response averaged across species of a particular photosynthetic type within each subfamily. (b) Individual species responses. Abbreviations*: Pa = P. aequinerve, Pe = P. ecklonii, As C_3 = A. semialata subsp. eckloniana, Kc = K. curva, Md = M. disticha, Pp = P. pallida, Pc = P. curvifolia, Hc = H. contortus, Hh = H. hirta, Tt = T. triandra, As C_4 = A. semialata subsp. semialata, Ac = A. congesta, Aj = A. junciformis, Ad = A. diffusa.* (n = 3-4 for subfamily and n= 5-6 per individual species. Vertical bars represent standard errors). Different letters indicate significant differences between means at $P < 0.05$ (Tukey HSD test).

3.3.5. Flammability

For the architectural burns, the Panicoideae were significantly more flammable than the Panicoideae (Table 3.3 and Fig. 3.5a). However at the level of the individual species, flammability differed within each photosynthetic type. Within the photosynthetic type, *A. semialata* subsp. *eckloniana* was significantly less flammable than *P. aequinerve* and *P. ecklonii* and within the photosynthetic type all three species were significantly different from each other (Table 3.3 and Fig. 3.5 b).

For the non-architectural burns, the species were significantly more flammable than the species (Table 3.3 and Fig. 3.5c). However at the level of the individual species, flammability again differed within each photosynthetic type. Within the photosynthetic type, *P. ecklonii* was significantly lower than *P. aequinerve* and *A. semialata* subsp. *semialata* (Table 3.3 and Fig. 3.5 d). Within the photosynthetic type, *H. contortus* was significantly less flammable than *H. hirta*.

These results imply that some species are more flammable than others and that architecture does influence flammability.

Table 3.3: GLM results for differences in flammability between and Panicoideae photosynthetic types with species nested in photosynthetic type**.** n.s., not significant; P > 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001.

Treatment	Species (Photosynthetic Type)	Photosynthetic type
Architecture	****** $_{,30}$= 7.4	*** $_{,30}$= 58.8
Non-architecture	** $_{,24}$= 19.4	** $_{,24}$= 4.5

3.3.6. Summary of Results

The pre-fire characteristics showed both a photosynthetic and a phylogenetic response. The species had a lower moisture content and their canopy died back at a faster rate than the species implying that the species had a larger fuel load than the species. However, a comparison of the dead biomass at the end of winter showed the Panicoideae, in particular the Panicoideae, to have a proportionally larger dead standing biomass suggesting that these species accumulated the dead material while the dead material of the non-Panicoideae fell off and decayed. In addition to this the Panicoideae also had a proportionally larger below-ground biomass. Both the large dead standing biomass and the large below-ground biomass suggest that the Panicoideae shunt carbon below-ground during winter to reduce the impact of fire and to support their fast recovery at the onset of the growing season.

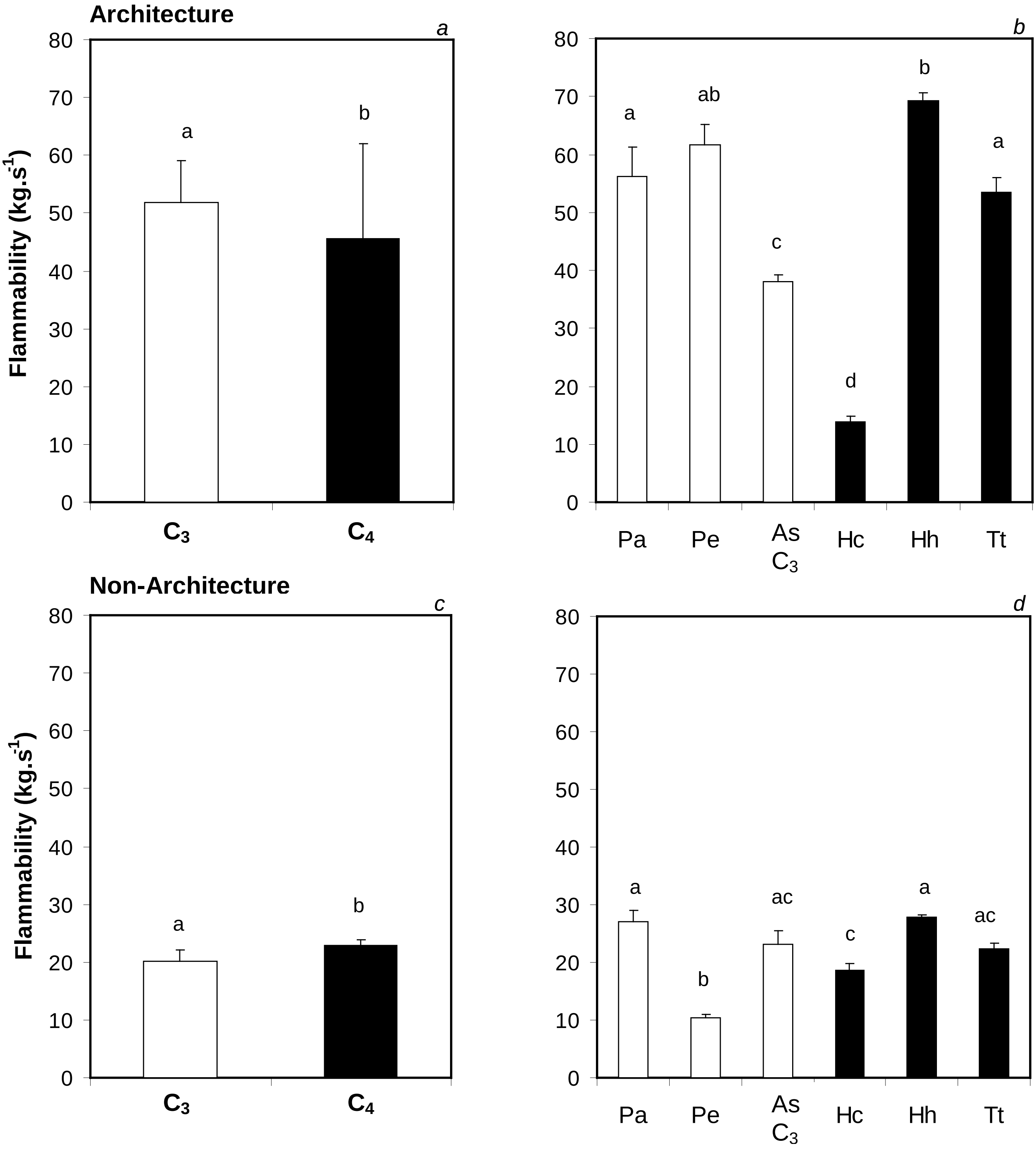

Figure 3.5: Flammability of the Panicoid species based on plant architecture (a and b) and non-architecture (c and d). The left column indicates the average response across species and the right column indicates individual species response. Abbreviations: n.s. = not significant, *Pa = P. aequinerve, Pe = P. ecklonii, As* C_3 *= A. semialata subsp. eckloniana, Hc = H. contortus, Hh = H. hirta, Tt = T. triandra,* (n = 3 for subfamily and n= 5-6 per individual species. Vertical bars represent standard errors). Different letters indicate significant differences between means at $P < 0.05$ (Tukey HSD test).

CHAPTER 4: POST FIRE RECOVERY

4.1. INTRODUCTION

The successful recovery of a plant after being burnt is determined by a combination of pre-fire characteristics and the efficiency with which the plant can utilise the resources which become available in the post-fire environment (Bond and van Wilgen, 1996; Morgan, 1996; 1999). Pre-fire characteristics include a large below ground storage organ to enable rapid re-growth after a fire (Bellingham 2000; Bond and van Wilgen, 1996; Morgan, 1996) and protection of the perrenating buds, which are either buried below the soil surface or are surrounded by closely packed leaf sheaths to prevent fire damage (Gibson, 2009; Morgan, 1999; Sarmiento, 1992).

Conditions associated with the post-fire environment, such as increased light intensity and soil temperatures and decreased nitrogen concentrations, also affect species recovery (Knapp, 1984; Manson *et al.,* 2007; Ojima *et al.*, 1994). Grasses, such as those that possess the pathway, are physiologically better adapted at taking advantage of these conditions than their counterparts which is why it has been suggested that grasses are able to recover more quickly after a fire (Long, 1999) and as a consequence dominate in frequently burnt ecosystems (Bond, 2008). For example, intensive research on the Konza Prairie in north-eastern Kansas has found that the above ground primary production of grasses increased in regions that were frequently burnt while forbs (largely perennial herbs) were inhibited in frequently burnt sites. Although most studies compare the post-fire recovery between grasses and dicots, it is predicted that a similar pattern will be observed when comparing and grasses, with the grasses being unaffected or even stimulated by fire while the grasses will be inhibited by fire.

While a / response is predicted, recent studies have illustrated the importance of accounting for a species' phylogenetic history (Edwards and Still, 2008; Edwards *et al.*, 2007; Taub, 2000). For example, Edwards and Still (2008) found that the restriction of grasses to warmer habitats was mainly a result of their evolutionary history but that the pathway provided a competitive advantage to the grasses in more arid environments. In South Africa the distribution of grasses is found to occur mainly in the summer rainfall areas (Vogel *et al.,*1978). However, if the analysis of distribution is taken a step further, and differences between subfamilies (or phylogenetic history) compared, it is found that the Andropogoneae (NADP-ME) dominate in ecosystems that are mainly fire dependent (ecosystems where fire results in changes in species composition) while the Chloridoideae (NAD-ME) are found to dominate in climate dependent ecosystems (reviewed in Bond *et al.,* 2003a). Climate dependent ecosystems are defined as ecosystems where fire either results in structural changes or has no effect, implying that these ecosystems are affected by changes in climate rather than fire. Bond *et al.* (2003a) attributed these differences in distributions between the Andropogoneae and Chloridoideae to differences in biochemical pathways since the Andropogoneae have the NADP-ME pathway which is characterised as having the highest quantum yield, making them more efficient than the Chloridoideae which have the NAD-ME pathway. Species evolutionary history is therefore important to consider when examining post-fire recovery between and species as well as between subfamilies.

The aim of this study was to investigate whether plant recovery after burning was due to the advantage conferred by physiology or whether it was a result of the plants evolutionary history. This distinction was made by examining the post-fire recovery of field and pot grown and grass species, measuring the impact burning had on the recovery of the canopy leaf area, leaf mass and above-ground biomass. The cost of recovery to the plant was determined by examining specific leaf area (SLA) and the impact of post-fire recovery on the below-ground biomass.

4.2. METHODS AND MATERIALS

4.2.1. Post Fire Recovery: Field and Pot Experiment

As discussed in detail in Chapter 2 (Methods and Materials), the plants from both the field and the pot experiment were harvested, separated into their different components, dried in an oven at 70°C until constant weight was obtained and the weights recorded.

The field experiment tracked the recovery of the plants that had been subjected to a natural fire on 26/07/2007 with twelve harvests that were completed between August 2007 and July 2008. In addition to the harvest of recovering burnt plants, control plants were harvested on the same day from an unburned adjacent area. Harvest intervals were planned to coincide with the initial high rate of re-growth and were then diminished over time.

For the pot experiment (See Chapter 2 for details) four sets of six plants of each species were harvested at three week intervals. For the plants harvested subsequent to the experimental burn, plants were either allowed to recover under normal conditions in the greenhouse or were recovered in the dark. This was done to allow the contributions of photosynthesis and reallocation to be determined.

The absolute values for each plant component were used to compare the recovery of the field and pot grown species over time. Specific leaf area (SLA), which is a measure of leaf area per unit of leaf biomass, was also calculated by dividing the living canopy leaf area by the leaf mass (Poorter and Nagel, 2000). SLA can be used to determine the cost of the leaves to the plant as it is a measure of leaf density and thickness.

Data Analysis

A 2-level nested general linear model (GLM) was used to measure differences between species, subfamilies, photosynthetic types and phylogenetic groups on the absolute values for canopy leaf area, leaf mass, SLA, above-ground biomass and below-ground biomass. In each case, three separate analyses were run to determine whether the major effects could be attributed to photosynthetic type, phylogenetic group or subfamily. The first analysis compared the phylogenetic groups (Panicoideae verse non-Panicoideae) with species nested in group, the second analysis compared photosynthetic types (verse) with species nested in type while the third analysis compared subfamilies (Panicoideae, Danthonioideae, Panicoideae and Aristidoideae) with species nested in subfamily.

If both the phylogenetic and photosynthetic analyses were significant, then the values were compared to determine which model was better at explaining the observed variance.

The raw data was transformed where required and statistical differences between means were determined by Tukey HSD *post-hoc* tests when the results were significant. The results from the *post-hoc* tests were included on the graphs and indicated by an *, however in some instances the three-way interaction (e.g. Group x Treatment x Time) was not significant and so the *post-hoc* tests from the two way-interaction (e.g. Group x Treatment) were included and indicated as “Treatment * ” or “Treatment n.s.”.

4.3. RESULTS

4.3.1 Field Experiment

As only six of the fourteen selected species (three and three) occur on the hill surrounding Grahamstown, the field experiment was used only to compare trends between photosynthetic types as opposed to a full comparison between photosynthetic types, phylogenetic groups and subfamilies, as was conducted in Chapter 3 and for the data from the pot experiment.

Since this experiment was conducted on individuals grown in the field, there was no control over the timing of the natural fire in relation to spring growth i.e. despite the fire occurring at the end of July, conditions may have been favourable for spring growth to have started before the fire occurred. In addition, the plant sizes were also varied due to uncontrolled growth history, making the averaging across species, within a photosynthetic type, problematic. Lastly, only the above-ground biomass could be quantified in the field study.

4.3.1.1 Canopy Area

The field comparisons between photosynthetic types showed that the species, when compared to controls, recovered their canopy area by the third harvest date while the species took on average two weeks longer to replace their canopy area (Table 4.1 and Fig. 4.1.1). The fast recovery of the canopy area of *Panicum aequinerve* and the slow recovery of *T. triandra* exerted an influence on the data, skewing the results so that the species appeared to recover more quickly than the species. When these species were removed from the analysis both the and species recovered their canopy area by the third harvest date.

Despite the photosynthetic type taking longer to replace the leaf canopy area compared to the photosynthetic type, fire did not have an impact on the final leaf canopy area produced over the course of the year and both types had the highest canopy areas around 26 weeks after the fire (autumn). Subsequently the canopy areas decreased at the end of the season as winter approached (Fig. 4.1.1).

As expected, there were differences between individual species with the growth of canopy area attaining maximum size at different times. While the growth curves of both the burnt *P. aequinerve* and *T. triandra* were stimulated by fire, the growth curves of *T. triandra* imply that the control plants had already begun to re-grow before the fire occurred with the fire resulting in the loss of new tissue, explaining the delayed recovery of *T. triandra.* Fire did not have an effect on the growth curves of the other four species. The results imply that the timing of the fire in relation to re-growth can have an impact on recovery and subsequent carbon gain.

Table 4.1: GLM results for differences in canopy area, leaf mass, SLA and Above-ground biomass between and photosynthetic types of field species with species nested in photosynthetic type. n.s., not significant; P > 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001.

	Canopy Area	**Leaf**	**SLA**	**Above-ground Biomass**
Type	*** $_{,826}$ = 31.8	*n.s.* $_{,826}$ = 1.5	*** $_{,826}$ = 67.9	*** $_{,826}$ = 420.1
Species (Type)	*** $_{,826}$ = 615.2	*** $_{,826}$ = 600.1	*** $_{,826}$ = 198.4	*** $_{,826}$ = 878.2
Treatment	*** $_{,826}$ = 30.8	*** $_{,826}$ = 143.3	*** $_{,826}$ = 133.1	*** $_{,826}$ = 840.0
Time	*** $_{,826}$ = 107.4	*** $_{,826}$ = 146.0	*** $_{,826}$ = 128.7	*** $_{,826}$ = 210.4
Type X Treatment	*n.s.* $_{,826}$ = 0.03	* $_{,826}$ = 5.4	*** $_{,826}$ = 14.6	*** $_{,826}$ = 197.9
Species (Type) X Treatment	*** $_{,826}$ = 33.8	*** $_{,826}$ = 44.4	*** $_{,826}$ = 6.0	*** $_{,826}$ = 46.5
Type X Time	*** $_{,826}$ = 12.9	*** $_{,826}$ = 18.8	*** $_{,826}$ = 8.8	*** $_{,826}$ = 10.1
Species (Type) X Time	*** $_{,826}$ = 28.2	** $_{,826}$ = 23.3	*** $_{,826}$ = 29.1	*** $_{,826}$ = 17.4
Type X Treatment X Time	*** $_{,826}$ = 11.05	*** $_{,826}$ = 20.3	*** $_{,826}$ = 8.1	*** $_{,826}$ = 70.0
Species (Type) X Treatment X Time	*** $_{,826}$ = 6.9	*** $_{,826}$ = 7.0	*** $_{,826}$ = 5.1	*** $_{,826}$ = 5.8

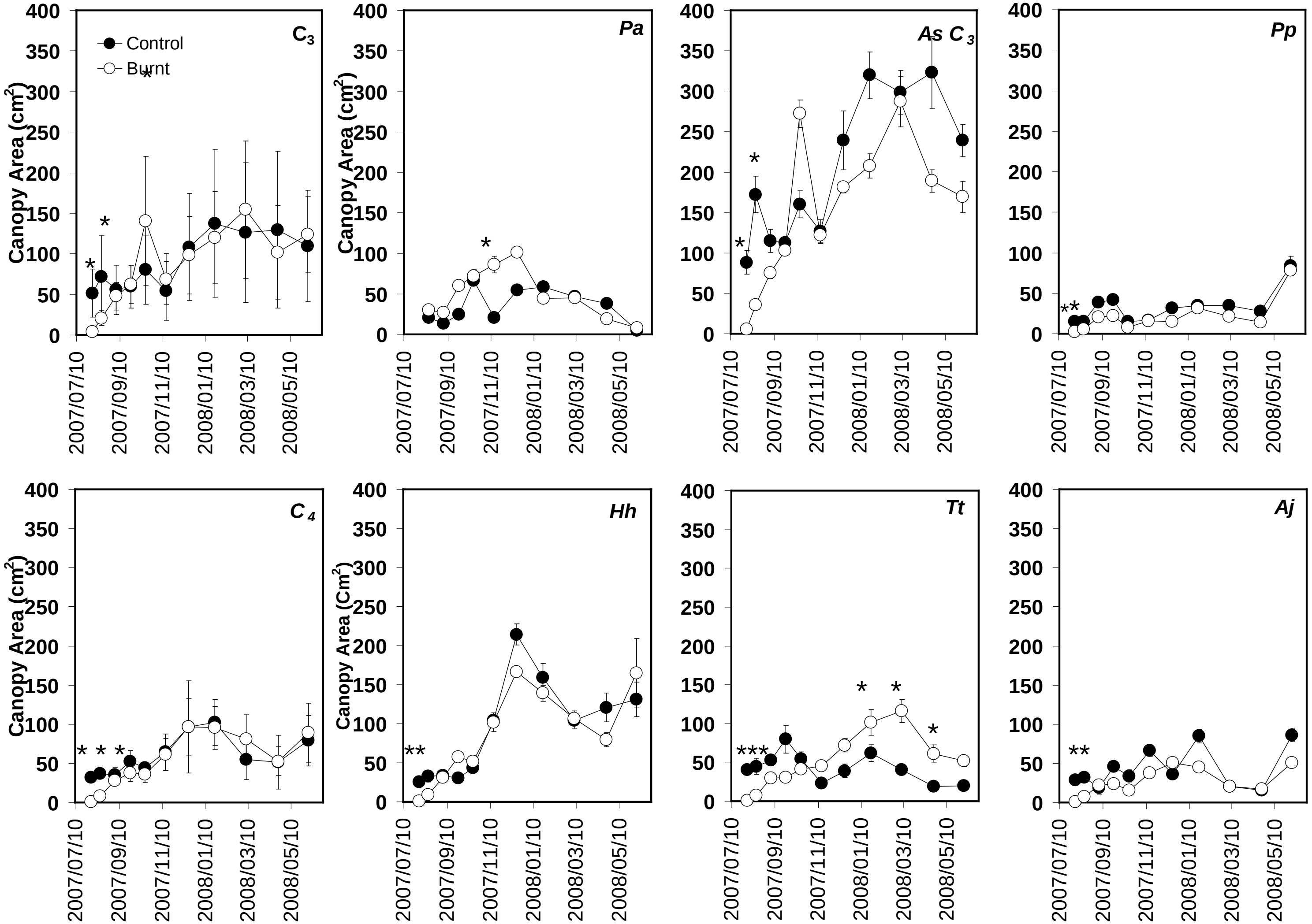

Figure 4.1.1: Canopy Area of the field grown C_3 and C_4 species. The left column represents the response averages across species of a particular photosynthetic type. Individual species responses are indicated as *Pa = P. aequinerve, As C*$_3$ *= A. semialata subsp. eckloniana, Pp = P. pallida, Hh = H. hirta, Tt = T. triandra, Aj = A. junciformis.* (n=3 per photosynthetic type and n= 8 per individual species. Vertical bars represent standard errors). * indicates significant differences between means of the control and burnt plants at $P<0.05$ (Tukey HSD test).

4.3.1.2 Leaf Mass

A similar trend was seen when comparing the leaf mass of the and species with the species recovering on average after ~9 weeks while the species recovered after ~16 weeks (Table 4.1 and Fig. 4.1.2). Again the results were skewed by the fast recovery of *P. aequinerve* and the slow recovery of *T. triandra* with both the and species recovering after nine weeks when these species were removed from the analysis.

As with the canopy area, the growth curves for the leaf mass between treatments for each photosynthetic type remained unaffected with both treatments reaching maximum leaf mass at the same time (Fig. 4.1.2). Again, *T. triandra* and *P. aequinerve* produced a larger maximum leaf mass after burning implying that they were stimulated by fire but that *T. triandra* was delayed in reaching maximum leaf mass as a result of re-growth that occurred before they were burned, highlighting the importance of the timing of the end of season fires.

4.3.1.3 Specific Leaf Area (SLA)

The fast recovery of the canopy area was with leaves of reduced mass and hence increased SLA relative to the species (Table 4.1 and Fig. 4.1.3). Subsequently, SLA declined over subsequent harvests and this recovery was more rapid in the than the species implying that from the beginning of recovery the species invested larger amounts of carbon in thicker leaves than the species.

Species responses did not vary with the exception of *P. pallida* which had the highest SLA and showed the slowest recovery implying that this species was not as well adapted to fire as the other species.

4.3.1.4 Above-ground Biomass

The species also recovered their total above-ground biomass more quickly than the species (Table 4.1 and Fig. 4.1.4). This was probably a result of differences in growth forms with the field species investing very few resources in support tissue such as vegetative stems while the species, especially *H. hirta*, invest a larger proportion of resources in the support tissue. Since the plants recovered their photosynthetic tissue (i.e. leaves) first, the recovery of the stems was delayed thus explaining why the species took longer to recover their above-ground biomass.

