

Phenotypic plasticity and ecological memory in *Andropogon gerardii*: responses to simulated grazing pressure

by

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Abstract

This thesis explores the effects of ecological memory on plants responses to subsequent stressors, specifically focusing on the physiological and biochemical responses of *Andropogon gerardii* (big bluestem) to grazing pressures. By examining the concept of ecological memory, this research highlights how past environmental stressors, such as grazing, influence future plant responses. Utilizing a multifaceted approach that spans predictive modeling, physiological assessments, and biochemical analyses, this study rigorously tests the hypothesis that the legacy effects of grazing, embodied in ecological memory, profoundly impact *A. gerardii*'s physiological fabric and biochemical mechanisms.

Structured into three chapters, the thesis first explores the biochemical and physiological foundations of stress responses, investigating the role of flavonoids and phenolic compounds in mediating *A. gerardii*'s response to simulated grazing stress (Chapter 2). It then examines how these biochemical responses underpin post-defoliation growth and biomass allocation, revealing the echoes of ecological memory in plant growth patterns and resource allocation strategies (Chapter 3).

Our findings reveal that ecological memory influences *A. gerardii*'s response to simulated grazing, manifesting in nuanced physiological adaptations. Despite initial hypotheses, the interaction between plant memory and clipping treatments showed a complex effect on chlorophyll content and photosynthetic rates, with plants exhibiting a history of grazing demonstrating enhanced photosynthetic performance post-defoliation. Interestingly, the study underscores the significant role of flavonoids in mediating physiological responses to stress, independent of ecological memory. These secondary metabolites were pivotal in optimizing photosynthetic

efficiency and gas exchange, contributing to the plant's defensive responses and recovery post-clipping.

Furthermore, the research delves into biomass allocation strategies in response to defoliation, revealing that early-season biomass removal does not significantly affect end-of-season growth traits across plants with and without grazing memory. However, a notable finding was the consistent influence of relative growth rates (RGR) during early and late growth stages on end-of-season biomass, underscoring the inherent growth dynamics as a critical determinant of plant vigor.

Contrary to the anticipated benefits of grazing memory on growth enhancement, the study suggests that *A. gerardii*'s immediate physiological responses to defoliation play a more pivotal role than previously hypothesized. While grazing memory appears to confer some degree of adaptive advantage, it does not significantly modulate the correlation between RGR and end-of-season biomass attributes, indicating that innate growth responses are paramount.

In conclusion, this thesis illuminates the complexity of ecological memory and its impact on the physiological and growth responses of *A. gerardii* to grazing. The findings highlight the crucial role of secondary metabolites, particularly flavonoids, in mediating plant responses to stress and suggest that managing the timing and intensity of defoliation may be more effective than leveraging ecological memory in grassland management practices.

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Dedication

To Photosystem II, for its remarkable and unique ability to generate oxygen, sustaining life.

Chapter 1: Introduction

'Ecological memory' (Ogle et al., 2015), sometimes referred to as 'the history or legacy effects of environmental stressors' (Cuddington, 2011; Kannenberg et al., 2020) encompasses the mechanisms through which past environmental conditions and organismal experiences influence future responses and adaptations of ecosystems and individual species (Canarini et al., 2021). For ease of reference throughout this study, these concepts will be collectively termed as 'memory.' It is an evolving concept within the field of ecology, providing a framework for understanding how historical interactions with the environment, such as grazing, can prime an organism or ecosystem to respond to similar future stresses in a more robust manner (Conrath et al., 2006; Hilker et al., 2016). This memory encompasses an array of adaptive physiological and biochemical responses that prime the plant to cope more effectively with subsequent herbivores and grazing pressures (Galviz et al., 2022; Garai & Sopory, 2023; Hilker et al., 2016). These responses encompass a range of adaptations, including phenotypic plasticity, epigenetic alterations, and variations in gene expression (Hilker et al., 2016). Additionally, there's a noted plasticity in the synthesis and accumulation of secondary metabolites (Metlen et al., 2009), alongside adjustments in the metabolisms of abscisic acid and jasmonic acid, which contribute to the strengthening of stomatal closure and plant defense mechanisms (Qu et al., 2022). This type of memory has been observed to last for various durations in plants, ranging from hours to days in species such as *Arabidopsis thaliana*, which exhibited an increase in the transcript levels of genes induced by drought conditions (Ding et al., 2012). Nykänen & Koricheva (2004) demonstrated that trees can retain a memory of past stress for several months to increase their defenses against herbivores.

In grassland ecosystems, grazing is not only a common disturbance but also a fundamental ecological process that shapes species composition (Fahnestock & Knapp, 1994; Vinton et al., 1993), community structure (Eom et al., 2001; Milchunas et al., 1988), and ecosystem function (Li et al., 2018; Teague & Kreuter, 2020). The interplay between grass species, including big bluestem, and their grazers has been the subject of extensive research, and the effects of grazing on various aspects of grass physiology, community composition, and ecosystem processes have been documented in numerous studies (Vinton & Hartnett, 1992; Woodis & Jackson, 2008). However, these effects of grazing are not isolated events; they create a cascade of ecological consequences that ultimately shape the adaptive responses of grassland species (de Vries et al., 2019; Ren et al., 2017). In the quintessential tallgrass prairie ecosystem, *Andropogon gerardii* (big bluestem) stands as a prime example of a species molded by the evolutionary pressures of grazing. The ecological memory that arises from such interactions is postulated to significantly impact physiological and biochemical responses (Alves de Freitas Guedes et al., 2019; Wang et al., 2022), leading to variations in biomass, growth rate, and primary productivity (Briggs et al., 1994; Cass et al., 2015; Ogle et al., 2015). These parameters are not just reflective of a plant's immediate survival and fitness but are also indicative of its long-term competitive stance within the ecosystem (Alward & Joern, 1993; Oesterheld & McNaughton, 1991). Thus, the legacy of grazing is embedded within the very fabric of grassland ecology, influencing the resilience and adaptability of species like big bluestem over successive generations (Ogle et al., 2015; Qu et al., 2022).

While this thesis delves into the concept of ecological memory in the context of grazing, it is built upon the foundational work of several key studies that have explored the multifaceted nature of grazing effects on big bluestem. Notable among these is the work by Vinton & Hartnett,

(1992), which examines the interactions between grazers and compensatory growth of big bluestem.

In addition, the studies of over-grazing induced memory on grass species including seminal works by Oosterheld & McNaughton, (1991); Qu et al., (2022); Ren et al., (2017, 2018); and Wang et al., (2022), employed transcriptomic analyses to identify the most sensitive and conservative mechanisms, examining pathways such as carbohydrate metabolism, photosynthesis, and secondary metabolite production via the propanoid pathway. Further studies (Bazzaz et al., 1987; Canarini et al., 2021; Cass et al., 2015; Hilker et al., 2016; Palazzi et al., 2014) have enhanced our understanding of the growth and defense responses to grazing and defoliation. They all have collectively advanced our understanding of grazing and ecological memory, particularly induced-grazing memory. The insights from these studies serve as the intellectual groundwork for this thesis, providing a framework for investigating the lasting impacts of grazing on the physiological and ecological dynamics of C4 grass species, *A. gerardii*. Such insights encompass the long-term consequences of intense grazing, which include an upsurge in photosynthetic rates, and the stimulation of phenylpropanoid, enriched carbohydrate, and secondary metabolic pathways.

The overarching objective of this thesis is to rigorously test the hypothesis that the legacy effects of grazing (referred to memory in this study) impart a long-lasting influence on the plant's physiology and biochemical fabric of *A. gerardii*, shaping its growth rates, biomass accumulation, photosynthetic efficiency, and the production of defense-induced compounds including phenolic compounds. The plant's responses to grazing are not merely immediate reactions but are part of a continuum shaped by past experiences (Ogle et al., 2015; Qu et al., 2022). This ecological memory might confer an advantage in the competitive prairie ecosystem, allowing *A. gerardii* to anticipate

and mitigate the impacts of grazing (Agrawal, 2000; Alward & Joern, 1993; Oesterheld & McNaughton, 1991).

To interrogate this hypothesis, the thesis is structured into two thematic chapters, each addressing a core aspect of the plant's response to grazing:

The Biochemical Foundations of Stress Response

Chapter 2 investigates the influence of ecological memory on the physiological and biochemical responses of *Andropogon gerardii* under simulated grazing stress through clipping, with an acute focus on total phenols, flavonoids, and phenylalanine ammonia-lyase (PAL) activity. This chapter is particularly concentrated on flavonoids and their role as potential mediators of photosynthesis after defoliation. Acknowledged for their ability to regulate stomatal conductance and modulate light signaling pathways (Brunetti et al., 2019; Gai et al., 2020), and provide antioxidant benefits (Agati et al., 2012; Pourcel et al., 2007), flavonoids are hypothesized to enhance photosynthetic efficiency in response to stress. Key indicators, including the maximum electron transport rate (ETR_m), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and the overall rate of photosynthesis (A_{net}), serve as metrics to gauge the enhancement in photosynthetic performance, a critical factor in the plant's recovery and energy reallocation post biomass loss (Busch et al., 2024; Knapp, 1993; Mantlana et al., 2009).

The research explores whether a history of grazing (memory) intensifies flavonoid synthesis, thereby influencing the plant's compensatory capabilities following defoliation. This aspect is pivotal to understanding how *A. gerardii* might leverage flavonoids to amplify photosynthetic output and hasten recovery from grazing-induced stress. The findings from this study could reveal coping mechanisms that enable *A. gerardii* to thrive amidst the repetitive stress

of grazing, offering insights into the resilience mechanisms of grassland species and informing sustainable grassland management practices.

Post-Defoliation Growth and Biomass Allocation—Echoes of Ecological Memory

Chapter 3, the concluding experimental chapter, focuses on how memory of previous grazing influences growth responses and biomass distribution in *A. gerardii*. By comparing the growth patterns and resource allocation strategies of plants with and without a grazing history, this chapter explores the hypothesis that ecological memory facilitates adaptive growth and resilience, thus affecting the plant's ability to compensate for biomass loss due to grazing.

In conclusion, this thesis endeavors to provide a comprehensive evaluation of the legacy effects of grazing on *A. gerardii*, including physiological and biochemical analyses, shedding light on the mechanisms of ecological memory and their implications for grassland management and conservation. This study offers novel insights into the dynamics of grassland ecosystems, highlighting the role of historical grazing and plant memory in shaping future ecosystem resilience and productivity. This work underscores the importance of interdisciplinary approaches in addressing ecological questions, paving the way for informed conservation and management practices that support the health and sustainability of grassland ecosystems.

References

- Agati, G., Azzarello, E., Pollastri, S., & Tattini, M. (2012). Flavonoids as antioxidants in plants: Location and functional significance. *Plant Science: An International Journal of Experimental Plant Biology*, 196, 67–76. <https://doi.org/10.1016/j.plantsci.2012.07.014>
- Agrawal, A. (2000). Overcompensation of plants in response to herbivory and the by-product benefits of mutualism. *TRENDS IN PLANT SCIENCE*, 5(7), 309–313. [https://doi.org/10.1016/S1360-1385\(00\)01679-4](https://doi.org/10.1016/S1360-1385(00)01679-4)
- Alves de Freitas Guedes, F., Menezes-Silva, P. E., DaMatta, F. M., & Alves-Ferreira, M. (2019). Using transcriptomics to assess plant stress memory. *Theoretical and Experimental Plant Physiology*, 31(1), 47–58. <https://doi.org/10.1007/s40626-018-0135-0>
- Alward, R. D., & Joern, A. (1993). Plasticity and overcompensation in grass responses to herbivory. *Oecologia*, 95(3), 358–364. <https://doi.org/10.1007/BF00320989>
- Bachle, S., & Nippert, J. B. (2022). Climate variability supersedes grazing to determine the anatomy and physiology of a dominant grassland species. *Oecologia*, 198(2), 345–355. <https://doi.org/10.1007/s00442-022-05106-x>
- Bazzaz, F. A., Chiariello, N. R., Coley, P. D., & Pitelka, L. F. (1987). Allocating Resources to Reproduction and Defense: New assessments of the costs and benefits of allocation patterns in plants are relating ecological roles to resource use. *BioScience*, 37(1), 58–67. <https://doi.org/10.2307/1310178>
- Briggs, J. M., Fahnestock, J. T., Ward, L., & Knapp, A. K. (1994). *Aboveground biomass in tallgrass prairie: Effect of time since last fire.*

Brunetti, C., Sebastiani, F., & Tattini, M. (2019). Review: ABA, flavonols, and the evolvability of land plants. *Plant Science*, 280, 448–454.

<https://doi.org/10.1016/j.plantsci.2018.12.010>

Busch, F. A., Ainsworth, E. A., Amtmann, A., Cavanagh, A. P., Driever, S. M., Ferguson, J. N., Kromdijk, J., Lawson, T., Leahey, A. D. B., Matthews, J. S. A., Meacham-Hensold, K., Vath, R. L., Vialet-Chabrand, S., Walker, B. J., & Papanatsiou, M. (2024). A guide to photosynthetic gas exchange measurements: Fundamental principles, best practice and potential pitfalls. *Plant, Cell & Environment*, n/a(n/a). <https://doi.org/10.1111/pce.14815>

Canarini, A., Schmidt, H., Fuchslueger, L., Martin, V., Herbold, C. W., Zezula, D., Gündler, P., Hasibeder, R., Jecmenica, M., Bahn, M., & Richter, A. (2021). Ecological memory of recurrent drought modifies soil processes via changes in soil microbial community.

Nature Communications, 12(1), Article 1. <https://doi.org/10.1038/s41467-021-25675-4>

Cass, C. L., Peraldi, A., Dowd, P. F., Mottiar, Y., Santoro, N., Karlen, S. D., Bukhman, Y. V., Foster, C. E., Thrower, N., Bruno, L. C., Moskvina, O. V., Johnson, E. T., Willhoit, M. E., Phutane, M., Ralph, J., Mansfield, S. D., Nicholson, P., & Sedbrook, J. C. (2015). Effects of *PHENYLALANINE AMMONIA LYASE* (*PAL*) knockdown on cell wall composition, biomass digestibility, and biotic and abiotic stress responses in *Brachypodium*. *Journal of Experimental Botany*, 66(14), 4317–4335. <https://doi.org/10.1093/jxb/erv269>

Conrath, U., Beckers, G. J. M., Flors, V., García-Agustín, P., Jakab, G., Mauch, F., Newman, M.-A., Pieterse, C. M. J., Poinssot, B., Pozo, M. J., Pugin, A., Schaffrath, U., Ton, J., Wendehenne, D., Zimmerli, L., & Mauch-Mani, B. (2006). Priming: Getting Ready for Battle. *Molecular Plant-Microbe Interactions®*, 19(10), 1062–1071.

<https://doi.org/10.1094/MPMI-19-1062>

- Cuddington, K. (2011). Legacy Effects: The Persistent Impact of Ecological Interactions. *Biological Theory*, 6(3), 203–210. <https://doi.org/10.1007/s13752-012-0027-5>
- de Vries, J., Evers, J., Dicke, M., & Poelman, E. (2019). Ecological interactions shape the adaptive value of plant defence: Herbivore attack versus competition for light. *FUNCTIONAL ECOLOGY*, 33(1), 129–138. <https://doi.org/10.1111/1365-2435.13234>
- Ding, Y., Fromm, M., & Avramova, Z. (2012). Multiple exposures to drought “train” transcriptional responses in Arabidopsis. *Nature Communications*, 3(1), 740. <https://doi.org/10.1038/ncomms1732>
- Eom, A.-H., Wilson, G. W., & Hartnett, D. C. (2001). Effects of ungulate grazers on arbuscular mycorrhizal symbiosis and fungal community structure in tallgrass prairie. *Mycologia*, 93(2), 233–242.
- Fahnestock, J. T., & Knapp, A. K. (1994). Plant responses to selective grazing by bison: Interactions between light, herbivory and water stress. *Vegetatio*, 115(2), 123–131. <https://doi.org/10.1007/BF00044867>
- Gai, Z., Wang, Y., Ding, Y., Qian, W., Qiu, C., Xie, H., Sun, L., Jiang, Z., Ma, Q., Wang, L., & Ding, Z. (2020). Exogenous abscisic acid induces the lipid and flavonoid metabolism of tea plants under drought stress. *Scientific Reports*, 10(1), Article 1. <https://doi.org/10.1038/s41598-020-69080-1>
- Galviz, Y., Souza, G. M., & Lüttge, U. (2022). The biological concept of stress revisited: Relations of stress and memory of plants as a matter of space–time. *Theoretical and Experimental Plant Physiology*, 34(2), 239–264. <https://doi.org/10.1007/s40626-022-00245-1>

- Garai, S., & Sopory, S. K. (2023). Memory of plants: Present understanding. *The Nucleus*, 66(1), 47–51. <https://doi.org/10.1007/s13237-022-00399-y>
- Hilker, M., Schwachtje, J., Baier, M., Balazadeh, S., Bäurle, I., Geiselhardt, S., Hinch, D. K., Kunze, R., Mueller-Roeber, B., Rillig, M. C., Rolff, J., Romeis, T., Schmülling, T., Steppuhn, A., van Dongen, J., Whitcomb, S. J., Wurst, S., Zuther, E., & Kopka, J. (2016). Priming and memory of stress responses in organisms lacking a nervous system. *Biological Reviews*, 91(4), 1118–1133. <https://doi.org/10.1111/brv.12215>
- Kannenberg, S. A., Schwalm, C. R., & Anderegg, W. R. L. (2020). Ghosts of the past: How drought legacy effects shape forest functioning and carbon cycling. *Ecology Letters*, 23(5), 891–901. <https://doi.org/10.1111/ele.13485>
- Knapp, A. K. (1993). Gas Exchange Dynamics in C³ and C⁴ Grasses: Consequence of Differences in Stomatal Conductance. *Ecology*, 74(1), 113–123. <https://doi.org/10.2307/1939506>
- Li, W., Li, X., Zhao, Y., Zheng, S., & Bai, Y. (2018). Ecosystem structure, functioning and stability under climate change and grazing in grasslands: Current status and future prospects. *Current Opinion in Environmental Sustainability*, 33, 124–135. <https://doi.org/10.1016/j.cosust.2018.05.008>
- Mantlana, K. B., Veenendaal, E. M., Arneith, A., Grispen, V., Bonyongo, C. M., Heitkonig, I. G., & Lloyd, J. (2009). Biomass and leaf-level gas exchange characteristics of three African savanna C₄ grass species under optimum growth conditions. *African Journal of Ecology*, 47(4), 482–489. <https://doi.org/10.1111/j.1365-2028.2008.00961.x>

- Metlen, K. L., Aschehoug, E. T., & Callaway, R. M. (2009). Plant behavioural ecology: Dynamic plasticity in secondary metabolites. *Plant, Cell & Environment*, 32(6), 641–653. <https://doi.org/10.1111/j.1365-3040.2008.01910.x>
- Milchunas, D. G., Sala, O. E., & Lauenroth, W. K. (1988). A Generalized Model of the Effects of Grazing by Large Herbivores on Grassland Community Structure. *The American Naturalist*, 132(1), 87–106. <https://doi.org/10.1086/284839>
- Nykänen, H., & Koricheva, J. (2004). Damage-induced changes in woody plants and their effects on insect herbivore performance: A meta-analysis. *Oikos*, 104(2), 247–268. <https://doi.org/10.1111/j.0030-1299.2004.12768.x>
- Oosterheld, M., & McNaughton, S. J. (1991). Effect of Stress and Time for Recovery on the Amount of Compensatory Growth after Grazing. *Oecologia*, 85(3), 305–313.
- Ogle, K., Barber, J. J., Barron-Gafford, G. A., Bentley, L. P., Young, J. M., Huxman, T. E., Loik, M. E., & Tissue, D. T. (2015). Quantifying ecological memory in plant and ecosystem processes. *Ecology Letters*, 18(3), 221–235. <https://doi.org/10.1111/ele.12399>
- O’Keefe, K., & Nippert, J. B. (2017). Grazing by bison is a stronger driver of plant ecohydrology in tallgrass prairie than fire history. *Plant and Soil*, 411(1), 423–436. <https://doi.org/10.1007/s11104-016-3048-1>
- Palazzi, E., Currò, F., Reverberi, A., & Fabiano, B. (2014). Resilience Engineering Strategy Applied to an Existing Process Plant. *Chemical Engineering Transactions*. <https://www.semanticscholar.org/paper/Resilience-Engineering-Strategy-Applied-to-an-Plant-Palazzi-Curr%C3%B2/e59ebb983e38f06477cd7f5e762680170d24be57>

- Pourcel, L., Routaboul, J.-M., Cheynier, V., Lepiniec, L., & Debeaujon, I. (2007). Flavonoid oxidation in plants: From biochemical properties to physiological functions. *Trends in Plant Science*, 12(1), 29–36. <https://doi.org/10.1016/j.tplants.2006.11.006>
- Qu, K., Cheng, Y., Gao, K., Ren, W., Fry, E. L., Yin, J., & Liu, Y. (2022). Growth-Defense Trade-Offs Induced by Long-term Overgrazing Could Act as a Stress Memory. *Frontiers in Plant Science*, 13. <https://doi.org/10.3389/fpls.2022.917354>
- Ren, W., Hou, X., Wu, Z., Kong, L., Guo, H., Hu, N., Wan, D., & Zhang, J. (2018). De novo transcriptomic profiling of the clonal *Leymus chinensis* response to long-term overgrazing-induced memory. *Scientific Reports*, 8(1), Article 1. <https://doi.org/10.1038/s41598-018-35605-y>
- Ren, W., Hu, N., Hou, X., Zhang, J., Guo, H., Liu, Z., Kong, L., Wu, Z., Wang, H., & Li, X. (2017). Long-Term Overgrazing-Induced Memory Decreases Photosynthesis of Clonal Offspring in a Perennial Grassland Plant. *Frontiers in Plant Science*, 8. <https://doi.org/10.3389/fpls.2017.00419>
- Teague, R., & Kreuter, U. (2020). Managing Grazing to Restore Soil Health, Ecosystem Function, and Ecosystem Services. *Frontiers in Sustainable Food Systems*, 4. <https://www.frontiersin.org/articles/10.3389/fsufs.2020.534187>
- Vinton, M. A., & Hartnett, D. C. (1992). Effects of Bison Grazing on *Andropogon gerardii* and *Panicum virgatum* in Burned and Unburned Tallgrass Prairie. *Oecologia*, 90(3), 374–382.
- Vinton, M. A., Hartnett, D. C., Finck, E. J., & Briggs, J. M. (1993). The interactive effects of fire, bison (*Bison bison*) grazing and plant community composition in tallgrass prairie. *American Midland Naturalist*, 129, 10–18. <https://doi.org/10.2307/2426430>

- Wang, Y., Zhu, W., Ren, F., Zhao, N., Xu, S., & Sun, P. (2022). Transcriptional Memory in *Taraxacum mongolicum* in Response to Long-Term Different Grazing Intensities. *Plants*, *11*(17), Article 17. <https://doi.org/10.3390/plants11172251>
- Woodis, J. E., & Jackson, R. D. (2008). The effects of clipping height and frequency on net primary production of *Andropogon gerardii* (C4 grass) and *Bromus inermis* (C3 grass) in greenhouse experiments. *Grass and Forage Science*, *63*(4), 458–466. <https://doi.org/10.1111/j.1365-2494.2008.00653.x>

Chapter 2: Flavonoids-Mediated Stress Responses in *Andropogon gerardii*: Decoupling Biochemical Adaptations from Ecological Memory in Response to Clipping Stress

Abstract

This chapter explores the biochemical and physiological adaptations of *Andropogon gerardii* using simulated grazing stress to study the effect of ecological memory, focusing on the decoupling of flavonoid-mediated stress responses from ecological memory. The study was conducted in a greenhouse environment replicating natural conditions of the Konza Prairie. The physiological and biochemical responses of *A. gerardii*, a dominant species in North American tallgrass prairies, was assessed through the lens of photosynthetic efficiency, secondary metabolite production, and enzyme activity. The central biochemical markers analyzed included total phenolic compounds, flavonoids, and phenylalanine ammonia-lyase (PAL) activity, all critical to the plant's defensive strategy and stress tolerance. The investigation revealed that while ecological memory plays a role in the plant's response to the history of grazing, flavonoids levels significantly impact photosynthesis and gas exchange, with their concentration increasing in response to clipping stress and correlating positively with photosynthetic performance. Notably, this adaptive response was not solely reliant on ecological memory, as flavonoids independently enhanced the plant's recovery mechanisms. These findings suggest a complex interaction between environmental history, biochemical pathways, and current stressors that contribute to *A. gerardii*'s responses. By highlighting the nuanced role of flavonoids and challenging the presumed dominance of ecological memory in stress response, this chapter expands our understanding of

plant adaptability and suggests new avenues for research into the mechanisms of plant-environment interactions.

Introduction

Grassland ecosystems, pivotal to global biodiversity, endure numerous environmental stressors that shape their flora's physiological and biochemical landscape (Brown & Stuth, 1993; Brunsell et al., 2017; Ren et al., 2017, 2018), with grazing notably influencing the traits and competitive interactions of species such as *Andropogon gerardii* (Díaz et al., 2007; Fahnestock & Knapp, 1994). This dominant species in North American tallgrass prairies demonstrates remarkable resilience, shaped by both its ecological interactions, such as preferential grazing by bison, and its capacity for compensation following defoliation (Lima et al., 2020; Vinton & Hartnett, 1992, 1992; Woodis & Jackson, 2008). These adaptive responses not only affect individual species but also drive community dynamics within these ecosystems (Knapp et al., 1999).

Photosynthesis and gas exchange, fundamental to plant vitality and growth, are profoundly influenced by environmental factors such as grazing (Parsons et al., 1983; Pfanz et al., 2007). In response to the reduction of photosynthetic tissues from grazing, plants engage in a delicate balance of compensatory mechanisms (Anten et al., 2003; Figueroa-Macias et al., 2021). These mechanisms aim to reallocate nutrients towards either regrowth or defense, with the production of secondary metabolites being a key strategy in this adaptive response (Abegaz & Kinfé, 2019; Albrecht & Argueso, 2017).

Our investigation centers on the measurement of key biochemical markers that are integral to plant physiological adaptation and defense: total phenolic compounds, flavonoids, and

phenylalanine ammonia-lyase (PAL) activity. These markers are vital in a plant's defense mechanisms and stress responses (Barros & Dixon, 2020; Chen et al., 2017). Phenolic compounds and flavonoids, in particular, are known for their robust antioxidant properties, which arm the plant against oxidative stress, a frequent outcome of environmental stresses (Barros & Dixon, 2020; Chen et al., 2017). Their capacity to scavenge reactive oxygen species (ROS) is a cornerstone of the plant's defensive strategy, mitigating damage and preserving cellular integrity (Chalker-Scott & Fuchigami, 2018; Harborne, 1989).

