THE EFFECT OF CLIMATE CHANGE ON ABOVE AND BELOWGROUND PLANT-HERBIVORE INTERACTIONS

By
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To my parents for teaching me that with hard work and persistence I could do so much with so little. To my sisters and husband for their unconditional love and support.
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>4</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>8</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>9</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>10</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>11</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>1  INTRODUCTION</td>
<td>13</td>
</tr>
<tr>
<td>Scope of the Dissertation</td>
<td>15</td>
</tr>
<tr>
<td>2  IS THERE A TEMPERATE BIAS IN OUR UNDERSTANDING OF HOW CLIMATE CHANGE WILL ALTER PLANT-HERBIVORE INTERACTIONS? A META-ANALYSIS OF EXPERIMENTAL STUDIES</td>
<td>17</td>
</tr>
<tr>
<td>Overview</td>
<td>17</td>
</tr>
<tr>
<td>Background</td>
<td>18</td>
</tr>
<tr>
<td>Methods</td>
<td>23</td>
</tr>
<tr>
<td>Meta-analysis: Data Collection</td>
<td>23</td>
</tr>
<tr>
<td>Meta-Analysis: Statistical Analyses and Tests for Publication Bias</td>
<td>25</td>
</tr>
<tr>
<td>Results</td>
<td>26</td>
</tr>
<tr>
<td>What Components of Climate Change and Plant-Herbivore Interactions Are Being Investigated?</td>
<td>27</td>
</tr>
<tr>
<td>Effect of Climate Change on Plant Growth, Nutritional Quality, Defenses and Interactions</td>
<td>28</td>
</tr>
<tr>
<td>Support for Predictions: Effects on Tropical vs. Temperate Plant Species</td>
<td>29</td>
</tr>
<tr>
<td>Effects of experimental climate change belowground</td>
<td>30</td>
</tr>
<tr>
<td>Discussion</td>
<td>30</td>
</tr>
<tr>
<td>Concluding Remarks</td>
<td>35</td>
</tr>
<tr>
<td>3  A WHOLE-PLANT PERSPECTIVE REVEALS UNEXPECTED IMPACTS OF ABOVE AND BELOWGROUND HERBIVORES ON PLANT GROWTH AND DEFENSE</td>
<td>46</td>
</tr>
<tr>
<td>Overview</td>
<td>46</td>
</tr>
<tr>
<td>Background</td>
<td>46</td>
</tr>
<tr>
<td>Methods</td>
<td>49</td>
</tr>
<tr>
<td>Results</td>
<td>54</td>
</tr>
</tbody>
</table>
PLANT RESPONSES TO ISOLATED AND SIMULTANEOUS HERBIVORY AND PRECIPITATION VARIATION REVEALS THE COMPLEXITY OF PLANT-HERBIVORE INTERACTIONS IN CHANGING ENVIRONMENTS

Overview ............................................................................................................................................... 67
Background ............................................................................................................................................. 68
Methods .................................................................................................................................................. 71
Study System .......................................................................................................................................... 71
Experimental Design .............................................................................................................................. 72
Statistical Analyses ............................................................................................................................... 75
Results .................................................................................................................................................... 76
Survival ................................................................................................................................................... 76
Growth ..................................................................................................................................................... 77
Defense ................................................................................................................................................... 78
Discussion ............................................................................................................................................... 79

Decreased Water Drastically Reduces Seedling Survivorship; This Is Further Exacerbated by Belowground Herbivory ................................................................. 80
Herbivory Has a Greater Effect on Plant Growth Responses than Changes in Precipitation; However, Responses to AG and BG Herbivores Were Highly Idiosyncratic ........................................................................................................................................ 81
While Precipitation and Herbivory Can Significantly Alter Plant Defensive Chemistry, These Changes Are Not Consistent in Direction or Magnitude .......................................................................................................................... 82
The Impacts of Belowground Herbivores Are Unique and Context-Dependent ................................................................................................................................. 83
Plant-Herbivore Interactions in a Changing World ............................................................................... 84
Concluding Remarks ............................................................................................................................ 85

CONCLUSIONS ..................................................................................................................................... 95

APPENDIX

A WATER TREATMENTS EXPLANATION FOR CHAPTER 4 .................................................................. 96

LIST OF REFERENCES ............................................................................................................................ 98