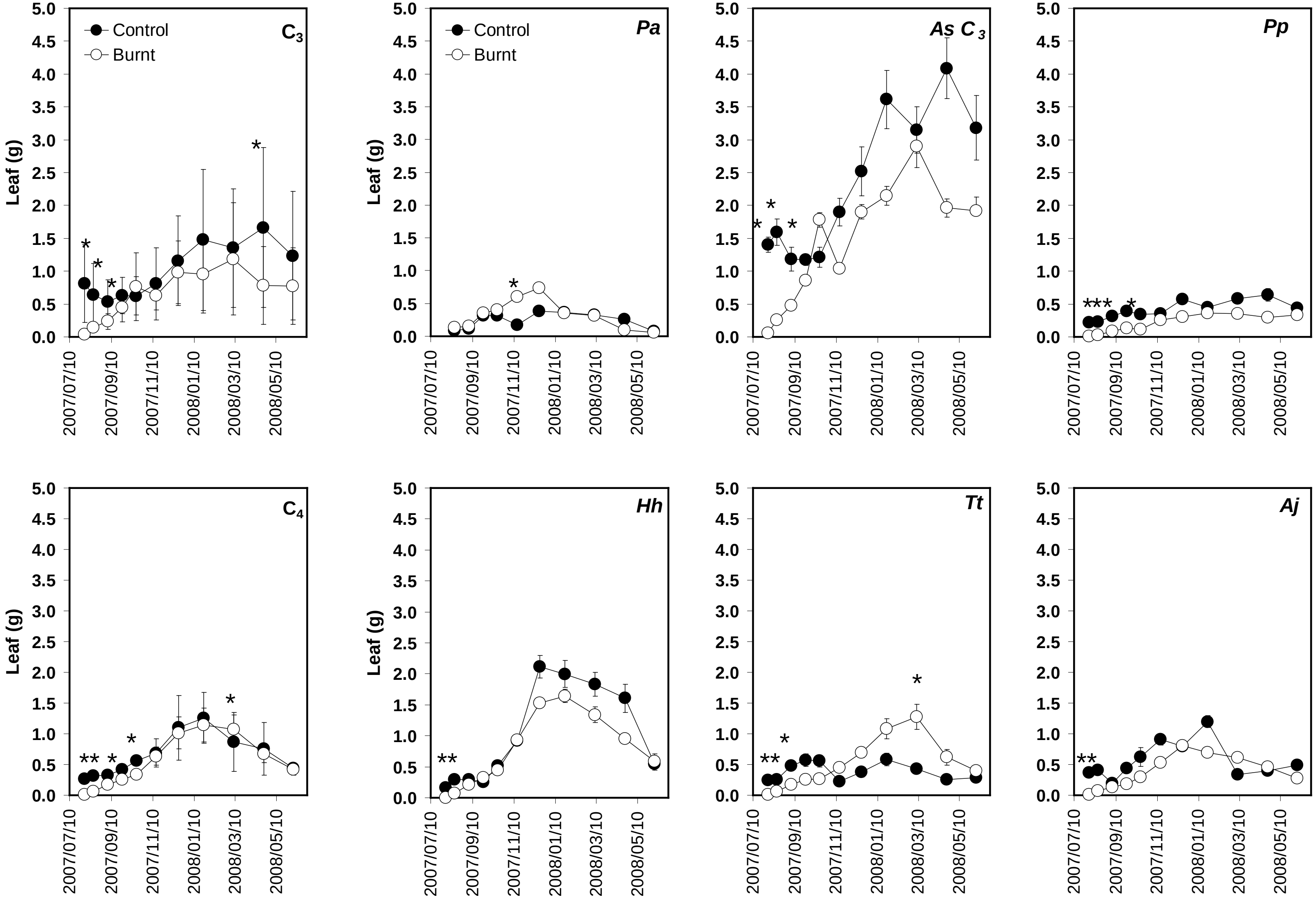

Figure 4.1.2: Leaf mass of the field grown C_3 and C_4 species. The left column represents the response averages across species of a particular photosynthetic type. Individual species responses are indicated as *Pa = P. aequinerve, As C_3 = A. semialata subsp. eckloniana, Pp = P. pallida, Hh = H. hirta, Tt = T. triandra, Aj = A. junciformis.* (n=3 per photosynthetic type and n= 8 per individual species. Vertical bars represent standard errors). * indicates significant differences between means of the control and burnt plants at $P<0.05$ (Tukey HSD test).

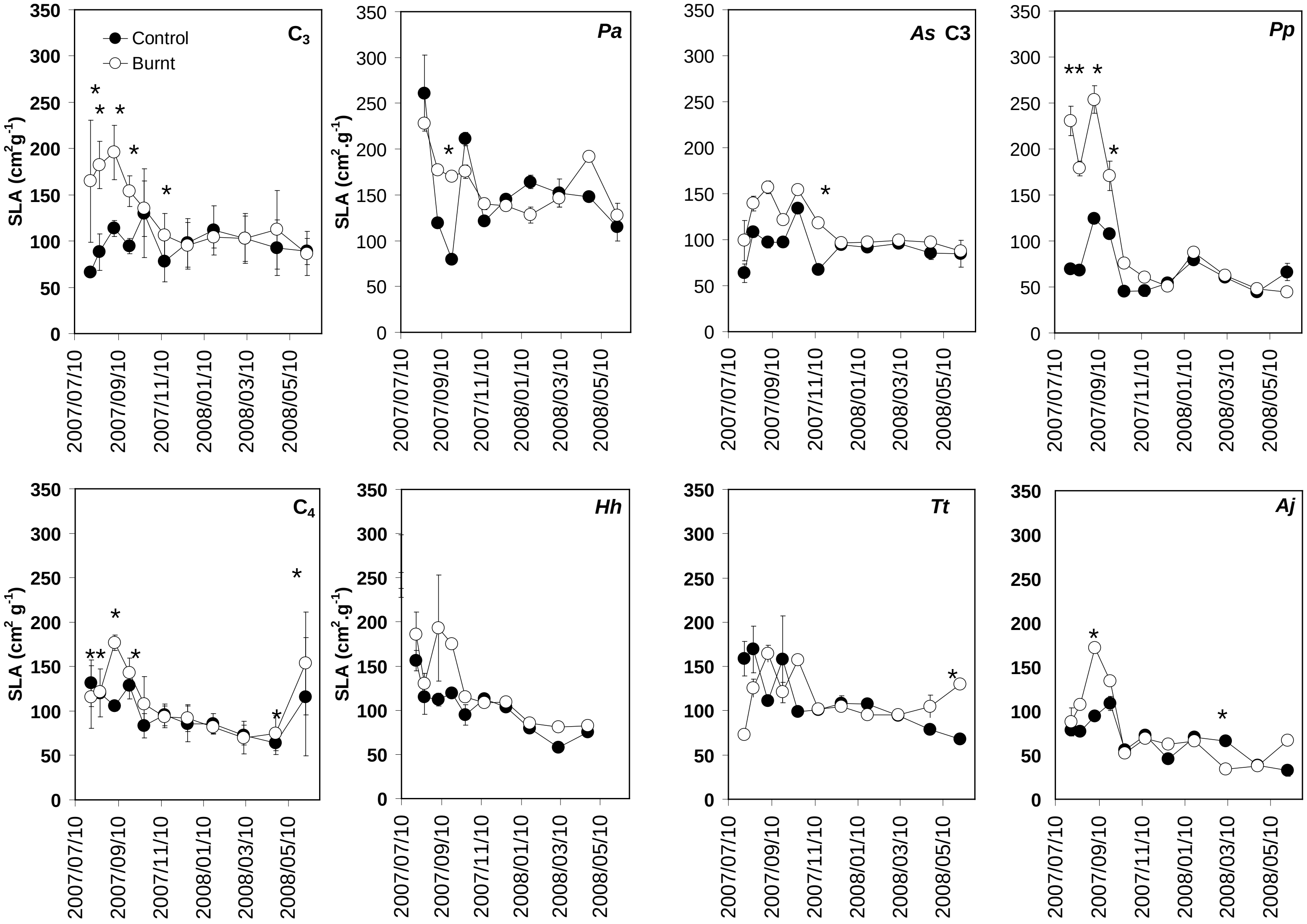

Figure 4.1.3: Specific Leaf Area (SLA) of the field grown C_3 and C_4 species. The left column represents the response averages across species of a particular photosynthetic type. Individual species responses are indicated as *Pa = P. aequinerve, As C_3 = A. semialata subsp. ecklonian*a, *Pp = P. pallida, Hh = H. hirta, Tt = T. triandra, Aj = A. junciformis.* (n= 3 per photosynthetic type and n= 8 per individual species. Vertical bars represent standard errors). * indicates significant differences between means of the control and burnt plants at P<0.05 (Tukey HSD test).

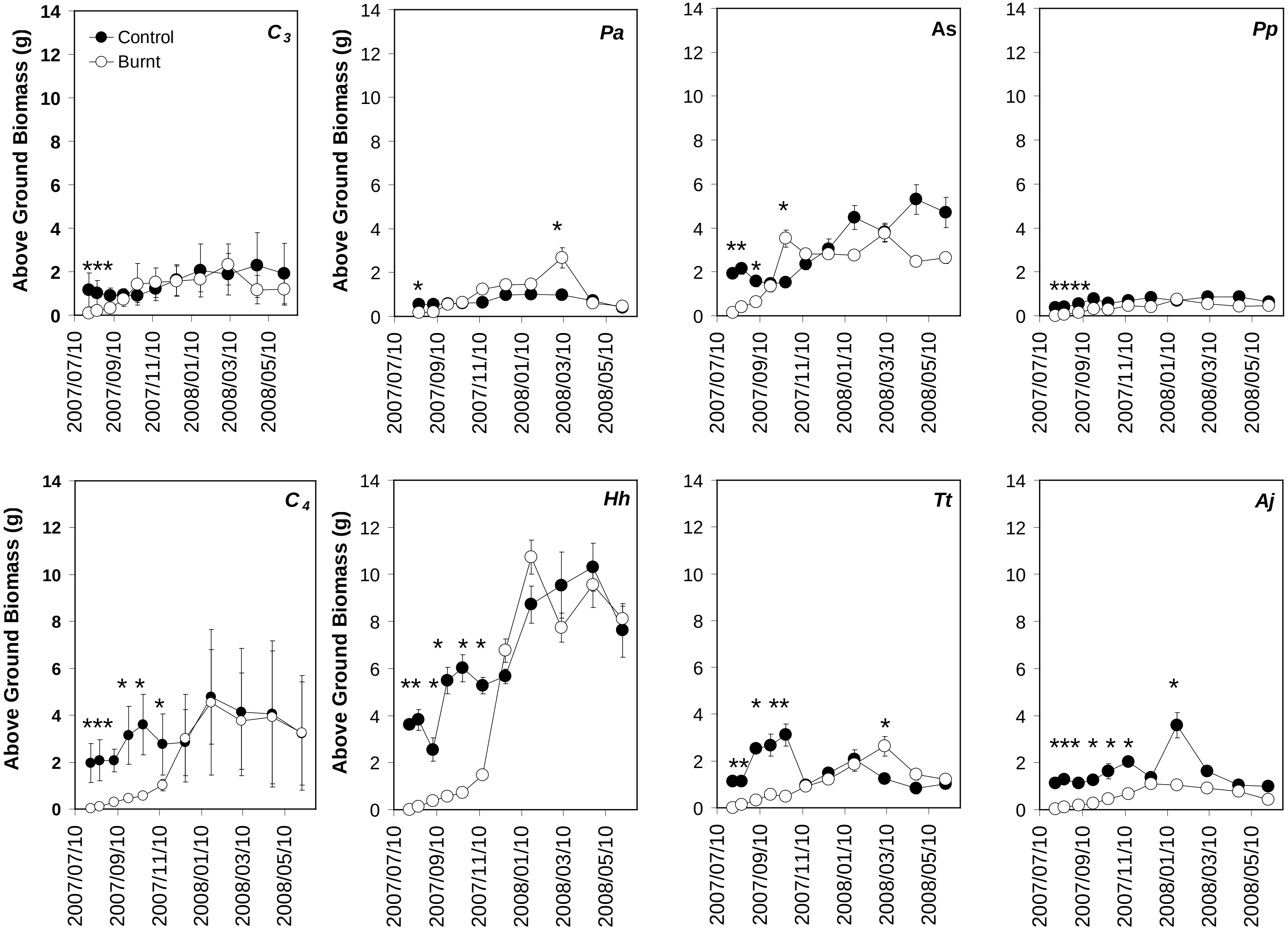

Figure 4.1.4: Above-ground biomass of the field grown C_3 and C_4 species. The left column represents the response averages across species of a particular photosynthetic type. Individual species responses are indicated as *Pa = P. aequinerve, As C_3 = A. semialata subsp. eckloniana, Pp = P. pallida, Hh = H. hirta, Tt = T. triandra, Aj = A. junciformis*. (n=3 per photosynthetic type and n= 8 per individual species. Vertical bars represent standard errors). * indicates significant differences between means of the control and burnt plants at P<0.05 (Tukey HSD test).

4.3.2 Pot Experiment

4.3.2.1. Canopy Area

The Panicoideae recovered their canopy area more quickly and completely than the non-Panicoideae (Table 4.2.1 and Fig. 4.2.1.1) implying that the Panicoideae species recover from fire irrespective of their photosynthetic pathway. In contrast, when the species were grouped according to photosynthetic type, both the and photosynthetic types showed a significant difference in canopy area between treatments with the burnt treatments being significantly lower than the controls (Table 4.2.2 and Fig. 4.2.1.1).

Model values showed that the phylogenetic grouping explained more variance than the photosynthetic grouping (Table 4.2.1 and 4.2.2) and this was supported by the subfamily responses. The and Panicoideae recovered their canopy area while the Danthonioideae and Aristidoideae never recovered their canopy area (Table 4.2.3 and Fig. 4.2.1.2).

There was, however, some variation between species response within subfamilies (Table 4.2.3 and Fig. 4.2.1.2). While each species within the Danthonioideae showed a similar trend of not recovering their canopy area, it was only *K. curva* and *M. disticha* that did not show a significant difference between the burnt and control plants. In contrast, within the Panicoideae, *T. triandra* was the only species to show a significant difference in the recovery of the burnt plants. None of the three species within the Aristidoideae showed a significant difference between treatments, although the trends observed for *A. congesta* and *A. diffusa* suggest that they never recovered their canopy area. Despite these variances between species, the response between treatments for each subfamily was still significant when species were nested in subfamily.

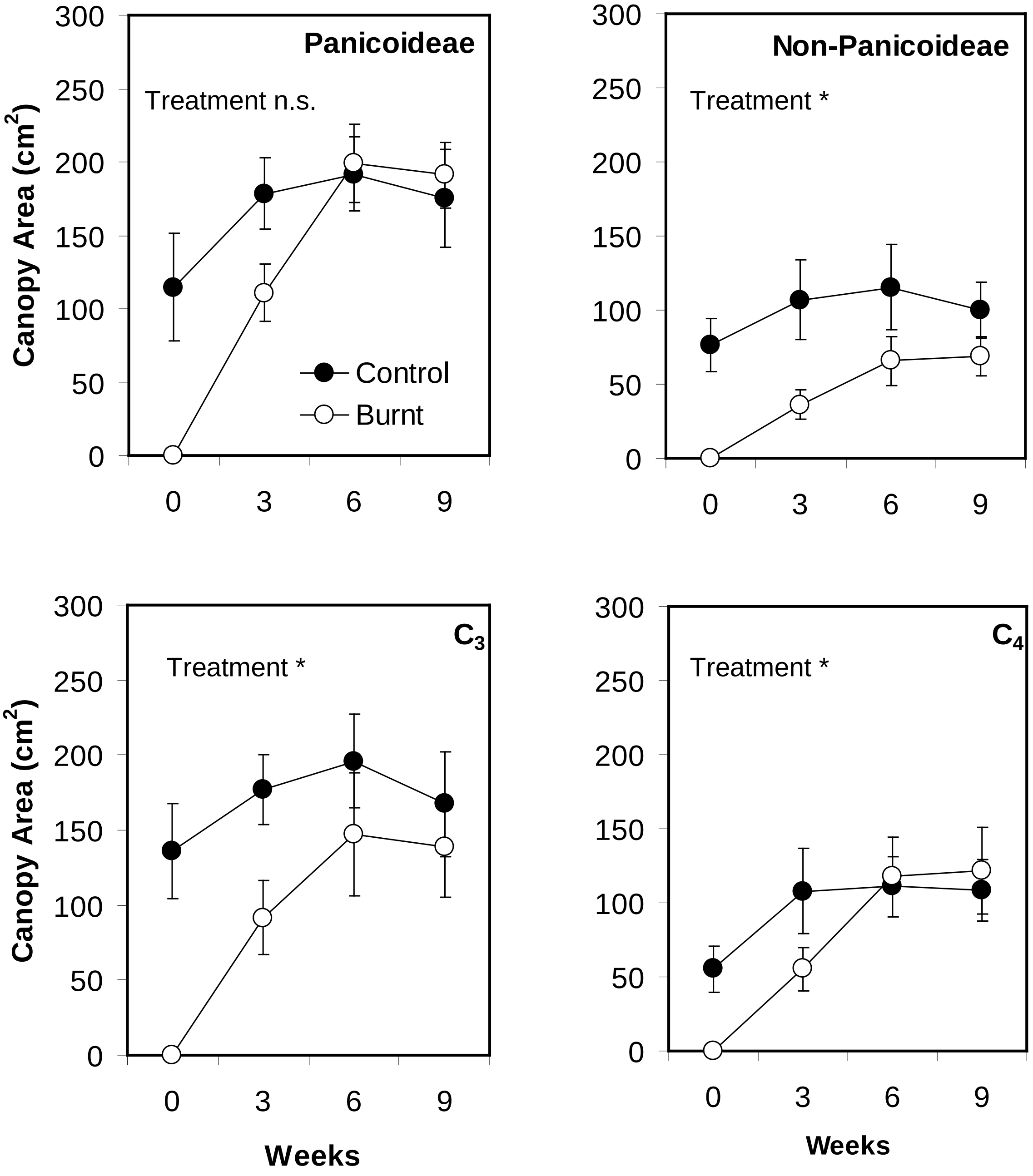

Figure 4.2.1.1: Average canopy area of control and burnt plants grouped by phylogenetic group and photosynthetic type. The above-ground biomass was completely removed by the fire at 0 and statistical comparisons were made on data from plants harvested at weeks 3, 6 and 9. (n=6-7 and vertical bars represent standard errors). * indicates significant differences between treatments at $P < 0.05$ (Tukey HSD test).

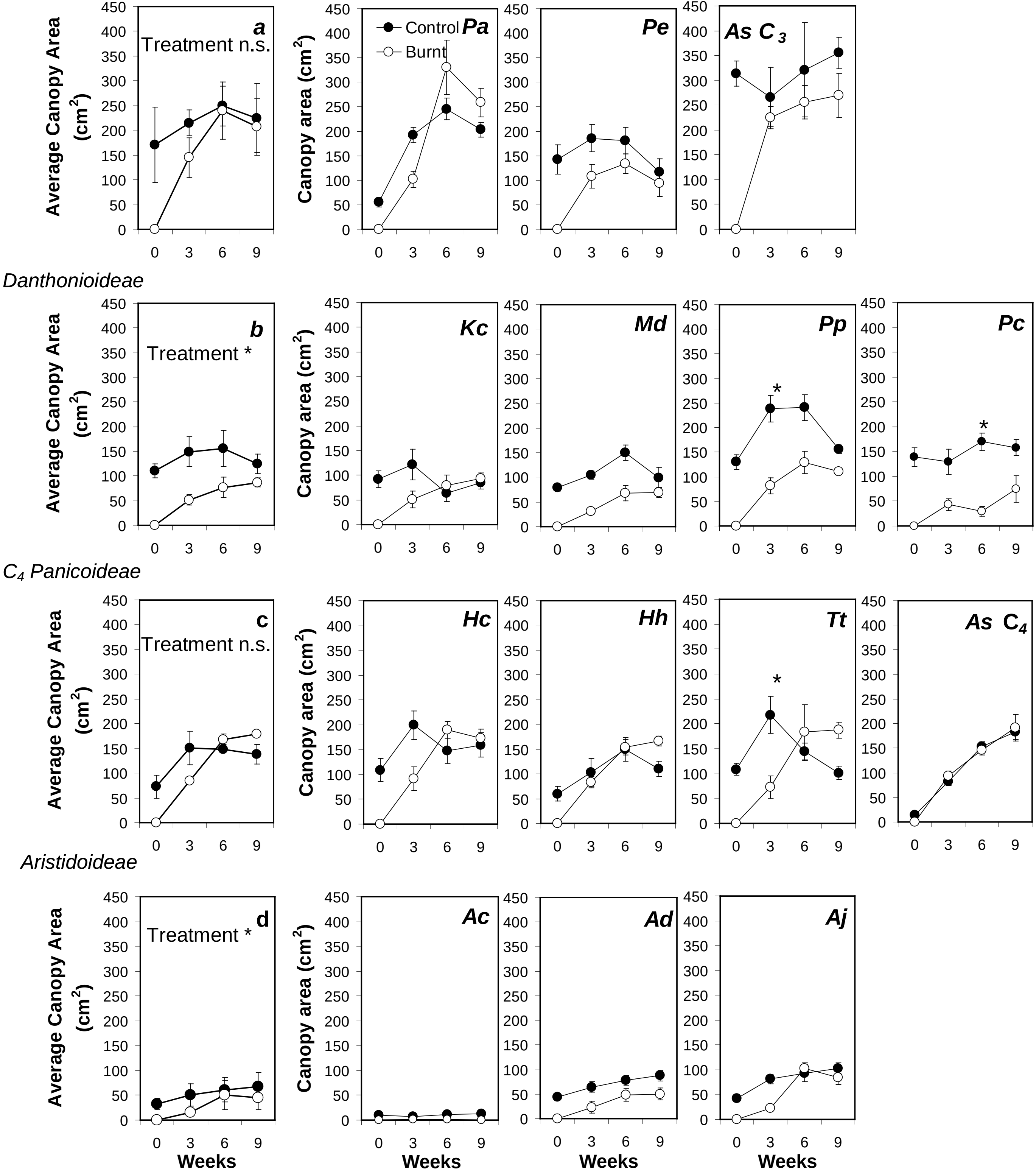

Figure 4.2.1.2: Canopy area of pot-cultivated control and burnt and grasses belonging to the indicated subfamilies. The above-ground biomass of the burnt treatment was completely removed by the fire at time 0 and statistical comparisons were made on data from plants harvested at weeks 3, 6 and 9. Panels a, b c and d indicate the responses average across species of a particular photosynthetic type, within each subfamily. Individual species responses are indicated as *Pa = P. aequinerve, Pe = P. ecklonii, As = A. semialata* subsp. *eckloniana*, *Kc = K. curva, Md = M. disticha, Pp = P. pallida, Pc = P. curvifolia, Hc = H. contortus, Hh = H. hirta, Tt = T. triandra, As = A. semialata* subsp. *semialata, Ac = A. congesta, Aj = A. junciformis, Ad = A. diffusa.* (n = 3-4 for subfamily, n= 5-6 per individual species and vertical bars represent standard errors). * indicates significant differences between treatments for each subfamily and between means of the control and burnt plants for individual species at $P < 0.05$ (Tukey HSD test).

Table 4.2.1: GLM results for differences in canopy leaf area, leaf mass, SLA, above-ground biomass and below-ground biomass between Panicoideae and non-Panicoideae phylogenetic groups with species nested in phylogenetic group. Statistical comparisons were made on data from plants harvested at weeks 3, 6 and 9. n.s., not significant; P > 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001.