Beyond defense, flavonoids are deeply involved in the regulation of growth and development, owing to their dual role as antioxidants and signaling molecules (Erb & Kliebenstein, 2020). Their spectrum of physiological functions is broad, extending from providing a photoprotection barrier (Close & McArthur, 2002; Pourcel et al., 2007) to modulating auxin transport and acting as an endogenous growth regulator, as observed in the *Arabidopsis thaliana* chalcone synthase mutant (Brown et al., 2001). Notably, diminished flavonoids' levels have been linked to an accumulation of ROS, particularly in guard cells, leading to phenotypes that exhibit accelerated abscisic acid-induced stomatal closure and altered gas exchange (Watkins et al., 2014). Additionally, a correlation has been observed between lower flavonoid concentrations and inhibited root growth (Brown et al., 2001), highlighting their significance beyond mere stress response to fundamental aspects of plant development. While the majority of existing literature highlights the role of phenolic compounds in safeguarding against photodamage, some of the studies suggest alternative functions, including their capacity to act as carbon sinks, which effectively act to absorb excess photosynthetic carbon (Close & McArthur, 2002; Waterman et al., 1984).

PAL is essential in the phenylpropanoid pathway (Dixon et al., 2002), catalyzing the synthesis of phenolic compounds, including flavonoids, and linking primary to secondary metabolism in response to stress (Pourcel et al., 2007; Yadav et al., 2020). Decreased PAL activity results in lower biomass and reduced root growth, making plants more susceptible and less resilient (Cass et al., 2015b). Furthermore, PAL activity is crucial for secondary metabolite production in plants under long-term grazing stress, with previous stress exposure enhancing PAL's response to intensive grazing (Ren et al., 2017; Yadav et al., 2020).

Central to the understanding of these adaptive strategies is the concept of ecological memory (Canarini et al., 2021; Zhang et al., 2023), which allows plants to utilize past environmental experiences to inform current and future responses to similar stressors (Hilker et al., 2016; Kafle & Wurst, 2019). This memory manifests both genetically (Chen et al., 2014; Dang et al., 2021; Ren et al., 2018; Wang et al., 2022) and as acquired physiological adjustments (Ren et al., 2017; West-Eberhard, 2008), enabling species like *A. gerardii* to optimize defense pathways and resource allocation in anticipation of recurring stress events such as grazing (Bazzaz et al., 1987; Siemens et al., 2010). Thus, the interplay between the immediate physiological impacts of grazing and the more temporally extended influence of ecological memory provides a comprehensive framework for understanding plant responses and adaptation in dynamic grassland environments (Avramova, 2019; Hilker et al., 2016).

Collectively, these biochemical markers can affect plant growth (Erb, 2018; Erb & Kliebenstein, 2020) and resilience (Rama & Vinutha, 2019), potentially by reinforcing the effects of ecological memory (Sharma et al., 2022), enhancing stress tolerance, and facilitating recovery from damage (Abegaz & Kinf, 2019; Divekar et al., 2022). Given this intricate relationship between these factors, this study aims to elucidate the role of secondary metabolite production in

response to simulated grazing in *A. gerardii*. Specifically, we seek to dissect the role of ecological memory in enhancing the plant's physiological performance and response to stress and examine how photosynthetic parameters and the production of secondary metabolites contribute to *A. gerardii*'s adaptive strategies. Based on these objectives, we propose the following hypotheses:

H1. Ecological memory of grazing positively influences plant physiological performance under similar subsequent stress, clipping, suggesting that previous grazing experiences enhance *A. gerardii*'s adaptive capabilities.

H2. The synthesis of phenolic compounds (including flavonoids) and increased PAL enzyme activity are significantly affected by ecological memory and will impact plant responses toward the end of the growing season, highlighting the importance of these secondary metabolites and PAL activity in physiological stress responses modulated by ecological memory.

H3. Ecological memory and secondary metabolite production are hypothesized to be pivotal in shaping plants responses, compensation, and recovery mechanisms in *A. gerardii* in response to environmental stress, with historical stress exposure fundamentally determining the plant's adaptive capacity.

This research aims to explore the intricate role of secondary metabolite production in *A. gerardii* in response to simulated grazing, with a particular focus on the contributions of ecological memory.

Methods

Site Description

Research was conducted in spring and summer 2023 by transferring plant rhizomes with early grown leaves from the Konza Prairie to the greenhouse space. The Konza Prairie Biological Station (KPBS), a 3,487-ha native C4 grassland in northeastern Kansas, USA (39°05' N, 96°35' W) is located in the tallgrass prairie within the Flint Hills, one of the largest continuous stretches of unplowed prairies remaining in North America.

Plant Materials and Treatments

For this investigation, two watersheds, N01A and N01B, were selected, both of which are subject to the same fire regime (annual burning) and bison grazing treatment. Rhizome collection occurred within similar upland topographic regions of both watersheds. Established in 1988, four exclosures, with a total area of 50 m² each, in the upland areas of each watershed (Hartnett, 2023), amounting to eight in total, facilitated a controlled sampling approach. The vegetation within these exclosures remained ungrazed by bison, effectively representing plants devoid of grazing-induced ecological memory. Conversely, to sample plants with a history of grazing, four additional regions in the uplands of each watershed were identified, totaling eight across both watersheds. From each of the 16 total regions representing both types of ecological memory, a minimum of two plants were carefully transplanted to the greenhouse. There, a clipping experiment simulated heavy grazing pressure. The experiment featured two treatments: clipped (G) and unclipped/control (C), with one plant from each region assigned to each treatment. This resulted in four distinct groups with eight biological replicates per group, delineating the effects of past field grazing memory and current clipping treatments as follows:

1. No grazing-induced ecological memory (M-)/clipped (G): M-/G
2. With grazing-induced ecological memory (M+)/clipped (G): M+/G
3. No grazing-induced ecological memory /control (C): M-/C
4. With grazing-induced ecological memory (M+)/control (C): M+/C

As such, treatment groups will be designated using more simplified abbreviations: M-G, M+G, M-C, and M+C. 'Ecological memory' will also be referred to more succinctly as 'memory'.

Clipping treatments and measurements

The research was conducted in the greenhouse facilities of the Department of Agronomy at Kansas State University, located in Manhattan, KS, USA. Following collection, the rhizomes were planted in containers measuring 24 cm diameter and 23 cm height, filled with a uniform mixture of greenhouse and Konza Prairie soil, to maintain consistent soil conditions and control for environmental variability (Ren et al., 2018; Wang et al., 2022). The greenhouse conditions included maintaining temperatures between 24°C to 32°C during the day and 15°C to 24°C at night. Humidity levels were kept between 50% to 70%. Adequate natural light exposure was ensured to provide full sunlight conditions. Air circulation was maintained using fans, and watering was performed as needed to maintain consistent soil moisture without waterlogging. After an acclimatization period of one month, a simulation of heavy grazing was implemented through a clipping process, where 70% of the plant aboveground biomass were removed on a height basis to mimic grazing pressure, as described by Alber et al. (2014). The height of each plant was measured prior to each clipping, and 70% of the measured height was removed by clipping to mimic heavy grazing pressure in the greenhouse. This process was carried out twice, in mid-June and mid-July.

Before and after the clipping events, we collected data on plant physiology including photosynthetic rates, Rapid Light Curves (RLCs), and chlorophyll content using nondestructive methods. Please refer to their specific section in the methods of chapter two for more details. For the analysis of biochemical markers, total phenols, flavonoids, and PAL activity, sampling was conducted at three intervals during each clipping event for the M-G and M+G groups: immediately before (before), immediately after (after), and 8 hours post-clipping (8 hours). Within this chapter, these sampling times are collectively termed 'timing' and encompass three levels as mentioned. Samples were also collected from the control groups (M-C and M+C) once in June and July, coinciding with the sampling times of the clipped plants. Unlike the clipped groups, which were sampled thrice to coincide with the clipping events, the unclipped control plants were sampled only once due to the absence of clipping treatment at each clipping event. Further sampling occurred in early August and September across all groups to monitor marker variability across the growing season. All collected samples were instantly flash-frozen in liquid nitrogen and subsequently stored at -80°C in the laboratory's ultra-low temperature freezer.

Photosynthetic measurements

Net photosynthetic rates were measured from late June to mid-September using a LI-COR 6400XT infrared gas analyzer (LI-COR Inc., Lincoln, NE, USA). Reference CO₂ concentration was set to 400 $\mu\text{mol mol}^{-1}$, PAR was set to 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and relative humidity was maintained between 50-65% during measurements to approximate ambient conditions at this site. Measurements were taken between the hours of 9:30 and 12:00 on clear, sunny days.

Instantaneous water-use efficiency (iWUE) was calculated using the net photosynthetic

rate (A_{net}) and stomatal conductance (g_s) in a given formula (Farquhar et al., 1989):

$$A_{\text{net}}/g_s$$

For the variables related to photosynthesis and measured by Licor 6400 (A_{net} , g_s , transpiration (E), intercellular concentration of CO_2 (c_i), and $i\text{WUE}$), there is a missing data for plants with the memory ($M+$) that underwent clipping treatment (G) (group $M+G$) on 16 July right before applying the second round of clipping (Figure 1).

Rapid Light Curves

Using Photosynthesis Yield Analyzer MINI-PAM (Walz GmbH, Effeltrich, Germany), data on photosynthetic performance of plants were collected throughout the project in 7 courses starting from Mid-June 23 (after applying the first clipping treatment) to Mid Sep 23. Data were exported from WinControl into Sigmaplot 2001 (v7.0, SPSS Inc.). Rapid Light Curves (RLCs) were measured using actinic light incremented in nine steps from 0 to 1354 with a prescribe duration of 10 sec. Overall, RLCs took 90 s (Ralph & Gademann, 2005) and spanned a range of $1400 \mu\text{mol photon m}^{-2} \text{s}^{-1}$.

RLCs fitting

To conduct a quantitative comparison of RLCs, we employed a methodological approach grounded in the foundational work of Platt et al., (1980), which has been further elaborated upon by Ralph & Gademann, (2005). The Platt formulation offers a robust description of the photosynthetic response as a function of varying light intensities, encapsulating both the initial light response curve and the photoinhibitory phase at elevated irradiance levels (Platt et al., 1980; Ralph & Gademann, 2005).

For automated RLC analysis, the ‘`ssplatt.my`’ function from the ‘`platt`’ package in R has been developed by SWotherspoon and is publicly accessible via the GitHub repository. This code was further modified by Ricardo, (2020/2021). In this study, in transitioning from R to Python, we adapted the code to employ the `curve_fit` function from the `scipy.optimize` library to fit RLCs (Gommers et al., 2022; Müller-Bravo et al., 2022).

The analytical workflow commenced with the computation of relative Electron Transport Rate (rETR) by integrating the effective quantum yield of Photosystem II (YII) with Photosynthetically Active Radiation (PAR), where ETR represents an estimation of the electron flow through the photosynthetic electron transport chain as posited by Beer et al., (1998).

The empirical data were mathematically modeled to a double exponential decay function, as originally described by Platt et al., (1980), utilizing the Marquardt-Levenberg regression algorithm. The key equations central to this analysis were:

$$rETR_{max} = Ps(\alpha/[\alpha + \beta])(\beta/[\alpha + \beta])^{\beta/\alpha}$$

$$E_k = ETR_{max}/\alpha$$

The curve-fitting process was finalized by first establishing initial parameter values. Subsequent application of nonlinear models facilitated the extraction of a comprehensive set of RLCs’ parameters, which included the initial slope (α), the photoinhibitory coefficient (β), the onset of light saturation (E_k), the maximum electron transport rate (E_m), and the maximal relative electron transport rate (ETR_m). Although all derived RLC parameters were extracted, only ETR_m , α , and E_k are included as the primary parameters of interest to describe photosynthetic capacity of leaves in this chapter.

Chlorophyll Content using SPAD meter.

Chlorophyll (chl) content was quantified utilizing a SPAD 502 meter. To ensure consistency across all treatments and groups, measurements were uniformly taken on the half part of the leaves. The results have been expressed as the SPAD index (Cotrozzi et al., 2020; Shibaeva et al., 2020).

Total Phenolic Compounds, Flavonoids Concentration, and PAL activity

Total phenolic content was determined using the procedures described by Singleton et al., 1999 with modifications (Juan & Chou, 2010). An aliquot of 0.1 mL plant extracts or standard (gallic acid (Sigma-Aldrich Co., St. Louis, MO, USA)) was mixed with 0.1 mL FolineCiocalteu phenol reagent 10% v/v (Sigma-Aldrich Co., St. Louis, MO, USA) and allowed to react for 5 min. Then 800 µl of 7% (w/v) Na₂CO₃ (Sigma-Aldrich Co., St. Louis, MO, USA) was added and allowed to react for 90 min at room temperature. The concentrations employed for the standard solution ranged from 0 to 125 nmol/mL of solvent, with intervals of 25 nmol/mL. Absorbance was measured at 725 nm using a microplate reader (SPECTRAmax 190 Microplate Spectrophotometer, Molecular Devices Co., Sunnyvale, CA, USA). The results were expressed as mmol gallic acid/g fresh weight of tissues.

Total flavonoids were extracted and determined using the method described by Zhishen et al., 1999 by modifications (Juan & Chou, 2010). 250 µl of standard (quercetin (Sigma-Aldrich Co., St. Louis, MO, USA) (Jain et al., 2021; Sulastri et al., 2018)) or plant leaf extracts obtained using 90% methanol (50 mg of fresh weight) was added to 1.25 ml deionized water and then was

mixed with 75 μ l of 5% (w/v) NaNO₂ (Sigma-Aldrich Co., St. Louis, MO, USA). After standing for 6 min, this mixture was combined with 10% (w/v) AlCl₃ (150 μ l) (Sigma-Aldrich Co., St. Louis, MO, USA). Then 500 μ l of 1 N NaOH (Sigma-Aldrich Co., St. Louis, MO, USA) and 275 μ l of deionized water was added to the mixture. The tubes were kept at room temperature and in the dark for 5 min before reading the absorbance. The concentrations used for the standard were 0, 100, 200, 400, 600, 800, and 1000 μ g quercetin/ml of solvent. Absorbance was recorded using a microplate reader (SPECTRAmax 190 Microplate Spectrophotometer, Molecular Devices Co., Sunnyvale, CA, USA) at 510 nm. The concentration of flavonoids was reported as mmol quercetin/g FW tissues.

The activity of PAL, which catalyzes the conversion of L-phenylalanine to trans-cinnamic acid and ammonia (Barros & Dixon, 2020), was quantified by monitoring the increase in optical density (OD) at 290 nm, using a PAL assay kit (Catalogue E-BC-K522-S, Elabscience Biotechnology Co. Ltd, China) (Wang et al., 2022).

To analyze the data related to these biochemical markers, initially, we investigated the influence of timing, memory, and date on phenols, flavonoids, and PAL activity focusing exclusively on plants subjected to clipping treatments (G), both with (M+) and without (M-) prior grazing memory. For a detailed explanation of timing and its corresponding intervals, please refer to the “clipping treatments and measurements” from methods section of this chapter.

Subsequently, we assessed the monthly fluctuations of PAL, phenols, and flavonoid concentrations for July, August, and September (Figure 2.5). The three-way ANOVA incorporated memory, treatments, and date as predictors, with July data derived from samples collected before applying the simulated heavy grazing pressure, denoted as "before" in this study.

Statistical methods

To model RLCs, the `curve_fit` function from the `scipy.optimize` module was employed (Virtanen et al., 2020), which is part of the SciPy library, a Python-based ecosystem of open-source software for mathematics, science, and engineering (Oliphant, 2007).

Each step of our data preprocessing, assumption checks, statistical analysis, and machine learning techniques was thoroughly documented within our Python scripts and Jupyter notebooks. We chose Visual Studio Code (VSCode) as our primary integrated development environment (IDE) (Speight, 2021). `statsmodels` was applied for the ANOVA, Tukey's HSD test, and multiple regression analysis (OLS) (Seabold & Perktold, 2010). `pandas` and `numpy` were used for data manipulation, handling, and transformation (Harris et al., 2020; McKinney, 2010). `Matplotlib` (Hunter, 2007) and `seaborn` (Lemenkova, 2020; Waskom, 2021) were utilized for data visualization. All necessary assumptions for the valid application of ANOVA, post-hoc Tukey's HSD test, and regression analyses were thoroughly examined, such as normality, independence, homoscedasticity, and multicollinearity (by examining the Variance Inflation Factor (VIF)), using appropriate tests and diagnostic plots.

Due to unmet linear regression assumptions and ineffective data transformations, a generalized linear model (GLM) was employed to analyze the relationship between PAL activity and ETRm.

Results

Photosynthesis: Statistical analysis revealed that the date of measurement had a highly significant effect on the rate of photosynthesis ($p < 0.001$). Additionally, there were significant

interaction effects between memory condition and date ($p = 0.0162$), and between treatment condition and date ($p = 0.0000115$) on photosynthesis rates. The three-way interaction between memory condition, treatment condition, and date also showed a potential significance ($p = 0.0247$). There were significant effects of memory condition alone ($p = 0.0014$), but no significant effect of clipping treatment alone ($p > 0.05$) on the rate of photosynthesis.

As showed in Figure 2.1.A, after the first round of clipping in June, A_{net} was lower in plants lacking the memory of previous grazing events (M-G) which displays the negative effect of the absence of memory on the rate of photosynthesis.

In the first interval between the first clipping in June and second clipping in July, plants in all groups reduced the rate of photosynthesis, and plants lacking the memory in control treatment performed better significantly ($p = 0.0014$). In the second interval, after the second round of clipping, all plants in 4 different groups increase A_{net} regardless of clipping treatment. However, our analysis shows that plants with the memory of previous grazing events perform better while they started flowering. Also, they all decreased A_{net} by the end of the season in Sep (Figure 2.1.A).

Stomatal conductance (g_s): The most significant factors affecting g_s are the date of measurement ($p = 2.97\text{E-}26$) and the interaction between memory and date ($p = 4.47 \times 10^{-3}$). Both these finding reflect the effect of the date we applied the clipping treatments which resulted in photosynthetic tissue loss. The clipping treatment alone (G vs C) ($p > 0.05$), memory alone ($p = 8.03\text{E-}02$), and the other interaction terms are not statistically significant in influencing g_s (Table 2.1, Figure 2.1.B).

Transpiration rate (E): The date of measurement is the most significant factor ($p = 9.44\text{E-}54$). There are also significant interaction effects between memory and date reflecting the effect of memory at different clipping time ($p = 1.64\text{E-}02$), as well as between treatment condition and date

($p=6.80E-04$). However, the memory condition alone, clipping treatment alone, and the three-way interaction did not significantly affect transpiration rate. Overall, transpiration decreases in all plants regardless of their memory and clipping treatment, indicating non-significant effect of the interaction of memory and treatment ($p>0.05$) (Figure 2.1.C).

iWUE: The date of measurement shows a highly significant effect ($p=9.17E-23$), and there are significant interaction effects between memory and date ($p=4.37E-05$) and between treatment condition and date ($p=5.45E-04$). However, the memory alone ($p=2.80E-01$), treatment alone ($p>0.05$), and the three-way interaction between memory, treatment, and date ($p=8.64E-01$) did not significantly affect iWUE. After the first clipping treatment, plants with no memory have the lowest iWUE (group: M-G, Figure 2.1, D), and plants without the memory (regardless of the clipping treatment) have higher iWUE (M-G and M-C, Figure 2.1, D).

C_i: For C_i, both the memory alone ($p=1.34E-03$) and the date of measurement ($p=1.16E-26$) are highly significant factors. There were also significant interaction effects between memory and date ($p=4.37E-05$), or between treatment condition and date ($p=8.10E-17$). The treatment condition alone and the three-way interaction between memory, treatment, and date had no significant effect on C_i (Table 2.1). After applying the first clipping, C_i increased in clipping plants compared to control and plants without the memory has a higher level of C_i (Figure 2.1.E). Plants in M-G had the highest C_i after the first clipping in June and by the end of the season, C_i dropped in them. However, this decreased at the end of the season is not significant indicating that plants regardless of the treatment and memory status decrease C_i by the end of the growing season.

RLCs

ETRm: Memory and treatments were not significant predictors (p values >0.05). The interaction between memory and treatment was significant ($p= 1.85E-02$). Date was an important predictor for ETRm ($p= 1.04E-16$) (Figure 2.C). Similarly, the interaction between memory and date ($p= 2.36E-03$) and treatment and date ($p= 3.61E-02$) were also significant. The three-way interaction of them is not significant ($p= 2.59E-01$) (Table 2.2, and Figure 2.2.C).

For **Alpha**, the initial slope of the RLCs, date had a significant effect ($p=5.18E-07$), but none of the other main effects were significant at the 0.05 level, and the interaction terms did not reach conventional levels of significance (Table 2.2, and Figure 2.2.A).

Lastly, for **Ek**, date again was a highly significant predictor ($p= 9.14E-12$), while both memory ($p= 3.03E-01$) and clipping treatments ($p= 1.08E-01$) were not significant. However, the interaction between memory and date ($p= 2.52E-02$) and treatment and date ($p= 2.03E-02$) were significant (Table 2.2 and Figure 2.2.B).

Chl using SPAD-502

Three-way ANOVA revealed that memory had no significant effect on chl ($p= 0.28$). Different clipping treatments (clipped (G) vs control (C)) did not have significant effects on chl either. However, date was highly significant ($p < 0.001$). The interactions of date and memory ($p < 0.001$), date and treatment ($p= 0.003$) were significant as well. However, the interaction of memory and treatment was not significant ($p= 0.196$), indicating that there was no interaction effect between memory and treatment on Chl content, contrary to our initial hypothesis (Table 2.3 and Figure 2.3).

Total Phenols, Flavonoids, and PAL activity

Phenols-June: As two-way ANOVA revealed (Table 2.4), phenols were not affected by memory or timing after applying the first round of clipping in June. However, their interaction was significant (0.005). Plants with the memory (M+) had lower amount of phenols especially 8 hours after clipping. While plants without memory (M-), in fact, increased the amount of phenols within 8 hours after clipping in June, but this change was not significant (p value > 0.05) (Table 2.4, Figure 2.4).

Flavonoids- June: 'Timing' had a highly significant effect on flavonoid levels in June (p= 0.0002). Memory of previous grazing did not significantly affect flavonoid levels (p= 0.97). Also, there was no significant interaction effect of timing and memory (p= 0.82). We found that there was a significant change in flavonoids in M- plants between before and right after clipping (p= 0.027). This trend was visible in M+ too; however, that enhancement was not significant in M+ plants (p value= 0.1). Also, within 8 hours, they reduced flavonoids. This change in both group was not significant (p value for M+ 0.25 and for M- 0.109) (Table 2.4, Figure 2.4).

PAL-June: 'Timing' (p = 0.15) did not have a significant effect on PAL activity in June. Although, 'memory' of previous grazing had a highly significant effect. Plants with memory of previous grazing (M+) affected PAL activity significantly (p= 0.0003). Also, there was a significant interaction between 'timing' and 'memory' (p= 0.002) in June. Plants with memory (M+) had a higher PAL activity especially within 8 hours after clipping from 30 to 50 U/g tissues (p= 0.0009) and this was significantly higher than PAL activity in plants without the memory at the same level of timing (8 hours) (p value> 0.0001) (Table 2.4, Figure 2.4).

Phenols-July: After second round of clipping in July, the main effect of timing ($p = 0.008$) had a significant effect on phenol levels. Memory still did not have a significant effect on the amount of phenols ($p = 0.47$), and there was no significant effect of the interactions of the two main predictors ($p = 0.79$). Also, the amount of phenols in July (before clipping) was less than amount of phenols produced in June (Table 2.4, Figure 2.4). Plants reduced their phenols between the two clipping treatments in June and July by 50%. After applying the second round of clipping, both memory groups of plants increased their phenols significantly and reduced them within 8 hours and there were no significant differences between the mean of phenols within 8 hours after clipping in 2 different memory statuses (Table 2.4).

Flavonoids-July: Similar to June, timing had a highly significant effect on flavonoid levels in July ($p = 0.00014$). Memory did not significantly affect flavonoid levels ($p = 0.11$). There was no significant interaction between the two factors of timing and memory ($p = 0.97$) on flavonoids in July. Flavonoids showed the same trend as phenols after the second round of clipping. The increase in flavonoids right after the clipping was significantly different from the level of flavonoids before clipping for plants without the memory (p value 0.029) (Table 2.4, Figure 2.4).

PAL-July: The effect of timing was highly significant ($p = 1.04E-09$). Memory and the interaction of memory and timing were not significant. Both groups of plants with and without memory significantly decreased PAL activity within 8 hours after clipping ($p > 0.0001$). However, the differences between the means in PAL activity after 8 hours was not significant in plants with and without memory (p value 0.87) (Table 2.4, Figure 2.4).

Monthly changes in biochemical markers

The statistical analysis revealed significant main effects of treatment and date on all three biochemical markers (Table 2.5), with p-values well below the 0.05 threshold. Notably, clipping treatments' impacts (G vs C) on flavonoids and phenols were extremely pronounced (p-values of 4.29E-18 and 4.15E-10, respectively). Date effects also produced very low p-values for all compounds.

Contradicting our initial hypothesis, memory did not exhibit a significant impact on any individual biochemical markers, as reflected by p-values exceeding 0.05 (Table 2.5). Additionally, interactions between memory and clipping treatments were non-significant with p-values above 0.05 for all compounds (Table 2.5 and Figure 2.5).

The interactions among physiological and biochemical features

ETRm and Phenols: Linear regression Model's adjusted R^2 displayed a moderately significant relationship (adjusted $R^2 = 0.53$) (Figure 2.6.C, Table 2.9). Contrary to our hypothesis, memory and treatments did not exert significant effects within the model (p values of 0.98, 0.066, respectively), indicating a lack of support for our initial hypothesis. However, the interactions between memory and treatments played a significant role ($p = 0.022$), negatively affecting ETRm with a coefficient of -13.25, meaning that in plants lacking memory when subjected to clipping treatment (M-G), there is a 13.25 unit decrease in ETRm. Date played a pivotal role, particularly in September and June (p values > 0.0001) (Figure 2.6.C, Table 2.9). More importantly, our results confirmed the hypothesis, demonstrating that the concentrations of phenolic compounds in leaves significantly increase ETRm ($p = 0.029$) (Figure 2.6.C, , Table 2.9). Every unit increase in the

concentration of phenolic compounds in leaves is associated with an increase of 10.301 units in ETRm (Figure 2.6.C).