BIOGRAPHICAL SKETCH ..................................................................................................................... 116
<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-1</td>
<td>Spearman’s rank correlations between effect sizes ($d$) and sample sizes</td>
<td>38</td>
</tr>
<tr>
<td>2-2</td>
<td>Heterogeneity ($Q_b$) for the overall plant species between CO$_2$ and the other climate change factors</td>
<td>39</td>
</tr>
<tr>
<td>3-1</td>
<td>Measurements of the aboveground (AG) and belowground (BG) plant parameters</td>
<td>61</td>
</tr>
<tr>
<td>3-2</td>
<td>Results of the analyses from linear mixed models for the effects of the herbivory treatments on growth and terpenoid concentration</td>
<td>62</td>
</tr>
<tr>
<td>3-3</td>
<td>Abundance of <em>Meloidogyne incognita</em> eggs, J2 juveniles, and females in root egg masses of different sizes</td>
<td>63</td>
</tr>
<tr>
<td>4-1</td>
<td>Monthly average precipitation (mm) and temperature ($^\circ$C) for the past 10 years (2004-2013) and for the duration of the experiment (September 2013 to July 2014)</td>
<td>87</td>
</tr>
<tr>
<td>4-2</td>
<td><em>Solanum lycocarpum</em> general growth responses to water and herbivory treatments from linear mixed models</td>
<td>88</td>
</tr>
<tr>
<td>4-3</td>
<td>Measurements of the stem, leaf and root parameters after 300 days of experimentation</td>
<td>89</td>
</tr>
<tr>
<td>4-4</td>
<td>Biomass (g dw) and terpenoid concentration (µmol/g dw) responses to water, herbivory and the interactive treatments from linear mixed models</td>
<td>91</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-1</td>
<td>Framework for predicted trends and results of how climate change will affect plants and plant-herbivore interactions</td>
</tr>
<tr>
<td>2-2</td>
<td>Funnel plots of effect sizes and sample sizes</td>
</tr>
<tr>
<td>2-3</td>
<td>Climate change effects on plant and plant-herbivore interactions</td>
</tr>
<tr>
<td>2-4</td>
<td>Influence of climate change on plant traits for different groups of species</td>
</tr>
<tr>
<td>2-5</td>
<td>Influence of climate change on plant-herbivore interactions for different groups of plant species</td>
</tr>
<tr>
<td>3-1</td>
<td>Results of experimental treatments showing A) Relative growth rate of leaf area, B) relative growth rate of total roots length, C) terpenoid concentration in leaves and D) terpenoid concentration in roots</td>
</tr>
<tr>
<td>3-2</td>
<td>Whole-plant terpenoid concentration of n=6 seedlings harvested at the start of herbivory treatments</td>
</tr>
<tr>
<td>3-3</td>
<td>Results of experimental treatments showing <em>Solanum lycocarpum</em> A) Total plant biomass and B) Root:Shoot ratio in the three different herbivory treatments</td>
</tr>
<tr>
<td>4-1</td>
<td>Effects of water treatments on the survivorship of <em>Solanum lycocarpum</em> seedlings</td>
</tr>
<tr>
<td>4-2</td>
<td><em>Solanum lycocarpum</em> seedlings Total Biomass (g dw) and Root:Leaf Biomass Ratio for plants exposed to (A and D) decreased water, (B and E) average water level and (C and F) increased water and also to aboveground herbivory (AG), belowground herbivory (BG), simultaneous herbivory (AGBG) and no-herbivory controls</td>
</tr>
<tr>
<td>4-3</td>
<td>Plant Total Terpenoid concentration (µmol/g dw) and Root:Leaf Terpenoid Ratio when plants were exposed to (A and D) decreased water, (B and E) average water level and (C and F) increased water and also to aboveground herbivory (AG), belowground herbivory (BG), simultaneous herbivory (AGBG) and no-herbivory controls</td>
</tr>
</tbody>
</table>
## LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AG</td>
<td>Aboveground</td>
</tr>
<tr>
<td>BG</td>
<td>Belowground</td>
</tr>
<tr>
<td>IPCC</td>
<td>Intergovernmental Panel on Climate Change</td>
</tr>
<tr>
<td>UFU</td>
<td>Universidade Federal de Uberlândia</td>
</tr>
<tr>
<td>RGR</td>
<td>Relative Growth Rate</td>
</tr>
</tbody>
</table>
Most ecological studies of plant-herbivore interactions to date have explored aboveground (AG) interactions. In doing so, they overlook a diverse fauna of belowground (BG) herbivores and their far-reaching impacts in both natural and agricultural systems. The identification of patterns and generalizations that explain AG and BG interactions has been challenging. A previously proposed conceptual model provides a framework for AG-BG interactions, but its generality has been questioned due to the lack of attention to other factors that can determine the outcome of the interaction such as the herbivore type and the abiotic variations such as nutrient availability, soil moisture and precipitation. Considering that in the coming decades, a major form of abiotic variation will come in the form of anthropogenic climate change, understanding the link between AG and BG communities requires experiments that test the model considering the type of herbivory in both increasing and decreasing precipitation scenarios. This study was conducted in the Neotropical savannas known as the Cerrado, a biodiversity hotspot with a rich community of above and belowground herbivores and for which climate change models predict major shifts in precipitation in
the coming century. I used shadehouse experiments as well as broad-scale inventory data to explore how the range of predicted changes in precipitation influences above and belowground plant herbivore interactions. First I provide a context and motivation for this study about AG and BG plant-herbivore interaction and climate change. Then I present a review of the existing experimental studies about how climate change factors will influence tropical and temperate plant-herbivore interactions. To understand how AG and BG herbivory changes the plant growth rates and defenses, I did an experiment challenging seedlings of the tropical shrub *Solanum lycocarpum*. Finally, I determine how biotic (herbivory type) and abiotic (precipitation variation) factors affect plant survival, growth and defense investment, by also experimenting with seedlings in a shadehouse.
CHAPTER 1
INTRODUCTION