	Canopy Leaf Area	**Leaf Mass**	**SLA**	**Above-ground Biomass**	**Below-ground Biomass**
Group	*** $_{,480}$ = 379.6	*** $_{,480}$ = 38.7	*** $_{,480}$ = 287.4	*** $_{,480}$ = 130.9	*** $_{,480}$ = 307.2
Species (Type)	*** $_{,480}$ = 35.3	*** $_{,480}$ = 35.9	*** $_{,480}$ = 40.7	*** $_{,480}$ = 29.1	*** $_{,480}$ = 43.5
Treatment	*** $_{,480}$ = 61.9	*** $_{,480}$ = 236.2	*** $_{,480}$ = 251.6	*** $_{,480}$ = 401.9	** $_{,480}$ = 7.3
Time	*** $_{,480}$ = 19.2	*** $_{,480}$ = 42.0	*** $_{,480}$ = 88.6	*** $_{,480}$ = 59.6	*n.s.* $_{,480}$ = 0.7
Time X Treatment	*** $_{,480}$ = 14.2	*** $_{,480}$ = 13.1	*** $_{,480}$ = 19.1	*** $_{,480}$ = 6.5	*n.s.* $_{,480}$ = 2.9
Group X Treatment	*** $_{,480}$ = 27.3	*** $_{,480}$ = 47.8	*** $_{,480}$ = 13.7	** $_{,480}$ = 8.9	** $_{,480}$ = 11.6
Group X Time	*n.s.* $_{,480}$ = 0.9	*n.s.* $_{,480}$ = 0.5	*n.s.* $_{,480}$ = 1.1	*n.s.* $_{,480}$ = 2.4	*n.s.* $_{,480}$ = 1.2
Group X Treatment X Time	*n.s.* $_{,480}$ = 0.6	*n.s.* $_{,480}$ = 0.2	*** $_{,480}$ = 5.0	*n.s.* $_{,480}$ = 0.6	*n.s.* $_{,480}$ = 0.9
	0.6672	0.6429	0.7359	0.6818	0.6417

Table 4.2.2: GLM results for differences in canopy leaf area, leaf mass, SLA, above-ground biomass and below-ground biomass between and photosynthetic types with species nested in photosynthetic type. Statistical comparisons were made on data from plants harvested at weeks 3, 6 and 9. n.s., not significant; P > 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001.

	Canopy Leaf Area	**Leaf Mass**	**SLA**	**Above-ground Biomass**	**Below-ground Biomass**
Type	*** $_{,480} = 97.4$	*** $_{,480} = 20.6$	*** $_{,480} = 75.4$	*n.s.* $_{,480} = 1.0$	*** $_{,480} = 17.8$
Species (Type)	*** $_{,480} = 56.5$	*** $_{,480} = 35.7$	*** $_{,480} = 58.6$	*** $_{,480} = 39.2$	*** $_{,480} = 66.8$
Treatment	*** $_{,480} = 59.8$	*** $_{,480} = 225.9$	*** $_{,480} = 236.9$	*** $_{,480} = 395.1$	** $_{,480} = 7.2$
Time	*** $_{,480} = 18.6$	*** $_{,480} = 40.2$	*** $_{,480} = 85.5$	*** $_{,480} = 58.5$	*n.s.* $_{,480} = 0.7$
Treatment x Time	*** $_{,480} = 13.7$	*** $_{,480} = 12.4$	*** $_{,480} = 18.1$	*** $_{,480} = 6.3$	*** $_{,480} = 6.3$
Type X Treatment	** $_{,480} = 9.5$	*** $_{,480} = 19.6$	** $_{,480} = 8.9$	*n.s.* $_{,480} = 0.5$	** $_{,480} = 7.4$
Type X Time	*n.s.* $_{,480} = 1.1$	* $_{,480} = 3.0$	*n.s.* $_{,480} = 1.7$	*n.s.* $_{,480} = 2.5$	*n.s.* $_{,480} = 0.9$
Type X Treatment X Time	*n.s.* $_{,420} = 0.5$	*n.s* $_{,480} = 0.3$	*n.s.* $_{,480} = 0.9$	*n.s.* $_{,480} = 0.5$	*n.s.* $_{,480} = 0.3$
	0.6553	0.6267	0.7293	0.6762	0.6374

Table 4.2.3: GLM results for differences in canopy leaf area, leaf mass, SLA, above - ground biomass and below-ground biomass between subfamilies with species nested in subfamily. Statistical comparisons were made on data from plants harvested at weeks 3, 6 and 9. n.s., not significant; P > 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001.

	Canopy Leaf Area	**Leaf**	**SLA**	**Above-ground Biomass**	**Below-ground Biomass**
Subfamily	*** $_{,420}$ = 213.5	*** $_{,420}$ = 51.0	*** $_{,420}$ = 248.7	*** $_{,420}$ = 101.9	*** $_{,420}$ = 180.4
Species (Subfamily)	*** $_{,420}$ = 28.1	*** $_{,420}$ = 46.4	*** $_{,420}$ = 35.1	*** $_{,420}$ = 36.5	*** $_{,420}$ = 32.3
Treatment	*** $_{,420}$ = 69.4	*** $_{,420}$ = 303.2	*** $_{,420}$ = 225.4	*** $_{,420}$ = 530.4	** $_{,420}$ = 8.7
Time	*** $_{,420}$ = 22.3	*** $_{,420}$ = 54.1	*** $_{,420}$ = 174.2	*** $_{,420}$ = 78.9	*n.s.* $_{,420}$ = 0.7
Time X Treatment	*** $_{,420}$ = 15.0	*** $_{,420}$ = 15.4	*** $_{,420}$ = 56.2	*** $_{,420}$ = 8.9	*n.s.* $_{,420}$ = 2.9
Subfamily X Treatment	*** $_{,420}$ = 12.8	*** $_{,420}$ = 26.8	*** $_{,420}$ = 16.4	*** $_{,420}$ = 12.4	*** $_{,420}$ = 7.1
Species (Subfamily) X Treatment	*** $_{,420}$ = 2.5	*** $_{,420}$ = 9.2	*** $_{,420}$ = 15.3	*** $_{,420}$ = 14.3	** $_{,420}$ = 2.3
Subfamily X Time	*n.s.* $_{,420}$ = 1.0	*n.s.* $_{,420}$ = 1.9	*** $_{,420}$ = 10.7	** $_{,420}$ = 3.3	*n.s.* $_{,420}$ = 0.8
Species (Subfamily) X Time	** $_{,420}$ = 2.2	** $_{,420}$ = 2.6	*** $_{,420}$ = 3.9	** $_{,420}$ = 1.8	*n.s.* $_{,420}$ = 1.2
Subfamily X Treatment X Time	*n.s.* $_{,420}$ = 1.7	* $_{,420}$ = 2.1	*** $_{,420}$ = 11.7	*n.s.* $_{,420}$ = 1.2	*n.s.* $_{,420}$ = 0.4
Species (Subfamily) X Treatment X Time	** $_{,420}$ = 2.1	** $_{,420}$ = 1.8	*** $_{,420}$ = 5.5	*n.s.* $_{,420}$ = 1.4	*n.s.* $_{,420}$ = 1.1

4.3.2.2. Leaf Mass

There was a significant difference between the burnt and control treatments for both the Panicoideae and non-Panicoideae; however the recovery of the leaf mass was more complete in the Panicoideae than the non-Panicoideae (Table 4.2.1 and Fig. 4.2.2.1). A similar pattern was observed between photosynthetic types with the leaf mass of the burnt treatment being significantly lower in both the and types but with the recovering more quickly than the (Table 4.2.2 and Fig. 4.2.2.1).

The values showed that the Panicoideae and non-Panicoideae model explained more variance than the photosynthetic model (Table 4.2.1 and 4.2.2) and this was supported by the subfamily response. The and Panicoideae recovered their leaf mass after six weeks whereas the Aristidoideae and Danthonioideae never recovered their leaf mass (Table 4.2.3 and Fig. 4.2.2.2). Species response within subfamilies varied within the Danthonioideae and the Aristidoideae subfamilies (Table 4.2.3 and Fig. 4.2.2.2). It was found that *K.* curva showed no significant difference between treatments which contrasted with the responses of the other three species. Within the Aristidoideae it was found that *A. congesta* showed no significant difference between treatments and *A. junciformis* was found to have recovered within six weeks. This contrasts the average subfamily response which showed that the Aristidoideae did not recover control leaf mass over the duration of the entire experiment. It is therefore possible that *A. diffusa* exerted a strong influence on the Aristidoideae subfamily response.

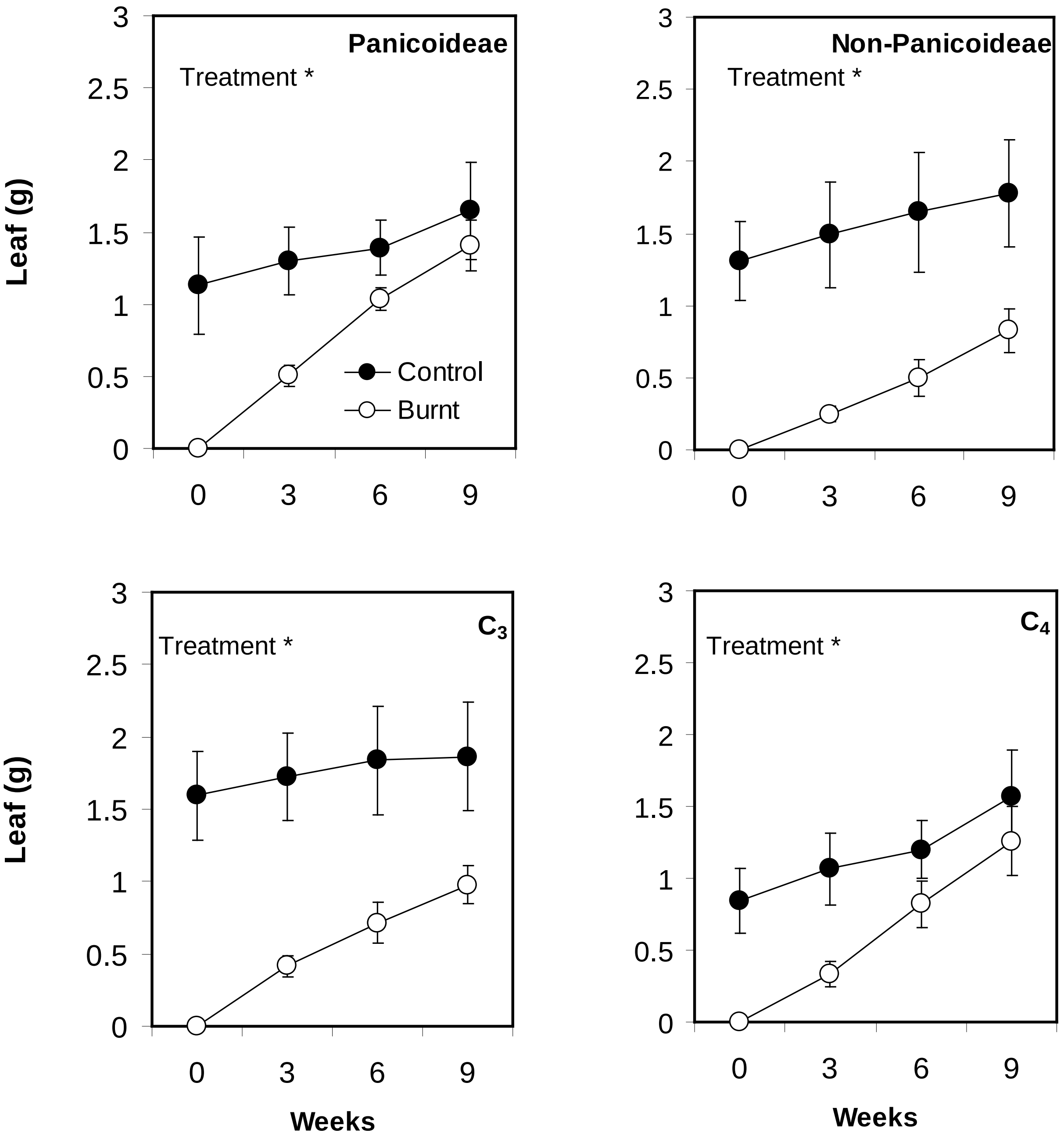

Figure 4.2.2.1: Average leaf mass of control and burnt plants grouped by phylogenetic group and photosynthetic type. The above-ground biomass was completely removed by the fire at 0 and statistical comparisons were made on data from plants harvested at weeks 3, 6 and 9. (n=6-7 and vertical bars represent standard errors). * indicates significant differences between treatments at $P < 0.05$ (Tukey HSD test).

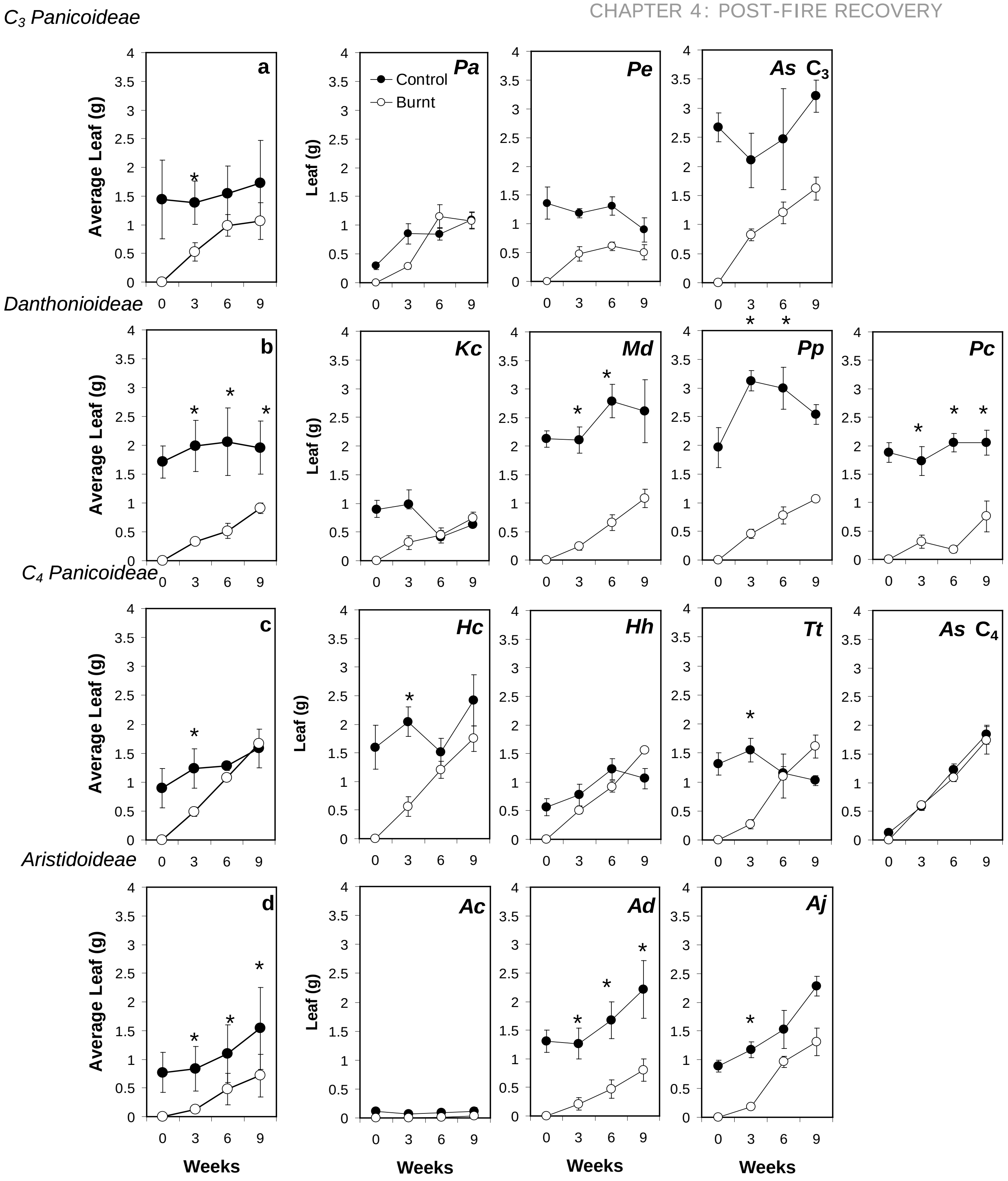

Figure 4.2.2.2: Leaf mass of pot-cultivated control and burnt and grasses belonging to the indicated subfamilies. The above-ground biomass of the burnt treatment was completely removed by the fire at time 0 and statistical comparisons were made on data from plants harvested at weeks 3, 6 and 9. Panels a, b c and d indicate the responses average across species of a particular photosynthetic type, within each subfamily. Individual species responses are indicated as *Pa = P. aequinerve, Pe = P. ecklonii, As = A. semialata* subsp. *ecklоniana*, *Kc = K. curva, Md = M. disticha, Pp = P. pallida, Pc = P. curvifolia, Hc = H. contortus, Hh = H. hirta, Tt = T. triandra, As = A. semialata* subsp. *semialata, Ac = A. congesta, Aj = A. junciformis, Ad = A. diffusa.* (n = 3-4 for subfamily, n= 5-6 per individual species and vertical bars represent standard errors). * indicates significant differences between means of the control and burnt plants at P < 0.05 (Tukey HSD test).

4.3.2.3. Specific Leaf Area (SLA)

The fast recovery of the Panicoideae canopy area was with leaves of reduced mass and hence increased SLA relative to the non-Panicoideae species (Table 4.2.1 and Fig. 4.2.3.1). SLA declined over subsequent harvests and the recovery was more rapid in the non-Panicoideae than the Panicoideae species implying that the non-Panioideae invested a larger amount of carbon in their leaves than the Panicoideae during the initial recovery. However, when compared between photosynthetic types, the SLA of the and species showed a significant difference between the burnt and control treatments respectively but the burnt treatments of both photosynthetic types appeared to recover to control values at similar rates (Table 4.2.2 and Fig. 4.2.3.1).

The value for the phylogenetic model was higher than that of the photosynthetic model. However, the subfamily responses showed that the Panicoideae and Danthonioideae did not recover their SLA, while the Panicoideae and Aristidoideae recovered their SLA by the ninth week (Table 4.2.3 and Fig. 4.2.3.2). The cost of recovery was therefore greater for the species than for the species despite the Panicoideae recovering their canopy area as fast as the Panicoideae.

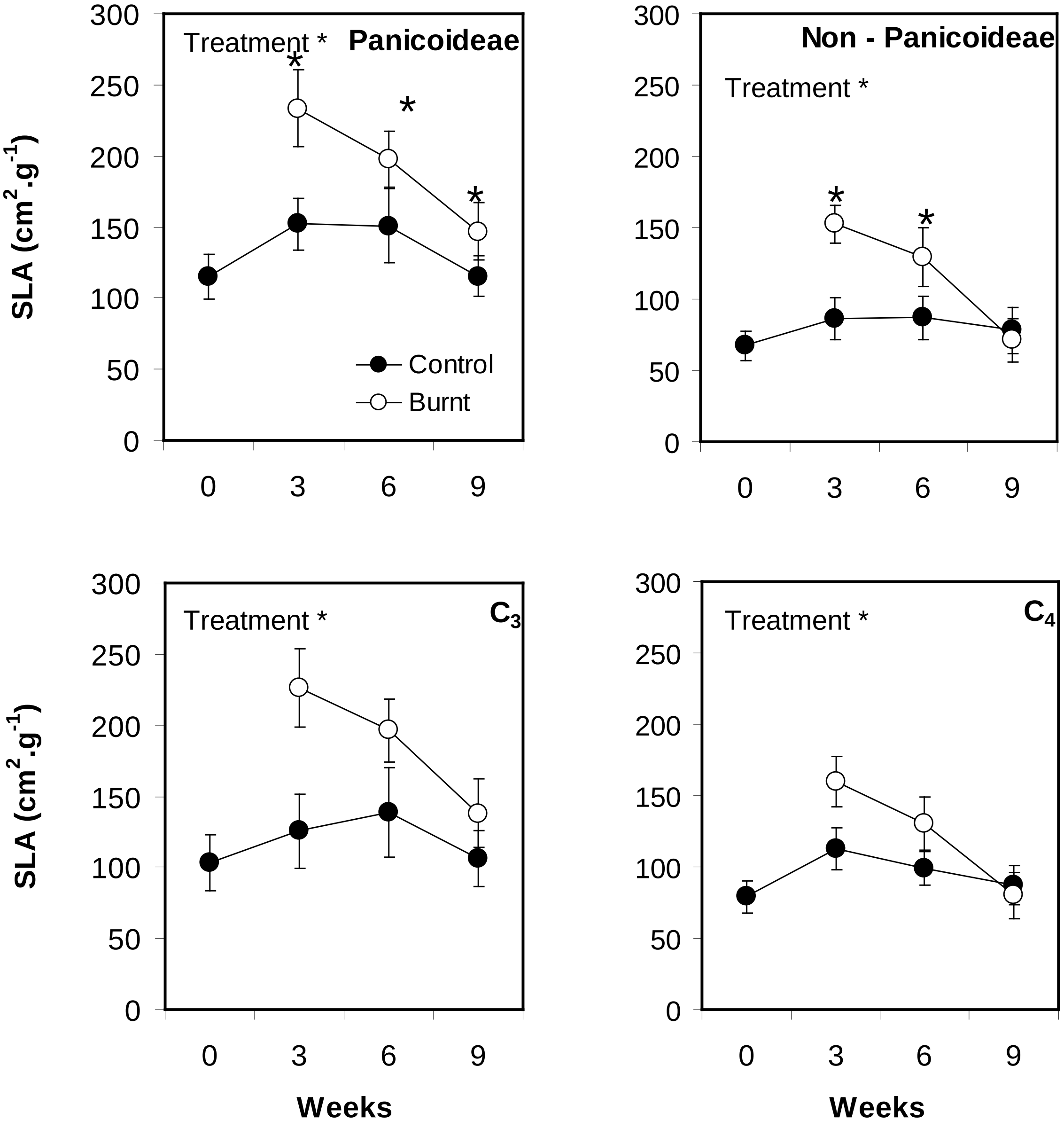

Figure 4.2.3.1: Average specific leaf area (SLA) of control and burnt plants grouped by phylogenetic group and photosynthetic type. The above-ground biomass was completely removed by the fire at 0 and statistical comparisons were made on data from plants harvested at weeks 3, 6 and 9. (n=6-7 and vertical bars represent standard errors). * indicates significant differences between means of the control and burnt plants at $P < 0.05$ (Tukey HSD test).