ETRm and Flavonoids: The adjusted R^2 value in the model is 0.53. The effect of flavonoids on ETRm was statistically significant ($p=0.03$, coefficient= 19.36) (Figure 2.6.B, Table 2.8). This finding corroborates our hypothesis that flavonoid concentration has a positive association with ETRm, where an increase in flavonoids corresponds to an increase of 19.36 units in ETRm, supporting the predicted relationship within our theoretical framework. Memory ($p=0.068$) and treatment ($p=0.054$) had no significant effect contrary to our hypothesis that memory will affect the response. However, the interaction between them ($p=0.031$) was significant and was associated with 12.36 unit decrease in ETRm in plants lacking memory and undergone clipping treatments (M-G) (Figure 2.6.B, , Table 2.8).

ETRm and PAL activity: The GLM regression analysis highlighted the significant influences of the interaction between memory and clipping treatments ($p=0.026$) on ETRm. PAL activity had a significant effect ($p=0.034$). Individual predictors including memory, clipping treatments, and PAL activity level showed less definitive impacts (0.66 and 0.41, respectively) contrary to our hypothesis (Figure 2.6.A, , Table 2.7).

Rate of Photosynthesis and PAL activity: The adjusted R^2 value of 0.547 suggests a strong fit of the model to the data (Figure 2.7.A, Table 2.10). The effect of memory approaches significance ($p=0.065$), suggesting a potential decrease (-1.96) in A_{net} associated with the lack of memory in plants, albeit not conclusively. Clipping treatment was associated with a positive effect on A_{net} (1.892), but this effect is marginally significant ($p=0.076$). The combined influence of memory and clipping treatments on A_{net} is significantly negative ($p=0.001$), meaning that plants lacking a 'memory' of past grazing events exhibit a decrease in A_{net} by approximately 5.06 units

following a clipping treatment. The PAL was not statistically significant ($p = 0.544$) (Figure 2.7.A, Table 2.10). Contrary to the hypothesis, PAL activity does not significantly influence A_{net} when controlling for other variables in the model.

Rate of Photosynthesis and Total Phenols: An adjusted R^2 of 0.546 indicated a strong fit while accounting for the number of predictors (Figure 7.C, Table 2.12). The model shows a negative trend (-2.001) with borderline significance ($p = 0.067$), hinting at a potential decrease in A_{net} associated with the absence of memory of previous grazing exposure in plants. Clipping treatment presents a positive but not statistically significant effect (1.83, $p = 0.139$). The interaction of memory and treatment is significantly negative (coefficient = -5.1002, $p = 0.001$), indicating a significantly decrease in A_{net} in plants without memory when they are clipped (M-G). The inclusion of total amount of phenols did not show a statistically significant effect on A_{net} (-0.04, $p = 0.974$), suggesting that within this model's context, phenol does not significantly influence A_{net} contrary to our hypothesis (Figure 2.7.C, Table 2.12).

Rate of Photosynthesis and Flavonoids: Adjusted R^2 of the model is 0.56. The absence of memory showed a negative effect (-1.89) with non-significant p value ($p = 0.070$), hinting at a possible decrease in A_{net} . Clipping treatments significantly and positively affected A_{net} ($p = 0.007$). 3.45 unit increase in A_{net} was associated with clipping treatments. Flavonoids showed a significant positive impact on A_{net} (coefficient = 5.08, $p = 0.029$) (Figure 2.7.B, Table 2.11), aligning with our hypothesis regarding the beneficial effects of flavonoids on plants' photosynthetic performance.

Rate of Photosynthesis and ETRm: The adjusted R^2 of 0.548 suggested a good fit (Figure 2.7.D, Table 2.13). The absence of memory showed a negative effect on A_{net} , but it was not significant ($p = 0.067$). Clipping treatment had a positive effect, suggesting a beneficial impact on A_{net} . However, this effect is significant ($p = 0.093$). The interaction of memory and clipping

treatments was significantly negative (coefficient= -4.89, $p = 0.002$), highlighting a specific combined effect of clipping in plants with no memory (M-G) decreased A_{net} . ETRm had a small positive effect on A_{net} (coefficient of 0.0195), but it was not statistically significant ($p= 0.414$) (Figure 2.7.D, Table 2.13), suggesting that within this model, ETRm did not have a clear influence on A_{net} contrary to our hypothesis.

C_i and Flavonoids: The adjusted R^2 of 0.47 suggested a good fit. The regression model suggested that there is no significant effects of memory and clipping treatments and the interaction of them in the level of C_i (0.96, 0.47, and 0.35, respectively); however, the level of flavonoids notably influenced C_i ($p= 0.015$) (Figure 2.8.A, Table 2.15). The concentration of flavonoids was associated with a decrease of 52.52 in C_i .

PAL and Stomatal Conductance: The model demonstrated a strong explanatory power with an adjusted R^2 value of 0.70 (Figure 2.8.B, Table 2.14). All predictors' effects were significant including memory, clipping treatments, date, and PAL activity with p values of 0.011, 0.004, 0.0001, and 0.024, respectively. Memory significantly negatively affected g_s (coefficient= -0.0254), indicating a decrease in g_s associated with the absence of memory. Clipping treatment was associated with an increase of 0.029 in g_s . The model indicated that higher PAL activity levels are associated with a decrease of 0.076 in g_s ($p= 0.024$). The interaction of memory and treatment was not significant ($p= 0.400$) (Figure 2.8.A, Table 2.14).

Discussion

Chlorophyll, Stomatal Conductance, Transpiration Rate, and iWUE: Contrary to initial hypotheses, the interaction of plant memory and clipping treatments did not significantly affect chlorophyll content as measured by SPAD-502, suggesting that chl concentration, a proxy

for photosynthetic potential, remains stable across different memory and stress conditions (Li et al., 2015; Lynham et al., 2021) (Table 2.3, Figure 2.3). However, temporal factors, including seasonal changes and plant developmental stages, play a critical role resulting in lower level of chl in plants lacking memory exposed to clipping treatment (M-G) which is in line with the findings of Lynham et al., 2021. Notably, plants lacking prior stress memory displayed lower efficiency post-clipping across transpiration rate and iWUE (Table 2.1) (Ashraf & Harris, 2013; Ren et al., 2018).

Photosynthetic Rate: In this study, plants with a history of grazing had a greater photosynthetic rate following clipping treatments (M+G) (Table 2.1 and Figure 2.1), suggesting an inherent plasticity which is in line with the findings of Ren et al., 2018. Such an inherent plasticity may involve physiological priming (Hilker et al., 2016), can include secondary metabolite production such as flavonoids allowing these plants to maintain or quickly regain photosynthetic capacity post-disturbance (Wang et al., 2022).

C_i and Flavonoids: Among photosynthetic parameters, the higher C_i that was observed in big bluestem without prior stress memory upon clipping (M-G), especially in June, may suggest that these plants are responding to the sudden stress of clipping by increasing their stomatal opening to absorb more CO₂ (Busch et al., 2024) (Figure 2.1.B). This might be an attempt to maximize carbon gain early in the growing season to recover from the loss of photosynthetic tissues due to clipping (Karabourniotis et al., 2014; Zhang et al., 2020) (Figure 2.1.E). It is an immediate compensatory mechanism, possibly driven by the need to sustain rapid growth, tissue regeneration, and development during this critical period (Agrawal, 2000; Alward & Joern, 1993), particularly in conditions where light and temperature are optimal for growth, which is later modulated by the accumulation of flavonoids (Figure 2.5.B). As the season progresses towards its

end, there is a natural decline in C_i with no significant effects of treatment and/or memory (Table 2.1), which aligns with the general decrease in photosynthetic activity commonly associated with plant senescence (Figure 2.1.E) (Chieppa et al., 2021; Sarath et al., 2014).

Concurrently, flavonoid levels were observed to be higher early in the season and decline towards the end (Figure 2.5.B). The absence of effects from plant memory and treatment on flavonoids' levels (Table 2.5) suggests that their production is primarily driven by developmental cues and seasonal rhythms rather than by stress history or current stress treatments (Erb & Kliebenstein, 2020; Titov et al., 2006) (as indicated by the p value well below 0.05 for the effect of date). More importantly, the significant negative impact of flavonoids on C_i across all treatments and memory conditions (Figure 2.8.B) could imply a regulatory role for these compounds in fine-tuning photosynthetic activity (McGinn & Morel, 2008). While it has been observed that lower flavonoids levels are associated with increased stomatal closure due to ROS accumulation in guard cells (Watkins et al., 2014), our findings reflect the multifaceted nature of flavonoids. They may be part of a broader spectrum of stress responses that not only include photoprotection and antioxidant defense (Elin Novia Sembiring et al., 2018; Titov et al., 2006) but also involve the modulation of enzyme activities and hormonal pathways, such as abscisic acid (ABA) (Bright et al., 2006), influencing stomatal function and thus impacting photosynthetic gas exchange. This is supported by Gai et al., (2020), who found that plants under drought stress increase flavonoid production in response to exogenous ABA application, leading to further stomatal closure. Additionally, the increased production of flavonols triggered by ABA suggests an interaction between ABA and light signaling pathways (Brunetti et al., 2019), highlighting flavonoids' crucial role in protecting the photosynthetic machinery (Erb, 2018; Pourcel et al., 2007).

Investigating the activity of carbonic anhydrases, enzymes that catalyze the interconversion of CO₂ and bicarbonate and thus directly influence C_i levels (by reducing C_i), may be worthwhile. Although it has been discovered that phenols, including flavonoids, act as competitive inhibitors for the CO₂ binding sites of carbonic anhydrases (Gulcin & Beydemir, 2013). Consequently, increased production of flavonoids could lead to lower carbonic anhydrase activity.

Rate of Photosynthesis and Flavonoids: Our study revealed that flavonoids exert a significant positive impact on the A_{net} (Figure 2.7.B), particularly in plants that undergo clipping without a history of grazing (M-G). This suggests that flavonoids play a key role in mitigating the impact of stress on photosynthesis, more so than the total phenolic compounds (Figure 2.7.C). The enhanced performance of flavonoid-rich plants post-clipping aligns with the observation that plants lacking prior stress memory displayed lower efficiency post-clipping (Avramova, 2019; Ren et al., 2018; Wang et al., 2022). The photosynthetic machinery itself might be adjusted in plants with stress memory to either protect the plant during stress or recover more rapidly afterward (Galviz et al., 2022; Thellier, 2017). Plants without this adjustment might take longer to return to optimal photosynthetic efficiency post-clipping (Züst & Agrawal, 2017). Phenolic compounds and flavonoids are known to enhance the efficiency of the photosynthetic machinery, possibly by protecting against photodamage or oxidative stress, thus improving the photosynthetic rate and ETR_m (Close & McArthur, 2002).

The evidence corroborates our second hypothesis, which posited that the production of flavonoids would bolster photosynthesis and gas exchange in response to stress, thus promoting growth and stress tolerance independently of ecological memory, the evidence strongly corroborates this assumption. Flavonoids significantly enhanced A_{net}, particularly in plants subjected to clipping without prior exposure to grazing (M-G). This underscores the pivotal role

of flavonoids in counteracting stress effects on the rate of photosynthesis, more so than total phenolic compounds.

ETR_m, Flavonoids, and Total Phenols: The ETR_m was generally low in the first clipping interval and higher between Mid-July and mid-August possibly reflecting a time-dependent adaptation, where the plant adjusts its photosynthetic machinery in anticipation of or in response to current stresses (Table 2.2 and Figure 2.2.C). Interestingly, the levels of phenolic compounds and flavonoids, which were highest in August and/or July and decreased in September (Figure 2.5.C & B), positively affected ETR_m (Figure 6. B & C). In the regression models assessing their impacts on ETR_m, while total phenolic compounds and flavonoids both exerted a positive effect, flavonoids, as a specific subgroup of phenolics, demonstrated a more pronounced enhancement of ETR_m. Total phenolic compounds led to a 10 unit increase in ETR_m, with flavonoids alone contributing to a larger 19 unit rise. This differential effect underscores the particular efficacy of flavonoids in augmenting ETR_m. Notably, both phenolic compounds and flavonoids appear to mitigate the negative impact on ETR_m caused by the interaction of the lack of stress memory and the act of clipping. This indicates that the presence of these compounds can alleviate some of the detrimental effects that arise from the absence of an adaptive stress response in plants subjected to clipping treatments. Such a finding reinforces the potential role of these secondary metabolites in bolstering plant tolerance against mechanical stressors, facilitating recovery and maintaining photosynthetic efficiency in challenging conditions. The subsequent decline in ETR_m in September aligns with the onset of senescence and a natural reduction in photosynthetic demands (Hensel et al., 1993; Sarath et al., 2014) and is of a function of the interaction of clipping treatment

and memory where plants lacking previous exposure grazing (M-) significantly reduced their ETRm when subjected to clipping treatments (G) (M-G) (Tabel 2.2).

In examining the effects of flavonoids on *A. gerardii* in response to clipping, our analysis focused on key indicators of plant photosynthetic performance such as C_i , A_{net} , and ETRm. We found these compounds to significantly contribute to the plant's ability to compensate for stress and recover efficiently (Figures 2.6.B & 2.7.B). Supporting the third hypothesis, our results showed that flavonoids help mitigate stress impacts by optimizing gas exchange, significant reduction of C_i and enhancing photosynthetic efficiency, crucial for the plant's rapid recovery post-clipping. However, there was no significant effect of memory in isolation in the model.

PAL Activity and Stomatal Conductance: PAL is a crucial enzyme in plant defense and secondary metabolism well known to increase the production of phenolic compounds including flavonoids and lignin (Ren & Sun, 2014; Wang et al., 2022). The higher activity of PAL has been linked to improved plant responses to various stresses (Wang et al., 2022). Interestingly, we found that PAL activity has a positive yet non-significant effect on the production of phenols and flavonoids in *A. gerardii* when they are subjected to clipping following exposure to prior grazing pressure (Tabel 2.6). Furthermore, the lack of significant effects from plant memory and clipping treatments in isolation on the monthly changes in PAL activity, as shown in Table 2.5, implies that the enzyme's activity is mainly driven by inherent seasonal developmental processes and enhanced plant defense perhaps through antioxidant activity (Ren & Sun, 2014) and is less affected by previous grazing incidents or recent clipping stress, even though there are studies demonstrating that the memory of intense grazing can stimulate PAL activity (Wang et al., 2022).

As thoroughly documented in the literature, in C_4 plants like big bluestem, g_s is closely linked to photosynthetic efficiency and water use (Lambers & Oliveira, 2019). Thus, seasonal

decrease in g_s shown in Figure 2.1.B, despite the absence of water deficit in this experiment, coincides with slower plant growth and a shift towards energy saving before dormancy (Cornelissen & Fernandes, 2001; Reich, 2014).

Moreover, the observed increase in PAL activity throughout the season indicates a strategic realignment in the plant's priorities, shifting from a focus on growth and leaf area expansion to enhance photosynthesis and compensate for tissue loss, as discussed in this chapter and supported by Alward & Joern, (1993); Pfanz et al., (2007); and Züst & Agrawal (2017), towards emphasizing defense (Albrecht & Argueso, 2017; Yadav et al., 2020) and reallocating resources as the season moves towards senescence (Bazzaz et al., 1987). This shift also aligns with the findings of Cass et al., (2015), who demonstrated a clear association between reduced PAL activity and decreased root growth.

Furthermore, increased PAL activity may suggest a distinct signaling pathway, independent of phenolic compounds, that limits stomatal opening and thus reduces g_s . As previously mentioned, the levels of flavonoids and phenols decrease towards the season's end (Figure 2.5.B & C), yet in this study, they showed a weak positive correlation with PAL (Table 2.6). This supports the idea that the elevated PAL activity observed towards the season's end could be linked to some roles of PAL in defense mechanisms beyond mere phenylpropanoid biosynthesis, aiming to redirect resources to rhizomes and support regrowth in the upcoming growing season. Perennial plants rely on dormancy and the survival of belowground parts like crowns, rhizomes, and meristems, essential for tiller production, to endure. Thus, preserving meristem health is crucial for plant survival (Sarath et al., 2014). This finding lends partial support to the second hypothesis of our study, which proposed an increase in PAL activity throughout the season; however, this increase was not influenced by the effect of memory. Furthermore,

consistent with this hypothesis, PAL activity appears to enhance plant performance towards the end of the growing season (Figure 2.6.A). While the study found no significant effects of plant memory and treatment in isolation on the monthly changes in PAL activity, the mention of studies indicating that intense grazing can stimulate PAL activity (Ren et al., 2017, 2018; Wang et al., 2022) suggests a complex relationship between past stress exposures and PAL activity, which partially supports our third hypothesis by indicating that ecological memory might influence metabolic responses, although not as straightforwardly as hypothesized.

Dissecting the Impact: Flavonoids Within Total Phenols - Isolating Their Unique Contribution to Photosynthetic Efficiency

The observation that flavonoids significantly affect A_{net} while total phenolic compounds do not, despite flavonoids being included in the measurement of total phenols, might seem paradoxical at first. However, this outcome can be explained by several factors related to the complex roles of different phenolic compounds in plants, the specific actions of flavonoids, and the measurements used in this study.

Total phenols, which include a variety of compounds besides flavonoids (Harborne, 1989), can obscure the latter's beneficial effects on photosynthesis, resulting in inconclusive overall impacts. Flavonoids, however, specifically bolster photosynthesis through protective and light-enhancing mechanisms (Brunetti et al., 2019; Gai et al., 2020), unlike other phenolic compounds that may not contribute to or might even impede this process (Treutter, 2006). Furthermore, the presence of other phenolic compounds could counteract the favorable effects of flavonoids, adding complexity to their overall influence. Additionally, the efficacy of flavonoids on photosynthesis is concentration-dependent, which may not be achieved within the mixed context of total phenols, thus influencing detectable results (Agati et al., 2012; Nakabayashi & Saito, 2015). In essence, the

pronounced beneficial impact of flavonoids on photosynthesis, as opposed to the ambiguous effect of total phenols, highlights the intricate interplay of phenolic compounds and underscores the importance of a focused analysis on their distinct roles in plant biochemistry.

Conclusion

In conclusion, this study has unraveled the complex interplay between ecological memory, secondary metabolite production, and photosynthetic responses in *Andropogon gerardii* under simulated grazing conditions. By analyzing photosynthetic performance indicators, levels of flavonoids and phenolic compounds, and PAL activity, we've deepened our understanding of the physiological and biochemical adaptations that underpin responses to environmental stress in this keystone tallgrass prairie species.

Central to our findings is the critical role of flavonoids in enhancing the plant's responses to clipping treatment (G vs C) regardless of the ecological memory, significantly mitigating the adverse effects of clipping on photosynthesis and playing a vital role in regulating key physiological processes like stomatal conductance and carbon assimilation.

Although plants with a grazing history, indicative of ecological memory, demonstrated certain inherent plasticity, such as potentially enhanced photosynthetic responses to subsequent stress, the response and recovery mechanisms were profoundly influenced by the presence of flavonoids. These findings underscore the complexity of response mechanisms, extending beyond ecological memory to include the timing and context of stress exposure. This refined perspective suggests that immediate biochemical responses, particularly flavonoid production, alongside the specific temporal context of stress exposure, are paramount in determining a plant's response and its ability to recover from stress.

Moving forward, future research endeavors could delve into the intricate molecular crosstalk between ABA and flavonoids, unraveling how these interactions influence plant response and stress recovery mechanisms. This line of investigation may reveal how flavonoids affect ABA signaling pathways, which play a critical role in stomatal closure and other stress responses. Moreover, examining the interplay between flavonoids and carbonic anhydrases could provide valuable insights into their combined effect on stomatal conductance and photosynthesis. Uncovering the nuances of this relationship could identify new strategies to optimize water use efficiency and carbon assimilation, further enhancing plant response. Such studies promise not only to enrich our understanding of plant physiological processes but also to offer practical applications for ecosystem management, particularly in the context of climate change and its impact on grassland ecosystems. This chapter lays the groundwork for such investigations, highlighting the importance of flavonoids in the adaptive strategies of *A. gerardii* and inviting a deeper exploration of their role within the broader framework of plant-environment interactions.

References

- Abegaz, B. M., & Kinf, H. H. (2019). Secondary metabolites, their structural diversity, bioactivity, and ecological functions: An overview. *Physical Sciences Reviews*, 4(6).
<https://doi.org/10.1515/psr-2018-0100>
- Agati, G., Azzarello, E., Pollastri, S., & Tattini, M. (2012). Flavonoids as antioxidants in plants: Location and functional significance. *Plant Science: An International Journal of Experimental Plant Biology*, 196, 67–76. <https://doi.org/10.1016/j.plantsci.2012.07.014>
- Agrawal, A. (2000). Overcompensation of plants in response to herbivory and the by-product benefits of mutualism. *TRENDS IN PLANT SCIENCE*, 5(7), 309–313.
[https://doi.org/10.1016/S1360-1385\(00\)01679-4](https://doi.org/10.1016/S1360-1385(00)01679-4)
- Alber, N. B., Brink, G. E., & Jackson, R. D. (2014). Temperate grass response to extent and timing of grazing. *Canadian Journal of Plant Science*, 94(5), 827–833.
<https://doi.org/10.4141/cjps2013-404>
- Albrecht, T., & Argueso, C. (2017). Should I fight or should I grow now? The role of cytokinins in plant growth and immunity and in the growth-defence trade-off. *ANNALS OF BOTANY*, 119(5), 725–735. <https://doi.org/10.1093/aob/mcw211>
- Alward, R. D., & Joern, A. (1993). Plasticity and overcompensation in grass responses to herbivory. *Oecologia*, 95(3), 358–364. <https://doi.org/10.1007/BF00320989>
- Anten, N. P. R., Martínez-Ramos, M., & Ackerly, D. D. (2003). Defoliation and Growth in an Understory Palm: Quantifying the Contributions of Compensatory Responses. *Ecology*, 84(11), 2905–2918. <https://doi.org/10.1890/02-0454>
- Ashraf, M., & Harris, P. J. C. (2013). Photosynthesis under stressful environments: An overview. *Photosynthetica*, 51(2), 163–190. <https://doi.org/10.1007/s11099-013-0021-6>

- Avramova, Z. (2019). Defence-related priming and responses to recurring drought: Two manifestations of plant transcriptional memory mediated by the ABA and JA signalling pathways. *Plant, Cell & Environment*, 42(3), 983–997. <https://doi.org/10.1111/pce.13458>
- Barros, J., & Dixon, R. A. (2020). Plant Phenylalanine/Tyrosine Ammonia-lyases. *Trends in Plant Science*, 25(1), 66–79. <https://doi.org/10.1016/j.tplants.2019.09.011>
- Bazzaz, F. A., Chiariello, N. R., Coley, P. D., & Pitelka, L. F. (1987). Allocating Resources to Reproduction and Defense: New assessments of the costs and benefits of allocation patterns in plants are relating ecological roles to resource use. *BioScience*, 37(1), 58–67. <https://doi.org/10.2307/1310178>
- Beer, S., Vilenkin, B., Weil, A., Veste, M., Susel, L., & Eshel, A. (1998). Measuring photosynthetic rates in seagrasses by pulse amplitude modulated (PAM) fluorometry. *Marine Ecology Progress Series*, 174, 293–300. <https://doi.org/10.3354/meps174293>
- Bright, J., Desikan, R., Hancock, J. T., Weir, I. S., & Neill, S. J. (2006). ABA-induced NO generation and stomatal closure in Arabidopsis are dependent on H₂O₂ synthesis. *The Plant Journal*, 45(1), 113–122.
- Brown, D. E., Rashotte, A. M., Murphy, A. S., Normanly, J., Tague, B. W., Peer, W. A., Taiz, L., & Muday, G. K. (2001). Flavonoids Act as Negative Regulators of Auxin Transport in Vivo in Arabidopsis. *Plant Physiology*, 126(2), 524–535. <https://doi.org/10.1104/pp.126.2.524>
- Brown, J. R., & Stuth, J. W. (1993). How Herbivory Affects Grazing Tolerant and Sensitive Grasses in a Central Texas Grassland: Integrating Plant Response across Hierarchical Levels. *Oikos*, 67(2), 291–297. <https://doi.org/10.2307/3545474>

- Brunetti, C., Sebastiani, F., & Tattini, M. (2019). Review: ABA, flavonols, and the evolvability of land plants. *Plant Science*, 280, 448–454.
<https://doi.org/10.1016/j.plantsci.2018.12.010>
- Brunsell, N. A., Van Vleck, E. S., Nosschi, M., Ratajczak, Z., & Nippert, J. B. (2017). Assessing the Roles of Fire Frequency and Precipitation in Determining Woody Plant Expansion in Central U.S. Grasslands. *Journal of Geophysical Research: Biogeosciences*, 122(10), 2683–2698. <https://doi.org/10.1002/2017JG004046>
- Busch, F. A., Ainsworth, E. A., Amtmann, A., Cavanagh, A. P., Driever, S. M., Ferguson, J. N., Kromdijk, J., Lawson, T., Leakey, A. D. B., Matthews, J. S. A., Meacham-Hensold, K., Vath, R. L., Vialet-Chabrand, S., Walker, B. J., & Papanatsiou, M. (2024). A guide to photosynthetic gas exchange measurements: Fundamental principles, best practice and potential pitfalls. *Plant, Cell & Environment*, n/a(n/a). <https://doi.org/10.1111/pce.14815>
- Canarini, A., Schmidt, H., Fuchslueger, L., Martin, V., Herbold, C. W., Zezula, D., Gündler, P., Hasibeder, R., Jecmenica, M., Bahn, M., & Richter, A. (2021). Ecological memory of recurrent drought modifies soil processes via changes in soil microbial community. *Nature Communications*, 12(1), Article 1. <https://doi.org/10.1038/s41467-021-25675-4>
- Cass, C. L., Peraldi, A., Dowd, P. F., Mottiar, Y., Santoro, N., Karlen, S. D., Bukhman, Y. V., Foster, C. E., Thrower, N., Bruno, L. C., Moskvina, O. V., Johnson, E. T., Willhoit, M. E., Phutane, M., Ralph, J., Mansfield, S. D., Nicholson, P., & Sedbrook, J. C. (2015). Effects of PHENYLALANINE AMMONIA LYASE (PAL) knockdown on cell wall composition, biomass digestibility, and biotic and abiotic stress responses in *Brachypodium*. *Journal of Experimental Botany*, 66(14), 4317–4335.
<https://doi.org/10.1093/jxb/erv269>