Herbivory directly and indirectly affects plant fitness (Karban and Strauss 1993, Trlica and Rittenhouse 1993). Consequently, plants have evolved a diverse array of defensive adaptations to prevent herbivores from feeding, some of which are always present (i.e., constitutive responses) while others are augmented only after the plant is attacked (i.e., induced responses) (Karban and Baldwin 1997). The availability of resources and other abiotic factors in locations where plants are growing can influence these defenses and their allocation (Coley et al. 1985, Kursar and Coley 2003, Fine et al. 2004); ultimately this can translate into strong direct and indirect effects on herbivore fitness, host choice, and rates of attack (Diamond and Kingsolver 2010).

Most ecological studies of plant-herbivore interactions to date have explored aboveground (AG) interactions (e.g., Price et al. 1980, Karban and Strauss 1993, Simms and Triplett 1994, Karban and Baldwin 1997, Fine et al. 2004). In doing so, they overlook a diverse fauna of belowground (BG) herbivores and their far-reaching impacts in both natural and agricultural systems (Bardgett and Wardle 2010, Rasmann et al. 2011a). As is the case with AG herbivores, BG herbivores can have strong and negative impacts on plant fitness (Rasmann et al. 2011a) and primary production (Stanton 1988), reduce a species’ competitive ability (Bardgett and Wardle 2010), and ultimately alter vegetation dynamics and community composition (Bardgett and Wardle 2010). Similarly, belowground interactions between plants and herbivores can also be influenced by changes in abiotic conditions. For example, the abundance and species richness of root-feeders is reduced in low moisture and nutrient-poor environments (Johnson et al. 2011).
Above and belowground plant-herbivore interactions has been suggested to have important effects on the structure and functioning of terrestrial ecosystems (Bardgett and Wardle 2010, Erb et al. 2011, Johnson et al. 2011). Despite some studies addressing AG and BG interactions, the identification of patterns and generalizations that explain these interactions has so far proved challenging. Masters et al. (1993) proposed a conceptual model suggesting a contramensalism between AG and BG interactions, favoring AG herbivores over BG herbivores. While the model provides an approach for AG-BG interactions, its generality has been questioned due to other factors that can determine the outcome of the interaction, such as (i) the sequence of herbivore arrival (Erb et al. 2011), (ii) the herbivore type (Kaplan et al. 2008, Hopkins et al. 2009, Erb et al. 2011), and (iii) the abiotic variations such as nutrient availability (Engelkes et al. 2008, Bardgett and Wardle 2010), soil moisture and precipitation (Bardgett and Wardle 2010, Erb et al. 2011, Johnson et al. 2011). For instance, decreases in precipitation and soil moisture can alter plant defenses and the survival, performance, and diversity of AG and BG herbivores (White 1984, Koricheva et al. 1998, Huberty and Denno 2004, Johnson et al. 2011), which is perhaps why most studies to date have focused on drought-related responses (Koricheva et al. 1998, Bardgett and Wardle 2010). However, understanding the link between AG and BG communities requires experiments that test the model considering the herbivory type in both increasing and decreasing precipitation scenarios. Failing to do so ignores a wide range of potential interactions between AG and BG communities and their consequences for plant-herbivore interactions (Johnson et al. 2011).
In the coming decades, a major form of abiotic variation will come in the form of anthropogenic climate change. Current predictions suggest there will be elevated concentrations of CO$_2$ and O$_3$, increased temperatures, and greater spatial variation in precipitation (Parmesan 2006, Christensen et al. 2007). Not surprisingly, these changes are predicted to have important consequences for AG and BG plant-herbivore interactions, as well as for the link between these two subsystems (Bidart-Bouzat et al. 2005, Bidart-Bouzat and Imeh-Nathaniel 2008, Bardgett and Wardle 2010). For instance, elevated CO$_2$ and O$_3$ can increase chemical defenses and modify plant-herbivore relationships, though the magnitude and direction of these changes depend on the ecosystem and host plant (Kuokkanen et al. 2001, Coviella et al. 2002, Bidart-Bouzat et al. 2005, Bidart-Bouzat and Imeh-Nathaniel 2008). Similarly, increased temperature can influence plant growth and physiology (Shi et al. 2010) and change the distribution of AG and BG herbivores (Hunter 1992, VanDongen et al. 1997). However, despite the fact that changes in precipitation are predicted to be among the most widespread consequences of changing climate, surprisingly few studies have simultaneously explored the effects of altered precipitation on plants, herbivores, and AG/BG linkages – an essential step in understanding of how ongoing global change phenomena influence plant-herbivore interactions.