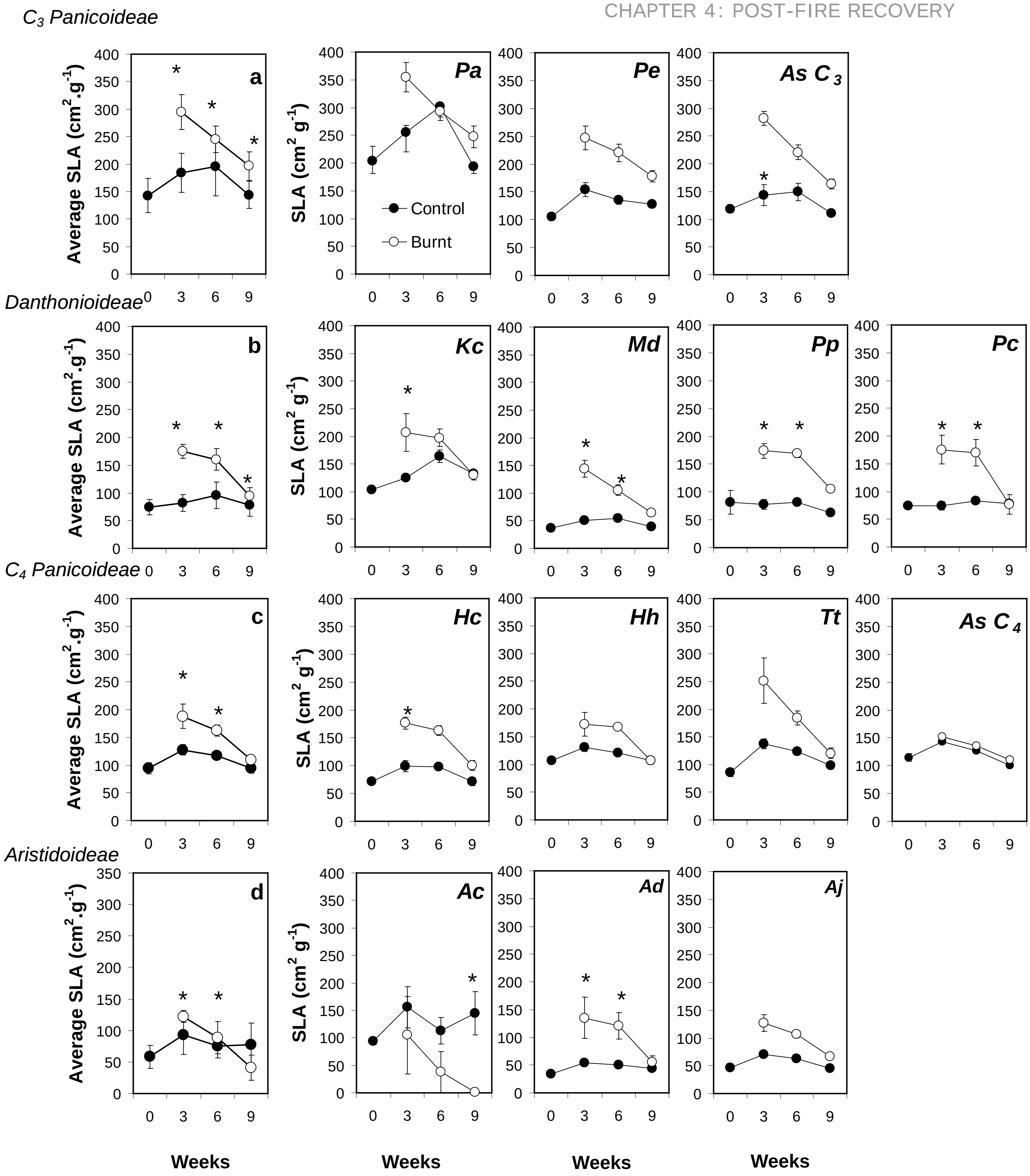

Figure 4.2.3.2: Specific Leaf Area (SLA) of pot-cultivated control and burnt and grasses belonging to the indicated subfamilies. The above-ground biomass of the burnt treatment was completely removed by the fire at time 0 and statistical comparisons were made on data from plants harvested at weeks 3, 6 and 9. Panels a, b c and d indicate the responses average across species of a particular photosynthetic type, within each subfamily. Individual species responses are indicated as *Pa = P. aequinerve, Pe = P. ecklonii, As = A. semialata* subsp. *eckloniana*, *Kc = K. curva, Md = M. disticha, Pp = P. pallida, Pc = P. curvifolia, Hc = H. contortus, Hh = H. hirta, Tt = T. triandra, As = A. semialata* subsp. *semialata, Ac = A. congesta, Aj = A. junciformis, Ad = A. diffusa.* (n = 3-4 for subfamily, n= 5-6 per individual species and vertical bars represent standard errors). * indicates significant differences between means of the control and burnt plants at P < 0.05 (Tukey HSD test).

4.3.2.4. Above-ground Biomass

Neither the Panicoideae nor the non-Panicoideae group recovered their above-ground biomass (Table 4.2.1 and Fig. 4.2.4.1) despite recovering their canopy area and leaf mass. This difference between the burnt and control plants was a result of the burnt species not investing in support tissue such as culms. In contrast, there was no significant difference between the and photosynthetic types (Table 4.2.2 and Fig. 4.2.4.1) adding further support that the recovery was based on phylogeny rather than physiology.

None of the subfamilies recovered their average above-ground biomass within the duration of the experiment (Table 4.2.3 and Fig. 4.2.4.2). Individual species responses were similar with the exception of *P. ecklonii*, *K. curva* and *A. semialata* subsp. *semialata* which all recovered their above-ground biomass (Table 4.2.3 and Fig. 4.3.4.2), probably because neither of these species invest resources in vegetative culms.

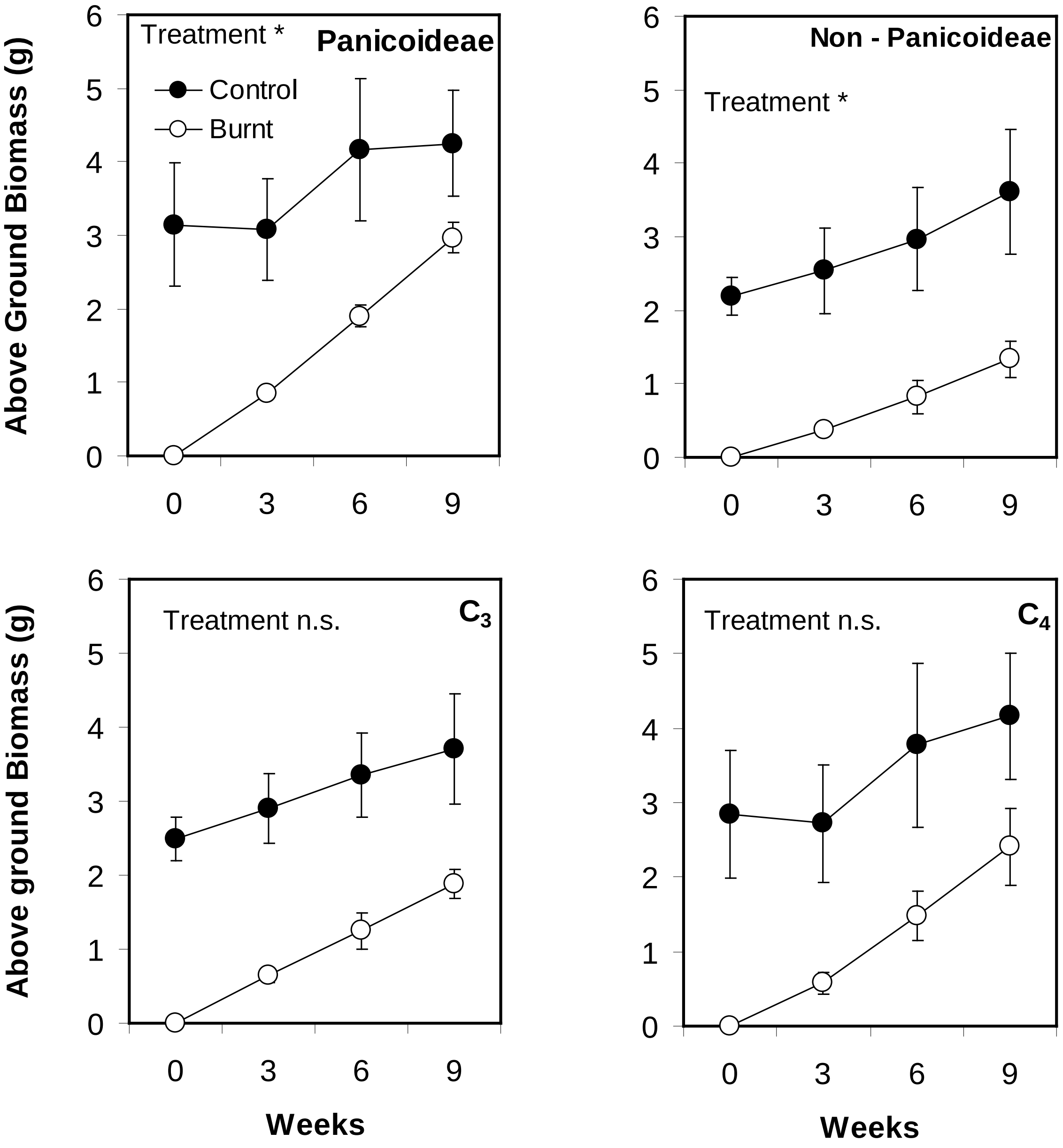

Figure 4.2.4.1: Average above-ground biomass of control and burnt plants grouped by phylogenetic group and photosynthetic type. The above-ground biomass was completely removed by the fire at 0 and statistical comparisons were made on data from plants harvested at weeks 3, 6 and 9. (n=6-7 and vertical bars represent standard errors). * indicates significant differences between treatments at $P < 0.05$ (Tukey HSD test).

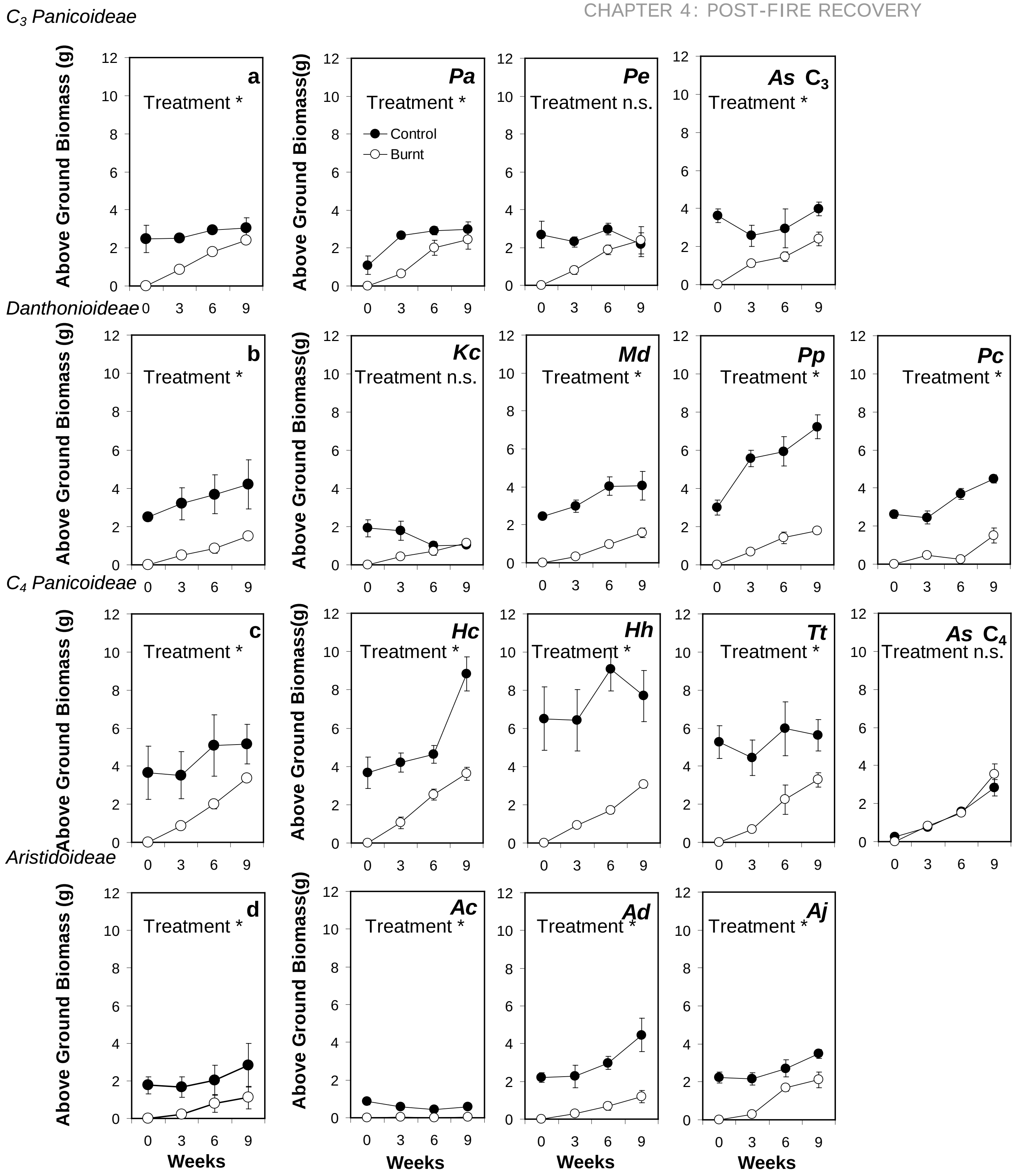

Figure 4.2.4.2: Above-ground biomass of pot-cultivated control and burnt and grasses belonging to the indicated subfamilies. The above-ground biomass of the burnt treatment was completely removed by the fire at time 0 and statistical comparisons were made on data from plants harvested at weeks 3, 6 and 9. Panels a, b c and d indicate the responses average across species of a particular photosynthetic type, within each subfamily. Individual species responses are indicated as *Pa* = *P. aequinerve, Pe* = *P. ecklonii, As* = *A. semialata* subsp. *eckloniana*, *Kc* = *K. curva, Md* = *M. disticha, Pp* = *P. pallida, Pc* = *P. curvifolia, Hc* = *H. contortus, Hh* = *H. hirta, Tt* = *T. triandra, As* = *A. semialata* subsp. *semialata, Ac* = *A. congesta, Aj* = *A. junciformis, Ad* = *A. diffusa.* (n = 3-4 for subfamily, n= 5-6 per individual species and vertical bars represent standard errors). * indicates significant differences between treatments at P < 0.05 (Tukey HSD test).

4.3.2.5. Below-ground Biomass

The Panicoideae group showed no significant change in the below-ground biomass of the burnt and control treatments suggesting that there was no reallocation from below ground reserves in the burnt plants (Table 4.2.1 and Fig. 4.2.5.1). In contrast, there was a significant decrease in the below-ground biomass of the non-Panicoideae suggesting that there was a high reallocation cost to these species.

The photosynthetic types showed the same pattern with the species not being affected by fire while there was a cost of reallocation to the species (Table 4.2.2 and Fig. 4.2.5.1).

However, at the subfamily level only the Danthonioideae showed a significant decrease in below ground-biomass while the other three subfamilies showed no change over time (Table 4.2.3 and Fig. 4.2.5.2). Individual species responses generally showed a decline in below-ground biomass of both the control and burnt treatments (Fig. 4.2.5.2), implying that the species reallocate resources from below ground to the above-ground biomass at the onset of the growing season, irrespective of whether or not they’ve been burnt. The only species that was significantly affected by fire was *P. curvifolia* as this was the only species that showed a significant difference between the control and burnt treatments.

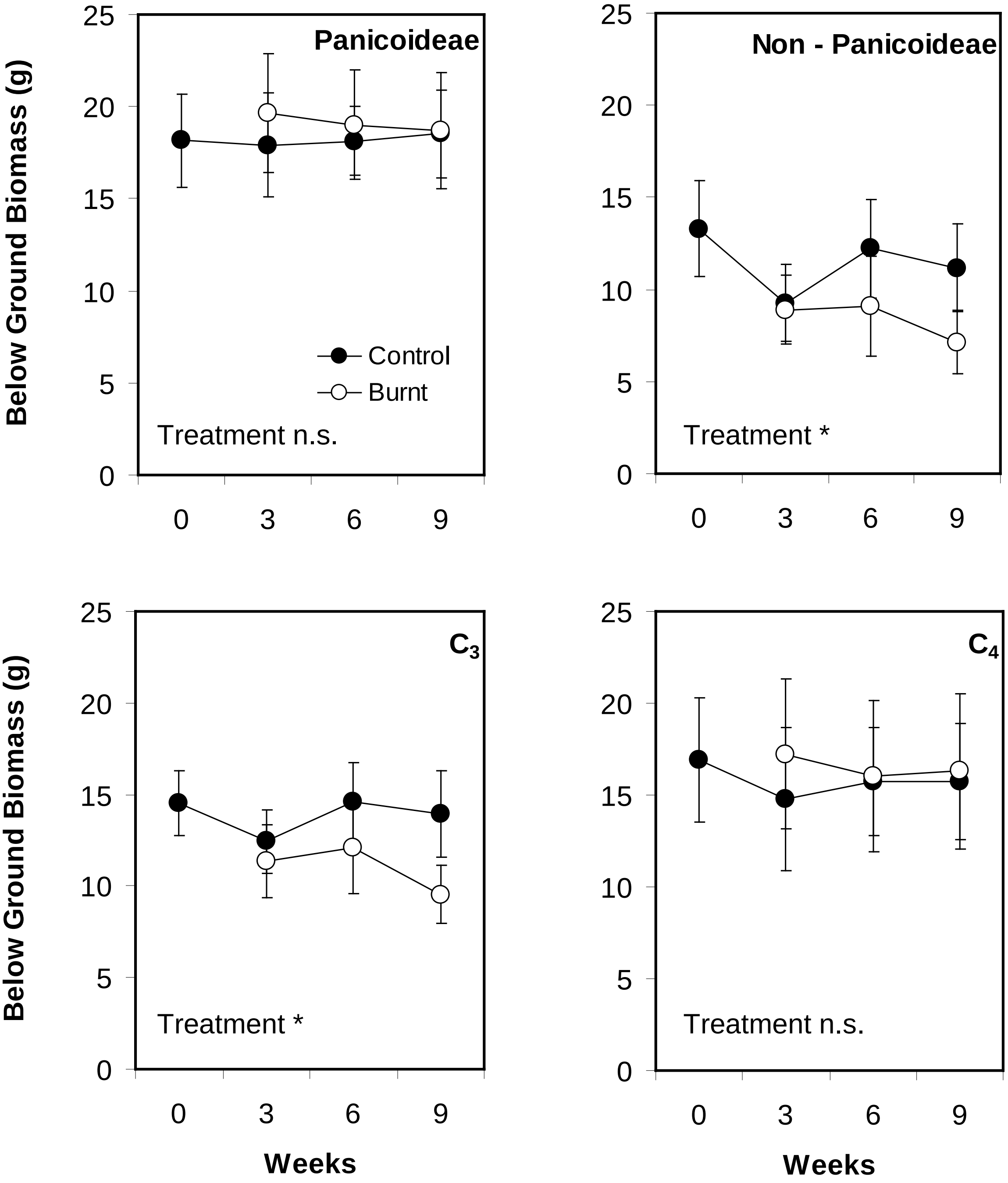

Figure 4.2.5.1: Average below-ground biomass of control and burnt plants grouped by phylogenetic group and photosynthetic type. Statistical comparisons were made on data from plants harvested at weeks 3, 6 and 9. (n=6-7 and vertical bars represent standard errors). * indicates significant differences between treatments at $P < 0.05$ (Tukey HSD test).

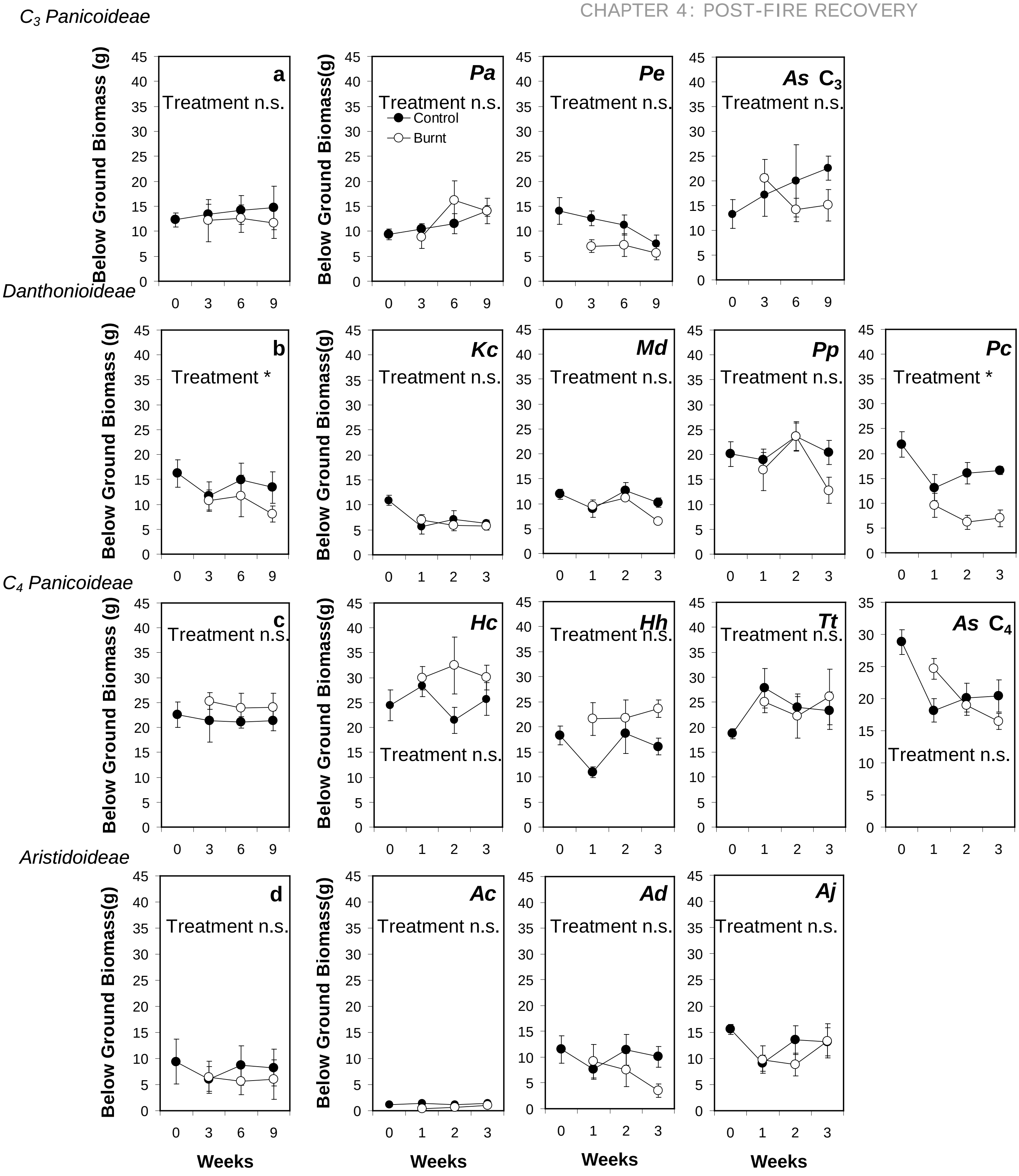

Figure 4.2.5.2: Below ground-biomass of pot-cultivated control and burnt and grasses belonging to the indicated subfamilies. Statistical comparisons were made on data from plants harvested at weeks 3, 6 and 9. Panels a, b c and d indicate the responses average across species of a particular photosynthetic type, within each subfamily. Individual species responses are indicated as *Pa = P. aequinerve, Pe = P. ecklonii, As = A. semialata* subsp. *eckloniana*, *Kc = K. curva, Md = M. disticha, Pp = P. pallida, Pc = P. curvifolia, Hc = H. contortus, Hh = H. hirta, Tt = T. triandra, As = A. semialata* subsp. *semialata, Ac = A. congesta, Aj = A. junciformis, Ad = A. diffusa.* (n = 3-4 for subfamily, n= 5-6 per individual species and vertical bars represent standard errors). * indicates significant differences between treatments for each subfamily and individual species at P < 0.05 (Tukey HSD test)

4.3.3 Summary of Results

The field experiment showed the species to recover more quickly than the species but when *P. aequinerve* and *T. triandra* were removed the recovery of the and species was found to be the same. *P. aequinerve* showed a fast recovery and can possibly be explained by this species being opportunistic under favourable conditions, while *T. triandra* showed a slow recovery. The delayed recovery of *T. triandra* could be due to *T. triandra* already having begun spring regrowth at the time of the fire highlighting the adverse effects of a mid-season fire on the recovery of a species.

In contrast, the pot experiment showed the species to recover more quickly than the species but when phylogenetic history was accounted for it was found that the Panicoideae recovered more quickly and completely than the non-Panicoideae. The above-ground recovery of the Panicoideae showed no impact on the below-ground reserves and can possibly be explained by the Panicoideae reallocating reserves above-ground irrespective of whether or not they have been burnt, since they would need to replace the live tissue that died back during the dry season. However, the burnt non-Panicoideae showed a decline in below-ground biomass possibly as a result of having to replace the live tissue that was removed during the fire.