- Chalker-Scott, L., & Fuchigami, L. (2018). The role of phenolic compounds in plant stress responses. In *Low temperature stress physiology in crops* (pp. 67–80). CRC Press.
- Chen, S., Cai, Y., Zhang, L., Yan, X., Cheng, L., Qi, D., Zhou, Q., Li, X., & Liu, G. (2014). Transcriptome analysis reveals common and distinct mechanisms for sheepgrass (*Leymus chinensis*) responses to defoliation compared to mechanical wounding. *PloS One*, 9(2), e89495. <https://doi.org/10.1371/journal.pone.0089495>
- Chen, Y., Li, F., Tian, L., Huang, M., Deng, R., Li, X., Chen, W., Wu, P., Li, M., Jiang, H., & Wu, G. (2017). The Phenylalanine Ammonia Lyase Gene LjPAL1 Is Involved in Plant Defense Responses to Pathogens and Plays Diverse Roles in Lotus japonicus-Rhizobium Symbioses. *Molecular Plant-Microbe Interactions*®, 30(9), 739–753. <https://doi.org/10.1094/MPMI-04-17-0080-R>
- Chieppa, J., Brown, T., Giresi, P., Juenger, T. E., Resco de Dios, V., Tissue, D. T., & Aspinwall, M. J. (2021). Climate and stomatal traits drive covariation in nighttime stomatal conductance and daytime gas exchange rates in a widespread C4 grass. *New Phytologist*, 229(4), 2020–2034. <https://doi.org/10.1111/nph.16987>
- Close, D. C., & McArthur, C. (2002). Rethinking the role of many plant phenolics – protection from photodamage not herbivores? *Oikos*, 99(1), 166–172. <https://doi.org/10.1034/j.1600-0706.2002.990117.x>
- Cornelissen, T., & Fernandes, G. (2001). Defence, growth and nutrient allocation in the tropical shrub *Bauhinia brevipes* (Leguminosae). *AUSTRAL ECOLOGY*, 26(3), 246–253. <https://doi.org/10.1046/j.1442-9993.2001.01109.x>

- Cotrozzi, L., Peron, R., Tuinstra, M. R., Mickelbart, M. V., & Couture, J. J. (2020). Spectral Phenotyping of Physiological and Anatomical Leaf Traits Related with Maize Water Status. *Plant Physiology*, 184(3), 1363–1377. <https://doi.org/10.1104/pp.20.00577>
- Dang, Z., Jia, Y., Tian, Y., Li, J., Zhang, Y., Huang, L., Liang, C., Lockhart, P. J., Matthew, C., & Li, F. Y. (2021). Transcriptome-Wide Gene Expression Plasticity in *Stipa grandis* in Response to Grazing Intensity Differences. *International Journal of Molecular Sciences*, 22(21), 11882. <https://doi.org/10.3390/ijms222111882>
- Díaz, S., Lavorel, S., McINTYRE, S., Falczuk, V., Casanoves, F., Milchunas, D. G., Skarpe, C., Rusch, G., Sternberg, M., Noy-Meir, I., Landsberg, J., Zhang, W., Clark, H., & Campbell, B. D. (2007). Plant trait responses to grazing ? A global synthesis. *Global Change Biology*, 13(2), 313–341. <https://doi.org/10.1111/j.1365-2486.2006.01288.x>
- Divekar, P. A., Narayana, S., Divekar, B. A., Kumar, R., Gadratagi, B. G., Ray, A., Singh, A. K., Rani, V., Singh, V., Singh, A. K., Kumar, A., Singh, R. P., Meena, R. S., & Behera, T. K. (2022). Plant Secondary Metabolites as Defense Tools against Herbivores for Sustainable Crop Protection. *International Journal of Molecular Sciences*, 23(5). Scopus. <https://doi.org/10.3390/ijms23052690>
- Dixon, R. A., Achnine, L., Kota, P., Liu, C. J., Reddy, M. S., & Wang, L. (2002). The phenylpropanoid pathway and plant defence-a genomics perspective. *Mol Plant Pathol*, 3(5), 371–390. <https://doi.org/10.1046/j.1364-3703.2002.00131.x>
- Elin Novia Sembiring, Berna Elya, & Rani Sauriasari. (2018). Phytochemical Screening, Total Flavonoid and Total Phenolic Content and Antioxidant Activity of Different Parts of *Caesalpinia bonduc* (L.) Roxb. *Pharmacognosy Journal*, 10(1). <http://fulltxt.org/article/408>

- Erb, M. (2018). Plant Defenses against Herbivory: Closing the Fitness Gap. *Trends in Plant Science*, 23(3), 187–194. <https://doi.org/10.1016/j.tplants.2017.11.005>
- Erb, M., & Kliebenstein, D. J. (2020). Plant Secondary Metabolites as Defenses, Regulators, and Primary Metabolites: The Blurred Functional Trichotomy. *Plant Physiology*, 184(1), 39–52. <https://doi.org/10.1104/pp.20.00433>
- Fahnestock, J. T., & Knapp, A. K. (1994). Plant responses to selective grazing by bison: Interactions between light, herbivory and water stress. *Vegetatio*, 115(2), 123–131. <https://doi.org/10.1007/BF00044867>
- Farquhar, G. D., Ehleringer, J. R., & Hubick, K. T. (1989). Carbon Isotope Discrimination and Photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology*, 40(1), 503–537. <https://doi.org/10.1146/annurev.pp.40.060189.002443>
- Figuerola-Macias, J., Garcia, Y., Nunez, M., Diaz, K., Olea, A., & Espinoza, L. (2021). Plant Growth-Defense Trade-Offs: Molecular Processes Leading to Physiological Changes. *INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES*, 22(2). <https://doi.org/10.3390/ijms22020693>
- Gai, Z., Wang, Y., Ding, Y., Qian, W., Qiu, C., Xie, H., Sun, L., Jiang, Z., Ma, Q., Wang, L., & Ding, Z. (2020). Exogenous abscisic acid induces the lipid and flavonoid metabolism of tea plants under drought stress. *Scientific Reports*, 10(1), Article 1. <https://doi.org/10.1038/s41598-020-69080-1>
- Galviz, Y., Souza, G. M., & Lüttge, U. (2022). The biological concept of stress revisited: Relations of stress and memory of plants as a matter of space–time. *Theoretical and Experimental Plant Physiology*, 34(2), 239–264. <https://doi.org/10.1007/s40626-022-00245-1>

- Gommers, R., Virtanen, P., Burovski, E., Weckesser, W., Oliphant, T. E., Haberland, M., Cournapeau, D., Reddy, T., alexbrc, Peterson, P., Nelson, A., Wilson, J., endolith, Mayorov, N., Polat, I., Walt, S. van der, Roy, P., Brett, M., Laxalde, D., ... Moore, E. (2022). *scipy/scipy: SciPy 1.9.0 (v1.9.0)* [Computer software]. Zenodo. <https://doi.org/10.5281/zenodo.6940349>
- Gulcin, I., & Beydemir, S. (2013). Phenolic Compounds as Antioxidants: Carbonic Anhydrase Isoenzymes Inhibitors. *Mini Reviews in Medicinal Chemistry*, 13(3), 408–430. <https://doi.org/10.2174/138955713804999874>
- Harborne, J. B. (1989). *Plant phenolics* (Vol. 1). Academic Press San Diego, CA, USA:
- Harris, C. R., Millman, K. J., van der Walt, S. J., Gommers, R., Virtanen, P., Cournapeau, D., Wieser, E., Taylor, J., Berg, S., Smith, N. J., Kern, R., Picus, M., Hoyer, S., van Kerkwijk, M. H., Brett, M., Haldane, A., del Río, J. F., Wiebe, M., Peterson, P., ... Oliphant, T. E. (2020). Array programming with NumPy. *Nature*, 585(7825), Article 7825. <https://doi.org/10.1038/s41586-020-2649-2>
- Hartnett, D. (2023). *PEB01 Aboveground net primary productivity of tallgrass prairie based on accumulated plant biomass in grazing excloures on bison-grazed watersheds* [dataset]. Environmental Data Initiative. <https://doi.org/10.6073/PASTA/CFBE5212EC4678F1A51EF36427354F1E>
- Hensel, L. L., Grbić, V., Baumgarten, D. A., & Bleecker, A. B. (1993). Developmental and age-related processes that influence the longevity and senescence of photosynthetic tissues in arabidopsis. *The Plant Cell*, 5(5), 553. <https://doi.org/10.1105/tpc.5.5.553>
- Hilker, M., Schwachtje, J., Baier, M., Balazadeh, S., Bäurle, I., Geiselhardt, S., Hinch, D. K., Kunze, R., Mueller-Roeber, B., Rillig, M. C., Rolff, J., Romeis, T., Schmölling, T.,

- Steppuhn, A., van Dongen, J., Whitcomb, S. J., Wurst, S., Zuther, E., & Kopka, J. (2016). Priming and memory of stress responses in organisms lacking a nervous system. *Biological Reviews*, 91(4), 1118–1133. <https://doi.org/10.1111/brv.12215>
- Hunter, J. D. (2007). Matplotlib: A 2D Graphics Environment. *Computing in Science & Engineering*, 9(3), 90–95. <https://doi.org/10.1109/MCSE.2007.55>
- Jain, A., Madu, C. O., & Lu, Y. (2021). Phytochemicals in Chemoprevention: A Cost-Effective Complementary Approach. *Journal of Cancer*, 12(12), 3686–3700. <https://doi.org/10.7150/jca.57776>
- Juan, M.-Y., & Chou, C.-C. (2010). Enhancement of antioxidant activity, total phenolic and flavonoid content of black soybeans by solid state fermentation with *Bacillus subtilis* BCRC 14715. *Food Microbiology*, 27(5), 586–591. <https://doi.org/10.1016/j.fm.2009.11.002>
- Kafle, D., & Wurst, S. (2019). Legacy effects of herbivory enhance performance and resistance of progeny plants. *Journal of Ecology*, 107(1), 58–68. <https://doi.org/10.1111/1365-2745.13038>
- Karabourniotis, G., Liakopoulos, G., Nikolopoulos, D., Bresta, P., Stavroulaki, V., & Sumbele, S. (2014). “Carbon gain vs. water saving, growth vs. defence”: Two dilemmas with soluble phenolics as a joker. *Plant Science*, 227, 21–27. <https://doi.org/10.1016/j.plantsci.2014.06.014>
- Knapp, A. K., Blair, J. M., Briggs, J. M., Collins, S. L., Hartnett, D. C., Johnson, L. C., & Towne, E. G. (1999). The Keystone Role of Bison in North American Tallgrass Prairie: Bison increase habitat heterogeneity and alter a broad array of plant, community, and ecosystem processes. *BioScience*, 49(1), 39–50. <https://doi.org/10.1525/bisi.1999.49.1.39>

- Lambers, H., & Oliveira, R. S. (2019). *Plant Physiological Ecology*. Springer International Publishing. <https://doi.org/10.1007/978-3-030-29639-1>
- Lemenkova, P. (2020). *Python Libraries Matplotlib, Seaborn and Pandas for Visualization Geospatial Datasets Generated by QGIS* (SSRN Scholarly Paper 3699706). <https://papers.ssrn.com/abstract=3699706>
- Li, X., Liu, Z., Wang, Z., Wu, X., Li, X., Hu, J., Shi, H., Guo, F., Zhang, Y., & Hou, X. (2015). Pathways of *Leymus chinensis* Individual Aboveground Biomass Decline in Natural Semiarid Grassland Induced by Overgrazing: A Study at the Plant Functional Trait Scale. *PLOS ONE*, 10(5), e0124443. <https://doi.org/10.1371/journal.pone.0124443>
- Lima, J. R. L., Rodrigues, R. C., Sousa, G. O. C., Costa, C. dos S., Parente, H. N., & Santos, F. N. de S. (2020). Ecophysiology of *Andropogon* grass subjected to different cutting frequencies and intensities. *Revista Brasileira de Engenharia Agrícola e Ambiental*, 24, 610–615. <https://doi.org/10.1590/1807-1929/agriambi.v24n9p610-615>
- Lynham, S., Freile, F. G., Puri, N. M., O'Reilly, N., Mitchell, G. H., Wells, T. N. C., Willcox, M., & Beatson, R. (2021). Identification of chlorophyll a-b binding protein AB96 as a novel TGFβ1 neutralizing agent. *Scientific Reports*, 11(1), Article 1. <https://doi.org/10.1038/s41598-021-87454-x>
- McGinn, P. J., & Morel, F. M. M. (2008). Expression and Inhibition of the Carboxylating and Decarboxylating Enzymes in the Photosynthetic C4 Pathway of Marine Diatoms. *Plant Physiology*, 146(1), 300–309. <https://doi.org/10.1104/pp.107.110569>
- McKinney, W. (2010). *Data Structures for Statistical Computing in Python*. 56–61. <https://doi.org/10.25080/Majora-92bf1922-00a>

- Müller-Bravo, T. E., Sullivan, M., Smith, M., Frohmaier, C., Gutiérrez, C. P., Wiseman, P., & Zontou, Z. (2022). PISCOLA: A data-driven transient light-curve fitter. *Monthly Notices of the Royal Astronomical Society*, 512(3), 3266–3283.
<https://doi.org/10.1093/mnras/stab3065>
- Nakabayashi, R., & Saito, K. (2015). Integrated metabolomics for abiotic stress responses in plants. *Current Opinion in Plant Biology*, 24, 10–16.
<https://doi.org/10.1016/j.pbi.2015.01.003>
- Oliphant, T. E. (2007). Python for Scientific Computing. *Computing in Science & Engineering*, 9(3), 10–20. <https://doi.org/10.1109/MCSE.2007.58>
- Parsons, A. J., Leafé, E. L., Collett, B., & Stiles, W. (1983). The Physiology of Grass Production Under Grazing. I. Characteristics of Leaf and Canopy Photosynthesis of Continuously-Grazed Swards. *Journal of Applied Ecology*, 20(1), 117–126.
<https://doi.org/10.2307/2403380>
- Pfanz, H., Vodnik, D., Wittmann, C., Aschan, G., Batic, F., Turk, B., & Macek, I. (2007). Photosynthetic performance (CO₂-compensation point, carboxylation efficiency, and net photosynthesis) of timothy grass (*Phleum pratense* L.) is affected by elevated carbon dioxide in post-volcanic mofette areas. *Environmental and Experimental Botany*, 61(1), 41–48. <https://doi.org/10.1016/j.envexpbot.2007.02.008>
- Platt, T., Gallegos, C. L., & Harrison, W. G. (1980). Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. *Journal of Marine Research*.
- Pourcel, L., Routaboul, J.-M., Cheynier, V., Lepiniec, L., & Debeaujon, I. (2007). Flavonoid oxidation in plants: From biochemical properties to physiological functions. *Trends in Plant Science*, 12(1), 29–36. <https://doi.org/10.1016/j.tplants.2006.11.006>

- Ralph, P. J., & Gademann, R. (2005). Rapid light curves: A powerful tool to assess photosynthetic activity. *Aquatic Botany*, 82(3), 222–237.
<https://doi.org/10.1016/j.aquabot.2005.02.006>
- Rama, P. G., & Vinutha, T. (2019). Metabolomic Profiling of Plants to Understand Reasons for Plant Stress Resilience to Abiotic Stress. In S. H. Wani (Ed.), *Recent Approaches in Omics for Plant Resilience to Climate Change* (pp. 57–74). Springer International Publishing. https://doi.org/10.1007/978-3-030-21687-0_3
- Reich, P. B. (2014). The world-wide ‘fast–slow’ plant economics spectrum: A traits manifesto. *Journal of Ecology*, 102(2), 275–301. <https://doi.org/10.1111/1365-2745.12211>
- Ren, S.-C., & Sun, J.-T. (2014). Changes in phenolic content, phenylalanine ammonia-lyase (PAL) activity, and antioxidant capacity of two buckwheat sprouts in relation to germination. *Journal of Functional Foods*, 7, 298–304.
<https://doi.org/10.1016/j.jff.2014.01.031>
- Ren, W., Hou, X., Wu, Z., Kong, L., Guo, H., Hu, N., Wan, D., & Zhang, J. (2018). De novo transcriptomic profiling of the clonal *Leymus chinensis* response to long-term overgrazing-induced memory. *Scientific Reports*, 8(1), Article 1.
<https://doi.org/10.1038/s41598-018-35605-y>
- Ren, W., Hu, N., Hou, X., Zhang, J., Guo, H., Liu, Z., Kong, L., Wu, Z., Wang, H., & Li, X. (2017). Long-Term Overgrazing-Induced Memory Decreases Photosynthesis of Clonal Offspring in a Perennial Grassland Plant. *Frontiers in Plant Science*, 8.
<https://doi.org/10.3389/fpls.2017.00419>
- Ricardo, G. (2021). *Gerard-ricardo/PAM-RLC-analysis* [R]. <https://github.com/gerard-ricardo/PAM-RLC-analysis> (Original work published 2020)

- Sarath, G., Baird, L. M., & Mitchell, R. B. (2014). Senescence, dormancy and tillering in perennial C4 grasses. *Plant Science*, 217–218, 140–151.
<https://doi.org/10.1016/j.plantsci.2013.12.012>
- Seabold, S., & Perktold, J. (2010). *Statsmodels: Econometric and Statistical Modeling with Python*. 92–96. <https://doi.org/10.25080/Majora-92bf1922-011>
- Sharma, M., Kumar, P., Verma, V., Sharma, R., Bhargava, B., & Irfan, M. (2022). Understanding plant stress memory response for abiotic stress resilience: Molecular insights and prospects. *Plant Physiology and Biochemistry*, 179, 10–24.
<https://doi.org/10.1016/j.plaphy.2022.03.004>
- Shibaeva, T. G., Mamaev, A. V., & Sherudilo, E. G. (2020). Evaluation of a SPAD-502 Plus Chlorophyll Meter to Estimate Chlorophyll Content in Leaves with Interveinal Chlorosis. *Russian Journal of Plant Physiology*, 67(4), 690–696.
<https://doi.org/10.1134/S1021443720040160>
- Siemens, D., Keck, A., & Ziegenbein, S. (2010). Optimal defense in plants: Assessment of resource allocation costs. *EVOLUTIONARY ECOLOGY*, 24(6), 1291–1305.
<https://doi.org/10.1007/s10682-010-9374-5>
- Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In *Methods in Enzymology* (Vol. 299, pp. 152–178). Academic Press.
[https://doi.org/10.1016/S0076-6879\(99\)99017-1](https://doi.org/10.1016/S0076-6879(99)99017-1)
- Speight, A. (2021). *Visual Studio Code for Python Programmers*. John Wiley & Sons.
- Sulastri, E., Zubair, M., Anas, N., Abidin, S., Hardani, R., Yulianti, R., & Yulianti, R. (2018). Total Phenolic, Total Flavonoid, Quercetin Content and Antioxidant Activity of

- Standardized Extract of *Moringa oleifera* Leaf from Regions with Different Elevation. *Pharmacognosy Journal*, 10(6s), s104–s108. <https://doi.org/10.5530/pj.2018.6s.20>
- Thellier, M. (2017). What Is the Need for a Plant to Have Memory? In M. Thellier (Ed.), *Plant Responses to Environmental Stimuli: The Role of Specific Forms of Plant Memory* (pp. 59–65). Springer Netherlands. https://doi.org/10.1007/978-94-024-1047-1_7
- Titov, S., Bhowmik, S. K., Mandal, A., Alam, M. S., & Uddin, S. N. (2006). Control of phenolic compound secretion and effect of growth regulators for organ formation from *Musa* spp. Cv. Kanthali floral bud explants. *Am. J. Biochem. Biotechnol*, 2(3), 97–104.
- Treutter, D. (2006). Significance of flavonoids in plant resistance: A review. *Environmental Chemistry Letters*, 4(3), 147–157. <https://doi.org/10.1007/s10311-006-0068-8>
- Vinton, M. A., & Hartnett, D. C. (1992). Effects of Bison Grazing on *Andropogon gerardii* and *Panicum virgatum* in Burned and Unburned Tallgrass Prairie. *Oecologia*, 90(3), 374–382.
- Virtanen, P., Gommers, R., Oliphant, T. E., Haberland, M., Reddy, T., Cournapeau, D., Burovski, E., Peterson, P., Weckesser, W., Bright, J., van der Walt, S. J., Brett, M., Wilson, J., Millman, K. J., Mayorov, N., Nelson, A. R. J., Jones, E., Kern, R., Larson, E., ... van Mulbregt, P. (2020). SciPy 1.0: Fundamental algorithms for scientific computing in Python. *Nature Methods*, 17(3), Article 3. <https://doi.org/10.1038/s41592-019-0686-2>
- Wang, Y., Zhu, W., Ren, F., Zhao, N., Xu, S., & Sun, P. (2022). Transcriptional Memory in *Taraxacum mongolicum* in Response to Long-Term Different Grazing Intensities. *Plants*, 11(17), Article 17. <https://doi.org/10.3390/plants11172251>
- Wang, Z.-L., Zhang, X., Fan, G.-J., Que, Y., Xue, F., & Liu, Y.-H. (2022). Toxicity Effects and Mechanisms of MgO Nanoparticles on the Oomycete Pathogen *Phytophthora infestans*

- and Its Host *Solanum tuberosum*. *Toxics*, 10(10), Article 10.
<https://doi.org/10.3390/toxics10100553>
- Waskom, M. (2021). seaborn: Statistical data visualization. *Journal of Open Source Software*, 6(60), 3021. <https://doi.org/10.21105/joss.03021>
- Waterman, P. G., Ross, J. A. M., & Mckey, D. B. (1984). Factors affecting levels of some phenolic compounds, digestibility, and nitrogen content of the mature leaves of *Barteria fistulosa* (Passifloraceae). *Journal of Chemical Ecology*, 10(3), 387–401.
<https://doi.org/10.1007/BF00988087>
- Watkins, J. M., Hechler, P. J., & Muday, G. K. (2014). Ethylene-Induced Flavonol Accumulation in Guard Cells Suppresses Reactive Oxygen Species and Moderates Stomatal Aperture. *Plant Physiology*, 164(4), 1707–1717.
<https://doi.org/10.1104/pp.113.233528>
- West-Eberhard, M. J. (2008). Phenotypic Plasticity. In S. E. Jørgensen & B. D. Fath (Eds.), *Encyclopedia of Ecology* (pp. 2701–2707). Academic Press.
<https://doi.org/10.1016/B978-008045405-4.00837-5>
- Woodis, J. E., & Jackson, R. D. (2008). The effects of clipping height and frequency on net primary production of *Andropogon gerardii* (C4 grass) and *Bromus inermis* (C3 grass) in greenhouse experiments. *Grass and Forage Science*, 63(4), 458–466.
<https://doi.org/10.1111/j.1365-2494.2008.00653.x>
- Yadav, V., Wang, Z., Wei, C., Amo, A., Ahmed, B., Yang, X., & Zhang, X. (2020). Phenylpropanoid Pathway Engineering: An Emerging Approach towards Plant Defense. *PATHOGENS*, 9(4). <https://doi.org/10.3390/pathogens9040312>

- Zhang, F., Zheng, J., Bennett, J. A., Zhang, B., Wang, Z., Li, Z., Li, H., Wang, J., Han, G., & Zhao, M. (2023). The legacy effects of grazing and precipitation variability on grassland productivity in a temperate steppe. *Agricultural and Forest Meteorology*, 343, 109788. <https://doi.org/10.1016/j.agrformet.2023.109788>
- Zhang, Z., Gong, J., Wang, B., Li, X., Ding, Y., Yang, B., Zhu, C., Liu, M., & Zhang, W. (2020). Regrowth strategies of *Leymus chinensis* in response to different grazing intensities. *Ecological Applications*, 30(5), e02113. <https://doi.org/10.1002/eap.2113>
- Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, 64(4), 555–559. [https://doi.org/10.1016/S0308-8146\(98\)00102-2](https://doi.org/10.1016/S0308-8146(98)00102-2)
- Züst, T., & Agrawal, A. A. (2017). Trade-Offs Between Plant Growth and Defense Against Insect Herbivory: An Emerging Mechanistic Synthesis. *Annual Review of Plant Biology*, 68, 513–534. <https://doi.org/10.1146/annurev-arplant-042916-040856>

Figures and Tables:

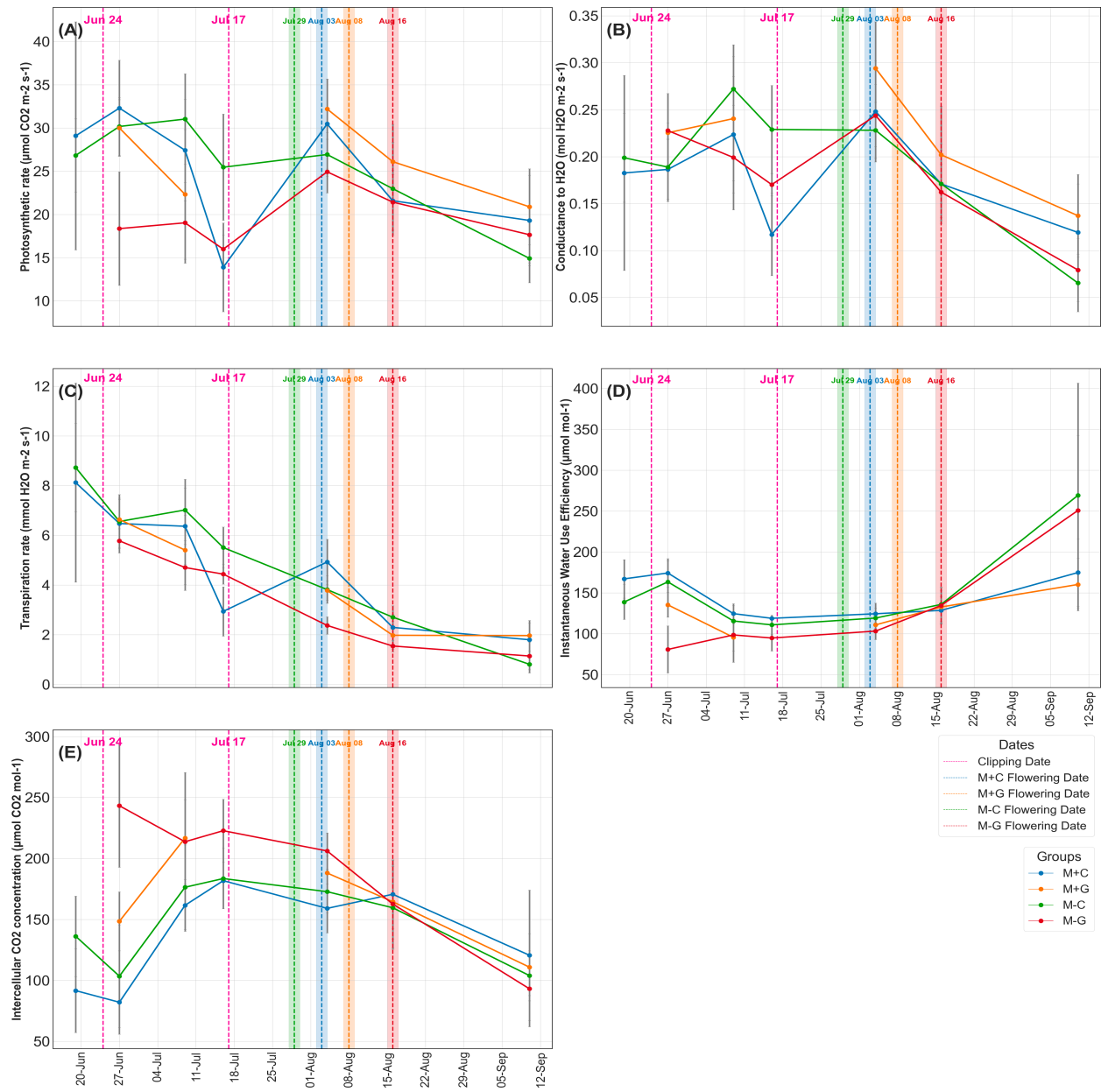


Figure 2. 1- Temporal trends of various physiological parameters (Mean $\pm$ standard error). (A) Photosynthesis rate (A_{net}) ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), (B) Conductance to H_2O ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), (C) Transpiration rate (E) ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), (D) Intercellular CO_2 concentration ($\mu\text{mol CO}_2 \text{ mol}^{-1}$), and (E) Instantaneous water-use efficiency ($i\text{WUE}$, $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$). The different line colors represent separate treatment groups: M+C (blue), M-C (green), M+G (orange), and M-G (red). Vertical dashed lines indicate specific dates of interest: deep pink dashed lines mark the dates of clipping treatments, while dashed lines in the same color as the treatment groups

denote flowering dates for each group. Some data points are absent from these plots due to equipment malfunctions that occurred at a specific time.