**Scope of the Dissertation**

Regardless of the growing literature on the interaction of AG-BG plant-herbivore systems, underlying patterns have been difficult to identify perhaps due to the lack of consideration of some important factors. Thus, the overall objective of this dissertation is to understand how the range of predicted changes in precipitation influences above
and belowground plant-herbivore interactions. First, I review the existing experimental studies about how climate change factors (CO₂, O₃, Temperature and precipitation) will influence tropical and temperate AG and BG plant-herbivore interactions. Then, in the following two chapters I did a series of experiments with seedlings of the tropical shrub *Solanum lycocarpum* in shadehouse. In chapter 2, I experimentally manipulated AG and BG herbivory to address (i) if AG herbivory result in the expression of BG defenses or vice-versa, (ii) the patterns of root growth and defense following BG herbivory are similar in leaves following AG herbivory, and finally (iii) if AG and BG herbivory result in similar patterns of plant growth and biomass allocation. In the chapter 3, I experimentally manipulated herbivory type and precipitation variation to address (i) how changes in precipitation patterns affect plant survival, root and leaf growth and defenses, (ii) if simultaneous effects of precipitation variation and above and belowground herbivory on whole-plant survival, growth and defense are antagonistic, synergistic or additive, (iii) if differences in growth and defenses between leaves and roots can be detected in response to simultaneous precipitation variation and herbivory, suggesting specificity in plant-part responses. Together, these Chapters address and evaluate the whole plant survival, defense and growth responses to both biotic and abiotic changes due to climate change.
CHAPTER 2
IS THERE A TEMPERATE BIAS IN OUR UNDERSTANDING OF HOW CLIMATE CHANGE WILL ALTER PLANT-HERBIVORE INTERACTIONS? A META-ANALYSIS OF EXPERIMENTAL STUDIES