CHAPTER 5: MECHANISMS OF POST FIRE RECOVERY

5.1. INTRODUCTION

Fire has been shown to stimulate the growth of some species while inhibiting others (Knapp, 1985; Knapp *et al.*, 1998; Pate, 1993; Pate *et al.*, 1991). Reallocation and photosynthetic efficiency, along with other characteristics (see Chapter 4 for more detail), play an important role in the recovery, and thus stimulation or inhibition, of a species after being burned (Bellingham, 2000; Bond and van Wilgen, 1996; Knapp, 1985; Morgan, 1999). For example, Knapp (1985) compared the photosynthetic rates of two co-occurring grass species. It was found that burning increased the photosynthetic rate of the dominant species, increasing leaf thickness and shoot biomass, but had no impact on the less dominant species. Similarly, studies in the Konza Prairie have shown that for a particular species, root biomass increased in burned regions compared to unburned regions (reviewed in Blair *et al.*, 1998) suggesting the importance of below ground storage in supporting above ground growth in frequently burnt ecosystems.

The aim of this study was to assess whether species growth after fire was inhibited or stimulated and to determine the contribution of photosynthesis and the reallocation of resources, to this response. The contribution of these processes was assessed using the control, burnt and dark-adapted treatments, of which the calculations and assumptions are described in detail below.

5.2. METHODS AND MATERIALS

5.2.1 Patterns of Reallocation, Photosynthesis, Fire Inhibition and Stimulation

As discussed in detail in Chapter 2 (Methods and Materials), the plants were harvested, separated into their different components, dried in an oven at 70°C until constant weight was obtained and the weights recorded.

Plant growth response after burning was attributed to four processes; reallocation, photosynthesis, fire stimulation and fire inhibition. The contribution of each process was measured using data from the pot experiment. For this experiment four sets of six plants of each species were harvested at three week intervals (See Chapter 2 for details). For the plants harvested subsequent to the experimental burn, plants were either allowed to recover under normal conditions (referred to as burnt plants) in the greenhouse or were recovered in the dark (referred to as dark plants). This was done to allow the contributions of photosynthesis and reallocation to be determined.

To determine the contribution of each process the following assumptions and calculations were made for the above-ground components:

1) The above-ground growth of the dark plants was due to reallocation from below-ground reserves only. Therefore, any above-ground biomass produced by these plants was an indication of how much

carbon was being reallocated from below-ground reserves i.e. Reallocation = Dark plants above-ground biomass

2) The above-ground growth of the burnt plants was due to both reallocation and photosynthesis. Therefore, the contribution of photosynthesis to above ground re-growth was calculated by subtracting the amount of above-ground biomass reallocated in the dark treatment from the amount accumulated by the plants recovered in the burnt treatment
   i.e. The Photosynthetic contribution = Burnt – Dark plant above-ground biomass

3) The above-ground growth of the control plants was due to reallocation and photosynthesis but this was influenced by fire which could potentially stimulate or inhibit growth. If stimulated then burnt plant biomass was greater than control biomass and Fire Stimulus = Burnt – Control biomass. Whereas, if inhibited then control biomass was greater than burnt biomass and Fire Inhibition = Control – Burnt biomass

To determine the effect of burning on below-ground allocation patterns the following assumptions were made:

1) The decrease in below-ground biomass of the dark-adapted plants was assumed to be due to reallocation and respiration
   i.e. Reallocation and Respiration = Burnt – Dark biomass

2) The burnt plants use their below-ground resources for reallocation and respiration but their re-growing leaves are also able to photosynthesise and can therefore “recharge” their below-ground biomass i.e. they increase their below-ground biomass and thus storage ability by shunting photosynthates below ground. This response could either be stimulated or inhibited by fire. If stimulated by fire then (Burnt - Dark) was greater than (Control - Dark) and Stimulated Recharge = (Burnt - Dark) – (Control - Dark). However, if (Control-Dark) was greater than (Burnt – Dark) then the response was inhibited by fire and Inhibited Recharge = (Control – Dark) – (Burnt – Dark).

5.2.2. Data Analysis

5.2.2.1 Above-ground Analyses

The data analysis of the canopy area, leaf mass and above-ground biomass was done in two parts. The first part was to determine whether it was valid to subtract one treatment from the other (as described above) based on whether differences between means of treatments were significant. Two analyses were used to determine this; one compared the burnt and the dark treatments to determine the photosynthetic contribution while the second one used the results from Chapter 4, which compared the burnt and control treatments, to determine patterns of fire stimulation and fire inhibition.

5.2.2.1a Analysis to determine whether the calculation of each process was significantly valid

To determine the photosynthetic contribution (Burnt – Dark treatment), a 2-level nested general linear model (GLM) was used to measure differences between species, subfamilies, phylogenetic groups and photosynthetic types on the absolute values for canopy leaf area, leaf mass, above-ground biomass and below-ground biomass. Three separate analyses were run in each case as partitions were calculated for averages of phylogenetic groups, photosynthetic types and subfamilies,. The first analysis compared the phylogenetic groups (Panicoideae verse non-Panicoideae) with species nested in group; the second analysis compared photosynthetic types (verse) with species nested in type while the third analysis compared subfamilies (Panicoideae, Danthonioideae, Panicoideae and Aristidoideae) with species nested in subfamily. A significant 3-way interaction (e.g. Group x Treatment x

Time) validated whether it was acceptable to subtract one treatment from the other to determine the photosynthetic contribution.

Using the same rationale, the statistical analyses from Chapter 4 were used to determine whether the control and burnt treatments were significantly different and hence validate whether fire stimulation and fire inhibition could be calculated (see Chapter 4 for details and tables).

5.2.2.1b Analyses used to determine whether the processes differed between phylogenetic groups, photosynthetic types and subfamilies

The second part of the analysis was used to determine whether there was a significant difference between phylogenetic groups, photosynthetic types and subfamilies for each process e.g. did the photosynthetic contribution to canopy area between the Panicoideae and non-Panicoideae differ? This analysis was only run if there was a significant difference between treatments, validating the calculations used to determine each component. For this analysis a two-way GLM using species averages, was used.

Since aboveground reallocation patterns were calculated based on the re-growth of the dark treatment (i.e. it was based on a single value), reallocation was compared between groups, types and subfamilies using a two-way GLM as described above.

5.2.2.2 Below-ground Analyses

The data analysis for the below-ground biomass was done in two parts as with the above ground components, except that all three treatments were compared in one analysis since all three were used to calculate recharge.

The raw data was transformed where required. Statistical differences between means were determined by Tukey HSD *post-hoc* tests when the results were significant.

5.3. RESULTS

5.3.1 Canopy Area

The Panicoideae showed a significantly larger reallocation of resources to the recovery of the canopy area than the non-Panicoideae (Table 5.2.1 and Fig. 5.1.1). In contrast, there was no significant difference between the and photosynthetic types. The *post-hoc* tests between subfamilies supported this result with the and Panicoideae reallocating a significantly larger portion of resources to the recovery of the canopy area than the Danthonioideae and Aristidoideae (Table 5.2.2 and Fig. 5.1.2). The Panicoideae were therefore better adapted than the non-Panicoideae at reallocating their below ground resources to support the recovery of the canopy area.

In addition to the reallocation of resources, there was a significant photosynthetic contribution to the recovery of the canopy area at the subfamily and species level (Table 5.1.3 and Fig. 5.1.2) with the and Panicoideae having a significantly larger contribution than the Aristidoideae (Table 5.2.2). The species within each subfamily all showed a similar response (Fig. 5.1.2).

Despite a significant photosynthetic effect at the subfamily level, there was no significant difference between treatments at the level of the phylogenetic groups and photosynthetic types and therefore the contribution from photosynthesis, as well as whether the plants were inhibited or stimulated by fire, could not be validated at this level (Table 5.1.1 and 5.1.2 and Fig. 5.1.1).

Table 5.1.1: GLM results for differences in canopy leaf area, leaf mass, above-ground biomass and below-ground biomass between Panicoideae and non-Panicoideae phylogenetic groups with species nested in phylogenetic group for the burnt and dark adapted treatments. n.s., not significant; P > 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001.

	Canopy Leaf Area	**Leaf Mass**	**Above-ground Biomass**	**Below-ground Biomass**
Group	*** $_{,480}$ = 459.5	*** $_{,480}$ = 183.9	*** $_{,480}$ = 228.8	*** $_{,726}$ = 447.2
Species (Group)	*** $_{,480}$ = 24.3	*** $_{,480}$ = 15.8	*** $_{,480}$ = 9.8	*** $_{,726}$ = 64.5
Treatment	*** $_{,480}$ = 379.7	*** $_{,480}$ = 532.2	*** $_{,480}$ = 547.4	*** $_{,726}$ = 24.7
Time	*n.s.* $_{,480}$ = 2.1	*** $_{,480}$ = 60.6	*** $_{,480}$ = 72.0	** $_{,726}$ = 5.6
Time X Treatment	*** $_{,480}$ = 14.1	*** $_{,480}$ = 40.3	*** $_{,480}$ = 63.7	*** $_{,726}$ = 5.5
Group X Treatment	*** $_{,480}$ = 11.3	** $_{,480}$ = 7.0	*** $_{,480}$ = 28.4	** $_{,726}$ = 6.3
Group X Time	*n.s.* $_{,480}$ = 1.3	* $_{,480}$ = 3.2	*** $_{,480}$ = 9.0	*n.s.* $_{,726}$ = 0.6
Group X Treatment X Time	*n.s.* $_{,480}$ = 1.1	*n.s.* $_{,480}$ = 0.2	* $_{,480}$ = 3.7	*n.s.* $_{,726}$ = 1.1

Table 5.1.2: GLM results for differences in canopy leaf area, leaf mass, above-ground biomass and below-ground biomass between and photosynthetic types with species nested in photosynthetic type for the burnt and dark adapted treatments. n.s., not significant; P > 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001.

	Canopy Leaf Area	**Leaf Mass**	**Above-ground Biomass**	**Below-ground Biomass**
Type	*n.s* $_{,480}$ = 0.1	*** $_{,480}$ = 13.1	*** $_{,480}$ = 12.6	*** $_{,726}$ = 32.4
Species (Type)	*** $_{,480}$ = 67.4	*** $_{,480}$ = 29.9	*** $_{,480}$ = 25.3	*** $_{,726}$ = 98.4
Treatment	*** $_{,480}$ = 409.1	*** $_{,480}$ = 530.0	*** $_{,480}$ = 498.6	*** $_{,726}$ = 25.5
Time	*n.s.* $_{,480}$ = 2.3	*** $_{,480}$ = 60.3	*** $_{,480}$ = 65.6	** $_{,726}$ = 5.5
Treatment x Time	*** $_{,480}$ = 15.2	*** $_{,480}$ = 40.1	*** $_{,480}$ = 58.0	*** $_{,726}$ = 5.4
Type X Treatment	*** $_{,480}$ = 46.4	*n.s.* $_{,480}$ = 3.5	*n.s.* $_{,480}$ = 0.4	* $_{,726}$ = 4.0
Type X Time	*n.s.* $_{,480}$ = 3.1	*n.s.* $_{,480}$ = 3.0	*n.s.* $_{,480}$ = 2.3	*n.s.* $_{,726}$ = 0.5
Type X Treatment X Time	*n.s.* $_{,420}$ = 0.9	*n.s* $_{,480}$ = 1.2	*n.s.* $_{,480}$ = 0.6	*n.s.* $_{,726}$ = 0.5

Table 5.1.3: GLM results for differences in canopy leaf area, leaf mass, above-ground biomass and below-ground biomass between subfamilies with species nested in subfamily for the burnt and dark adapted treatments. n.s., not significant; P > 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001.

	Canopy Leaf Area	**Leaf**	**Above-ground Biomass**	**Below-ground Biomass**
Subfamily	*** $_{,420} = 216.0$	*** $_{,420} = 101.9$	*** $_{,420} = 113.4$	*** $_{,630} = 276.3$
Species (Subfamily)	*** $_{,420} = 30.6$	*** $_{,420} = 22.5$	*** $_{,420} = 12.4$	*** $_{,630} = 46.3$
Treatment	*** $_{,420} = 475.7$	*** $_{,420} = 736.8$	*** $_{,420} = 713.4$	** $_{,630} = 26.7$
Time	*n.s.* $_{,420} = 2.1$	*** $_{,420} = 80.1$	*** $_{,420} = 90.6$	** $_{,630} = 5.4$
Time X Treatment	*** $_{,420} = 19.3$	*** $_{,420} = 55.2$	*** $_{,420} = 82.1$	*** $_{,630} = 5.9$
Subfamily X Treatment	*** $_{,420} = 20.8$	*** $_{,420} = 6.6$	*** $_{,420} = 14.7$	*** $_{,630} = 3.9$
Species (Subfamily) X Treatment	*** $_{,420} = 5.4$	*** $_{,420} = 11.9$	*** $_{,420} = 11.7$	*** $_{,630} = 2.1$
Subfamily X Time	** $_{,420} = 3.0$	*** $_{,420} = 5.1$	*** $_{,420} = 6.7$	*n.s.* $_{,630} = 0.8$
Species (Subfamily) X Time	* $_{,420} = 1.6$	*** $_{,420} = 2.7$	** $_{,420} = 2.0$	*n.s.* $_{,630} = 1.3$
Subfamily X Treatment X Time	* $_{,420} = 2.5$	** $_{,420} = 2.6$	**. $_{,420} = 3.4$	*n.s.* $_{,630} = 0.8$
Species (Subfamily) X Treatment X Time	*n.s.* $_{,420} = 1.4$	** $_{,420} = 2.1$	*n.s.* $_{,420} = 1.6$	*n.s.* $_{,630} = 1.2$

Table 5.2.1: GLM results for differences in reallocation, photosynthesis, fire stimulation and fire inhibition between phylogenetic groups and photosynthetic types for canopy leaf area, leaf mass and above-ground biomass. n.s., not significant; P > 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001.

		Phylogenetic Group			**Photosynthetic Type**		
		Group	**Time**	**Group x Time**	**Type**	**Time**	**Type x Time**
Canopy Area	**Reallocation**	*** $_{,36} = 41.7$	*n.s.* $_{,36} = 0.5$	*n.s.* $_{,36} = 0.5$	*n.s.* $_{,36} = 1.2$	*n.s.* $_{,36} = 0.2$	*n.s.* $_{,36} = 0.3$
Leaf Mass	**Reallocation**	** $_{,36} = 9.2$	*n.s.* $_{,36} = 0.4$	*n.s.* $_{,36} = 0.3$	*n.s.* $_{,36} = 2.4$	*n.s.* $_{,36} = 0.3$	*n.s.* $_{,36} = 0.1$
Above-ground Biomass	**Reallocation**	*** $_{,36} = 24.3$	*n.s.* $_{,36} = 0.2$	*n.s.* $_{,36} = 0.1$	*n.s.* $_{,36} = 1.5$	*n.s.* $_{,36} = 0.1$	*n.s.* $_{,36} = 0.1$
	Photosynthesis	*** $_{,36} = 12.9$	*** $_{,36} = 21.8$	*n.s.* $_{,36} = 2.1$	*n.s.* $_{,36} = 0.01$	*** $_{,36} = 15.1$	*n.s.* $_{,36} = 0.4$

Table 5.2.2: GLM results for differences in reallocation, photosynthesis, fire stimulation and fire inhibition between subfamilies for canopy leaf area, leaf mass and above-ground biomass. n.s., not significant; P > 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001.

		Subfamily	**Time**	**Subfamily x Time**
Canopy Area	**Reallocation**	*** $_{,30} = 13.6$	*n.s.* $_{,30} = 0.6$	*n.s.* $_{,30} = 0.6$
	Photosynthesis	*** $_{,30} = 7.8$	***. $_{,30} = 7.8$	*n.s.* $_{,30} = 0.6$
Leaf Mass	**Reallocation**	** $_{,30} = 4.0$	*n.s.* $_{,30} = 0.3$	*n.s.* $_{,30} = 0.2$
	Photosynthesis	*n.s* $_{,30} = 1.6$	*** $_{,30} = 11.6$	*n.s.* $_{,30} = 0.6$
	Fire Inhibition	** $_{,30} = 5.2$	*n.s.* $_{,30} = 1.0$	*n.s.* $_{,30} = 0.4$
Above-ground Biomass	**Reallocation**	*** $_{,30} = 8.2$	*n.s.* $_{,30} = 0.2$	*n.s.* $_{,30} = 0.2$
	Photosynthesis	** $_{,30} = 4.2$	*** $_{,30} = 18.8$	*n.s.* $_{,30} = 1.0$
	Fire Stimulation	*n.s.* $_{,30} = 1.7$	*n.s.* $_{,30} = 0.2$	*n.s.* $_{,30} = 0.2$
	Fire Inhibition	*n.s.* $_{,30} = 1.7$	*n.s.* $_{,30} = 0.2$	*n.s.* $_{,30} = 0.2$

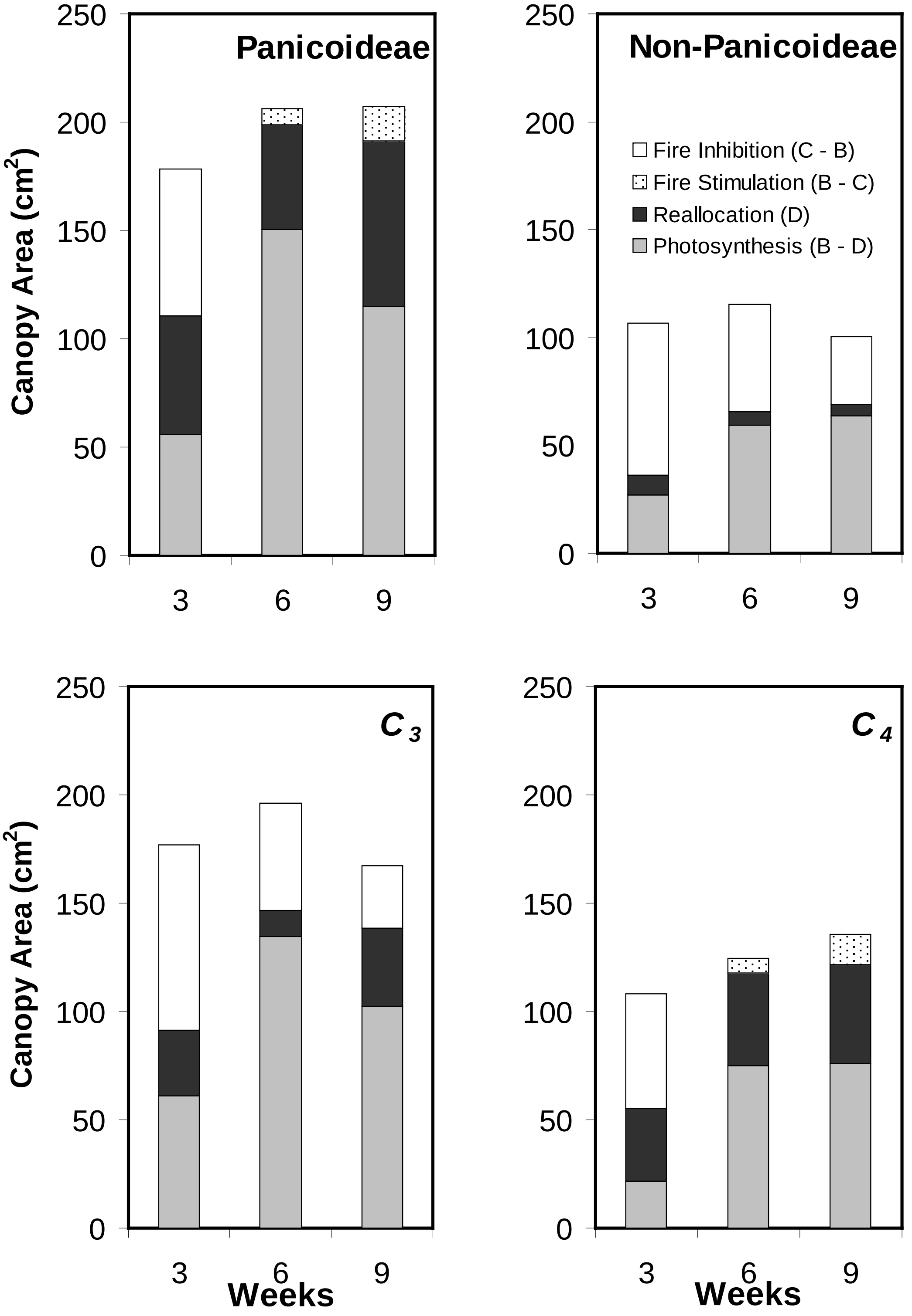

Figure 5.1.1: The contribution of photosynthesis (B-D), reallocation (D), fire stimulus (B-C) and fire inhibition (C-B) to the growth of the canopy area grouped by phylogenetic group and photosynthetic type, over three successive harvests. Abbreviations: Control Plants (C), burnt plants (B) and dark adapted plants (D). * indicates whether the subtraction one treatment from the other was valid based on whether differences between means of the treatments were significant P < 0.05 (Tukey HSD test).

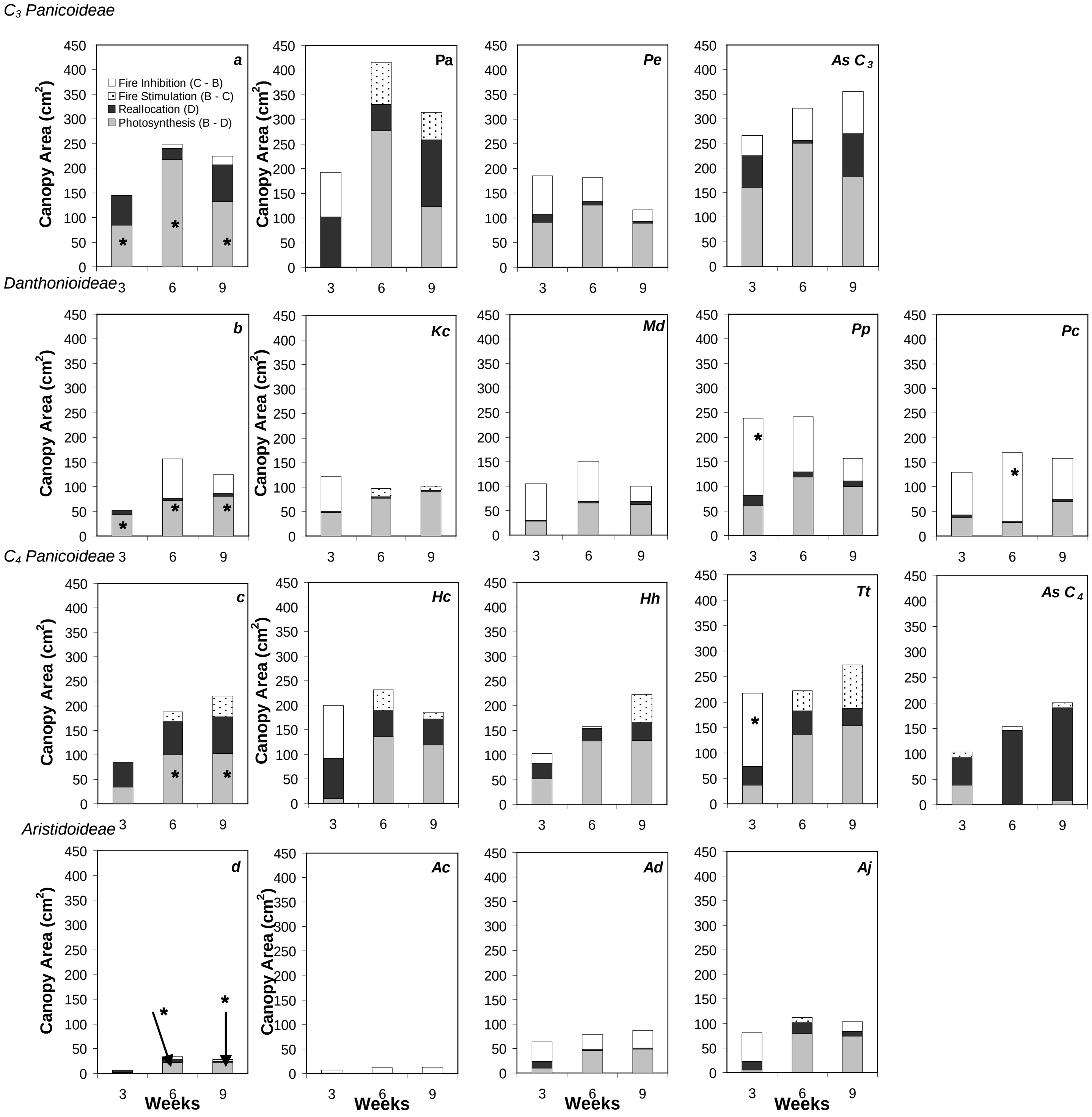

Figure 5.1.2: The contribution of photosynthesis (B-D), reallocation (D), fire stimulus (B-C) and fire inhibition (C-B) to the growth of the canopy area of and species grouped according to subfamily. Panels a, b c and d indicate the responses average across species of a particular photosynthetic type, within each subfamily. Individual species responses are indicated as *Pa = P. aequinerve, Pe = P. ecklonii, As = A. semialata* subsp. *eckloniana*, *Kc = K. curva, Md = M. disticha, Pp = P. pallida, Pc = P. curvifolia, Hc = H. contortus, Hh = H. hirta, Tt = T. triandra, As = A. semialata* subsp. *semialata, Ac = A. congesta, Aj = A. junciformis, Ad = A. diffusa.* Abbreviations: Control Plants (C), burnt plants (B) and dark adapted (D). (n= 5-6 per individual species). * indicates whether the subtraction one treatment from the other was valid based on whether differences between means of the treatments were significant P < 0.05 (Tukey HSD test).