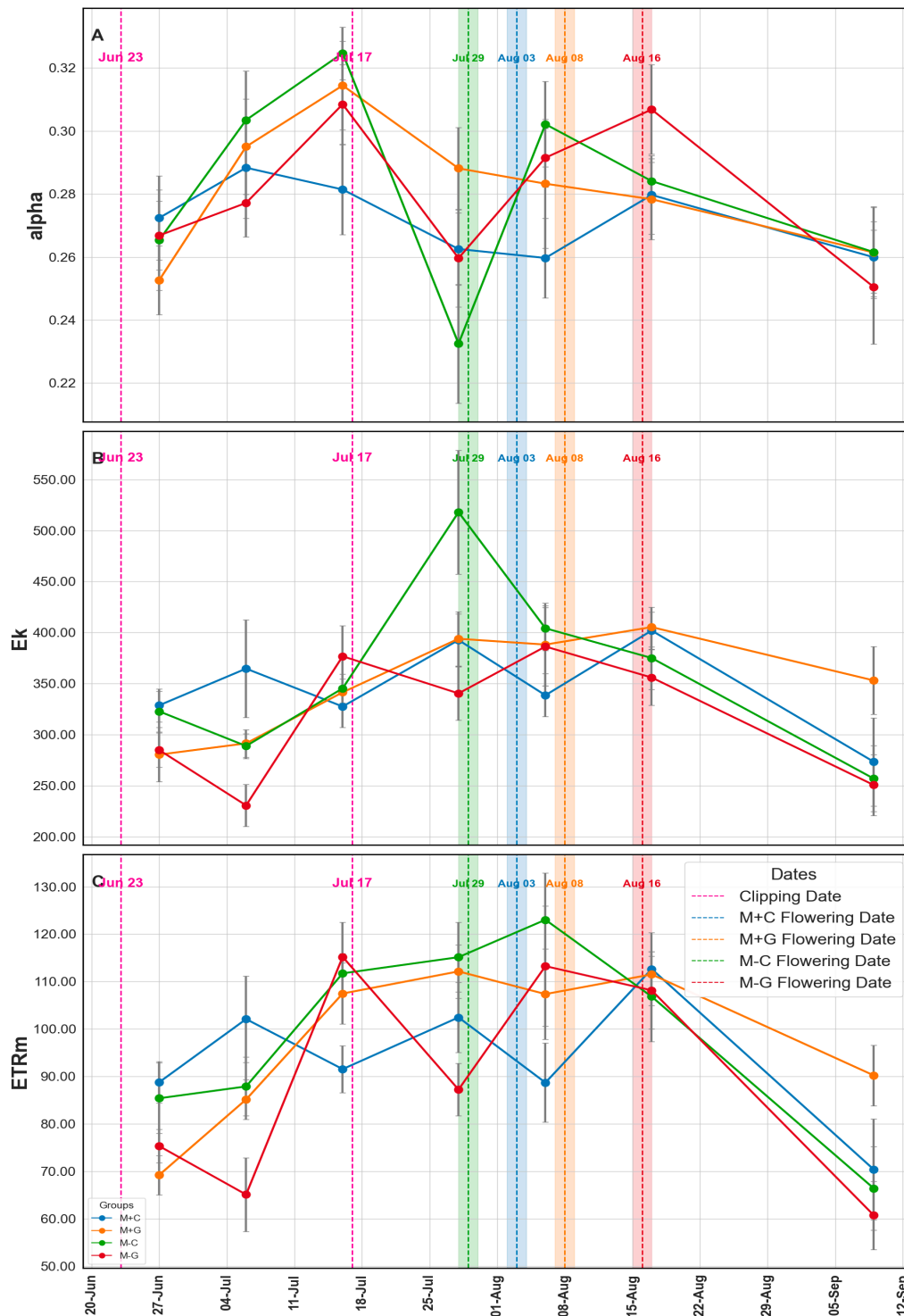


Figure 2. 2- Temporal trends of various photosynthetic parameters derived from RLCs (Mean $\pm$ standard error). (A) Alpha, the initial slope of the RLCs, (B) E_k , (C) Maximum Electron Transport Rate (ETRm) (a.u). The different line colors represent separate treatment groups: M+C

(blue), M-C (green), M+G (orange), and M-G (red). Vertical dashed lines indicate specific dates of interest: deep pink dashed lines mark the dates of clipping treatments, while dashed lines in the same color as the treatment groups denote flowering dates for each group.

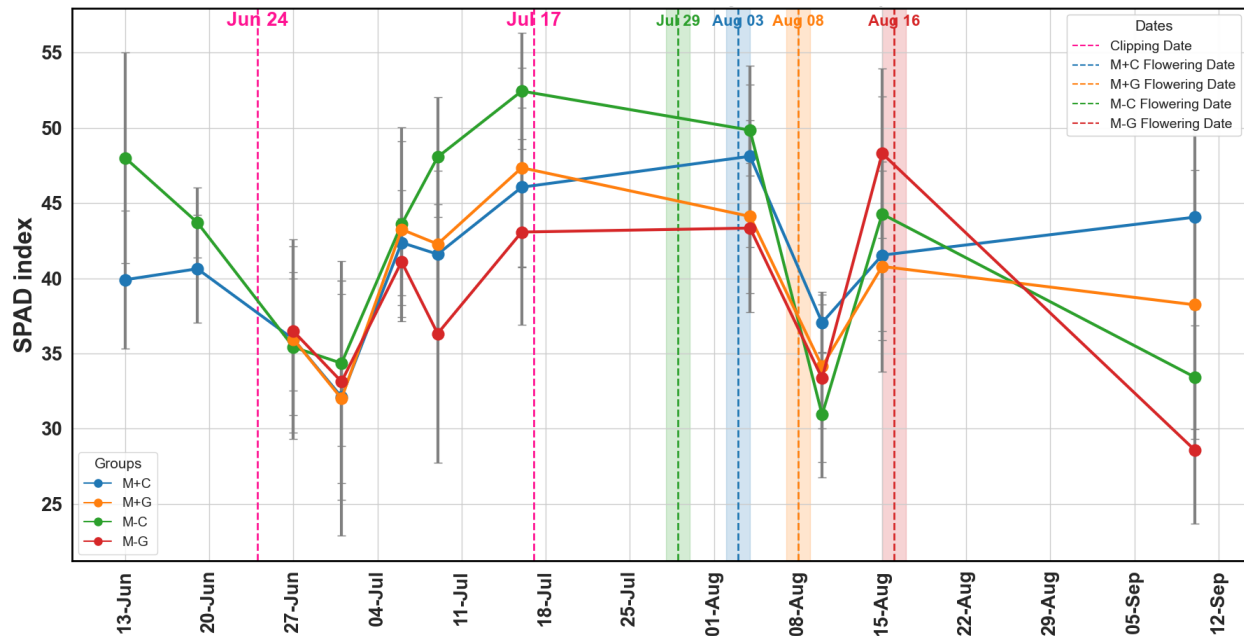


Figure 2. 3- Temporal trends of Chl (SPAD index) (Mean $\pm$ standard error). The different line colors represent separate treatment groups: M+C (blue), M-C (green), M+G (orange), and M-G (red). Vertical dashed lines indicate specific dates of interest: deep pink dashed lines mark the dates of clipping treatments, while dashed lines in the same color as the treatment groups denote flowering dates for each group.

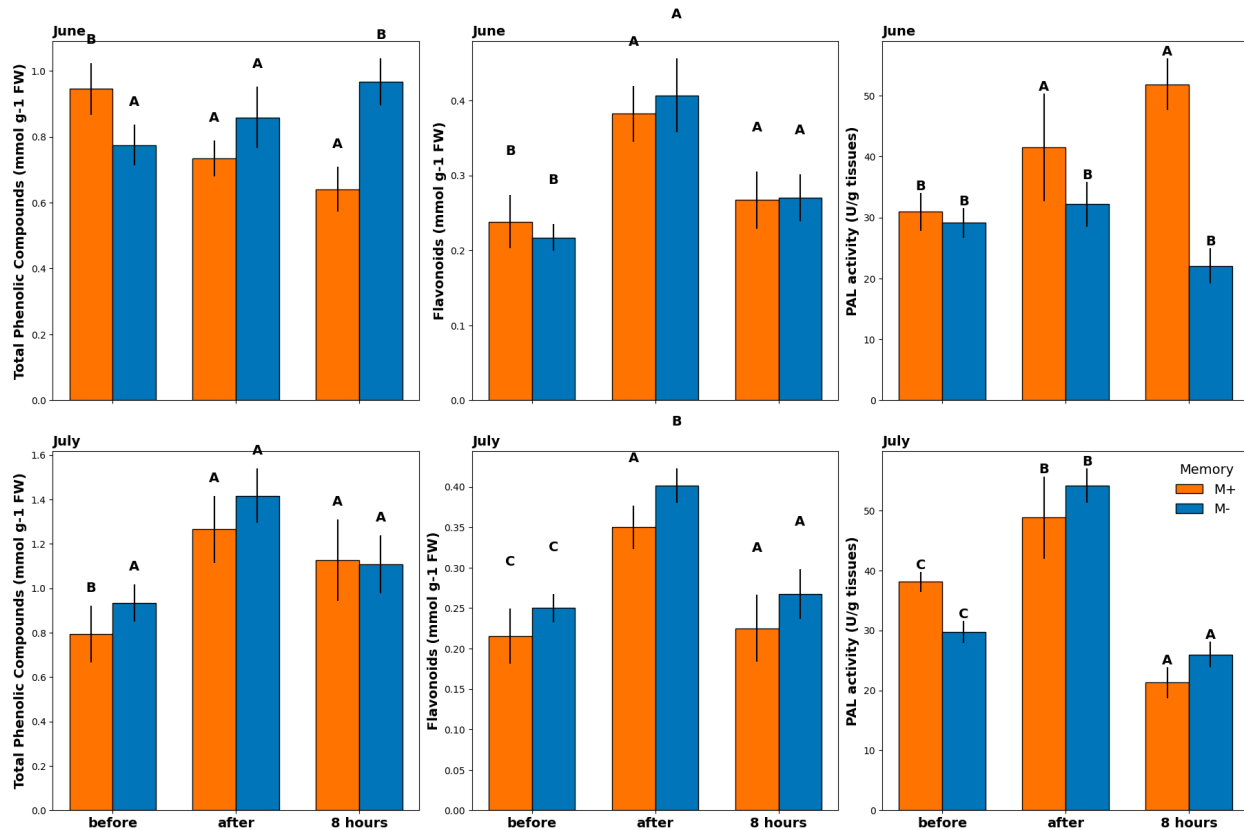


Figure 2. 4- Impact of Clipping Treatment on Biochemical Responses in *Andropogon gerardii* over time. This figure displays the changes in Total Phenolic Compounds, Flavonoids, and PAL Activity for the months of June and July, at three different timings: before (pre-clipping), after, and 8 hours post-clipping treatment. The bars represent the mean values, and the error bars indicate the standard errors for each group. The data are categorized by 'Memory' with two levels: M+ (with memory of previous grazing) and M- (without memory), depicted in blue and orange, respectively. Letters, positioned above the bars, denote statistical differences as determined by Tukey's honest significance test.

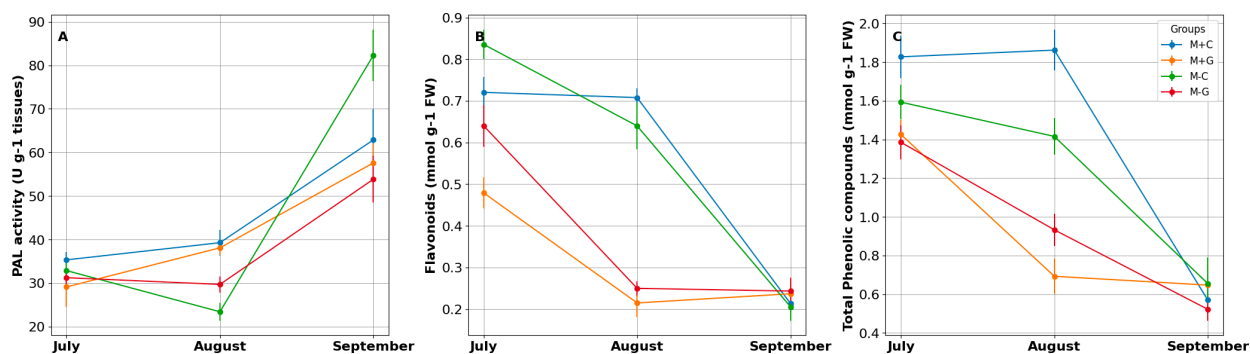


Figure 2. 5- Monthly Dynamics of Biochemical Markers in *Andropogon gerardii*, with Mean Values and Standard Error Bars Plotted. A) PAL activity is shown over months from July to September, illustrating the enzymatic response to treatment and memory conditions. B) Flavonoid

concentration is tracked over the same period, reflecting changes. C) Total phenolic compounds over time. Different colors correspond to distinct treatment and memory groups.

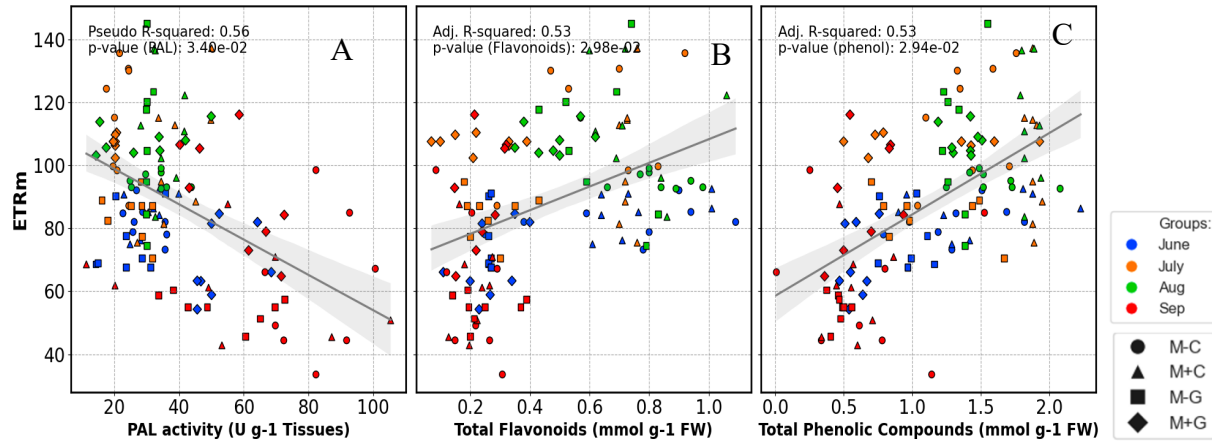


Figure 2. 6- Relationship Between ETRm and Biochemical Markers in the Context of Grazing Memory and Treatment Across Different Dates in *Andropogon gerardii*. Panel A presents a Generalized Linear Model (GLM) illustrating the relationship between PAL activity (U g^{-1} Tissues) and Electron Transport Rate (ETRm) with a Pseudo R-squared of 0.56 and a p-value of 0.034 for PAL activity. Panels B and C depict Linear Regression models showing the relationship of Total Flavonoids (mmol g^{-1} FW) and Total Phenolic Compounds (mmol g^{-1} FW) with ETRm, respectively, both with an Adjusted R-squared value of 0.53. Flavonoids exhibit a p-value of 0.029 and Phenolics a p-value of 0.0294. The models include the effects of memory, clipping treatments, and their interaction, as well as the date of measurements. Data points are differentiated by shape and color to represent various memory and treatment groups and different months, respectively, and the solid lines indicate the model's fitted values, with shaded areas showing the confidence intervals. The model formula used is $\text{ETRm} \sim [\text{biochemical markers}] + \text{memory} * \text{treatment} + \text{date}$, highlighting the combined effects of biochemical markers and the interaction between plant memory and clipping treatment over time.

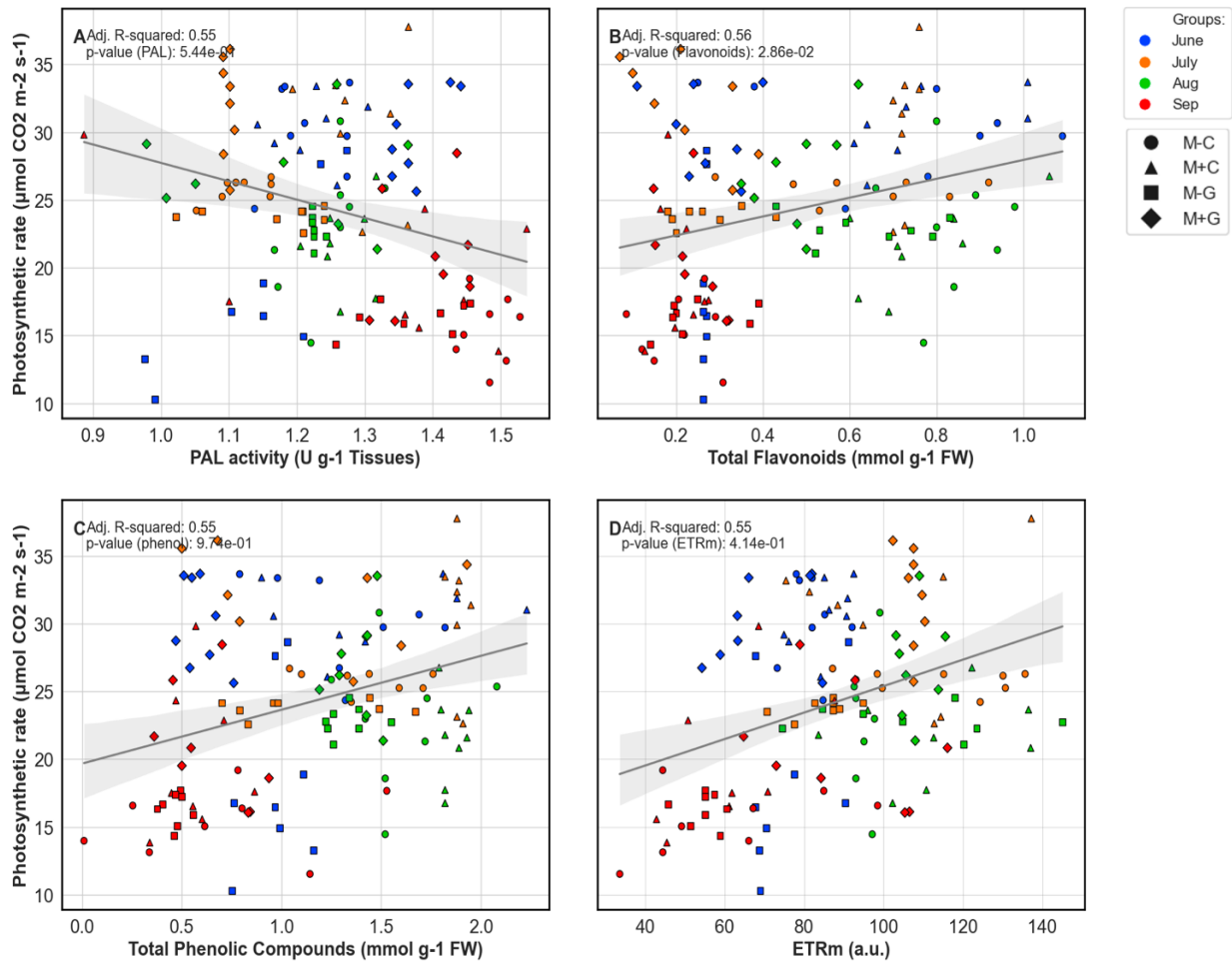


Figure 2. 7- Linear Regression Analysis of Photosynthetic Rate Influenced by Biochemical Markers or ETRm in *A. gerardii* Accounting for Grazing Memory, Clipping Treatments, and Date. A) PAL activity vs. Photosynthetic Rate: Adjusted $R^2 = 0.55$, p (PAL) = $5.44e-01$. B) Total Flavonoids vs. Photosynthetic Rate: Adjusted $R^2 = 0.56$, p (Flavonoids) = $2.66e-02$. C) Total Phenolic Compounds vs. Photosynthetic Rate: Adjusted $R^2 = 0.55$, p (Phenolics) = $9.9e-01$. D) ETRm vs. Photosynthetic Rate: Adjusted $R^2 = 0.55$, p (ETRm) = $1.41e-01$. The model formula used is $A_{net} \sim [\text{biochemical marker/ETRm}] + \text{memory} * \text{treatment} + \text{date}$, highlighting the combined effects of biochemical markers/ETRm and the interaction between plant memory and clipping treatment over time. Data points are differentiated by shape and color to represent different months and various memory and treatment groups, respectively, and the solid lines indicate the model's fitted values, with shaded areas showing the confidence intervals.

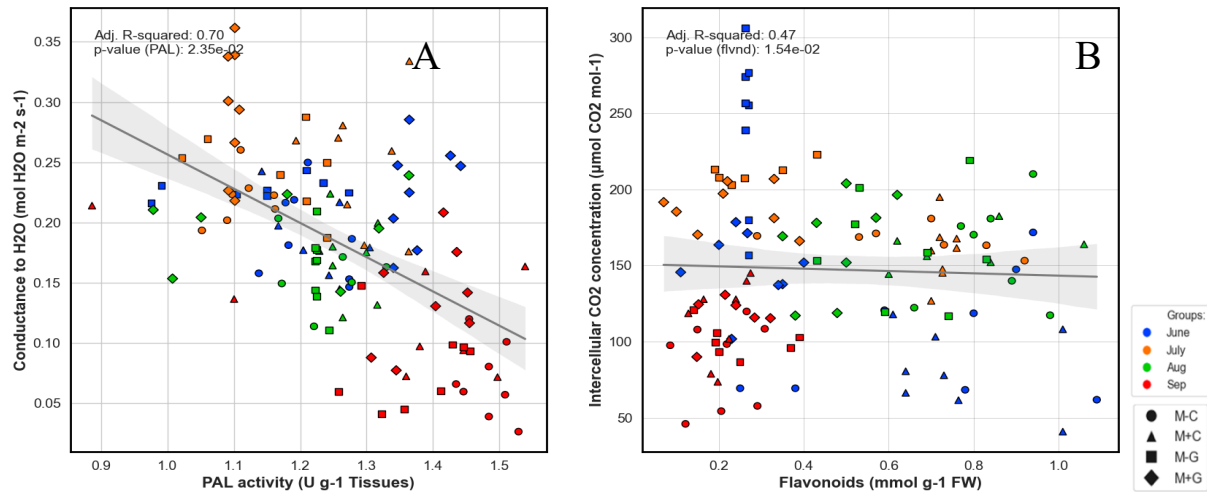


Figure 2. 8- Relationships between Stomatal Conductance, Intercellular CO₂ Concentration, and Biochemical Markers in *A. gerardii* Accounting for Grazing Memory, Treatment, and Date. **Panel A:** The relationship between PAL activity (U g⁻¹ Tissues) and stomatal conductance to H₂O (mol H₂O m⁻² s⁻¹), with an Adjusted R-squared of 0.70 and p-value for PAL of 2.35e-02. **Panel B:** The relationship between flavonoid concentration (mmol g⁻¹ FW) and intercellular CO₂ concentration (μmol CO₂ mol⁻¹), with an Adjusted R-squared of 0.47 and p-value for flavonoids of 1.54e-02. Both models incorporate the interaction of memory and treatment with the date as covariates. Data points are color-coded by months and shaped to represent different memory and treatment conditions, with gray areas showing confidence intervals. The model formula used is C_i or $g_s \sim [\text{biochemical markers}] + \text{memory} * \text{treatment} + \text{date}$, highlighting the combined effects of biochemical markers and the interaction between plant memory and clipping treatment over time. Data points are differentiated by shape to represent different months and by color to indicate various treatment groups, with solid lines depicting the model's fitted values and shaded areas representing the confidence intervals.