Overview

Climate change can drive major shifts in community composition and interactions between resident species. However, the magnitude of these changes depends on the type of interactions and the biome in which they take place. We review the existing conceptual framework for how climate change will influence tropical plant-herbivore interactions and formalize a similar framework for the temperate zone. We then conduct the first biome-specific tests of how plant-herbivore interactions change in response to climate-driven changes in temperature, precipitation, ambient CO$_2$, and ozone. We used quantitative meta-analysis to compare predicted and observed changes in experimental studies. Empirical studies were heavily biased towards temperate systems, so testing predicted changes in tropical plant-herbivore interactions was virtually impossible. Furthermore, most studies investigated the effects of CO$_2$ with limited plant and herbivore species. Irrespective of location, most studies manipulated only one climate change factor, despite that different factors can act in synergy to alter responses of plants and herbivores. Finally, studies on belowground plant-herbivore interactions were also rare; those conducted suggest climate change could have major impacts on belowground subsystems. Our results suggest there is a disconnection between the growing literature proposing how climate change will influence plant-herbivore interactions and the studies testing these predictions. General conclusions will also be hampered without better integration of above- and belowground systems, assessing the
impacts of multiple climate change factors simultaneously, and using greater diversity of species in experiments.

**Background**

Understanding the ecological impacts of climate change is an increasingly important area of theoretical and empirical research (Gilman et al. 2010, Wu et al. 2011). Much recent work on climate change effects has focused on changes in species distributions (Pauli et al. 2012, Pateman et al. 2012, Amissah et al. 2014, Sheppard et al. 2014), the loss of biodiversity (Bellard et al. 2012), changes in plant phenology (Wolkovich et al. 2013) and temporal mismatches between interacting species (Singer and Parmesan 1993, Durant et al. 2007, Kudo and Ida 2013). These effects have been observed over a relatively short time scale (IPCC 2014) and it is thought they could ultimately lead to drastic changes in interspecific interactions with community-level consequences (van der Putten et al. 2004, Tylianakis et al. 2008).

Herbivores and plants comprise the majority of terrestrial biodiversity. Their complex interactions can influence population and community dynamics (Lurgi et al. 2012) and have major effects on agricultural systems (Oerke 2006), making them central to the responses of terrestrial systems to climate change (Mulder et al. 1999). The highest diversity of herbivore species and feeding types is found in insects (Class Insecta) (Speight et al. 2008), which evidence to date suggests are extremely sensitive to changing climatic conditions (Bale et al. 2002). This is because there are multiple pathways by which climate change can exert effects — for example, directly via climate-driven changes in physiology and behavior or indirectly by changes in host plant characteristics or abundance (Bale et al. 2002). Understanding how plant-herbivore
interactions respond to changing climates is therefore critical to improving predictions for how climate change effects ecological and agricultural systems.

A rich literature has documented the diversity of ways with which plants interact with herbivores aboveground (e.g., leaf-feeders) and belowground (e.g., root-feeders). Aboveground (AG) and belowground (BG) herbivores can influence each other, their host-plants, and local communities and ecosystem properties (van der Putten et al. 2009). For example, BG herbivores can cause changes in leaf physiology, thereby affecting AG attackers and their predators (van Dam et al. 2005, Soler et al. 2005, Rasmann and Turlings 2007, Erb et al. 2011). The opposite is also true -- AG herbivores can drive changes in root physiology and resistance (Masters 1995, Soler et al. 2005, Kaplan et al. 2008, Erb et al. 2011). Finally, recent research has revealed unexpected ways plants themselves mediate interactions between belowground and aboveground herbivores (Bardgett and Wardle 2003, Wardle et al. 2004). Although there is mounting evidence that global climate change can alter AG interactions, how it influences BG interactions and the link between AG-BG subsystems has rarely been explored.

Because the distribution of organisms is geographically heterogeneous (Cramer et al. 2001, Walther et al. 2002), the magnitude of plant and herbivore responses to climate change and the magnitude of these effects might depend in large part on the biome in which their interactions take place (Walther et al. 2002). Although there is regional variability in model predictions, generally global increases in CO₂ will positively affect forest productivity (net primary production) and shift the species composition in the temperate zone (Reich and Frelich 2002, Reich et al. 2006, IPCC 2014). In the
tropical zone increases in CO$_2$ will increase net biomass, but could also cause higher fire incidence that can positively affect mortality and forest turnover (Cramer et al. 2004, Malhi and Phillips 2004, Phillips et al. 2004). Trees in the tropics are likely to be negatively affected by increases in temperature (Clark et al. 2003, 2010, Feeley et al. 2007, Way and Oren 2010) and may have limited ability to alter traits in response (Drake et al. 2015). In contrast, temperate trees will grow in response to warming (Carter 1996, Rehfeldt et al. 2002), and likely have greater flexibility to alter physiological and structural traits (Drake et al. 2015). Understanding how the effects of climate change influence plant-herbivore interactions across latitudes is therefore critical to predicting species responses.