5.3.2 Leaf Mass

As with the canopy area, the Panicoideae showed a significantly larger reallocation of resources to the recovery of the leaf mass than the non-Panicoideae (Table 5.2.1 and Fig. 5.2.1) suggesting that the Panicoideae were better adapted than the non-Panicoideae at reallocating resources from below-ground. In contrast to the phylogenetic comparison, there was no significant difference in reallocation patterns between the and photosynthetic types.

The photosynthetic contribution between phylogenetic groups and photosynthetic types could not be validated due to a non significant 3-way interaction.

At the subfamily level, the and Panicoideae reallocated a significantly larger portion of resources to the recovery of the leaf mass than the Danthonioideae and Aristidoideae, respectively (Table 5.2.2 and Fig. 5.2.2). This supported the phylogenetic model which showed that the Panicoideae reallocated a significantly larger proportion of resources above-ground. In addition a significant photosynthetic contribution was validated at the subfamily level (Table 5.1.3), however there was no difference in the photosynthetic contribution between subfamilies (Table 5.2.2).

The recovery of the leaf mass in the Danthonioideae was significantly inhibited by burning compared to the Panicoideae (Table 4.2.3 and Fig. 5.2.2) with species responses consistent with subfamily response. The results suggest that at the subfamily level, the Danthonioideae was severely affected by burning while the Panicoideae was the least affected.

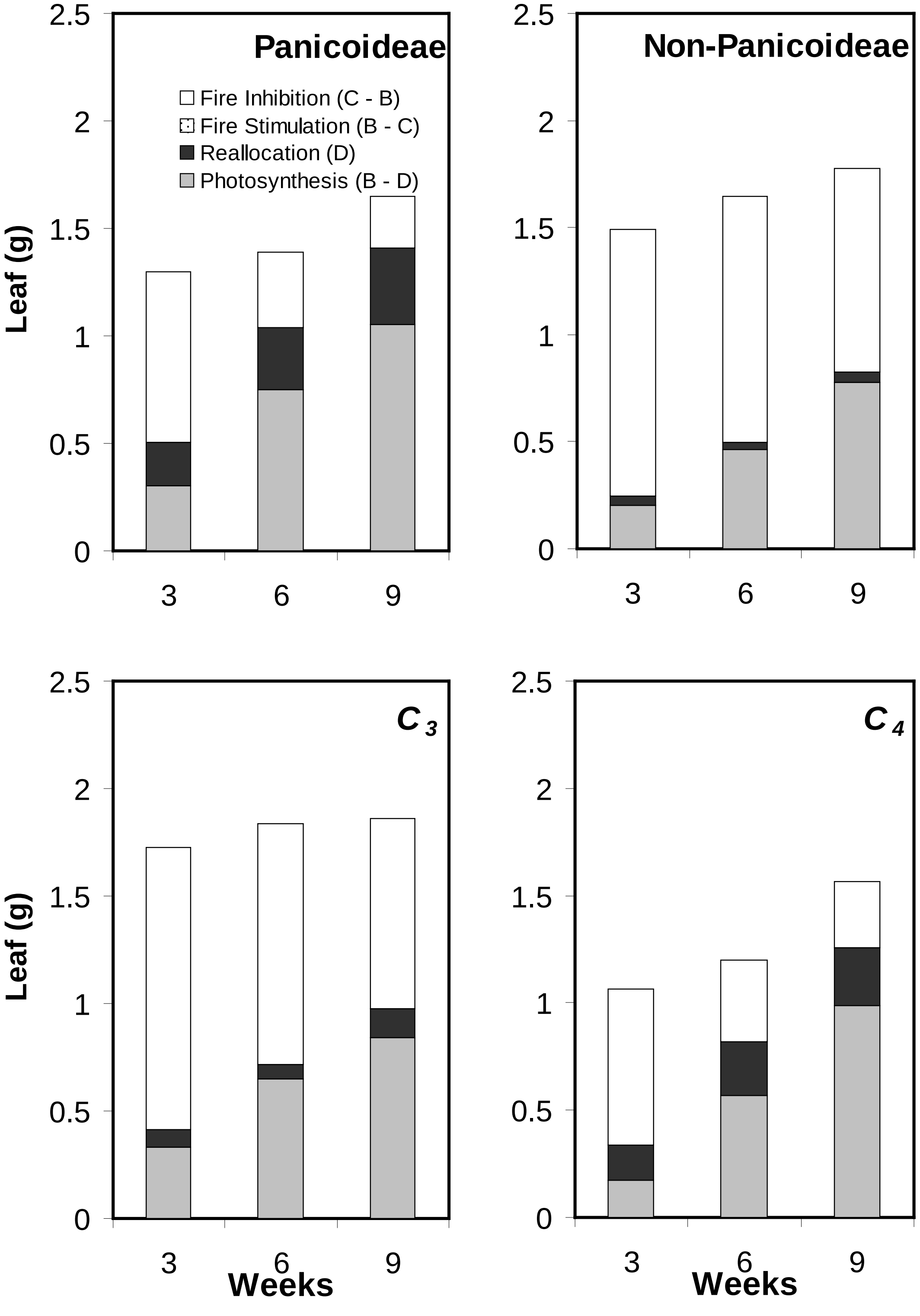

Figure 5.2.1: The contribution of photosynthesis (B-D), reallocation (D), fire stimulus (B-C) and fire inhibition (C-B) to the growth of the leaf mass grouped by phylogenetic group and photosynthetic type, over three successive harvests. Abbreviations: Control Plants (C), burnt plants (B) and dark adapted plants (D). * indicates whether the subtraction one treatment from the other was valid based on whether differences between means of the treatments were significant P < 0.05 (Tukey HSD test).

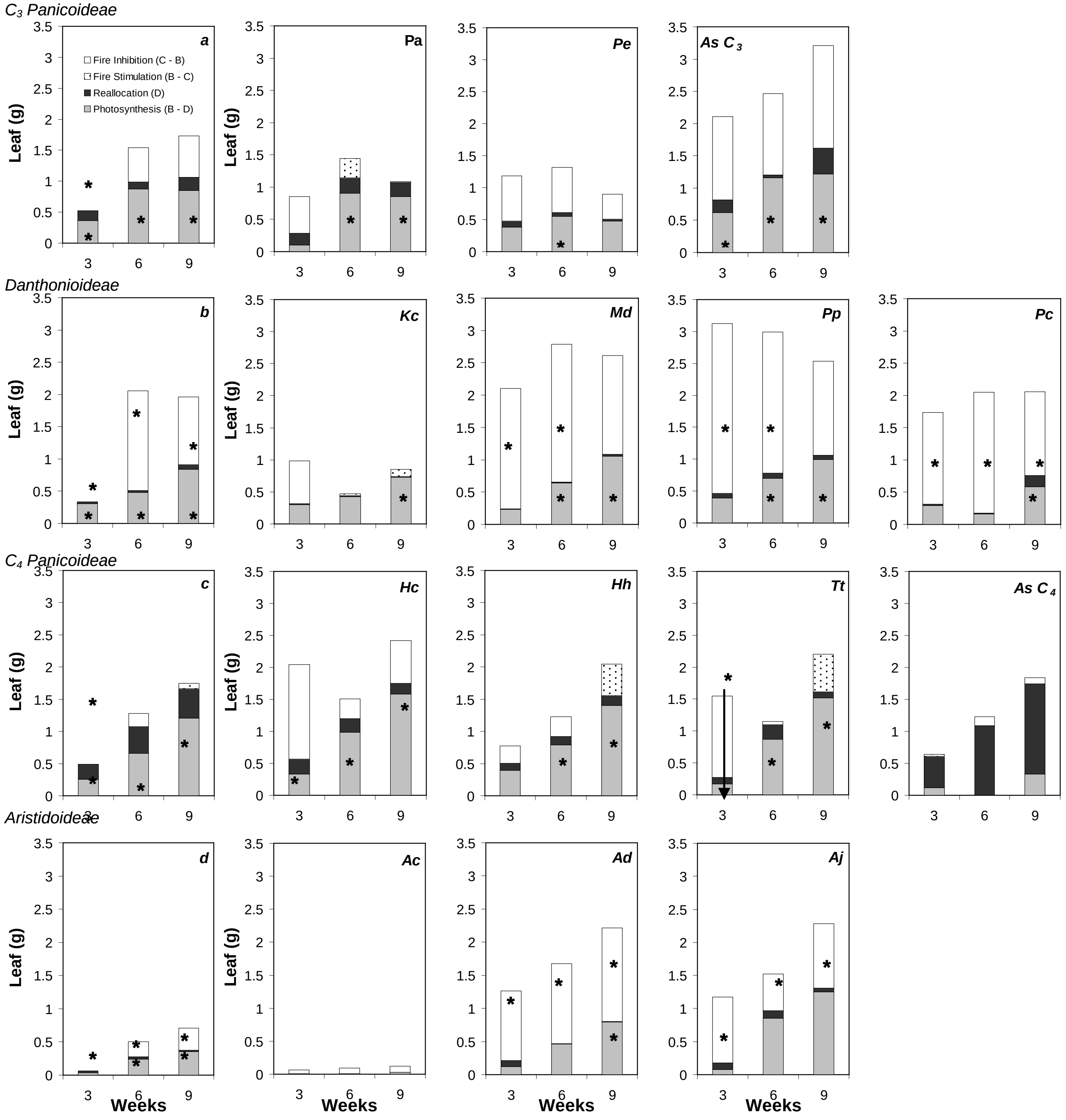

Figure 5.2.2: The contribution of photosynthesis (B-D), reallocation (D), fire stimulus (B-C) and fire inhibition (C-B) to the growth of the leaf mass of and species grouped according to subfamily. Panels a, b c and d indicate the responses average across species of a particular photosynthetic type, within each subfamily. Individual species responses are indicated as *Pa = P. aequinerve, Pe = P. ecklonii, As = A. semialata* subsp. *eckloniana*, *Kc = K. curva, Md = M. disticha, Pp = P. pallida, Pc = P. curvifolia, Hc = H. contortus, Hh = H. hirta, Tt = T. triandra, As = A. semialata* subsp. *semialata, Ac = A. congesta, Aj = A. junciformis, Ad = A. diffusa.* Abbreviations: Control Plants (C), burnt plants (B) and dark adapted (D). (n= 5-6 per individual species). * indicates whether the subtraction one treatment from the other was valid based on whether differences between means of the treatments were significant P < 0.05 (Tukey HSD test).

5.3.3 Above-ground Biomass

The Panicoideae reallocated a significantly larger portion of resources to the re-growth of the above-ground biomass than the non-Panicoideae (Table 5.2.1 and Fig. 5.3.1). However, the non-Panicoideae showed a significantly larger photosynthetic contribution to the re-growth than the Panicoideae (Table 5.1.1 and Table 5.2.1). In contrast, there was no difference between reallocation and photosynthetic patterns between the and photosynthetic types (Table 5.1.2 and Table 5.2.1).

The reallocation of resources was supported by the subfamily response. The Panicoideae reallocated a significantly larger portion of its resources to the above-ground growth than the Danthonioideae or Aristidoideae. However, the photosynthetic contribution at the subfamily level contradicted the above results since the Panicoideae had a significantly larger contribution than the Aristidoideae (Table 5.1.3 and 5.2.2 and Fig. 5.3.2).

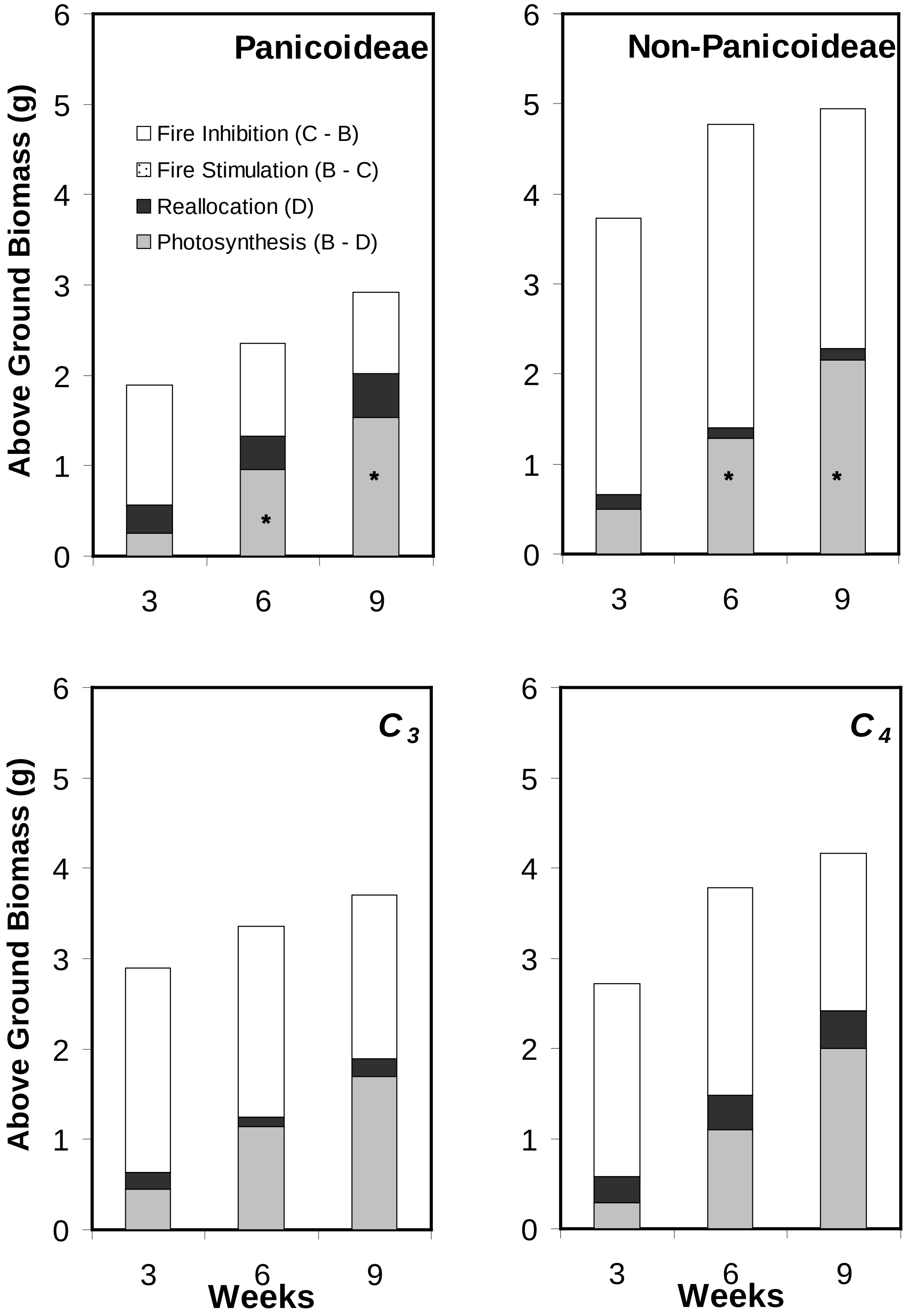

Figure 5.3.1: The contribution of photosynthesis (B-D), reallocation (D), fire stimulus (B-C) and fire inhibition (C-B) to the growth of the above-ground biomass grouped by phylogenetic group and photosynthetic type, over three successive harvests. Abbreviations: Control Plants (C), burnt plants (B) and dark adapted plants (D). * indicates whether the subtraction one treatment from the other was valid based on whether differences between means of the treatments were significant P < 0.05 (Tukey HSD test).

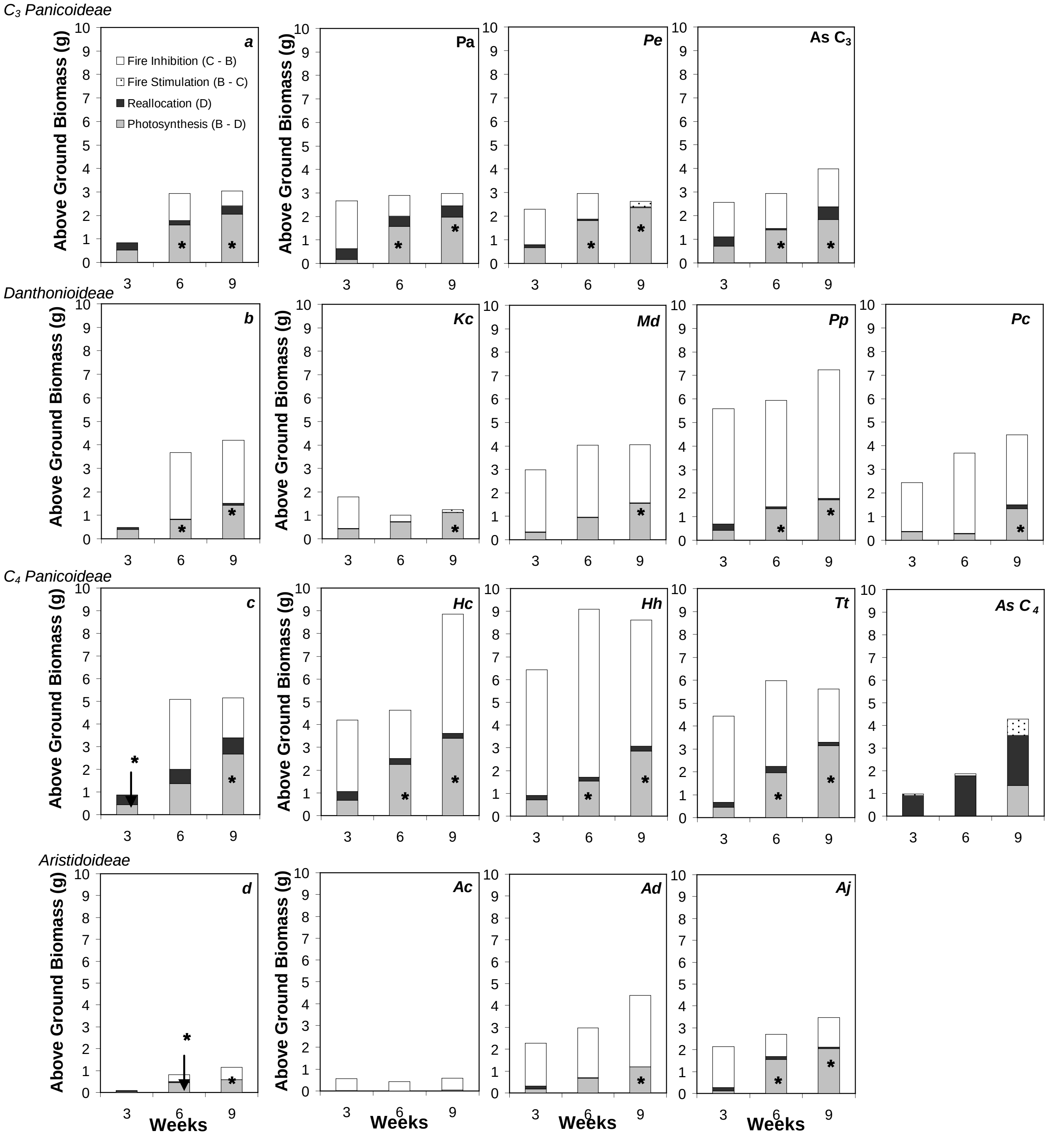

Figure 5.3.2: The contribution of photosynthesis (B-D), reallocation (D), fire stimulus (B-C) and fire inhibition (C-B) to the growth of the above-ground biomass of and species grouped according to subfamily. Panels a, b c and d indicate the responses average across species of a particular photosynthetic type, within each subfamily. Individual species responses are indicated as *Pa = P. aequinerve, Pe = P. ecklonii, As = A. semialata* subsp. *eckloniana*, *Kc = K. curva, Md = M. disticha, Pp = P. pallida, Pc = P. curvifolia, Hc = H. contortus, Hh = H. hirta, Tt = T. triandra, As = A. semialata* subsp. *semialata, Ac = A. congesta, Aj = A. junciformis, Ad = A. diffusa.* Abbreviations: Control Plants (C), burnt plants (B) and dark adapted (D). (n= 5-6 per individual species). * indicates whether the subtraction one treatment from the other was valid based on whether differences between means of the treatments were significant P < 0.05 (Tukey HSD test).

5.3.4 Below-ground Biomass

There was no significant difference between treatments at each time interval when compared between phylogenetic groups, photosynthetic types and subfamilies (Table 5.1.1, 5.1.2 and 5.2.1 and Fig. 5.4.1 and 5.4.2). It was therefore not valid to compare differences between phylogenetic groups, photosynthetic types and subfamilies.

However, despite a lack of significance the trends observed in the reallocation of below-ground resources supported the observed patterns of reallocation of above-ground resources with the Panicoideae reallocating more resources than the non-Panicoideae. These trends were supported by the subfamily responses with the and Panicoideae reallocating more resources from below-ground reserves than the Danthonioideae and Aristidoideae subfamilies.

5.3.5 Summary of Results

The Panicoideae showed a significantly larger reallocation of resources to the recovery of the canopy area, leaf mass and above-ground biomass compared to the non-Panicoideae. This implied that the Panicoideae were better adapted at utilising their below-ground reserves to recover from a fire than the non-Panicoideae. In addition, they also showed a greater photosynthetic contribution to the recovery of the canopy area. This was possibly as a result of the below-ground reserves initially supporting the fast recovery of the canopy area, creating a larger photosynthetic surface area which would have increased the photosynthetic contribution.