Table 2. 1- Results of Three-Way ANOVA for the Main Effects of Memory, Treatment, and Date and Their Interactions on Gas Exchange Measurements (Significance Level: $p < 0.05$)

Predictors	Anet	gs	E	iWUE	Ci
Memory	1.40E-03 *	8.03E-02	1.60E-01	2.80E-01	1.34E-03 *
Treatment	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00
Date	1.31E-09 ***	2.97E-26 ***	9.44E-54 ***	9.17E-23 ***	1.16E-26 ***
Memory: Treatment	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00
Memory: Date	1.62E-02 *	4.47E-03 **	1.64E-02 *	2.51E-08 ***	4.37E-05 **
Treatment: Date	1.15E-05 ***	6.77E-02	6.80E-04 **	5.45E-04 **	8.10E-17 ***
Memory: Treatment: Date	2.47E-02 *	5.72E-02	6.75E-01	8.64E-01	8.82E-02

$p < 0.001$: ***, $p < 0.01$: **, $p < 0.05$: *, No stars indicate $p \geq 0.05$ (not significant)

Table 2. 2- Results of Three-Way ANOVA for Main Effects and Interactions of Memory, Treatment, and Date on RLCs derived variables Including ETRm, Alpha, and Ek (Significance Level: $p < 0.05$)

Predictors	ETRm	alpha	Ek
Memory	5.96E-01	4.24E-01	3.03E-01
Treatment	2.36E-01	5.44E-01	1.08E-01
Date	1.04E-16 ***	5.18E-07*	9.14E-12***
Memory: Treatment	1.85E-02*	2.75E-01	5.09E-02
Memory: Date	2.36E-03**	1.19E-01	2.52E-02*
Treatment: Date	3.61E-02*	5.30E-01	2.03E-02*
Memory: Treatment: Date	2.59E-01	3.78E-01	1.87E-01

$p < 0.001$: ***, $p < 0.01$: **, $p < 0.05$: *, No stars indicate $p \geq 0.05$ (not significant)

Table 2. 3- Results of Three-Way ANOVA for Main Effects and Interactions of Memory, Treatment, and Date on Chl (Significance Level: $p < 0.05$)

Predictors	P value
Date	3.66E-45***
Memory	0.028*
Treatment	1.00E+00
Date: Memory	2.73E-09***
Date: Treatment	7.37E-05***
Memory: Treatment	0.196
Date: Memory: Treatment	0.027 *

$p < 0.001$: ***, $p < 0.01$: **, $p < 0.05$: *, No stars indicate $p \geq 0.05$ (not significant)

Table 2. 4- Results of Two-Way ANOVA for Main Effects and Interactions of Memory and Timing on Phenol and Flavonoids content and PAL activity (Significance Level: $p < 0.05$).

response variables	factors	PR(>F)
Phenol, June	C(timing)	0.69
	C(memory)	0.13
	C(timing):C(memory)	0.005**
Flavonoid, June	C(timing)	0.0002***
	C(memory)	0.97
	C(timing):C(memory)	0.82
PAL, June	C(timing)	0.151
	C(memory)	0.0003***
	C(timing):C(memory)	0.002**
Phenol, July	C(timing)	0.008**
	C(memory)	0.47

	C(timing):C(memory)	0.78
Flavonoid, July	C(timing)	0.00014***
	C(memory)	0.11
	C(timing):C(memory)	0.96
PAL, July	C(timing)	1.04E-09***
	C(memory)	9.08E-01
	C(timing):C(memory)	5.69E-02

p < 0.001: ***, p < 0.01: **, p < 0.05: *, No stars indicate p ≥ 0.05 (not significant)

Table 2. 5- Results of Three-Way ANOVA for Main Effects and Interactions of Memory, Treatment, and Date on Phenol and Flavonoids content and PAL activity (Significance Level: p < 0.05)

Predictors	PAL	Flavonoids	Phenol
Memory	5.77E-01	4.71E-01	4.19E-02
Treatment	5.26E-02	4.29E-18	4.15E-10
Date	3.41E-18	4.82E-19	1.47E-21
Memory: Treatment	5.21E-01	6.22E-01	2.43E-01
Memory: Date	4.65E-02	3.57E-01	7.70E-01
Treatment: Date	2.78E-02**	4.01E-09	3.03E-05
Memory: Treatment: Date	1.10E-01	5.58E-01	6.65E-02

p < 0.001: ***, p < 0.01: **, p < 0.05: *, No stars indicate p ≥ 0.05 (not significant)

Table 2. 6- Relationship between PAL activity and the production of Phenolic compounds and Flavonoids. Regression Models' formula: [biochemical markers (Phenols/Flavonoids)] ~ PAL + memory * treatment + date (Aug as the reference)

Predictors	Coefficient Phenolics	p value Phenolics	Coefficient flavonoids	p value flavonoids
Memory	-0.1875	0.017*	-0.0175	0.672
Treatment	-0.5032	0.000***	-0.3117	0.000***
Date [July]	-0.1437	0.072	-0.2095	0.000***
Date [June]	-0.4531	0.000***	-0.1767	0.000***
Date [September]	-0.9752	0.000***	-0.4751	0.000***
Memory: Treatment	0.2243	0.043*	0.0741	0.206
PAL	0.177	0.512	0.1223	0.393

p < 0.001: ***, p < 0.01: **, p < 0.05: *, No stars indicate p ≥ 0.05 (not significant)

Table 2. 7- Relationship between PAL activity and ETRm.

Predictors	Coefficient	P value
Memory	-1.7544	0.66
Treatment	3.2508	0.414
Date [July]	-4.7399	0.238
Date [June]	-27.521	0
Date [September]	-32.3861	0
Memory: Treatment	-12.6585	0.026
PAL	-0.2212	0.034

Table 2. 8- Relationship between Flavonoids and ETRm.

Predictors	Coefficient	P value
Memory	-1.6456	0.68
Treatment	9.4397	0.054
Date [July]	0.5972	0.893
Date [June]	-24.6592	0
Date [September]	-30.3528	0
Memory: Treatment	-12.3593	0.031
Flavonoids	19.359	0.03

Table 2. 9- Relationship between Phenolic compounds and ETRm.

Predictors	Coefficient	P value
Memory	-0.0588	0.989
Treatment	8.5804	0.066
Date [July]	-2.0105	0.62
Date [June]	-23.4077	0
Date [September]	-29.4166	0
Memory: Treatment	-13.2479	0.022
Phenols	10.3009	0.029

Table 2. 10- Relationship between PAL activity and Anet.

Predictors	Coefficient	P value
Memory	-1.9649	0.065
Treatment	1.892	0.076
Date [July]	4.5583	0
Date [June]	3.6668	0.001
Date [September]	-5.8458	0
Memory: Treatment	-5.059	0.001
PAL	2.2333	0.544

Table 2. 11- Relationship between Flavonoids and Anet.

Predictors	Coefficient	P value
Memory	-1.8966	0.07
Treatment	3.4488	0.007
Date [July]	5.5287	0
Date [June]	4.5796	0
Date [September]	-3.1672	0.033
Memory: Treatment	-5.4716	0
Flavonoids	5.0841	0.029

Table 2. 12- Relationship between Phenolic compounds and Anet.

Predictors	Coefficient	P value
Memory	-2.0008	0.067
Treatment	1.8334	0.139
Date [July]	4.4207	0
Date [June]	3.6688	0.003
Date [September]	-5.5192	0.001
Memory: Treatment	-5.1002	0.001
phenol	-0.04	0.974

Table 2. 13- Relationship between ETRm and Anet.

Predictors	Coefficient	P value
Memory	-1.954	0.067
Treatment	1.7881	0.093
Date [July]	4.4969	0
Date [June]	4.2332	0.001
Date [September]	-4.7189	0.001
Memory: Treatment	-4.8953	0.002
ETRm	0.0195	0.414

Table 2. 14- Relationship between PAL activity and Stomatal conductance.

Predictors	Coefficient	P value
Memory	-0.0254	0.011
Treatment	0.0286	0.004
Date [July]	0.0758	0
Date [June]	0.0366	0
Date [September]	-0.0556	0
Memory: Treatment	-0.0116	0.4
PAL	-0.076	0.024

Table 2. 15- Relationship between Flavonoids content and Ci.

Predictors	Coefficient	P value
Memory	-0.5293	0.956
Treatment	8.7197	0.471
Date [July]	10.1901	0.349
Date [June]	-25.4175	0.016
Date [September]	-80.0173	0
Memory: Treatment	30.9937	0.027
Flavonoids	-52.5214	0.015

Chapter 3: Dynamics of Growth Response and Biomass Allocation

in *Andropogon gerardii*: Interactions Between Simulated Grazing

and Its Analogous Plant Memory

Abstract

This chapter focuses on the adaptive responses of *Andropogon gerardii* to simulated grazing pressures, examining the influence of 'plant memory', the historical record of past grazing, on growth dynamics and biomass allocation. By employing controlled greenhouse experiments, we tested the hypothesis that *A. gerardii* with a prior history of grazing would exhibit differential growth responses compared to those lacking such memory, particularly in terms of compensatory growth mechanisms.

The study was conducted using rhizomes from grazed and ungrazed regions of Konza Prairie, which were then subjected to simulated grazing treatments in a greenhouse environment. The experiment focused on the removal of 70% aboveground biomass, emulating heavy grazing at two key growth stages. Subsequent measurements included leaf area, biomass of various plant fractions, and relative growth rates (RGR).

Our findings revealed significant main effects of grazing memory and time intervals on RGR, with a notable decrease in RGR during the transition from early to late growth stages. The presence of grazing memory was associated with higher RGR during early growth stages, suggesting that historical exposure to grazing could prime the plants for a more robust recovery post-defoliation. Additionally, mixed-effect model analyses demonstrated significant impacts of grazing memory on end-of-season biomass, especially in terms of total leaf area and specific leaf area (SLA), indicating a nuanced influence of historical grazing on plant growth patterns.

The results challenge the traditional view that previous grazing invariably enhances plant growth traits, showing instead that inherent growth rates following defoliation are more predictive of biomass outcomes than the memory of past grazing events. This suggests that immediate physiological responses to defoliation play a more decisive role in determining plant vigor than adaptive responses influenced by past experiences.

Introduction

The interaction between plants and their environment is a cornerstone of ecological research, with plant responses to biotic stressors such as grazing having profound effects on their growth dynamics and biomass allocation (Geng et al., 2007; Poorter et al., 2012). *Andropogon gerardii*, a dominant C4 species in North American tallgrass prairies, has evolved under periodic grazing pressures, which has influenced its adaptive strategies, shaping its survival tactics (Knapp et al., 2012). Reflecting this understanding, the response of plants to defoliation is found to be highly variable and context-dependent, exhibiting differences not only across species but within a single species as well. This variability is influenced by a range of factors, including the timing and frequency of herbivory events and the specific type of tissue that is removed (Brown & Stuth, 1993; Christiansen & Svejcar, 1988; Vinton & Hartnett, 1992).

Contrary to the long-held belief that loss of photosynthetic tissue due to herbivory by larger grazers invariably reduces plant growth, a perspective supported within the growth-defense trade-off, where plants are seen to invest in defense often at the expense of growth, an interaction that is further modulated by environmental conditions (Hilker & Schmülling, 2019), studies have highlighted the capacity for compensation. These studies reveal that plants can not only mitigate the adverse effects of such tissue loss but may also respond with enhanced growth under certain

conditions (Frank et al., 1998; McNaughton, 1986). Such interactions among plants and herbivores can sometimes result in defoliated plants producing more biomass than their nondefoliated counterparts, an outcome known as overcompensatory growth (Zhao et al., 2008). This surprising positive impact of grazing on plant productivity supports the grazing optimization hypothesis (Hilbert et al., 1981), which suggests that plants can 'overcompensate' for herbivory under certain conditions as a function of redistribution of plant hormones that trigger cell division and elongation and ultimately, increase leaf growth and tillering (Mipam et al., 2019; Siddappaji et al., 2015). Moreover, Ferraro & Oesterheld, 2002 demonstrated that the impact on biomass production was more closely related to the timing of the defoliation rather than the grazing event itself.

Grasses possess the remarkable ability to either compensate or overcompensate for herbivory, mitigating its detrimental effects (Knapp et al., 2012). This resilience, in line with the grazing optimization hypothesis, can be observed through increased net primary productivity, seed production, and plant longevity, all of which bolster overall plant fitness (Belsky, 1986). Belsky, 1986 also notes that such adaptive strategies may lead to the evolution of mutually beneficial relationships between herbivores and plants. In addition, the timing of defoliation and removal of the specific tissues due to herbivory can enhance these mutualistic interactions between plants and herbivores (Agrawal, 2000).

Building on the context of compensatory growth in response to grazing, the concept of 'priming' has been extensively investigated as another factor that may influence plant responses to grazing, potentially leading to an enhanced growth rate (Bandoly et al., 2016; Conrath et al., 2006; Hilker et al., 2016). Priming is characterized by a plant's exposure to subtle environmental signals that indicate potential stress, which then prepares the organism to more effectively cope with future adverse conditions (Hilker & Schmülling, 2019). This phenomenon enables even species without

a nervous system (Galviz et al., 2020; Hilker et al., 2016) to adjust their phenotypic stress responses in anticipation of environmental changes (Avramova, 2019; Garai & Sopory, 2023; Hilker & Schmülling, 2019; Sinha, 2023). This adaptive response, manifesting in response to different stressors and across different organisms, is known across scientific literature by several terms (Hilker et al., 2016), including 'inducible defenses' (Harvell, 1990). Despite the diverse nomenclature, these defenses share a common feature: they are activated by an organism's initial encounter with a stressor and subsequently enhance the organism's resistance to similar future stresses (Harvell, 1990; Hilker & Schmülling, 2019; Ren et al., 2017; Sinha, 2023).

As such, in exploring the mechanisms underlying grass responses to grazing, this study introduces the concept of grazing history, the 'memory' of past grazing events, as a potential factor influencing grasses' ability to recover and regrow. Simulated grazing, a method to replicate the effects of herbivory in a controlled setting, provides a unique opportunity to study these strategies and understand the underlying mechanisms of plant memory and response. This chapter investigates the dynamics of growth response and biomass allocation in *A. gerardii* in response to simulated grazing and its analogous plant memory. We explore how plants with a history of grazing respond differently from those without such a memory when subjected to analogous stress. We hypothesize that this memory may act as an enhancer, augmenting the plant's inherent potential to compensate for the loss of tissue and to thrive post-herbivory.

Based on the premise of plant memory and response to environmental stress, we propose the following hypotheses:

H1) *A. gerardii* with a memory of grazing will exhibit a different growth response to simulated grazing compared to those without such a memory, potentially showing greater resilience and compensatory growth mechanisms.

H2) The total biomass removed during early-season simulated grazing events will have a significant long-term impact on the end-of-season biomass allocation, with variations observed between plants with and without grazing memory.

H3) RGR during early and late growth stages will correlate with end-of-season biomass attributes, with the influence of plant memory modulating this relationship.

These hypotheses will be tested through a series of mixed-effects models, regression analyses, and ANOVA tests, assessing the interactions between simulated grazing, plant memory, and various growth and biomass parameters. Eventually, this study seeks to deepen our understanding of the adaptive significance of plant memory in response to grazing pressures, which may have implications for ecological management and conservation efforts.

Methods

Transplantation and Treatment of *A. gerardii* in a Controlled Greenhouse

Environment

A paired sampling strategy was employed to assess the differential growth responses of *A. gerardii*, considering the presence (M+) or absence (M-) of grazing memory. In mid-May 2023, early-season leaf-bearing rhizomes of *A. gerardii* were harvested from two watersheds at the Konza Prairie. Both areas are subject to annual burning and bison grazing. Within each watershed, four exclosures established in 1988 served as ungrazed controls against the grazed landscape. Plants without grazing history were sourced from these exclosures in upland areas (M-), while plants with a history of exposure to continuous bison grazing were collected from four plots in grazing areas within the same watersheds and topographic zones (M+). This setup provided a total of sixteen biological replicates for each memory condition across the two watersheds.

Following collection, the rhizomes were transplanted into pots (24 cm diameter in 23 cm height) under uniform soil conditions in a greenhouse, thus standardizing environmental variables. The plants received appropriate irrigation to ensure their acclimatization to this controlled environment. After a month, allowing for adjustment to greenhouse conditions, a simulated heavy grazing pressure was introduced in mid-June as the first clipping treatment.

This experiment incorporated two clipping treatments: clipping (G) to simulate grazing and control (no clipping, C), resulting in four distinct experimental groups: M+C, M-C, M+G, and M-G with eight biological replicates per each group, significantly enhancing the reliability of the data collected. This extensive replication enables a robust analysis of *A. gerardii*'s adaptive growth strategies under different grazing memories and current stress conditions. For details and more information regarding the experiment and treatments, please refer to the method section of chapter 2.

Clipping treatments and traits

Simulating Grazing Pressure

To simulate the effects of heavy grazing pressure on plant biomass, we adopted the procedure outlined by Alber et al., 2014 which involves the removal of 70% of the plant's biomass on a height basis. This simulation was conducted at two critical growth stages, in June and July, to mimic seasonal grazing impacts. Prior to the clipping treatments, the height of the plants was measured using a ruler (in centimeters) to ensure accurate biomass removal.

First clipping treatment in June

In June, corresponding to the early growth phase of grasses, the aboveground mass predominantly consisted of leaves without tillers. Clipping 70% of aboveground mass effectively removed all leaves from the experimental pots, leaving behind only the base of stems and a few leaves critical for subsequent non-destructive measurements such as photosynthesis rates, chlorophyll content, and rapid light curves (RLCs) as discussed in chapter 2.

Measurements were recorded for the total area (cm²) with a leaf area meter (LI-3100, LICOR), as well as the fresh and dry biomass (g) of the removed materials during each instance of simulated grazing pressure.

Subsequently, non-destructive techniques were utilized to assess the total leaf area remaining on the plants after clipping. For this purpose, the Leafscan application was employed. Leafscan operates by analyzing uploaded images of the leaves. Each image included a scale reference to ensure accuracy. The application calculates the leaf area (cm²) by recognizing the leaf edges against the contrasting background, providing precise measurements of the remaining foliar surface area. This method allowed for a rapid and reliable estimation of leaf area without further physical intervention with the plant material.

These data facilitated the calculation of both pre-clipped total leaf area and post-clipped total leaf area for the residual leaves.

Second clipping treatment in July and Tillers Observation

As the season progressed into July, a select few pots showed signs of stem development, indicating the onset of tillering. We observed the emergence of tillers in only three of the sampled pots. Two of these pots contained plants with a history of grazing, while the third pot had plants without prior exposure to grazing.

Adhering to the previously established protocol, we removed 70% of the plant aboveground biomass on a height basis, mirroring the simulated grazing treatment applied in June. This method ensured uniformity in the treatment across both periods. The height, area, fresh, and dry biomass of the tillers, which were among the plant materials clipped, were carefully documented. We measured these traits to accurately quantify the impact of simulated grazing, alongside the traits of the leaves that were also removed.

Post-clipping, the total leaf area of the foliage left on the plants was measured using the Leafscan application. This allowed for an accurate assessment of the total leaf area before and after the second clipping treatment.

Final Harvest Measurements

The final harvest in September entailed an extensive biomass assessment. Total area of leaves and flowering tillers were measured. We measured the total fresh mass of leaves, flowering tillers, and seeds. Subsequently, these samples were placed in an oven set at 60°C for 72 hours to ensure complete moisture removal and achieve a constant mass and weighed. After the drying period, we measured the dry mass of these components. This protocol was also applied to the belowground parts, encompassing total dry mass of rhizomes, fine roots, and total belowground dry weight. In addition to mass measurements, we recorded the lengths of tillers (for both clipped and control treatments) and roots (only for plants that underwent clipping treatment). The compiled data from these processes enabled us to calculate the fractional composition of the various plant parts, namely tillers, total roots, rhizomes, leaves, and seeds. These comprehensive metrics provided valuable insights into the plant's structural adaptations in response to the simulated grazing pressure by the effect of memory of grazing.

Calculation of derived variables

Utilizing these measured variables, we calculated the total dry matter (TDM) in grams for each plant. Further analyses included the determination of Specific Leaf Area (SLA), Shoot to Root ratio (based on dry mass), and Root Mass Fraction (RMF). We also quantified the mass fractions of tillers, leaves, seeds, and rhizomes (TMF, LMF, SMF, and RhMF, respectively). Total aboveground and belowground biomass were also calculated. These calculations were performed across all treatments, including plants with a memory of grazing and those without, which had undergone the clipping treatment in the greenhouse, as well as the unclipped plants serving as our control group.

In our analysis, we calculated these derived variables to assess plant biomass allocation and leaf economics traits using the following formulas:

Total Dry Mass (TDM) in grams represents the sum of all plant dry biomass and was calculated by adding the aboveground and belowground dry weight:

$$TDM = AGDW + BGDW$$

Root Mass Fraction (RMF) is the proportion of plant biomass located belowground and was calculated as the dry weight (DW) of belowground (BG) parts (BGDW) divided by the sum of aboveground (AGDW) and belowground dry weight:

$$RMF = BGDW / TDM$$

Similarly, we determined the mass fractions for flowering tillers, leaves, and seeds. The fraction for each component was calculated as its dry weight divided by the total dry mass.

Specific Leaf Area (SLA) ($\text{cm}^2.\text{g}^{-1}$) reflects the leaf area per unit dry weight and was determined by dividing the total leaf area (TLA) by the total leaf dry weight (TLDW):

$$SLA = TLA / TLDW$$

Shoot to Root (StoR) ratio indicates the balance between aboveground and belowground biomass and was computed as the ratio of aboveground dry weight to belowground dry weight:

$$StoR = AGDW : BGDW$$

These calculations follow the procedures outlined by Poorter et al., 2012, allowing for the comparison of biomass allocation strategies under different treatment conditions. By quantifying these variables, we were able to draw conclusions about the plant's growth patterns and structural adaptations in response to the simulated grazing. These metrics allowed us to dissect the allocation strategies of plants in response to simulated grazing and to understand how past experiences of grazing influenced their growth patterns and biomass distribution.

Calculation of Relative Growth Rate

To calculate the Relative Growth Rate (RGR), we initially considered the method proposed by Vinton & Hartnett, 1992:

$$RGR (L. day^{-1}) = (\ln L_2 - \ln L_1) / t_2 - t_1$$

Where L_x represents the length of leaves for a plant at time x , and t denotes the time at each measurement interval. Although leaf length has been shown to be a good predictor of leaf biomass for *A. gerardii* ($R^2 = 0.91$) (Vinton & Hartnett, 1992), our dataset for leaf length was incomplete. Consequently, as an alternative metric to calculate RGR, we turned to total leaf area, a variable we measured consistently before and after each clipping treatment and at the end of the season in September.

To establish the validity of using leaf area as a predictor for biomass, we first examined the correlation between leaf length and leaf area to see if leaf length can predict leaf area as the leaf length was initially suggested to be utilized to estimate RGR by Vinton & Hartnett, 1992. Our

analysis yielded a moderately strong relationship, with an R^2 of 0.60 when including both leaf length and width as predictors in the model to predict leaf area (Swemmer et al., 2006). Further, we investigated the relationship between leaf area and plant biomass, both fresh and dry. We found a strong correlation between leaf area and fresh weight (R^2 of 0.88), but a weaker relationship with dry weight (R^2 of 0.24). Although this information is not reported in this document. These findings indicate that total leaf area is a reliable predictor for fresh biomass.

Therefore, we relied on regression analysis to establish the relationship between leaf area and plant fresh biomass (FW). It was also deemed reasonable to use leaf area to estimate the fresh weight of leaves remaining on the plants after each clipping to calculate the fresh biomass before and after each treatment. Consequently, we adapted Hartnett's equation to calculate RGR using total leaf area for each interval:

$$RGR (cm^2 cm^{-2} day^{-1}) = (\ln A_2 - \ln A_1) / t_2 - t_1$$

Where A_x represents the total leaf area for a plant at time x , and t denotes the time at each measurement interval. This modification allowed us to utilize a complete dataset and maintain consistency in our RGR calculations across all plants in clipping treatment, including those with and without a memory of grazing.

We calculated RGR for two distinct growth intervals. The first interval, from June to July (RGR-June-July), spanned 24 days and followed the initial clipping treatment administered at the peak of vegetative growth. The second interval, from July to September (RGR-July-Sep), covered 54 days and came after the second round of clipping, when the removal of tissues aligned with the onset of the plant's reproductive phase. These durations were specifically chosen to analyze the growth response to simulated grazing during distinct developmental stages by the effect of grazing memory.

Statistical analyses

For the statistical analysis of our data, we employed mixed effects models where applicable. These models included the main effects of grazing memory, treatment applications, and time intervals, along with their interactions. Additionally, different sampling plots, denoted as 'sample' in this study, were incorporated as random effects to account for variability between plots.

ANOVA tests were conducted to determine the significance of the effects of grazing memory, treatment, and time interval, as well as their interactions, on the measured variables. Subsequent post hoc comparisons between groups were performed using Tukey's Honest Significant Difference (HSD) test to pinpoint specific group differences.

Regression analyses were utilized to examine the relationships among variables and to discern patterns and predictive capabilities within our dataset. This allowed us to quantify the strength and nature of associations between variables of interest.

Throughout our analyses, we checked the assumptions of each model to ensure the reliability of our results. These checks were comprehensive, ensuring normality of residuals, homogeneity of variance, and the absence of multicollinearity.

All coding and statistical computations were conducted using Python version 3.11.6 within the Visual Studio Code (VSCode) served as an advanced integrated development environment (IDE) that facilitated efficient coding, debugging, and management of our Python scripts, leveraging libraries such as Pandas for data manipulation, NumPy for numerical computations, and Statsmodels for conducting mixed effects models and ANOVA tests. The Scipy library facilitated our regression analyses and post hoc comparisons, while Matplotlib and Seaborn were utilized for data visualization.

Results

Relative Growth Rate (RGR)

In analyzing the data using mixed effect models, we assessed the influence of grazing memory and time intervals as the main effects, with 'sample' included as a random effect. The variance attributed to 'sample' was found to be minimal, suggesting that including it as a random effect does not significantly improve the model's fit. This indicated that 'sample' may not have a substantial effect on the outcome variables. Given that our primary interest lies in the effects of memory, time interval, and their interaction on the RGR, we opted for a two-way ANOVA as an appropriate alternative to understand these effects.

The ANOVA model indicates significant main effects of both memory and time interval on RGR (p values of 0.0027 and 3.31E-10, respectively), with both factors independently influencing RGR. However, the interaction between memory and interval was not significant ($p=0.161$), suggesting that the effect of memory on the change in RGR from the first to the second interval does not differ significantly between the memory groups (Table 3.1, Figure 3.1).

A statistically significant reduction in RGR was observed between the June-July and the July-September intervals for both groups of plants subjected to clipping treatments—those with memory of grazing (M+) and those without (M-). The Tukey HSD post-hoc analysis identified significant differences in RGR across various comparisons, detailed in Table 3.2.

Effects of time interval: Plants with memory of grazing (M+) in the June-July interval showed a significantly higher RGR compared to those in the July-September interval (M+) with a mean difference of 0.0689 ($p<0.05$). Also, plants without memory of grazing (M-) had a significantly lower RGR in the July-September interval compared to the June-July interval, with a mean difference of 0.092 ($p<0.05$).

Effect of memory: No significant difference was observed in RGR between plants with memory of grazing (M+) in the June-July interval and those without memory (M-) in the same interval, as indicated by a mean difference of -0.0162 ($p = 0.444$), which suggests that during the early growth stage, the presence of grazing memory does not significantly alter growth rates.

Furthermore, when comparing plants with memory of grazing (M+) in the July-September interval to those without memory (M-) in the same interval, we found that M- group had a significantly lower RGR, with a mean difference of 0.0394 ($p=0.0144$), suggesting that the presence of memory increased growth rate in the second interval.

Interaction effects of memory and interval: A comparison between plants with memory of grazing (M+) in the July-September interval and those without memory (M-) in the June-July interval showed a significant increase in RGR for M- with a mean difference of 0.0527 (p value of 0.0004).

Plants with memory of grazing (M+) in the June-July interval had a significantly higher RGR than those without memory (M-) in the July-September interval, with a substantial mean difference of 0.1083 ($p<0.001$).