Predictions about the outcome of plant-herbivore interactions under climate change have been based primarily on expected changes in plant nutritional content. Following Bryant’s (1983) hypothesized “carbon-nutrient balance”, Coley (1998) outlined a framework for how climate change should influence plant-herbivore interactions in the tropics. She argued that the increase in atmospheric CO$_2$ would result in increased mean carbon storage in soil organic matter (Schimel et al. 1994), ultimately leading to higher carbon absorption and lower concentrations of leaf nitrogen, i.e., nitrogen dilution (Fajer 1989, Coley 1998). Consequently, tropical plants would shift to having leaves with reduced nutritional quality (Fajer 1989, Coley 1998), reduced concentrations of nitrogen-based defenses, and elevated carbon-based defenses (Coley 1998). Herbivores would increase their per capita consumption rates due in part to increased CO$_2$ and the effects of reduced precipitation, ultimately leading to lower plant growth rates (Coley 1998).
Despite being almost twenty years since Coley put this comprehensive framework forward, a similar one for temperate plant-herbivore interactions has yet to emerge. Here we draw on prior empirical and theoretical work to develop a series of predictions for how climate change will influence plants, herbivores, and their interactions in temperate regions comparable to those put forward by Coley for the tropics. The complete framework can be found in Figure 2-1, but we briefly summarize it here. We hypothesize that if the climate in temperate regions changes as predicted (IPCC 2014), plants will have elevated levels of carbon-based defenses (Bryant et al. 1987, Gebauer et al. 1998, Bidart-Bouzat and Imeh-Nathaniel 2008). This will decrease leaf nutritional quality via the nitrogen dilution effect (Bryant et al. 1983). If as predicted increases in atmospheric CO$_2$ enhance reproduction and the development of herbivores, then herbivory in the temperate zone should increase with elevated CO$_2$ (Lincoln et al. 1993, Bezemer and Jones 1998). Drought would negatively affect plants by impeding the conductance or absorption of nutrients, thus decreasing plant growth and nutritional quality (Mattson and Haack 1987, Bartels and Sunkar 2005, Jactel et al. 2012, He and Dijkstra 2014). As growth declines, moderate drought will result in greater investment in physical and chemical N- and C-based defenses due to the increased levels of available carbon and nitrogen (Mattson and Haack 1987, Jactel et al. 2012). Thus, drought-stressed plants should be more attractive or acceptable to insects, causing herbivory to increase (Mattson and Haack 1987, Jactel et al. 2012). Because many plant and insect physiological processes are temperature-dependent, an increase in temperature should stimulate plant growth, leaf nutritional quality, and ultimately herbivory (Bale et al. 2002, Bidart-Bouzat and Imeh-Nathaniel 2008, Wu et al. 2011).
However, the effect of temperature on plant defenses will vary depending on the type of chemical produced (Bidart-Bouzat and Imeh-Nathaniel 2008). For example, N-containing compounds and some volatile organic compounds will be enhanced by increasing temperature (Sallas et al. 2003, Loreto et al. 2006) while some phenolic compounds will decrease (Kuokkanen et al. 2001). Ozone (O$_3$) causes stress in plant cells that results in decreased respiration and plant growth (Grantz et al. 2006, Lindroth 2010); it also reduces absorption and allocation of nutrients (Lindroth 2010). These physiological changes stimulate the induction of metabolic pathways that cause an increase in carbon-based defenses, resulting in decreased rates of herbivory (Sandermann 1996, Bidart-Bouzat and Imeh-Nathaniel 2008, Lindroth 2010).

Here we conduct the first biome-specific tests of predicted changes in plant-herbivore interactions resulting from climate change. Previous reviews of herbivory and climate have mostly been qualitative, focused on temperate species, and investigated the effects of rising CO$_2$ levels. We take a step forward by using a meta-analysis of experimental studies, for which we reviewed over 5000 published articles, to quantify the responses of tropical and temperate plant species, their above- and belowground subsystems, and both natural and agricultural systems. We then used data on the magnitude of the effects observed, variation in sample sizes, and the statistical power of individual studies to address the following questions: (1) How do different components of plant-herbivore interactions respond to experimental manipulation of climate change? (2) Do these patterns differ between tropical and temperate systems? (3) How do AG and BG plant-herbivore subsystems differ in their responses to manipulations of climate change variables?
Methods

Meta-analysis: Data Collection

We began by searching for articles in the Web of Science (ISI) on April 2014. We compiled all articles published between 1900 to 2013 that resulted from searches using the terms “climate change or global change”, “plant”, “herbivor* or insect”, “interaction”, “aboveground”, “belowground”, “response*” and “defense*” in different combinations. Data from book chapters, graduate theses and unpublished data were not included in our analyses.