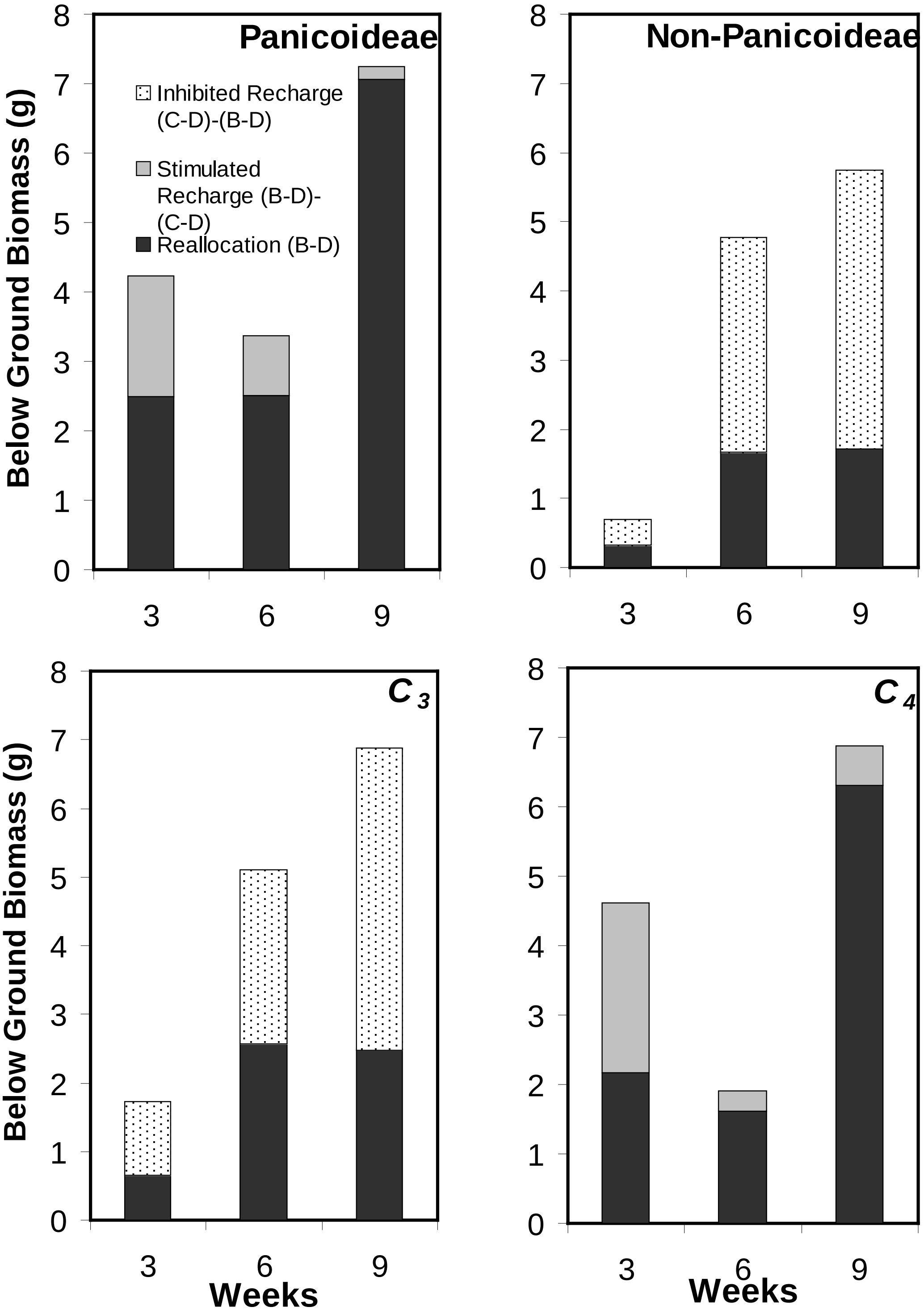

Figure 5.4.1: The contribution of reallocation (B-D), inhibited recharge (B-D)-(C-D) and stimulated recharge (C-D)-(B-D) from the below-ground biomass grouped by phylogenetic group and photosynthetic type, over three successive harvests. Abbreviations: Control Plants (C), burnt plants (B) and dark adapted plants (D). * indicates whether the subtraction one treatment from the other was valid based on whether differences between means of the treatments were significant P < 0.05 (Tukey HSD test).

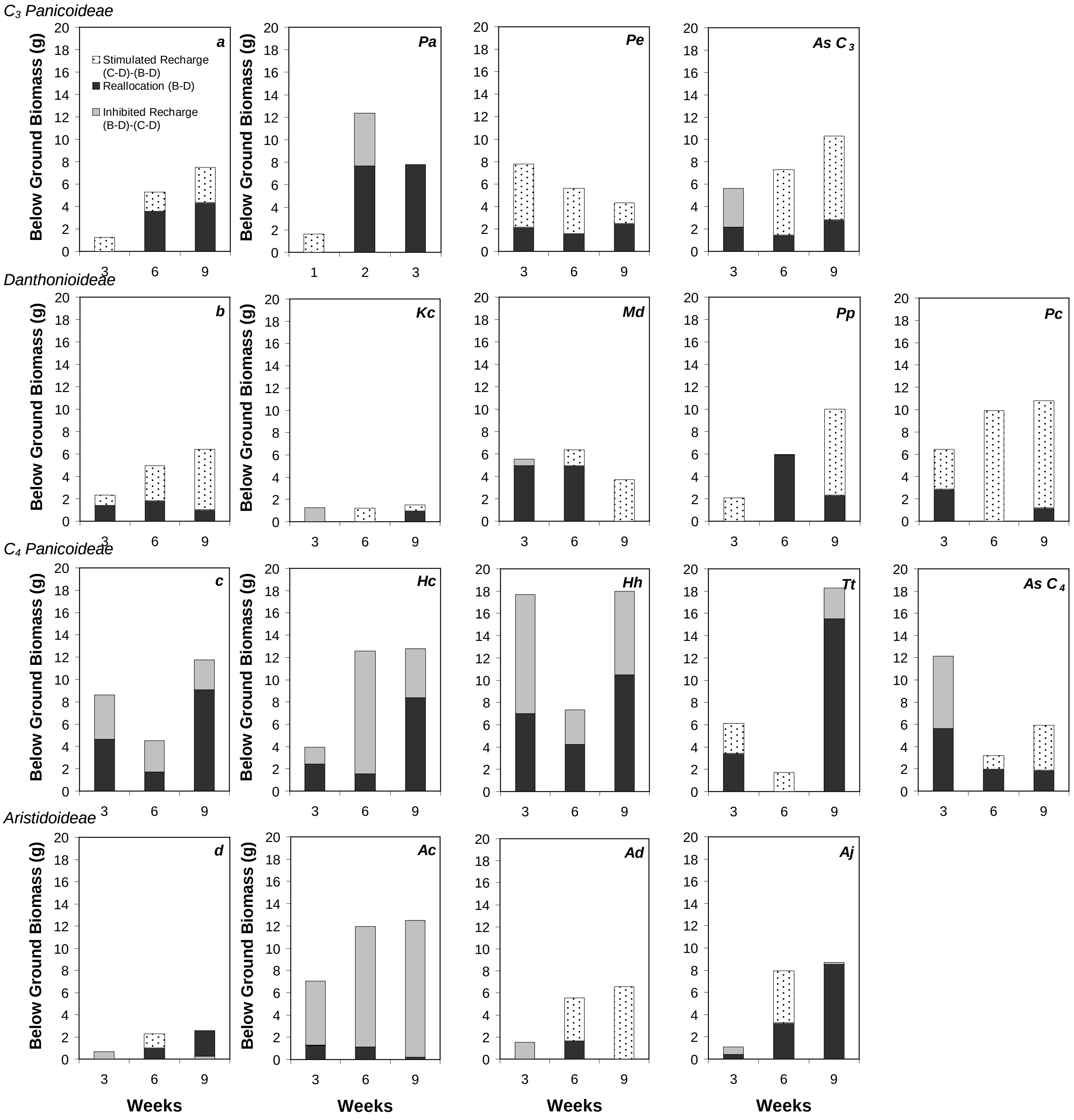

Figure 5.4.2: The contribution of reallocation (B-D), inhibited recharge (B-D)-(C-D) and stimulated recharge (C-D)-(B-D) from the below-ground biomass of and species grouped according to subfamily. Panels a, b c and d indicate the responses average across species of a particular photosynthetic type, within each subfamily. Individual species responses are indicated as *Pa = P. aequinerve, Pe = P. ecklonii, As = A. semialata* subsp. *eckloniana*, *Kc = K. curva, Md = M. disticha, Pp = P. pallida, Pc = P. curvifolia, Hc = H. contortus, Hh = H. hirta, Tt = T. triandra, As = A. semialata* subsp. *semialata, Ac = A. congesta, Aj = A. junciformis, Ad = A. diffusa.* Abbreviations: Control Plants (C), burnt plants (B) and dark adapted (D). (n= 5-6 per individual species* indicates whether the subtraction one treatment from the other was valid based on whether differences between means of the treatments were significant P < 0.05 (Tukey HSD test).

CHAPTER 6: DISCUSSION

6.1. INTRODUCTION

The aim of this study was to determine whether phylogenetic history or physiology determined recovery patterns of grass species after a burning event. This was achieved by doing a pair-wise comparison between and Panicoideae and non-Panicoideae (Danthonioideae and Aristidoideae) species. Pre-fire characteristics that would enhance fire frequency and assist with plant recovery after burning were compared across phylogenies and plant physiology. This was then followed by a field and pot comparison of post fire plant recovery which examined the re-growth of the leaf canopy area, leaf mass, above-ground biomass and the cost of this to the below-ground biomass. The contribution of reallocation and photosynthesis to the recovery of each above mentioned component was then analysed. It was hypothesised that the species would shunt carbon below ground during the dry season, reducing the impact of fire, have a larger potential fuel load and that they would show a fast recovery after burning as a result of their large below-ground biomass and photosynthetic efficiency.

6.2. PHYSIOLOGY OR PHYLOGENY?

Grass invasions into areas that were previously characterised by fire-intolerant species often initiate a grass/fire cycle (Hughes *et al.*, 1991). The invading grasses provide the fuel load necessary to start and spread fires and are also able to recover more quickly than the other species shifting the species composition towards a fire driven community (D’Antonio and Vitousek, 1992). Bond *et al.* (2003a) has shown that grasslands in South

Africa, in particular grasslands, are maintained by a frequent fire regime and that when these fires are excluded, there is a shift back towards a community dominated by woody vegetation. The carbon concentrating mechanism flourishes under warm, light saturated conditions brought about by the opening up of the canopy layer as a result of burning (Long, 1999; Sage, 2004). It was therefore predicted that species would be better adapted to surviving in frequently burnt ecosystems than their counterparts.

The results from the pot experiment supported this hypothesis with the species being better adapted to fire than the species. For example, the canopy of the species died back during winter while the species maintained their live canopy (Fig. 3.2.2a). The death of the canopy implied that the species were shunting carbon below-ground during winter and as a result were unaffected by burning since few live tissues were consumed by fire and therefore very little carbon was lost (Morgan, 1999). In contrast, the species lost live tissue during the burn which could have resulted in the loss of carbon, possibly explaining their delayed recovery.

In addition, while both the and the grass species burn when ignited, the live tissue and the higher moisture content (Fig. 3.4.2a) observed in the species would have retarded the fire more than in the species, which had less live tissue and a lower moisture content. The results imply that the dead canopy and lower moisture content of the species may provide a potentially more flammable fuel load that is more likely to ignite compared to the species.

As with the pre-fire characteristics, the post-fire recovery also showed the species from the pot-experiment to be better adapted to fire by recovering their canopy area, leaf mass and above-ground biomass more quickly than the species (Fig. 4.2.1.1, 4.2.2.1, 4.2.4.1). In addition, the recovery of the species did not have an impact on the below-ground reserves while the recovery of the burnt species resulted in a decrease in the below-ground biomass (Fig. 4.2.5.1). The implications of this will be discussed further on.

In contrast, the field data showed that the species recovered more quickly than the species. However, *P. aequinerve* and *T. triandra* exerted a strong influence on the results and when these species were removed, the recovery time of the and species were the same. The timing of the fire could have also delayed species recovery since the canopy area and leaf mass (Fig. 4.1.1 and Fig. 4.1.2) of *T. triandra* appeared to have already started spring growth at the time of the fire resulting in the loss of new leaves. The field experiment therefore highlights the importance of the timing of the fire, with late season fires that occur after re-growth has begun, resulting in the loss of carbon which could negatively affect species fitness.

Superimposed on the / comparison is the question of phylogeny. For example, examination of ecological distributions of species along fire, temperature and rainfall gradients have been largely attributed to variation in the photosynthetic pathways between species (Cerling *et al.*, 1993; 1997; Chazdon, 1978; Keeley and Rundel, 2003; Teeri and Stowe, 1976; Tieszen *et al.,* 1979). Approximately four studies have actually investigated the role of phylogenetic history on the current distributions of and species (Cabido *et al.,* 2007*;* Edwards and Still, 2008; Stock *et al.,* 2004 and Taub, 2000). While each of these studies showed a clear separation between and species, stronger patterns were observed when their evolutionary history was accounted for. For example, a recent study comparing the distribution of and species across temperature gradients in Hawaii found that the species response was due largely to their evolutionary history but that the pathway did confer a competitive advantage (Edwards and Still, 2008).

Since these studies highlight the importance of accounting for a species evolutionary history when comparing responses between species, this study examined the response between two evolutionary lineages superimposed over the photosynthetic types. It was found that the Panicoideae were better adapted than the non-Panicoideae at surviving in fire driven ecosystems and that there was stronger support for the phylogenetic comparison than the physiological one. The support was based on higher values for the phylogenetic model as well as by the individual sub-family responses with the and Panicoideae (Panicoideae) behaving similarly to each other but in contrast to the Danthonioideae and Aristidoideae subfamilies (non-Panicoideae) responses. However, the question that arises from these results is how there can be both a photosynthetic response as well as a phylogenetic response? It is probable that the Panicoideae exerted a strong influence on the models since this family was grouped with the Aristidoideae for the photosynthetic comparison and then with the Panicoideae for the phylogenetic comparison. Examination of the subfamily responses was therefore imperative to untangling whether the response was based on photosynthetic type or phylogenetic history.

Examination of the pre-fire characteristics for the phylogenetic comparison showed that despite the greater death of the canopy of the species compared to the species, it was in fact the Panicoideae that had a larger proportion of dead standing biomass at the end of winter compared to the non-Panicoideae (Fig. 3.3.1a). Further examination into the subfamily responses showed that it was the Panicoideae that had the greatest proportion of dead standing biomass compared to the other three subfamilies while the Aristidoideae subfamily had the lowest proportion of dead biomass (Fig. 3.3.2). Previous studies have found that the Andropogoneae subfamily (of which three of the four Panicoideae species fall into with the exception of *A. semialata* subsp. *semialata*) contain tannin-like compounds that accumulate in the leaves and prevent their decomposition (Ellis, 1990; Horner *et al.*, 1988). As a result, the dead leaves of the Panicoideae accumulated on the plant, possibly creating a potential fuel load that would increase their flammability, while the dead leaves of the Aristidoideae fell off and decayed.

Since the Panicoideae had a larger potential fuel load at the end of winter, possibly contributing to an increase in the frequency of fires, it is feasible that the Panicoideae confer traits that make them more flammable than their counterparts at the level of the individual plant. Bond and Midgley (1995) propose that flammability traits will only evolve in a species if they result in the death of neighbouring species, thus decreasing competition and increasing the fitness of the flammable species. The results show significant differences in flammability between species (Fig. 3.5), supporting Mutch's (1970) hypothesis that some species are more flammable than others and thus implying that some species have evolved traits to increase their flammability.

However, the architectural and non-architectural burns showed contrasting results with the Panicoideae being more flammable when architecture was intact and the Panicoideae being more flammable when the plant architectural effects were removed. Further studies, comparing the flammability of the Panicoideae and the non-Panicoideae subfamilies might provide more conclusive results as whether there are patterns of flammability between different photosynthetic types and phylogenetic groups.

As previously mentioned, in addition to a larger fuel load, the greater proportion of dead biomass observed in the Panicoideae (in particular the Panicoideae) meant that these species shunted carbon below-ground for storage and that as a result, fire had little impact on these species since few live tissues were removed. This was supported by the Panicoideae having a proportionally larger below-ground biomass at the end of the dry season compared to the non-Panicoideae (Fig. 3.1.1). The large below-ground biomass enabled the Panicoideae to reallocate significantly more carbon than the non-Panicoideae, to the recovery of the canopy area, leaf mass and above-ground biomass after the fire (Fig. 5.1.1, 5.2.1, 5.3.1) thus supporting the fast recovery of these components i.e. the canopy area, leaf mass and above-ground biomass (Fig. 4.2.1.1, 4.2.2.1, 4.2.4.1).

Despite the Panicoideae reallocating significantly more carbon to species recovery compared to the non-Panicoideae (as seen in Chapter 5), the results from chapter 4 showed that there was no difference between the below-ground biomass of the control and burnt treatments of the Panicoideae while the burnt treatment of the non-Panicoideae showed a significant decrease from the control (Fig. 4.2.5.1). As previously mentioned, a similar pattern was evident in the comparison between photosynthetic types with the below-ground biomass of the species remaining unaffected while the species showed a significant decrease over time. The results imply that the Panicoideae reallocated resources above ground to replace the dead tissue at the onset of the growing season, irrespective of whether or not they were burnt and thus explains why there was no difference in the below-ground reserves between the burnt and control plants (Fig. 4.2.5.1) despite the Panicoideae reallocating significantly more resources than the non-Panicoideae (Fig. 5.1.1, 5.2.1, 5.3.1). These results further imply that resources were shunted below ground during the dry season since the Panicoideae need to replace the above-ground tissue and that fire therefore had little impact on these species. In contrast, the non-Panicoideae species possibly had a different strategy whereby they maintained their live tissue (the Danthonioideae maintaining more than the Aristidoideae) and did not shunt carbon below-ground during the dry season as with the Panicoideae. As a result the below-ground biomass of the non-Panicoideae decreased significantly from the control values as it reallocated resources above-ground to replace the live tissue lost when burnt. The reallocation in the non-

Panicoideae would have resulted in the loss of carbon, which could have a negative impact on species fitness (Knapp, 1985).

In addition to the below-ground biomass supporting the fast and vigorous recovery of the canopy area in the Panicoideae it was also found that at the subfamily level, the and Panicoideae had a significantly larger photosynthetic contribution to the canopy area compared to the Aristidoideae subfamily (Fig. 5.1.2). This could not be tested between the Panicoideae and non-Panicoideae groups since the difference between the burnt and control treatments were not significant (see Chapter 5 for details). A fast recovery of the canopy area would allow species to take advantage of the high light intensity associated with recently burnt environments, possibly increasing their photosynthetic rate and thus their rate of recovery. For example, Knapp (1985) found that the post burn photosynthetic rates of *Andropogon gerardii,* a dominant prairie grass, were greater on burned than on unburned sites while the photosynthetic rates of *Panicum virgatum*, a less dominant prairie species, were not significantly different between burned and unburned sites.

As well as a larger canopy area, the Panicoideae were also found to have a higher SLA that decreased more slowly over time than did that of the non-Panicoideae (Fig. 4.2.3.1). A high SLA implies that the plants have produced leaves with a large canopy area relative to their mass to increase their rate of resource acquisition (such as and N) (Poorter and de Jong, 1999). This attribute would benefit the recovery of the Panicoideae species in recently burnt environments as it would enable a high rate of and N uptake per unit leaf area which could possibly increase their rate of recovery. The decrease over time of SLA might be associated with a need to increase mechanical support or decrease palatability through an increase in vasculature and fibre content (Evans and Poorter, 2001).

Another potential mechanism used by the Panicoideae species to recover quickly after burning could be linked to their growth form and the number and position of the perrenating buds (Benson *et al.,* 2004; Gibson, 2009). In grasses, the perrenating buds of species subjected to frequent burning are either protected by closely packed leaf sheaths or they are located below ground. The bud-bank, defined as the below ground population of meristems, also plays an important role in the regeneration of plants after fire, with species that have large bud-banks showing a faster recovery than those with smaller bud-banks (Gibson, 2009). Benson *et al.* (2004) found that annually burnt prairies had bud-banks that were twice as large as infrequently burnt prairies, suggesting that these plants have adapted to fire by increasing their bud-bank and as a result were able to recover more quickly than their infrequently burnt counterparts. Since the Panicoideae are adapted to surviving in frequently burnt environments, it is possible that their perrenating buds are well protected and that they have larger bud banks to enable a fast recovery. Further studies are needed to confirm whether these mechanisms are in fact used by the Panicoideae.

6.3. ECOLOGICAL IMPLICATIONS

If the Panicoideae are better adapted at recovering from fire than the non-Panicoideae, why then are mesic grasslands dominated by Panicoideae (particularly Andropogoneae) (Bond *et al.,* 2003) rather than by a mix of both and Panicoideae species? It is possible that despite there being no difference between the recovery of the and Panicoideae, the mechanism likely confers greater efficiencies of light, nitrogen use and water use on species relative to species (Long, 1999). As a result they would be better equipped to take advantage of conditions associated with the post-fire environment such as a high light intensity and low nitrogen and soil water content (Knapp, 1984). This would be possible as they would be able to achieve the same rate of carbon gain as the species while investing fewer resources in individual leaves thus allowing species flexibility to allocate carbon to other compartments such as root mass, storage, canopy area and reproduction (Long, 1999; Ripley *et al.,* 2008).

The implication is that while neither the nor the Panicoideae are affected by burning, fire alters the environmental conditions of an ecosystem shifting them towards conditions favourable to the mechanism. Since the Panicoideae are able to exploit the post-fire environment, they would be able to dominate in frequently burnt ecosystems (Bond *et al.,* 2003a; Vogel *et al.,* 1978). Therefore a high frequency of fire results in the Panicoideae being admitted to frequently burnt ecosystems with the species becoming dominant.

Another question that arises is why the Aristidoideae, which are also (NADP-ME) species, are not as capable at recovering after a fire as their Panicoideae counterparts? It is possible that since these species mostly occur at the more arid end of the rainfall gradient or at high altitudes (Gibbs Russell *et al.*, 1991; O' Connor and Bredenkamp, 1997), which are areas that are not frequently burnt (Bond, 1997), they were not adapted to fire driven ecosystems such as the Panicoideae and therefore did not invest large amounts of carbon in below-ground reserves. A smaller below-ground biomass would have meant that that were unable to recover quickly after being burnt. Conversely, it has been shown that the Panicoideae are drought sensitive (Frole, 2008; Ghannoum *et al.,* 2003) and would therefore be excluded from arid environments.

Bond *et al.* (2003a) provides evidence for this when comparing species occurrence in fire dependent ecosystems (characterised by a change in species composition after fire) with those in climate dependent ecosystems (characterised by either a structural change or no change at all after a fire) in South Africa. When the species were grouped according to their subfamilies, the Andropogoneae (NADP-ME) were found to dominate in mesic regions, areas characterised as being fire dependent ecosystems, while the Chloridoideae (NAD-ME) were found to dominate in the more arid regions, areas characterised as being climate driven ecosystems. Similar patterns have also been observed in North America with the Andropogoneae dominating in the mesic regions while the Chloridoideae dominate in the more arid regions (Barkworth and Capels, 2000 in Bond *et al.,* 2003a). It is possible that the distribution of the Aristidoideae, like the Chloridoideae, are also affected by climate rather than fire and that as a result the Aristidoideae were not adapted to recovering after being burnt like the Panicoideae were. Further research into the mechanisms that affect the species in the arid environments is required.

6.4. EVOLUTIONARY QUESTIONS

The Panicoideae evolved 16-22 MYA (Christin *et al.,* 2008) yet grasslands only expanded 8 MYA. Based on evidence in the oceanic charcoal sediments that indicate an increase in fire frequency and seasonality at this time, Keeley and Rundel (2005) suggest fire may have driven this sudden global expansion of grasslands. The results support this hypothesis in part since both the both the and Panicoideae were adapted to recovering after fire but it is the Panicoideae that dominate in frequently burnt ecosystems. It is therefore possible that the Panicoideae group were pre-adapted to fire driven ecosystems before the pathway evolved and that an increase in the frequency of fires resulted in the sudden expansion of grasslands since they were better able to take advantage of the post-fire environment.