Impact of Grazing Memory on Biomass and Growth Traits in Clipped *A. gerardii*

Mixed effect model analyses were conducted on clipped *A. gerardii* plants to determine the effect of grazing memory on several plant traits, with 'memory' treated as a fixed effect and 'sample' as a random effect. The model revealed that the absence of grazing memory had a statistically significant negative impact on total fresh weight (TtillerFW) and dry weight (TtillerDW) (g) of flowering tillers, with reductions evident in the memory-absent group as indicated by $p < 0.001$, and < 0.001 , respectively. Conversely, flowering tiller length (FtillerL)

showed a positive trend with the absence of memory, but this did not reach the statistical significance ($p = 0.053$) (Table 3.3, Figure 3.2.A, B, and C).

Further significant decreases associated with the absence of memory were observed in the total seed fresh weight (TSeFW) and dry weight (TSeDW), as well as aboveground dry weight (AGDW) (g), with p -values of <0.001 across these variables. Belowground dry weight (BGDW) also decreased significantly ($p = 0.003$). Specific leaf area (SLA) ($\text{cm}^2 \cdot \text{g}^{-1}$) was significantly lower in memory-absent plants ($p = 0.003$), and the shoot to root ratio (StoR) was similarly affected ($p = 0.014$) (Table 3.3, Figure 2.D, E, I, and J).

However, root mass fraction (RMF) (Table 3, Figure 2.K) and Root length (RL) (cm) (Table 3.3, not displayed in figure 3.2) were not significantly affected by the memory (p -values of 0.091 and 0.499, respectively). The total dry mass (TDM) was significantly lower in plants without grazing memory ($p < 0.001$) (Table 3, Figure 2.N), and there was a significant decrease in the total leaf area in September (TLA_sept) (Table 3.3, not displayed in figure 3.2) for memory-absent plants ($p < 0.001$).

In an effort to understand the long-term effects of grazing memory, we summed the values of total leaf area (TLA), total leaf fresh weight (TLFW), and total leaf dry weight (TLDW) for materials removed during the June and July clipping events. This summation provided a comprehensive measure of the biomass removed across different memory treatments in clipped *A. gerardii* plants.

The mixed effect model analysis revealed that, while some certain individual metrics, e.g., total leaf area in September (TLA_sept) showed significant differences based on memory, the cumulative biomass removed over the June and July intervals (TLA_summed, TLFW_summed,

TLDW_summed) did not demonstrate a significant difference when comparing plants with and without memory of grazing (p values of 0.157, 0.231, and 0.209, respectively) (Table 3.3).

Following the mixed effect model analysis which focused on plants subjected to clipping treatments, we expanded our statistical examination to include both clipped and control treatments, with and without memory. ANOVA tests were performed to compare the means of various growth and biomass variables across all groups, ensuring the assumptions were met for the validity of the test.

The ANOVA results demonstrated significant differences across several key variables when incorporating control groups into the analysis. The total fresh weight (FW) and dry weight (DW) of flowering tillers, as well as the length of flowering tillers, showed highly significant differences (p-values ranging from <0.0001 to <0.004) (Table 3.4, Figure 3.2. A and B), indicating that these traits are influenced by the treatment conditions. Similarly, the aboveground fresh and dry weights (AGFW and AGDW) (Table 3.4, Figure 3.2. H and I), and belowground dry weight (BGDW) were significantly different across the groups ($p < 0.01$) (Table 3.4, Figure 3.2.J), underscoring the treatment impact on plant biomass distribution.

Notably, the total dry mass (TDM) also exhibited a highly significant difference ($p = 0.0003$) (Table 3.4, Figure 3.2. N), while variables like root mass fraction (RMF), specific leaf area (SLA), and shoot to root ratio (StoR) did not show significant differences, with p-values exceeding the 0.05 threshold (Table 3.4, Figure 3.2. K, L, and M). These findings suggest that while certain biomass components are distinctly affected by the grazing memory and treatment conditions, others such as root-to-shoot allocations remain unaffected.

Analysis of Biomass Allocation Patterns in Response to Grazing Memory and Clipping Treatments

The ANOVA results for the derived fractional variables across four different groups highlight that among the fractional variables analyzed, only the leaf and rhizome mass fractions showed a response to the factors tested (Table 3.5, Figure 3.3). While the effect of treatment on the leaf mass fraction is not statistically significant, with a p-value of 0.058, it is on the edge of significance. This suggests a potential trend where treatment may influence the proportion of biomass allocated to leaves in *A. gerardii*, warranting further investigation. The rhizome mass fraction was influenced by the plant's memory of grazing ($p = 0.021$) (Table 3.5, Figure 3.3).

Impact of Relative Growth Rates and Grazing Memory on End-of-Season Biomass Attributes

Our regression analysis sought to understand the influence of early and late season growth rates, represented by RGR, on end-of-season biomass outcomes while accounting for the presence or absence of grazing memory. Two models stood out with significant results:

Specific Leaf Area (SLA): The RGR from the later July-September interval showed a significant positive effect on SLA, with an adjusted R^2 of 0.473 and a significant p-value ($p = 0.0072$). This relationship suggests that higher growth rates later in the season contribute to a larger specific leaf area. As with the previous model, the effect of grazing memory was not significant ($p = 0.35$) (Table 3.6, Figure 3.4.A), suggesting that RGR during this interval is a stronger determinant of SLA than the plant's memory of being grazed.

Total Leaf Fresh Weight (TLFW): This model, incorporating RGR from the June-July interval, demonstrated a strong positive relationship with TLFW measured in September, as

indicated by an impressive adjusted R^2 of 0.671 and a highly significant p-value ($p = 0.0005$). This suggests that vigorous early-season growth rates are a good predictor of greater leaf fresh biomass at the end of the growing season. The variable of grazing memory did not show a significant influence in this model ($p = 0.48$) (Table 3.6, Figure 3.4.B), indicating that the early growth rates themselves, regardless of grazing history, were more directly related to the final fresh weight outcomes.

Discussion

This study set out to explore the adaptive significance of plant memory in *A. gerardii*, focusing on its response to simulated grazing. Our findings offer novel insights into the complex interplay between past grazing experiences and plant growth dynamics, shedding light on the underlying mechanisms of biomass allocation and compensatory growth strategies.

RGR: The dynamics of RGR as influenced by grazing memory and time intervals are central to our understanding of plant adaptive responses. Our results indicate that plants with a history of grazing (M+) exhibit a significant decrease in RGR, about 48%, transitioning from the early to the late growing season. Conversely, plants without a grazing memory (M-) experience a larger decline, with their RGR reducing by approximately 72.6% over the same period. This result underscores the influence of grazing history on growth trajectories and the ability of this grass species to adjust their growth rates in response to past grazing events (Hilker & Schmölling, 2019; Kusch et al., 2022).

In addition, our findings regarding the RGR support the established principle that plants experiencing grazing must sustain an elevated RGR relative to ungrazed plants to fully recover from tissue loss (Barto & Cipollini, 2005; Harvell, 1990; Sinha, 2023; Vinton & Hartnett, 1992).

Specifically, *A. gerardii* individuals with a grazing history demonstrate a smaller decline in RGR after the second defoliation event, suggesting they can sustain higher growth rates to compensate for biomass loss (Hilbert et al., 1981). This capability is essential for not only survival but also for maintaining reproductive potential in the face of grazing pressures (Ferraro & Oosterheld, 2002). The observed trend in our data reflects the necessity of maintaining a robust RGR to counteract the effects of tissue removal, supporting the idea that a history of grazing can lead to an adaptive growth advantage in perennial grasses (Hilbert et al., 1981; Knapp et al., 2012; Vinton & Hartnett, 1992).

Moreover, the coping response of *A. gerardii* with grazing memory (M+) is evident from the less pronounced decline in RGR after both the first and second clipping events. In M+ plants, this could be seen as a strategic enhancement of growth post-defoliation, aiming to counterbalance biomass loss and taking advantage of higher light intensity and space (Knapp et al., 2012; McNaughton, 1986). The less pronounced decline in RGR in M+ plants across both intervals might be indicative of a priming effect, where previous grazing experiences have conditioned the plant for future stress events, enabling a rapid and efficient recovery through anticipatory resource allocation or physiological adjustments (Conrath et al., 2006; Hilker & Schmölling, 2019). The more marked decline in RGR for M- plants after each clipping event highlights their vulnerability and a potential deficit in adaptive priming mechanisms, which makes them more susceptible to the consequences of defoliation. These findings are also in line with the grazing optimization hypothesis, which posits that plants can compensate for herbivory under certain conditions, thereby enhancing their growth and reproductive output (Belsky, 1986; Hilbert et al., 1981).

Biomass allocation and growth traits: Our study's exploration into *A. gerardii* responses to grazing memory and defoliation presents intricate outcomes. The anticipated benefit of grazing

memory on growth traits was not universally observed. Clipping significantly impacted biomass, particularly the mass of flowering tillers and total dry mass, irrespective of grazing history (Tables 3.3 and 3.4). Seed biomass (DW) is slightly smaller in plants with the history of grazing when they are clipped and unclipped (M+G and M+C). Total root fraction, root length, and fine root mass do not show a significant effect of memory. These findings contrast with those reported in Guo et al., 2021, while they documented enhanced root development in *Agropyron cristatum*, a C3 grass in the family Poaceae, with a history of grazing, our results suggest a different outcome in *A. gerardii*. Additionally, our findings did not demonstrate an increase in fitness in plants with the memory of grazing (Thellier et al., 2018). However, we observed that plants with a history of grazing exhibited a higher RGR, indicative of the total leaf area's expansion over time in response to grazing. This could suggest a compensatory mechanism to cope with the loss of photosynthetic tissues (Oosterheld & McNaughton, 1991; Siddappaji et al., 2015).

The influence of grazing memory on *A. gerardii* becomes further apparent when considering the allocation of biomass among different plant fractions (Table 3.5). Specifically, the leaf and rhizome mass fractions were responsive to clipping treatments and memory, respectively, suggesting a differential allocation strategy that may be modulated by the plant's treatments and grazing history. The nearly significant effect of clipping on the leaf mass fraction hints at a trend where clipping could influence the proportion of biomass allocated to leaves, a critical component for photosynthesis and subsequent energy capture. This trend, while not reaching levels of significance, suggests a potential adaptive response that could optimize light capture (Alber et al., 2014; DeAngelis et al., 2012; A. Knapp et al., 2012), carbohydrate storage (Benot et al., 2019), and growth following defoliation events in plants with the memory (J. R. Brown & Stuth, 1993; Elson & Hartnett, 2017; Vinton & Hartnett, 1992).

Although plants without memory showed a potential for more robust belowground growth, as seen in higher rhizome mass fractions (Table 3.5, Figure 3.3), the expected advantages of memory in aboveground biomass recovery were not evident. Our results present a different perspective from the findings associated with *Leymus chinensis* possessing grazing history (X. Li et al., 2021; Qu et al., 2022), indicating that the effects of grazing memory on plant functional traits may vary among species. Instead, resource allocation patterns appeared complex and context-dependent.

The physiological importance of memory in *A. gerardii* could be multifaceted. Memory may confer an adaptive advantage in fluctuating environments by optimizing resource distribution between above- and belowground parts (Bazzaz et al., 1987; Geng et al., 2007), enhancing overall resilience and survival (Ingrisch & Bahn, 2018; Kusch et al., 2022).

Additional research is essential to elucidate how *A. gerardii* allocates resources in response to defoliation, particularly considering its historical exposure to grazing.

These results suggest that while grazing memory influences *A. gerardii*'s growth responses, the direct effects of defoliation and the plant's inherent growth mechanisms play a more substantial role in determining resilience and biomass production than previously hypothesized.

Impact of Relative Growth Rates and Grazing Memory on End-of-Season Biomass Attributes: Given the initial hypothesis that plant memory would positively influence the response of *A. gerardii* to improve growth following defoliation, and the correlation of RGR during early and late growth stages with end-of-season biomass attributes, we can draw the following conclusions from the data:

The significant correlation between RGR in both early (June-July) and late (July-September) growth stages with end-of-season total leaf fresh weight and SLA respectively

indicates that immediate growth rates post-defoliation are critical predictors of end-of-season biomass and leaf structure (Osone et al., 2008). This aligns with the part of the hypothesis predicting that RGR would correlate with end-of-season biomass attributes. The statistical analysis shows that memory does not significantly influence these correlations. This finding does not support the hypothesis that plant memory would modulate the relationship between RGR and end-of-season biomass attributes. Instead, it suggests that the plant's inherent growth response to defoliation is a more dominant factor than the memory of past grazing.

The strong innate growth response and recovery capability in *A. gerardii*, irrespective of grazing memory, highlight a potentially valuable trait for survival in environments with variable grazing pressures. The plant's ability to rapidly grow after defoliation events and produce significant biomass by the end of the season underscores its resilience (Ratajczak et al., 2022).

Conclusion

In conclusion, the comprehensive analysis of *A. gerardii* in response to grazing suggests that the plant's inherent physiological responses to defoliation are more predictive of end-of-season biomass and structural traits than the memory of past grazing. While plants with a history of grazing show a smaller decline in RGR throughout the season, indicating some degree of adaptive response, this memory does not significantly influence the total leaf fresh weight or SLA at the end of the growing season. These results challenge the initial hypothesis that grazing memory would enhance growth traits and suggest that the species' innate growth dynamics post-defoliation play a more critical role in determining plant vigor and survival. Consequently, grassland management practices may need to focus more on the timing and intensity of defoliation rather than on influencing plant memory to optimize the resilience and productivity of *A. gerardii* in grazing ecosystems.

References

- Agrawal, A. (2000). Overcompensation of plants in response to herbivory and the by-product benefits of mutualism. *TRENDS IN PLANT SCIENCE*, 5(7), 309–313.
[https://doi.org/10.1016/S1360-1385\(00\)01679-4](https://doi.org/10.1016/S1360-1385(00)01679-4)
- Alber, N. B., Brink, G. E., & Jackson, R. D. (2014). Temperate grass response to extent and timing of grazing. *Canadian Journal of Plant Science*, 94(5), 827–833.
<https://doi.org/10.4141/cjps2013-404>
- Avramova, Z. (2019). Defence-related priming and responses to recurring drought: Two manifestations of plant transcriptional memory mediated by the ABA and JA signalling pathways. *Plant, Cell & Environment*, 42(3), 983–997. <https://doi.org/10.1111/pce.13458>
- Bandoly, M., Grichnik, R., Hilker, M., & Steppuhn, A. (2016). Priming of anti-herbivore defence in *Nicotiana attenuata* by insect oviposition: Herbivore-specific effects. *PLANT CELL AND ENVIRONMENT*, 39(4), 848–859. <https://doi.org/10.1111/pce.12677>
- Barto, E. K., & Cipollini, D. (2005). Testing the optimal defense theory and the growth-differentiation balance hypothesis in *Arabidopsis thaliana*. *Oecologia*, 146(2), 169–178.
<https://doi.org/10.1007/s00442-005-0207-0>
- Bazzaz, F. A., Chiariello, N. R., Coley, P. D., & Pitelka, L. F. (1987). Allocating Resources to Reproduction and Defense: New assessments of the costs and benefits of allocation patterns in plants are relating ecological roles to resource use. *BioScience*, 37(1), 58–67.
<https://doi.org/10.2307/1310178>
- Belsky, A. J. (1986). Does Herbivory Benefit Plants? A Review of the Evidence. *The American Naturalist*, 127(6), 870–892. <https://doi.org/10.1086/284531>

- Benot, M.-L., Morvan-Bertrand, A., Mony, C., Huet, J., Sulmon, C., Decau, M.-L., Prud'homme, M.-P., & Bonis, A. (2019). Grazing intensity modulates carbohydrate storage pattern in five grass species from temperate grasslands. *Acta Oecologica*, 95, 108–115.
<https://doi.org/10.1016/j.actao.2018.11.005>
- Brown, J. R., & Stuth, J. W. (1993). How Herbivory Affects Grazing Tolerant and Sensitive Grasses in a Central Texas Grassland: Integrating Plant Response across Hierarchical Levels. *Oikos*, 67(2), 291–297. <https://doi.org/10.2307/3545474>
- Christiansen, S., & Svejcar, T. (1988). Grazing effects on shoot and root dynamics and above- and below-ground non-structural carbohydrate in *Caucasian bluestem*. *Grass and Forage Science*, 43(2), 111–119. <https://doi.org/10.1111/j.1365-2494.1988.tb01878.x>
- Conrath, U., Beckers, G. J. M., Flors, V., García-Agustín, P., Jakab, G., Mauch, F., Newman, M.-A., Pieterse, C. M. J., Poinssot, B., Pozo, M. J., Pugin, A., Schaffrath, U., Ton, J., Wendehenne, D., Zimmerli, L., & Mauch-Mani, B. (2006). Priming: Getting Ready for Battle. *Molecular Plant-Microbe Interactions®*, 19(10), 1062–1071.
<https://doi.org/10.1094/MPMI-19-1062>
- DeAngelis, D., Ju, S., Liu, R., Bryant, J., & Gourley, S. (2012). Plant allocation of carbon to defense as a function of herbivory, light and nutrient availability. *THEORETICAL ECOLOGY*, 5(3), 445–456. <https://doi.org/10.1007/s12080-011-0135-z>
- Elson, A., & Hartnett, D. C. (2017). Bison Increase the Growth and Reproduction of Forbs in Tallgrass Prairie. *The American Midland Naturalist*, 178(2), 245–259.
<https://doi.org/10.1674/0003-0031-178.2.245>
- Ferraro, D. O., & Oosterheld, M. (2002). Effect of defoliation on grass growth. A quantitative review. *Oikos*, 98(1), 125–133. <https://doi.org/10.1034/j.1600-0706.2002.980113.x>

- Frank, D. A., McNaughton, S. J., & Tracy, B. F. (1998). The Ecology of the Earth's Grazing Ecosystems: Profound functional similarities exist between the Serengeti and Yellowstone. *BioScience*, 48(7), 513–521. <https://doi.org/10.2307/1313313>
- Galviz, Y. C. F., Ribeiro, R. V., & Souza, G. M. (2020). Yes, plants do have memory. *Theoretical and Experimental Plant Physiology*, 32(3), 195–202. <https://doi.org/10.1007/s40626-020-00181-y>
- Garai, S., & Sopory, S. K. (2023). Memory of plants: Present understanding. *The Nucleus*, 66(1), 47–51. <https://doi.org/10.1007/s13237-022-00399-y>
- Geng, Y.-P., Pan, X.-Y., Xu, C.-Y., Zhang, W.-J., Li, B., & Chen, J.-K. (2007). Plasticity and ontogenetic drift of biomass allocation in response to above- and below-ground resource availabilities in perennial herbs: A case study of *Alternanthera philoxeroides*. *Ecological Research*, 22(2), 255. <https://doi.org/10.1007/s11284-006-0017-9>
- Guo, F.-H., Li, X.-L., Yin, J.-J., Jimoh, S. O., & Hou, X.-Y. (2021). Grazing-induced legacy effects enhance plant adaption to drought by larger root allocation plasticity. *Journal of Plant Ecology*, 14(6), 1024–1029. <https://doi.org/10.1093/jpe/rtab056>
- Harvell, C. D. (1990). The Ecology and Evolution of Inducible Defenses. *The Quarterly Review of Biology*, 65(3), 323–340. <https://doi.org/10.1086/416841>
- Hilbert, D. W., Swift, D. M., Detling, J. K., & Dyer, M. I. (1981). Relative growth rates and the grazing optimization hypothesis. *Oecologia*, 51(1), 14–18. <https://doi.org/10.1007/BF00344645>
- Hilker, M., & Schmölling, T. (2019). Stress priming, memory, and signalling in plants. *Plant, Cell & Environment*, 42(3), 753–761. <https://doi.org/10.1111/pce.13526>

- Hilker, M., Schwachtje, J., Baier, M., Balazadeh, S., Bäumle, I., Geiselhardt, S., Hinch, D. K., Kunze, R., Mueller-Roeber, B., Rillig, M. C., Rolff, J., Romeis, T., Schmölling, T., Steppuhn, A., van Dongen, J., Whitcomb, S. J., Wurst, S., Zuther, E., & Kopka, J. (2016). Priming and memory of stress responses in organisms lacking a nervous system. *Biological Reviews*, 91(4), 1118–1133. <https://doi.org/10.1111/brv.12215>
- Hill, M. O., Evans, D. F., & Bell, S. A. (1992). Long-Term Effects of Excluding Sheep from Hill Pastures in North Wales. *Journal of Ecology*, 80(1), 1–13. <https://doi.org/10.2307/2261058>
- Ingrisch, J., & Bahn, M. (2018). Towards a Comparable Quantification of Resilience. *Trends in Ecology & Evolution*, 33(4), 251–259. <https://doi.org/10.1016/j.tree.2018.01.013>
- James M., B., Joe, F., Mark J., S., Jonathan, S., Sarah J., C., Steve J., G., & Richard, T. (2001). A plant trait analysis of responses to grazing in a long-term experiment. *Journal of Applied Ecology*, 38(2), 253–267. <https://doi.org/10.1046/j.1365-2664.2001.00599.x>
- Knapp, A., Hoover, D. L., Blair, J., Buis, G., Burkepile, D. E., Chamberlain, A. J., Collins, S., Fynn, R. W. S., Kirkman, K. P., Smith, M. D., Blake, D., Govender, N., O’Neal, P., Schreck, T., & Zinn, A. (2012). A test of two mechanisms proposed to optimize grassland aboveground primary productivity in response to grazing. *Journal of Plant Ecology*, 5, 357–365. <https://doi.org/10.1093/jpe/rts020>
- Kusch, E., Davy, R., & Seddon, A. W. R. (2022). Vegetation-memory effects and their association with vegetation resilience in global drylands. *Journal of Ecology*, 110(7), 1561–1574. <https://doi.org/10.1111/1365-2745.13891>
- Li, J., Hou, F., & Ren, J. (2021). Grazing Intensity Alters Leaf and Spike Photosynthesis, Transpiration, and Related Parameters of Three Grass Species on an Alpine Steppe in the Qilian Mountains. *Plants*, 10(2), Article 2. <https://doi.org/10.3390/plants10020294>

- Li, X., Hu, N., Yin, J., Ren, W., & Fry, E. (2021). Historic grazing enhances root-foraging plasticity rather than nitrogen absorbability in clonal offspring of *Leymus chinensis*. *Plant and Soil*, 466(1), 65–79. <https://doi.org/10.1007/s11104-021-05033-5>
- McNaughton, S. J. (1986). On Plants and Herbivores. *The American Naturalist*, 128(5), 765–770.
- Mipam, T.-D., Zhong, L.-L., Liu, J.-Q., Miehe, G., & Tian, L.-M. (2019). Productive Overcompensation of Alpine Meadows in Response to Yak Grazing in the Eastern Qinghai-Tibet Plateau. *Frontiers in Plant Science*, 10. <https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2019.00925>
- Oosterheld, M., & McNaughton, S. J. (1991). Effect of Stress and Time for Recovery on the Amount of Compensatory Growth after Grazing. *Oecologia*, 85(3), 305–313.
- Osone, Y., Ishida, A., & Tateno, M. (2008). Correlation between relative growth rate and specific leaf area requires associations of specific leaf area with nitrogen absorption rate of roots. *New Phytologist*, 179(2), 417–427. <https://doi.org/10.1111/j.1469-8137.2008.02476.x>
- Poorter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P., & Mommer, L. (2012). Biomass allocation to leaves, stems and roots: Meta-analyses of interspecific variation and environmental control. *New Phytologist*, 193(1), 30–50. <https://doi.org/10.1111/j.1469-8137.2011.03952.x>
- Qu, K., Cheng, Y., Gao, K., Ren, W., Fry, E. L., Yin, J., & Liu, Y. (2022). Growth-Defense Trade-Offs Induced by Long-term Overgrazing Could Act as a Stress Memory. *Frontiers in Plant Science*, 13. <https://doi.org/10.3389/fpls.2022.917354>

- Ratajczak, Z., Collins, S. L., Blair, J. M., Koerner, S. E., Louthan, A. M., Smith, M. D., Taylor, J. H., & Nippert, J. B. (2022). Reintroducing bison results in long-running and resilient increases in grassland diversity. *Proceedings of the National Academy of Sciences*, *119*(36), e2210433119. <https://doi.org/10.1073/pnas.2210433119>
- Ren, W., Hu, N., Hou, X., Zhang, J., Guo, H., Liu, Z., Kong, L., Wu, Z., Wang, H., & Li, X. (2017). Long-Term Overgrazing-Induced Memory Decreases Photosynthesis of Clonal Offspring in a Perennial Grassland Plant. *Frontiers in Plant Science*, *8*. <https://doi.org/10.3389/fpls.2017.00419>
- Siddappaji, M. H., Scholes, D. R., Krishnankutty, S. M., Calla, B., Clough, S. J., Zielinski, R. E., & Paige, K. N. (2015). The role of invertases in plant compensatory responses to simulated herbivory. *BMC Plant Biology*, *15*(1), 278. <https://doi.org/10.1186/s12870-015-0655-6>
- Sinha, A. (2023). Plant Stress Memory: Struggle of Getting Better Each Time. *ScienceOpen Posters*. <https://doi.org/10.14293/S2199-1006.1.SOP-.PPUNR6C.v1>
- Swemmer, A. M., Knapp, A. K., & Smith, M. D. (2006). Growth Responses of Two Dominant C4 Grass Species to Altered Water Availability. *International Journal of Plant Sciences*, *167*(5), 1001–1010. <https://doi.org/10.1086/505611>
- Thellier, M., Lüttge, U., Norris, V., & Ripoll, C. (2018). Plant Accommodation to Their Environment: The Role of Specific Forms of Memory. In F. Baluska, M. Gagliano, & G. Witzany (Eds.), *Memory and Learning in Plants* (pp. 131–137). Springer International Publishing. https://doi.org/10.1007/978-3-319-75596-0_7
- Vanderklein, D. W., & Reich, P. B. (1999). The effect of defoliation intensity and history on photosynthesis, growth and carbon reserves of two conifers with contrasting leaf

lifespans and growth habits. *The New Phytologist*, 144(1), 121–132.

<https://doi.org/10.1046/j.1469-8137.1999.00496.x>

Vinton, M. A., & Hartnett, D. C. (1992). Effects of Bison Grazing on *Andropogon gerardii* and *Panicum virgatum* in Burned and Unburned Tallgrass Prairie. *Oecologia*, 90(3), 374–382.