For one of the resulting studies to be included in our meta-analysis it had to meet three criteria. First, the studies had to have both control and treatment levels (i.e., ambient conditions vs. experimental increases or decreases in a climatic variable). Second, studies had to be based on unique data sets; when studies by the same author(s) used subsets of the same dataset in separate papers only the most comprehensive study was included. Third, studies had to provide sufficient statistical information to allow the calculation of effect sizes. We calculated the effect sizes using means, sample sizes, standard errors or standard deviations extracted from control and experimental groups. For several publications there were discrepancies in the sample sizes reported in the methods, figures and tables; to standardize we used the ones reported in the methods. Since a large proportion of the studies presented the data graphically, we obtained the numeric values for responses directly from the figures.

We then used the statistical software MetaWin 2.1 to calculate effect sizes (ES) (Rosenberg et al. 2007) in each study. We calculate the effect sizes of each climate change factor only when a focal species had at least 3 measurements. These
measurements could be from the same study but with different species or from different studies with the same species. For studies where multiple measurements were recorded (e.g., monthly, yearly) only the first or last measurements were used to calculate effect sizes. The measurement has to have the most complete statistical information.

We assigned the articles to categories for analysis based on the climate change factors manipulated, plant species studied, subsystem in which the experiment and measurements took place, and plant response measured. The climate factors considered were levels of carbon dioxide (CO$_2$) and ozone (O$_3$), air temperature (T), and precipitation, both increases (iH$_2$O) and decreases (dH$_2$O). Plant species were divided into four groups: (1) crops, i.e., all species produced in agricultural systems; (2) grasses, i.e., all grass species independent of their geographic origin; (3) temperate species, i.e., all species from temperate region; and tropical, species, all species from the tropical zone. The two subsystems considered were AG – any insect feeding on leaves, with measurements of aboveground plant responses to herbivory and BG – nematodes or insects feeding on roots, and measurements of belowground plant responses. Plant responses measured were final AG or BG biomass, plant nutrients (i.e., nitrogen, carbohydrate, and sugar content, C:N ratio), and plant defenses (i.e., carbon-based defenses, nitrogen-based defenses, physical defenses). Finally, we used the change in herbivore body mass and rate of plant consumption in each climate change treatment to quantify the impact of climate change on the interactions between plants and herbivores.
Meta-Analysis: Statistical Analyses and Tests for Publication Bias

Because the majority of the studies reported a pair of means, variance, and had small sample sizes (Rosenberg et al. 2007), we used Hedges’ *d* to measure the effect size (ES) (Osenberg et al. 1999, Koricheva et al. 2013). It uses the treatment and control estimated means (\(\bar{Y}_1\) and \(\bar{Y}_2\)) and their associated sample sizes (\(n_1\) and \(n_2\)) and standard deviations (\(s^2_1\) and \(s^2_2\)) (Koricheva et al. 2013) to calculate the difference between the two groups, *d*, as:

\[
d = \frac{\bar{Y}_1 - \bar{Y}_2}{\sqrt{\frac{(n_1 - 1)s^2_1 + (n_2 - 1)s^2_2}{n_1 + n_2 - 2}}} \left(1 - \frac{3}{4(n_1 + n_2 - 2)}\right)
\]

It has the advantage of not being biased by unequal sampling variances in the paired groups, and includes a correction factor for small sample sizes (Rosenberg et al. 2007).