However, the evidence from this study implies that the expansion of grasslands would have been limited to mesic environments (where the Panicoideae occur) since not all the species were adapted to fire as has been shown by the Aristidoideae subfamily. It is therefore possible that the expansion of the grasslands was either driven by a few key species rather than by all the species or that fire was only one factor that contributed to their sudden expansion.

6.5. CONCLUSION

The study showed that the response of individual species to fire is determined more strongly by their evolutionary history rather than by their photosynthetic type with the Panicoideae recovering faster and more completely than the non-Panicoideae grasses. However, frequently burnt grasslands are dominated by the Panicoideae despite both the and Panicoideae being able to recover quickly after burning. This implies that while fire itself doesn't have an impact on the recovery of the and Panicoideae, it does alter the environmental conditions in favour of the mechanism which is better adapted at exploiting the conditions associated with the post-fire environment. While it is not disputed that fire may have played an important role in the expansion of the grasslands during the late Miocene, the results suggest that the influence of fire was limited to mesic environments and that other factors, such as drought, may have been a potential driver in xeric environments.

CHAPTER 7: REFERENCES

Abraham, T.I. (2007). *Photosynthetic and Growth Response of and Subspecies of Alloteropsis semialata to Nitrogen-Supply.* M.Sc. Thesis, Rhodes University, South Africa.

Barkworth, M.E. and Capels, K.M. (2000). Grasses in North America: a geographic perspective. IN Jacobs, S.W.L; Everett, J. (eds). *Grasses: Systematics and Evolution.* CSIRO, Melbourne. p 331-350. IN Bond, W.J.; Midgley, G.F. and Woodward, F.I. (2003a). What controls South African vegetation – climate or fire? *South African Journal of Botany.* 69(1): 79-91.

Beerling, D.J. and Osborne, C.P. (2006). The origin of the savanna biome. *Global Change Biology.* 12: 2023-2031.

Bell, T.L.; Pate, J.S. and Dixon, K.W. (1996). Relationships Between Fire Response, Morphology, Root Anatomy and Starch Distribution in South-west Australian Epcaridaceae. *Annals of Botany.* 77: 357-364.

Bellingham, P.J. (2000). Resprouting as a life history strategy in woody plant communities. *OIKOS*. 89(2): 409-416.

Benson, E.J.; Hartnett, D.C. and Mann, K.H. (2004). Belowground Bud Banks and Meristem Limitation in Tallgrass Prairie Plant Populations. *American Journal of Botany*. 91(3): 416-421.

Blair, J.M.; Seastedt, T.R.; Rice, C.W. and Ramundo, R.A. (1998). Terrestrial Nutrient Cycling in Tallgrass Prairie. In: Knapp, A.K.; Briggs, J.M. and Hartnett, D.C. eds. *Grassland Dynamics: Long term ecological research in tallgrass prairie.* New York, USA: Oxford

Bloom, A.J.; Chapin, F.S. and Mooney, H.A. (1985). Resource limitation in plants – an economic analogy. *Annual Review of Ecology and Systematics*. 16: 363-392.

Bond, W.J. (1997). Fire. IN: Cowling, R.M.; Richardson, D.M. and Pierce, S.M. eds. *Vegetation of Southern Africa*. Cambridge University Press, Cambridge. 421-442.

Bond, W.J. (2005). Large parts of the world are brown or black: A different view on the 'Green World' Hypothesis. *Journal of Vegetation Science.* 16: 261-266

Bond, W.J. (2008). What Limits Trees in Grasslands and Savannas? *Annual Review of Ecology, Evolution, and Systemtics.* 39: 641-659.

Bond, J.B. and Keeley, J.E. (2005). Fire as a global "herbivore": the ecology and evolution of flammable ecosystems. *TRENDS in Ecology and Evolution.* 20(7): 387-394.

Bond, W.J. and Midgley, J.J. (1995). Kill thy neighbour: an individualistic argument for the evolution of flammability. *OIKOS.* 73: 79-85.

Bond, W.J. and Van Wilgen, B.W. (1996). Plant demography and fire II: Event dependent effects. IN: *Fire and Plants.* Chapman and Hall, London. 88-122.

Bond, W.J.; Midgley, G.F. and Woodward, F.I. (2003a). What controls South African vegetation – climate or fire? *South African Journal of Botany.* 69(1): 79-91.

Bond, W.J.; Midgely, G.F. and Woodward, F.I. (2003b). The Importance of low atmospheric and fire in promoting the spread of grasslands and savannas. *Global Change Biology.* 9: 973-982.

Bond, W.J.; Woodward, F.I. and Midgley, G.F. (2005). The global distribution of ecosystems in a world without fire. *New Phytologist.* 165: 525-538.

Bouchenak-Khelladi, Y.; Salamin, N.; Savolainen, V.; Forest, F.; van der Bank, M.; Chase, M.W. and Hodkinson, T.R. (2008). Large multi-gene phylogenetic trees of the grasses (Poaceae): Progress towards complete tribal and generic level sampling. *Molecular Phylogenetics and Evolution*. 47: 488–505.

Bowen, B.J. and Pate, J.S. (1993). The significance of Root Starch in Post-fire Shoot Recovery of the Resprouter *Stirlingia latifolia* R. Br. (Proteaceae). *Annals of Botany*. 72: 7-16

Byram, G.M. (1959). Combustion of forest fuels. IN Brown, A.A and Davis, K.P. eds. *Forest Fire: Control and Use.* McGraw-Hill, New York. 61-89.

Cabido, M; Pons, E; Cantero, J.J.; Lewis, J.P. and Anton, A. (2007). Photosynthetic pathway variation among grasses along a precipitation gradient in Argentina. *Journal of Biogeography.* 35:131-140

Calvin, M and Benson, A.A. (1948). The Path of Carbon in Photosynthesis. *Science.* 107:476-480.

Cerling, T.E. (1999). Paleorecords of plants and ecosystems. In Sage, R.F. and Monson, R.K. (eds). *Plant Biology.* San Diego, CA, USA: Academic Press. pp. 445-469

Cerling, T.E.; Wang, Y. and Quade, J. (1993). Expansion of ecosystems as an indicator of global ecological change in the late Miocene. *Nature.* 361: 344-345.

Cerling, T.E.; Harris, J.M.; MacFadden, B.J.; Leakey, M.G.; Quade, J; Eisenmann, V and Ehleringer, J.R. (1997). Global vegetation change through the Miocene/Pliocene boundary. *Nature.* 389: 153-158.

Chazdon, R.L. (1978). Ecological Aspects of the Distribution of Grasses in Selected Habitats of Costa Rica. *Biotropica.* 10(4): 265-269.

Christin, P.A.; Besnard, G.; Samaritini, E.; Duvall, M.R.; Hodkinson, T.R.; Savolainen, V. and Salamin, N. (2008). Oligocene decline promoted photosynthesis in grasses. *Current Biology.* 18: 37-43.

D'Antonio, C.M. and Vitousek, P.M. (1992). Biological Invasions by Exotic Grasses, the Grass/Fire Cycle, and Global Change. *Annual Review of Ecological Systems.* 23: 63-87.

Dengler, N.G. and Nelson, T. (1999). Leaf Structure and Development in Plants. In: Sage R.F. and Monson, R.K. eds. *Plant Biology.* San Diego, CA, USA: Academic Press. 3-16.

Edwards, E.J. and Still, C.J. (2008). Climate, phylogeny and the ecological distribution of grasses. *Ecology Letters.* 11: 266-276.

Edwards, E.J.; Still, C.J. and Donoghue, M.J. (2007). The relevance of phylogeny to studies of global change. *TRENDS in Ecology and Evolution.* 22(5): 233-249

Ehleringer, J.R. (1978). Implications of Quantum Yield Differences on the Distributions of and Grasses. *Oecologia*. 31: 255-267.

Ehleringer, J.R. and Monson, R.K. (1993). Evolutionary and Ecological Aspects of Photosynthetic Pathway Variation. *Annual Review of Ecological Systematics.* 24: 411-439.

Ehleringer, J.R.; Cerling, T.E. and Helliker, B.R. (1997). Photosynthesis, atmospheric and climate. *Oecologia.* 112**:** 285-299.

Ehleringer, J.R.; Sage, R.F.; Flanagan, L.B. and Pearcy, R.W. (1991). Climate Change and the Evolution of Photosynthesis. *TRENDS in Ecology and Evolution.* 6 (3): 95-99

Ellis, R.P. (1977). Distribution of the Kranz syndrome in the southern African Eragrostoideae and Panicoideae according to bundle sheath anatomy and cytology. *Agroplantae* 9: 73-110

Ellis, R.P. (1990). Tannin-like substances in grass leaves. *Memoirs of the Botanical Survey of South Africa.* 59: 1-80.

Ellis, R.P.; Vogel, J.P. and Fuls A. (1980). Photosynthetic Pathways and the Geographical Distribution in South West Africa/Namibia. *South African Journal of Science.* 76: 307 – 314.

Evans, J.R. and Poorter, H. (2001). Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant, Cell and Environment.* 24: 755-767.

Frole, K.M. (2008). *Drought responses of and (NADP-ME) Panicoid Grasses.* M.Sc. Thesis, Rhodes University, South Africa.

Gambiza, J; Campbell, B.M.; Moe, S.R. and Frost, P.G.H. (2005). Fire behaviour in a semi-arid *Baikiaea plurijuga* savanna woodland on Kalahari sands in western Zimbabwe. *South African Journal of Science.* 101: 239 - 244.

Ghannoum, O.; Conroy, J.P., Driscoll, S.P.; Paul, M.J.; Foyer, C.H.; Lawlor, D.W. (2003). Nonstomatal limitations are responsible for drought induced photosynthetic inhibition in four grasses. *New Phytologist*. 159: 599-608.

Gibbs Russell, G.E.; Watson, L.; Koekemoer, M.; Smook, L.; Barker, N.P.; Anderson, H.M. and Dallwitz, M.J. (1991). *Grasses of Southern Africa*. National Botanical Gardens / Botanical Research Institute, South Africa.

Gibson, D.J. (2009). *Grasses and Grassland Ecology.* Oxford University Press Inc., New York.

Hansen, A.; Pate, J.S.; Hansen, A.P. (1991). Growth and Reproductive Performance of a Seeder and a Resprouter Species of *Bossiaea* as a Function of Plant Age After Fire. *Annals of Botany.* 67: 497 - 509.

Hartnett, D.C. (1991). Effects of Fire in Tallgrass Prairie on Growth and Reproduction of Prairie Coneflower (*Ratibida columnifera*: Asteraceae). *American Journal of Botany*. 78(3):429-435.

Hatch, M.D. (1987). Photosynthesis: a unique blend of modified biochemistry, anatomy and ultrastructure. *Biochemica Biophysica Acta.* 895:
81–106.

Hatch, M. D. and Slack, C. R**.** (1966). Photosynthesis by sugarcane leaves. *Biochemical Journal.* 101: 103-111.

Hoffman, W.A. (1999). Fire and Population dynamics of Woody Plants in a Neotropical Savanna: Matrix Model Projections. *Ecology.* 80(4): 1354-1369.

Horner, J.D.; Gosz, J.R. and Cates, R.G. (1988). The role of carbon-based plant secondary metabolites in decomposition in terrestrial ecosystems. *The American Naturalist.* 132: 869-883.

Huang, Y.; Street-Perrott, F.A.; Metcalfe, S.E.; Brenner, M.; Moreland, M. and Freeman, K.H. (2001) Climate Change as the Dominant Control on Glacial-Interglacial Variations in and Plant Abundance. *Science.* 293:1647-1651.

Hughes, F.; Vitousek, P.M. and Tunison, T. (1991). Alien Grass Invasion and Fire in the Seasonal Submontane Zone of Hawaii. *Ecology.* 72(2): 743-746.

Ibrahim, D.G.; Gilbert, M.E.; Ripley, B.S. and Osborne, C.P. (2008). Seasonal differences in photosynthesis between the and subspecies of *Alloteropsis semialata* are offset by frost and drought. *Plant, Cell and Environment.* 31(7): 1038-1050.

Kanai, R. and Edwards, G.E. (1999). The Biochemistry of photosynthesis. In: Sage R.F. and Monson, R.K. eds. *Plant Biology.* San Diego, CA, USA: Academic Press. 49-87.

Keeley, J.E. and Rundel, P.W. (2003). Evolution of CAM and Carbon-concentrating Mechanisms. *International Journal of Plant Sciences.* 164(3 Suppl.): S55-s77.

Keeley, J.E. and Rundel, P.W. (2005). Fire and the Miocene expansion of Grasslands. *Ecology Letters.* 8: 683-690.

Kellogg, E.A. (1999). Phylogenetic Aspects of the Evolution of Photosynthesis. In: Sage R.F. and Monson, R.K. eds. *Plant Biology.* San Diego, CA, USA: Academic Press. 3-16.

Kellogg, E.A. (2000). THE GRASSES: A Case Study in Macroevolution. *Annual Review of Ecological Systems.* 31:217–38

Knapp, A.K. (1984). Post-burn differences in solar radiation, leaf temperature and water stress influencing production in a lowland tallgrass prairie. *American Journal of Botany.* 71:220 - 227.

Knapp, A.K. (1985). Effect of Fire and Drought on the Ecophysiology of *Andropogon gerardii* and *Panicum virgatum* in a Tallgrass Prairie. *Ecology.* 66 (4):1309-1320.

Knapp, A.K. and Gilliam, F.S. (1985). Response of *Andropogon gerardii* (Poaceae) to fire-induced high vs. low irradiance environments in Tallgrass Prairie: Leaf Structure and Photosynthetic Pigments. *American Journal of Botany*. 72(11): 1668-1671.

Knapp, A.K. and Seastedt, T.R. (1986). Detritus Accumulation Limits Productivity of Tallgrass Prairie. *BioScience.* 36(10): 662-668.

Knapp, A.K.; Briggs, J.M.; Blair, J.M. and Turner, C.L. (1998). Patterns and Controls of Aboveground Net Primary Production in Tallgrass Prairie. In: Knapp, A.K.; Briggs, J.M. and Hartnett, D.C. eds. *Grassland Dynamics: Long term ecological research in tallgrass prairie.* New York, USA: Oxford University Press. 193-221.

Kruger, L.M.; Midgley, J.J. and Cowling, R.M. (1997). Resprouters vs reseeders in South African forest trees; a model based on forest canopy height. *Functional Ecology.* 11: 101-105.

Lean, J. and Warrilow, D.A. (1989). Simulation of the regional climatic impact of Amazon deforestation. *Nature.* 342: 411-413.

Long, S.P. (1999). Environmental Responses. In: Sage R.F. and Monson, R.K. eds. *Plant Biology.* San Diego, CA, USA: Academic Press. 215-249.

Lubke, R.A. and van Wijk, Y. (1998). Terrestrial Plants and coastal vegetation IN Eds: Lubke, R.A. and de Moor, I eds. *Field Guide to the Eastern and Southern Cape Coasts.* University of Cape Town Press, Cape Town. P 289-343.

Manson, A.D.; Jewitt, D. and Short, A.D. (2007). Effects of season and frequency of burning on soils and landscape functioning in a moist montane grassland. *African Journal of Range and Forage Science.* 24(1): 9-18.

Monson, R.K. (1999). The Origins of Genes and Evolutionary Pattern in the Metabolic Phenotype. In: Sage R.F. and Monson, R.K. eds. *Plant Biology.* San Diego, CA, USA: Academic Press. 377-410.

Morgan, J.W. (1996). Secondary juvenile period and community recovery following late-spring burning of a kangaroo grass *Themeda triandra* grassland. *Victorian Naturalist.* 113: 47-57.

Morgan, J.W. (1999). Defining grassland fire events and the response of perennial plants to annual fire in temperate grasslands of south-eastern Australia. *Plant Ecology.* 144: 127-144.

Mucina L., Rutherford M.C. (2006). *The vegetation of South Africa, Lesotho and Swaziland.* South African National Biodiversity Institute (SANBI) Pretoria, South Africa.

Murphy, B.P. and Bowman, D.M.J.S. (2007) Seasonal water availability predicts the relative abundance of and C4 grasses in Australia. *Global Ecology and Biogeography.* 16: 160-169.

Mutch, RW. (1970). Wildland Fires and Ecosystems – A Hypothesis. *Ecology*. 51(6): 1046-1051.

O' Connor, T.G. and Bredenkamp, G.J. (1997). Grassland. In: Cowling, R.M.; Richardson, D.M. and Pierce, S.M. *Vegetation of Southern Africa.* Cambridge University Press. 215-248

Ojima, D.S.; Schimel, D.S.; Parton, W.J. and Owensby, C.E. (1994). Long- and Short-Term Effects of Fire on Nitrogen Cycling in Tallgrass Prairies. *Biogeochemistry.* 24 (2): 67-84.

Osborne, C.P. and Beerling, D.J. (2006). Natures green revolution: the remarkable evolutionary rise of plants. *Philosophical Transactions of the Royal Society.* 361: 173-194.

Pagani, M.; Freeman, K.H. and Arthur, M.A. (1999). Late Miocene Atmospheric Concentrations and the Expansion of Grasses. *Science.* 285: 876-879.

Pate, J.S. (1993). Structural and functional responses to fire and nutrient stress: case studies from the sandplains of South-West Australia. In: Fowden, L.; Mansfield, T. and Stoddart, J. eds. *Plant Adaptation to Environmental Stress.* London, UK: Chapman and Hall. 189-205

Pate, J.S.; Froend, R.H.; Bowen, B.J.; Hansen, A. and Kuo, J. (1990). Seedling Growth and Storage Characteristics of Seeder and Resprouter Species in Mediterranean-type Ecosystems of S.W. Australia. *Annals of Botany.* 65: 585-601.

Pate, J.S.; Meney, K.A. and Dixon, K.W. (1991). Contrasting Growth and Morphological Characteristics of Fire sensitive (Obligate Seeder) and Fire-resistant (Resprouter) Species of Restionaceae (S. Hemisphere Restiads) from South-western Western Australia. *Australian Journal of Botany*. 39: 505-525.

Poorter, H and de Jong, R. (1999). A comparison of specific leaf area, chemical composition and leaf construction costs of field plants from 15 habitats differing in productivity. *New Phytologist*. 143: 163-176.

Poorter, H. and Nagel, O. (2000). The role of biomass allocation in the growth response of plants to different levels of light, , nutrients and water: a quantitative review. *Australian Journal of Plant Physiology.* 27: 595-607.

Poorter, H. and Remkes, C. (1990). Leaf Area Ratio and Net Assimilation Rate of 24 Wild Species Differing in Relative Growth Rate. *Oecologia*. 83: 553-559.

Poorter, H.; Remkes, C. and Lambers, H. (1990). Carbon and Nitrogen Economy of 24 Wild Species Differing in Relative Growth Rate. *Plant Physiology.* 94: 621-627.

Quade, J.; Cerling, T.E; Bowman, J.R. (1989). Development of Asian monsoon revealed by marked ecological shift during the latest Miocene in northern Pakistan. *Nature.* 342: 163-166.

Rawsthorne, S. (1992). C3-C4 intermediate photosynthesis: linking physiology to gene expression. *The Plant Journal.* 2 (3): 267-274

Reich, P.B.; Abrams, M.D.; Ellsworth, D.S.; Kruger, E.L. and Tabone, T.J. (1990). Fire Affects Ecophysiology and Community Dynamics of Central Wisconsin Oak Forest Regeneration. *Ecology.* 71 (6): 2179-2190.

Ripley, B.S. Abraham, T.I. and Osborne, C.P. (2008). Consequences of photosynthesis for the partitioning of growth: a test using and subspecies of *Alloteropsis semialata* under nitrogen-limitation. *Journal of Experimental Botany.* 59 (7): 1705-1714.

Roalson, E.H. (2008). Photosynthesis: Differentiating Causation and Coincidence. *Current Biology.* 18 (4): 167-168.

Rundel, P.W. (1980). The Ecological Distribution of and Grasses in Hawaiian Islands. *Oecologia.* 45: 354-359.

Sage, R.F. (2004). The Evolution of Photosynthesis. *New Phytologist.* 161: 341-370.

Sage, R.F.; Meirong, L.; Monson, R.K. (1999a). The Taxonomic Distribution of Photosynthesis. In: Sage R.F. and Monson, R.K. eds. *Plant Biology.* San Diego, CA, USA: Academic Press. 551-582.

Sage, R.F.; Wedin, D.A. and Li, M.R. (1999b). The biogeography of photosynthesis: patterns and controlling factors. In: Sage R.F. and Monson, R.K. eds. *Plant Biology.* San Diego, CA, USA: Academic Press. 313 – 373.

Sanchez-Ken, J.B.; Clark, L.G.; Kellogg, E.A. and Kay, E.E. (2007). Reinstatement and Emendation of Subfamily Micrairoideae (Poaceae). *Systematic Biology.*32 (1): 71-80.

Sarmiento, G. (1992). Adaptive Strategies of Perennial Grasses in South American Savannas. *Journal of Vegetation Science.* 3 (3): 325-336.

Schulze, R.E. (1997). Climate. In: Cowling, R.M.; Richardson, D.M. and Pierce, S.M. *Vegetation of Southern Africa.* Cambridge, United Kingdom: Cambridge University Press. 21-42.

Shukla, J.; Nobre, C. and Sellers, P.(1990). Amazon Deforestation and Climate Change. *Science.* 247: 1322-1325.

Sokal, R.R. and Rohlf, F.J. (1995). *Biometry: The Principles and Practice of Statistics in Biological Research (Third Edition)*. W.H. Freeman and Company, New York. p. 272-275.

Stock, W.D., Chuba, D.K. and Verboom, G.A. (2004). Distribution of South African and species of Cyperaceae in relation to climate and Phylogeny. *Australian Ecology .* 29: 313-319

Stone, A.W., Weaver, A.B. and West, W.O. (1998). Climate and weather IN Eds: Lubke, R.A. and de Moor, I eds.*Field Guide to the Eastern and Southern Cape Coasts.* University of Cape Town Press, Cape Town. p 41-49.

Svejcar, T.J. and Browning, J.A. (1988). Growth and gas exchange of *Andropogon gerardii* as influenced by burning. *Journal of Range Management*. 41 (3): 239-244.

Taub, D.R. (2000). Climate and the U.S. Distribution of Grass subfamilies and Decarboxylation variants of photosynthesis. *American Journal of Botany.* 87 (8): 1211-1215.

Teeri, J. A. and Stowe, L.G. (1976). Climatic Patterns and the Distribution of Grasses in North America. *Oecologia.* 23**:** 1-12.

Tieszen, L.L.; Senyimba, M.M.; Imbamba, S.K. and Troughton, J.H. (1979). The Distribution of and Grasses and Carbon Isotope Discrimination along an Altitudinal and Moisture Gradient in Kenya. *Oecologia*. 37: 337-350.

Trollope, W.S.W. (1981). Recommended terms, definitions and units to be used in fire ecology in Southern Africa. *Proceedings of the Grassland Society of southern Africa.* 16: 107-109.

Uys, RG; Bond, WJ and Everson, TM. (2004). The effect of different fire regimes on plant diversity in southern African grasslands. *Biological Conservation.* 118: 489-499.

Van Oudtshoorn, F. (2004). *Guide to Grasses of Southern Africa.* Briza Publications. Pretoria, South Africa.

Vogel, J.C.; Fuls, A. and Ellis, R.P. (1978). The Geographical Distribution of Kranz Grasses in South Africa. *South African Journal of Science.* 74: 209 – 215.

von Caemmerer, S. (2000). *Biochemical Models of Leaf Photosynthesis*. CSIRO Publishing. Canberra, Australia.