Zhao, W., Chen, S.-P., & Lin, G.-H. (2008). Compensatory growth responses to clipping defoliation in *Leymus chinensis* (*Poaceae*) under nutrient addition and water deficiency conditions. *Plant Ecology*, 196(1), 85–99. <https://doi.org/10.1007/s11258-007-9336-3>

Figures and Tabels

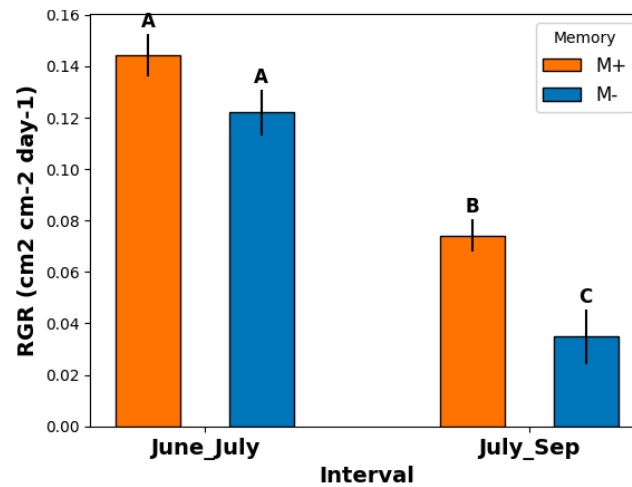


Figure 3. 1- Bar Plot of RGR by Grazing Memory and Time Interval with Tukey's HSD Mean comparison annotations for clipped *A. gerardii* plants. Groups sharing the same letter are not significantly different, whereas distinct letters indicate a statistically significant difference in the mean values.

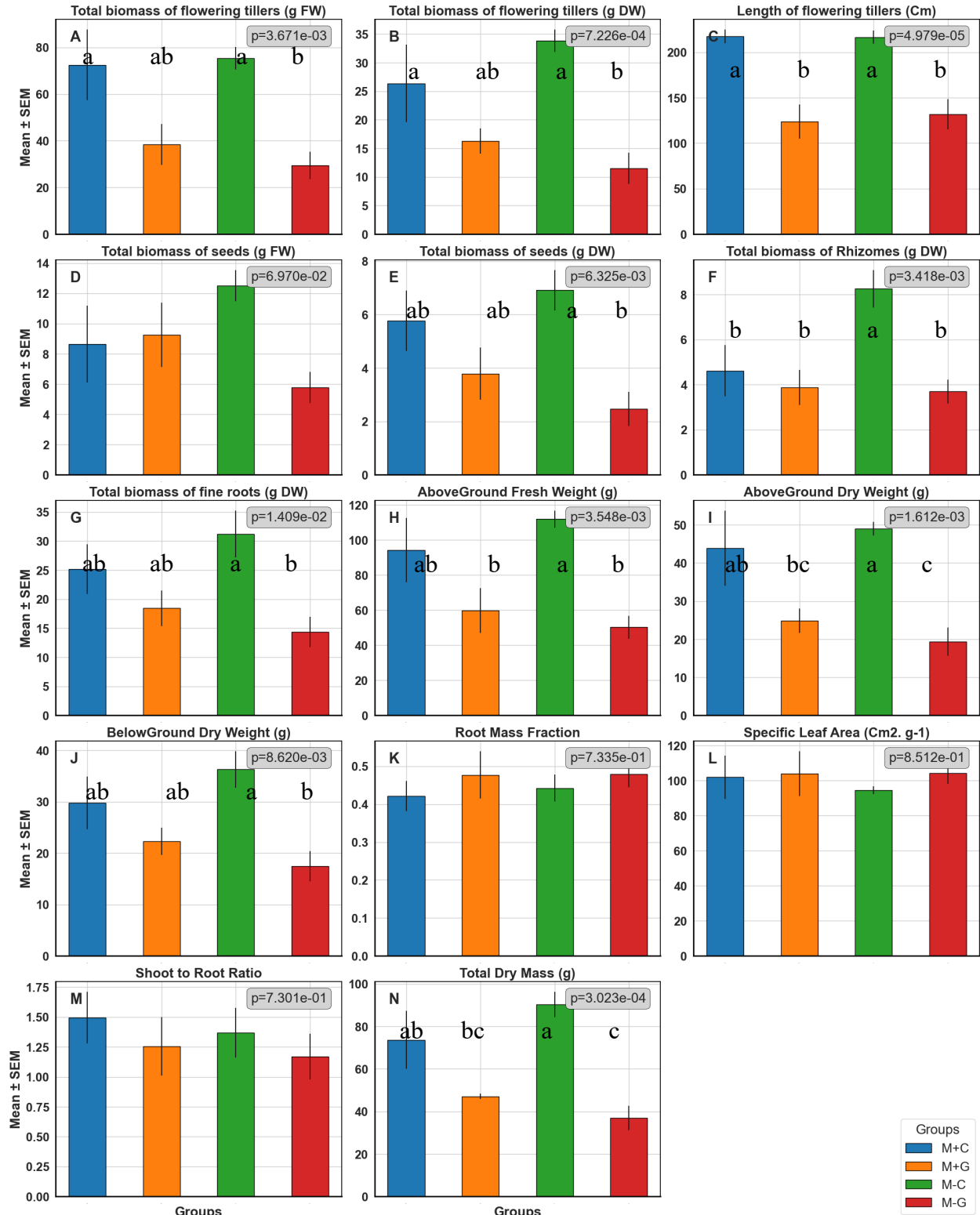


Figure 3. 2- Comparative Analysis of Biomass and Growth Traits in *A. gerardii* Plants Subject to Different Treatment Groups. The bar plots represent mean values with standard error (SEM) for various biomass and growth parameters measured across four groups: plants from the control treatment with and without the grazing memory (M+C and M-C, respectively), plants from the

clipping treatment with and without the memory of grazing (M+G and M-G, respectively). Statistical significance from ANOVA tests is annotated above each plot, with p-values indicating the level of significance between groups. Significant p-values (< 0.05) suggest substantial differences in means between different groups across the measured variables. The annotations of Tukey's Honestly Significant Difference (HSD) post-hoc test denoted by letters above each bar. Groups sharing the same letter are not significantly different, whereas distinct letters indicate a statistically significant difference in the mean values.

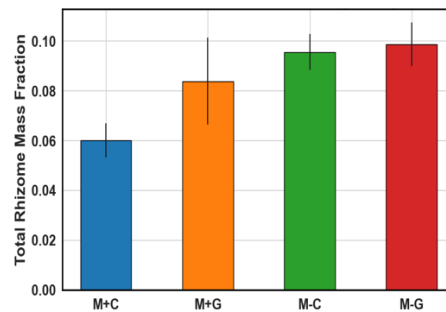


Figure 3. 3- Influence of Memory and Clipping Treatment on *A. gerardii* Rhizome Biomass Allocation. This bar chart illustrates the total rhizome mass fraction for *A. gerardii* under different treatment conditions at the season's end. Treatments include memory without clipping (M+C), memory with clipping (M+G), no memory without clipping (M-C), and no memory with clipping (M-G). Error bars represent standard errors. Marginally non-significant effect of memory on rhizome biomass allocation ($p = 0.055$).

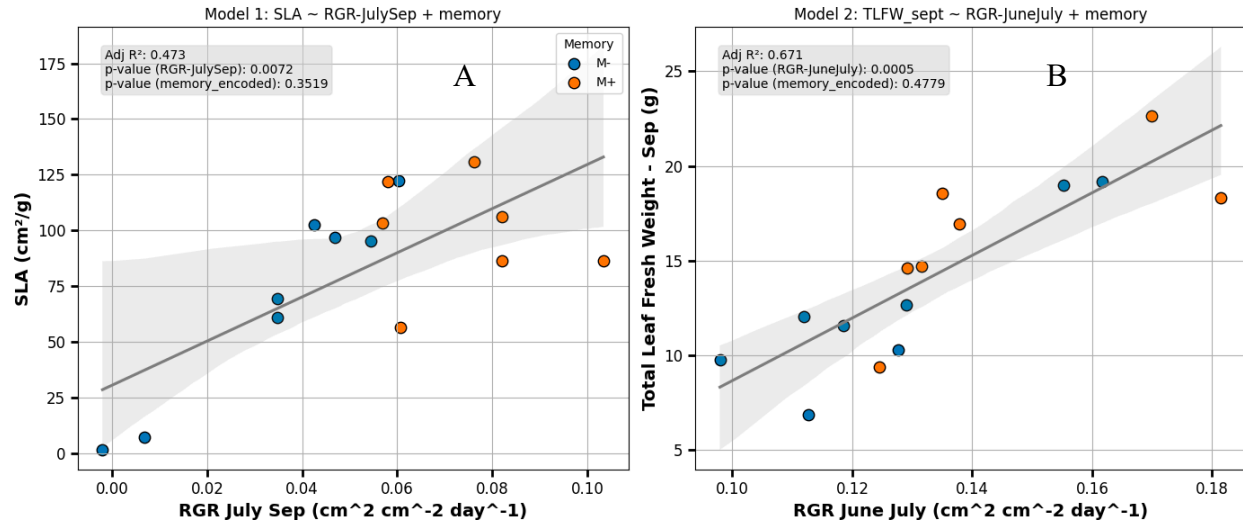


Figure 3. 4 - Relationship Between RGR and End-of-Season Biomass Variables in *A. gerardii*. The plots illustrate the regression analyses for total leaf fresh weight (TLFW) against RGR from the June-July interval (A) and specific leaf area (SLA) against RGR from the July-September interval (B), differentiated by groups with and without memory of grazing (M+ and M-). The significance of the RGR effect and memory is indicated by the p-values, with the adjusted R² value reflecting the strength of the model fit.

Table 3. 1- Two-way ANOVA assessing the effects of grazing memory, time interval, and their interaction on the Relative Growth Rate (RGR) of *A. gerardii*. The number of asterisks denotes the level of significance, with more asterisks indicating higher statistical significance ($p < 0.05$).

Predictors	Response Variable	PR(>F)
Memory	RGR	0.0027**
Time interval	RGR	3.31E-10***
Memory: Time interval	RGR	0.161

Table 3. 2- Tukey's HSD Test Results for RGR Comparisons Among Clipped *A. gerardii* Plants Across Different Grazing Memories and Growth Intervals.

group1	group2	Mean difference	p-adj	lower	upper	reject
M+JulySep	M+JuneJuly	0.0689	0	0.0384	0.0994	TRUE
M+JulySep	M-JulySep	-0.0394	0.0144	-0.0722	-0.0066	TRUE
M+JulySep	M-JuneJuly	0.0527	0.0004	0.0221	0.0832	TRUE
M+JuneJuly	M-JulySep	-0.1083	0	-0.1401	-0.0764	TRUE
M+JuneJuly	M-JuneJuly	-0.0162	0.444	-0.0457	0.0132	FALSE
M-JulySep	M-JuneJuly	0.092	0	0.0602	0.1239	TRUE

Table 3. 3- Impact of Grazing Memory on Biomass and Growth Traits in Clipped *A. gerardii* using Mixed effect models. For each variable, Memory was used as the main effect and sample as a random effect. The number of asterisks denotes the level of significance, with more asterisks indicating higher statistical significance ($p < 0.05$).

Variable	Effect of Memory	P-Value
TtillerFW	-13.697	0.000***
TtillerDW	-8.394	0.000***
FtillerL	6.267	0.053
TSeFW	-4.529	0.000***
TSeDW	-1.463	0.000***
TRhiDW	-0.293	0.543
FineRootDW	-4.906	0.058
RL	-5.180	0.499
AGFW	-9.510	0.238
AGDW	-10.131	0.000***
BGDW	-6.907	0.003**
RMF	0.062	0.091
SLA	-38.058	0.003**
StoR	-0.235	0.014*
TDM	-10.261	0.000***
TLA_summed	-215.577	0.157
TLFW_summed	-4.808	0.231
TLDW_summed	-1.409	0.209
TLA_sept	-146.037	0.000***
TLFW_sept	-3.778	0.000***
TLDW_sept	-0.773	0.206

Table 3. 4- ANOVA Analysis of Growth and Biomass Variables in Clipped and Control *A. gerardii* Plants Across Different Grazing Memory and Treatments. The number of asterisks denotes the level of significance, with more asterisks indicating higher statistical significance ($p < 0.05$).

	Variable		p-Value
1	TtillerFW	Total biomass of flowering tillers (g FW)	0.0037***
2	TtillerDW	Total biomass of flowering tillers (g DW)	0.0007***
3	FtillerL	Length of flowering tillers (Cm)	0.0001***
4	TSeFW	Total biomass of seeds (g FW)	0.0697
5	TSeDW	Total biomass of seeds (g DW)	0.0063**
6	TRhiDW	Total biomass of Rhizomes (g DW)	0.0034**
7	FineRootDW	Total biomass of fine roots (g DW)	0.0141*
8	AGFW	AboveGround Fresh Weight (g)	0.0035**
9	AGDW	AboveGround Dry Weight (g)	0.0016**
10	BGDW	BelowGround Dry Weight (g)	0.0086**
11	RMF	Root Mass Fraction	0.7335
12	SLA	Specific Leaf Area (Cm ² . g ⁻¹)	0.8512
13	StoR	Shoot to Root Ratio	0.7301
14	TDM	Total Dry Mass (g)	0.0003***

Table 3. 5- ANOVA Results for Derived Fractional Biomass Variables in *A. gerardii* Across Different Clipping Treatments and Memory Groups. The number of asterisks denotes the level of significance, with more asterisks indicating higher statistical significance ($p < 0.05$).

	Variable	memory p	Treatment p	Interaction p
1	TillerMassFraction	0.730	0.157	0.679
2	SeedMassFraction	0.375	0.443	0.834
3	LeafMassFraction	0.443	0.058*	0.801
4	RhizMassFraction	0.021**	0.211	0.332

Table 3. 6- Regression Model Analysis of RGR and Memory Effects on End-of-Season Biomass Variables in *A. gerardii*. The number of asterisks denotes the level of significance, with more asterisks indicating higher statistical significance ($p < 0.05$).

Model	Adj R2	p value RGR	p value memory
TLFW~RGR-June,July+memory	0.671	0.0005***	0.48
SLA~RGR-JulySep +memory	0.67	0.0004***	0.127

Chapter 4: Conclusion

Ecological memory, a term synonymous with the lasting effects of environmental stressors (Canarini et al., 2021; Ogle et al., 2015), plays a pivotal role in how plants like *Andropogon gerardii* respond and adapt to grazing (S. Chen et al., 2014; Y. Wang et al., 2022), a key disturbance in grassland ecosystems (Knapp et al., 2012; Knapp et al., 1999; Vinton et al., 1993). This memory is not just an archive of past interactions but an active influence that primes plants for future encounters with similar stresses (Galviz et al., 2022; Hilker et al., 2016). It encompasses a series of adaptive physiological and biochemical responses that are believed to enhance a plant's ability to cope with and recover from subsequent grazing pressures (Qu et al., 2022; Ren et al., 2024).

The objective of this research was to probe for the potential occurrence of the depth and breadth of this ecological memory's effects on *A. gerardii*. Ecological memory hypothesizes that the legacy of past grazing shapes the plant's current physiological state, influencing growth rates and biomass accumulation (chapter 2 and 3), photosynthetic efficiency, and the synthesis of defensive compounds (chapter 3). Through this lens, the thesis aimed to dissect the continuum of responses to grazing, from immediate reactions to long-term adaptations, and how these responses confer resilience in a competitive prairie ecosystem.

The findings from multiple chapters illuminates the complex interactions between time, physiological processes, and ecological memory. It reveals how these factors collectively influence grassland productivity and the resilience of prairie species, including *A. gerardii*.

Ecological Memory and Plant Stress Responses

The second significant finding is the nuanced role of ecological memory in plant resilience. The physiological and biochemical analyses revealed that while *A. gerardii* with a grazing history exhibited certain adaptive advantages in photosynthetic responses to stress, the presence of secondary metabolites, particularly flavonoids, was paramount in determining the plant's resilience and recovery. Flavonoids are crucial regulators of photosynthetic activity, stomatal conductance (Brunetti et al., 2019; Gai et al., 2020), and overall stress mitigation (Erb & Kliebenstein, 2020), illustrating that immediate biochemical responses play a critical role in a plant's ability to withstand and recover from defoliation stress (Agati et al., 2012).

Plant Growth Dynamics and Memory

The third major conclusion pertains to plant growth dynamics in response to defoliation. While plants with a history of grazing demonstrated a smaller decline in Relative Growth Rate (RGR), indicating some degree of inherent plasticity, the research concluded that intrinsic growth rates and immediate physiological responses to defoliation are more predictive of end-of-season biomass than the memory of past grazing events. This finding suggests that the coping mechanism in *A. gerardii* is primarily a result of immediate physiological adaptations rather than an evolved response influenced by historical grazing.

Implications for Grassland Management and Future Research

The implications of these findings are twofold. They challenge the assumed impact of ecological memory on plant resilience, recovery, and compensation (Agrawal, 2000; Galviz et al.,

2022; Zhang et al., 2023) and advocate for a more climate-centric approach in grassland management (Olimb & Robinson, 2019), emphasizing the need to understand and respond to the immediate and projected impacts of climate change on ecosystem productivity (Bachle & Nippert, 2022; De Boeck et al., 2018; Fay et al., 2008). Secondly, they highlight the potential for leveraging plant physiological traits, such as the production of flavonoids and the optimization of photosynthetic efficiency (Brunetti et al., 2019), as adaptive strategies to bolster plant resilience against environmental stressors.

Future research endeavors are encouraged to focus on unraveling the intricate molecular crosstalk between secondary metabolites and hormonal pathways within plants, with a special emphasis on how these interactions dictate plant adaptive responses to abiotic stresses, such as defoliation. An area of interest that has emerged from this thesis involves the role of flavonoids in regulating stomatal closure. Our findings contribute to an ongoing debate and suggest that higher levels of flavonoids are associated with lower intercellular CO₂ concentration (C_i), which could be indicative of increased stomatal closure. This aligns with the findings of Brunetti et al., (2019) and Gai et al., (2020), where the synthesis of flavonoids, potentially regulated by abscisic acid, ABA, was linked to enhanced stomatal closure. Contrary to Watkins et al., (2014), who proposed that a flavonoid deficiency leads to increased stomatal closure due to the accumulation of reactive oxygen species, our research supports the notion that an increase in flavonoid concentration can induce the same effect. This may reflect a complex biological feedback mechanism where flavonoids play a role in optimizing water use efficiency while maintaining photosynthetic capacity through regulated stomatal behavior (Brown et al., 2001; Erb & Kliebenstein, 2020; Pourcel et al., 2007). This apparent contradiction presents a compelling avenue for further investigation to clarify the relationship between flavonoids, ABA signaling, and stomatal

regulation. Understanding these pathways is essential for piecing together the broader picture of how plants manage water loss and gas exchange under stress conditions and could reveal novel insights into the enhancement of plant resilience through metabolic and hormonal modulation.

In conclusion, this thesis revealed that while ecological memory has a role in plant responses to grazing, its influence is less than that of immediate physiological reactions. This finding encourages a shift in focus towards understanding and managing the immediate biochemical responses to environmental changes for grassland conservation.

References

- Agati, G., Azzarello, E., Pollastri, S., & Tattini, M. (2012). Flavonoids as antioxidants in plants: Location and functional significance. *Plant Science: An International Journal of Experimental Plant Biology*, 196, 67–76. <https://doi.org/10.1016/j.plantsci.2012.07.014>
- Agrawal, A. (2000). Overcompensation of plants in response to herbivory and the by-product benefits of mutualism. *TRENDS IN PLANT SCIENCE*, 5(7), 309–313. [https://doi.org/10.1016/S1360-1385\(00\)01679-4](https://doi.org/10.1016/S1360-1385(00)01679-4)
- Bachle, S., & Nippert, J. B. (2022). Climate variability supersedes grazing to determine the anatomy and physiology of a dominant grassland species. *Oecologia*, 198(2), 345–355. <https://doi.org/10.1007/s00442-022-05106-x>
- Brown, D. E., Rashotte, A. M., Murphy, A. S., Normanly, J., Tague, B. W., Peer, W. A., Taiz, L., & Muday, G. K. (2001). Flavonoids Act as Negative Regulators of Auxin Transport in Vivo in Arabidopsis. *Plant Physiology*, 126(2), 524–535. <https://doi.org/10.1104/pp.126.2.524>
- Brunetti, C., Sebastiani, F., & Tattini, M. (2019). Review: ABA, flavonols, and the evolvability of land plants. *Plant Science*, 280, 448–454. <https://doi.org/10.1016/j.plantsci.2018.12.010>
- Canarini, A., Schmidt, H., Fuchslueger, L., Martin, V., Herbold, C. W., Zezula, D., Gündler, P., Hasibeder, R., Jecmenica, M., Bahn, M., & Richter, A. (2021). Ecological memory of recurrent drought modifies soil processes via changes in soil microbial community. *Nature Communications*, 12(1), Article 1. <https://doi.org/10.1038/s41467-021-25675-4>
- Chen, S., Cai, Y., Zhang, L., Yan, X., Cheng, L., Qi, D., Zhou, Q., Li, X., & Liu, G. (2014). Transcriptome analysis reveals common and distinct mechanisms for sheepgrass (*Leymus*

- chinensis*) responses to defoliation compared to mechanical wounding. *PloS One*, 9(2), e89495. <https://doi.org/10.1371/journal.pone.0089495>
- De Boeck, H. J., Hiltbrunner, E., Verlinden, M., Bassin, S., & Zeiter, M. (2018). Legacy Effects of Climate Extremes in Alpine Grassland. *Frontiers in Plant Science*, 9. <https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2018.01586>
- Erb, M., & Kliebenstein, D. J. (2020). Plant Secondary Metabolites as Defenses, Regulators, and Primary Metabolites: The Blurred Functional Trichotomy. *Plant Physiology*, 184(1), 39–52. <https://doi.org/10.1104/pp.20.00433>
- Fay, P. A., Kaufman, D. M., Nippert, J. B., Carlisle, J. D., & Harper, C. W. (2008). Changes in grassland ecosystem function due to extreme rainfall events: Implications for responses to climate change. *Global Change Biology*, 14(7), 1600–1608.
- Gai, Z., Wang, Y., Ding, Y., Qian, W., Qiu, C., Xie, H., Sun, L., Jiang, Z., Ma, Q., Wang, L., & Ding, Z. (2020). Exogenous abscisic acid induces the lipid and flavonoid metabolism of tea plants under drought stress. *Scientific Reports*, 10(1), Article 1. <https://doi.org/10.1038/s41598-020-69080-1>
- Galviz, Y., Souza, G. M., & Lüttge, U. (2022). The biological concept of stress revisited: Relations of stress and memory of plants as a matter of space–time. *Theoretical and Experimental Plant Physiology*, 34(2), 239–264. <https://doi.org/10.1007/s40626-022-00245-1>
- Hilker, M., Schwachtje, J., Baier, M., Balazadeh, S., Bäurle, I., Geiselhardt, S., Hinch, D. K., Kunze, R., Mueller-Roeber, B., Rillig, M. C., Rolff, J., Romeis, T., Schmölling, T., Steppuhn, A., van Dongen, J., Whitcomb, S. J., Wurst, S., Zuther, E., & Kopka, J. (2016).

- Priming and memory of stress responses in organisms lacking a nervous system. *Biological Reviews*, 91(4), 1118–1133. <https://doi.org/10.1111/brv.12215>
- Knapp, A., Hoover, D. L., Blair, J., Buis, G., Burkepile, D. E., Chamberlain, A. J., Collins, S., Fynn, R. W. S., Kirkman, K. P., Smith, M. D., Blake, D., Govender, N., O’Neal, P., Schreck, T., & Zinn, A. (2012). A test of two mechanisms proposed to optimize grassland aboveground primary productivity in response to grazing. *Journal of Plant Ecology*, 5, 357–365. <https://doi.org/10.1093/jpe/rts020>
- Knapp, A. K., Blair, J. M., Briggs, J. M., Collins, S. L., Hartnett, D. C., Johnson, L. C., & Towne, E. G. (1999). The Keystone Role of Bison in North American Tallgrass Prairie: Bison increase habitat heterogeneity and alter a broad array of plant, community, and ecosystem processes. *BioScience*, 49(1), 39–50. <https://doi.org/10.1525/bisi.1999.49.1.39>
- Ogle, K., Barber, J. J., Barron-Gafford, G. A., Bentley, L. P., Young, J. M., Huxman, T. E., Loik, M. E., & Tissue, D. T. (2015). Quantifying ecological memory in plant and ecosystem processes. *Ecology Letters*, 18(3), 221–235. <https://doi.org/10.1111/ele.12399>
- Olimb, S. K., & Robinson, B. (2019). Grass to grain: Probabilistic modeling of agricultural conversion in the North American Great Plains. *Ecological Indicators*, 102, 237–245. <https://doi.org/10.1016/j.ecolind.2019.02.042>
- Pourcel, L., Routaboul, J.-M., Cheynier, V., Lepiniec, L., & Debeaujon, I. (2007). Flavonoid oxidation in plants: From biochemical properties to physiological functions. *Trends in Plant Science*, 12(1), 29–36. <https://doi.org/10.1016/j.tplants.2006.11.006>
- Qu, K., Cheng, Y., Gao, K., Ren, W., Fry, E. L., Yin, J., & Liu, Y. (2022). Growth-Defense Trade-Offs Induced by Long-term Overgrazing Could Act as a Stress Memory. *Frontiers in Plant Science*, 13. <https://doi.org/10.3389/fpls.2022.917354>

- Ren, H., Xie, J., Ke, Y., Yu, Q., Lkhagva, A., & Liu, M. (2024). Moderate grazing weakens legacy effects of grazing history on primary productivity and biodiversity in a meadow grassland. *Agriculture, Ecosystems & Environment*, 362, 108825. <https://doi.org/10.1016/j.agee.2023.108825>
- Vinton, M. A., Hartnett, D. C., Finck, E. J., & Briggs, J. M. (1993). The interactive effects of fire, bison (*Bison bison*) grazing and plant community composition in tallgrass prairie. *American Midland Naturalist*, 129, 10–18. <https://doi.org/10.2307/2426430>
- Wang, Y., Zhu, W., Ren, F., Zhao, N., Xu, S., & Sun, P. (2022). Transcriptional Memory in *Taraxacum mongolicum* in Response to Long-Term Different Grazing Intensities. *Plants*, 11(17), Article 17. <https://doi.org/10.3390/plants11172251>
- Watkins, J. M., Hechler, P. J., & Muday, G. K. (2014). Ethylene-Induced Flavonol Accumulation in Guard Cells Suppresses Reactive Oxygen Species and Moderates Stomatal Aperture. *Plant Physiology*, 164(4), 1707–1717. <https://doi.org/10.1104/pp.113.233528>
- Zhang, F., Zheng, J., Bennett, J. A., Zhang, B., Wang, Z., Li, Z., Li, H., Wang, J., Han, G., & Zhao, M. (2023). The legacy effects of grazing and precipitation variability on grassland productivity in a temperate steppe. *Agricultural and Forest Meteorology*, 343, 109788. <https://doi.org/10.1016/j.agrformet.2023.109788>