The influence of climate change factors on plants and plant-herbivore interaction traits was quantified by calculating the ES for each of the pairwise comparisons (i.e., control vs. experimental treatment). The mean effect sizes were computed and compared using MetaWin 2.1 (Rosenberg et al. 2007). A positive ES indicates that a given CC factor enhances the response of a given plant trait (Rosenberg et al. 2007, Koricheva et al. 2013), while a negative ES indicates a detrimental effect on the performance of the trait. A CC factor effect was considered statistically significant if the 95% confidence interval of the trait’s mean ES did not include zero (Rosenberg et al. 2007, Koricheva et al. 2013), with the 95% CI calculated as mean ± 1.96 standard deviation/ \(\sqrt{\text{sample size}}\). Comparisons of CC effects on plants and plant-herbivore interactions both within and among factors was explored by calculating the between-group heterogeneity (Qb), which we then compared using a chi-square test statistic.
(Koricheva et al. 2013) to determine whether there were significant differences between ESs for different climate change factors. In sum, all analyses were performed to determine if the observed variance in ES was significantly different from that expected by sampling error (Koricheva et al. 2013).

Publication bias occurs when the effect sizes included in the meta-analysis generate false conclusions compared to those obtained when the effect sizes come from studies with appropriate statistical tests (Koricheva et al. 2013). Because of the wide range of sample sizes in the studies we reviewed we initially used the funnel plot method to test for publication bias (Rosenberg et al. 2007, Koricheva et al. 2013) by plotting the effect sizes of different CC factors on plant and plant-herbivore interactions against sample size. We also tested for a relationship between these with Spearman rank correlations. In the absence of bias the plots should show symmetry around the mean effect size for each group and effect sizes should not correlate with sample sizes (Rosenberg et al. 2007, Koricheva et al. 2013).

**Results**

The initial literature search resulted in 5644 publications, of which only 120 published between 1993-2013 met our criteria. The full data set is deposited in the Dryad Digital Repository ([http://dx.doi.org/10.5061/dryad.dn048](http://dx.doi.org/10.5061/dryad.dn048)). Of the 120 papers, n = 80 evaluated the effect of one climate change factor, n = 39 evaluated the effect of two, and only one evaluated the combined effects of three factors in the same experiment. Funnel plots of effect size vs. sample size for all Plant and PHI traits measured indicate a possible selection bias favoring studies with small sample sizes over those with larger ones (Figure 2-2). However, studies with small sample sizes showed more variation
around the mean effect size (Figure 2-2), and because Spearman’s rank correlations showed no significant relationships (except for sugars and carbohydrates; Table 2-1), the bias in the meta-analysis is negligible.

**What Components of Climate Change and Plant-Herbivore Interactions Are Being Investigated?**

There were 92 studies that measured AG traits after AG herbivory, while only one study considered AG traits after BG herbivory. Eleven studies considered AG traits in the absence of herbivores. No study measured BG traits after exclusively AG herbivory, two studies measured BG traits after BG herbivory, and one study measured BG traits in the absence of herbivores. Only 13 studies evaluated AG-BG linkages by measuring traits in both subsystems after AG and/or BG herbivory (Figure 2-3B).

The most frequently evaluated climate change factor was increasing CO₂ (n = 101), followed by decreased precipitation and increasing levels of ozone (n = 17, and n =16, respectively; Figure 2-3A). It is therefore not surprising the most common experimental frameworks were large open top chambers (OTC; e.g., Gao et al. 2008), small outdoor chambers (e.g., Flynn et al. 2006), and Free-Air CO₂ enrichment experiments (FACE, e.g., Hillstrom et al. 2010); 22.5%, 20%, and 18.33% of studies, respectively).

Only n = 4 publications investigated tropical species, (see Figure 2-3C and 2-3D), while n = 61 studies were conducted with temperate taxa. Crop species were used in n = 44 studies while n= 11 studies used grasses and their herbivores as the model systems (Figure 2-3C). In total the studies we reviewed investigated the responses of n = 106 plant species: n = 18 crop species, n = 22 grasses, n = 54 temperate plants, and
n = 12 tropical plants. The most frequently studied species were *Populus tremuloids* (7.37%), *Gossypium hirsutum* (6.84%), *Betula papyrifera* (4.74%) and *Acer saccharum* (3.68%). No tropical species was included in more than 2 studies.

**Effect of Climate Change on Plant Growth, Nutritional Quality, Defenses and Interactions**

When considering responses for all species combined, the effect size of the CO\(_2\) influence on AG biomass was positive and significant (i.e., did not cross the zero line), while the ones from changes in precipitation (i.e., dH\(_2\)O and iH\(_2\)O) were significant but negative. The between-factors heterogeneity (Q\(_b\)) shows that the CO\(_2\) effect differs significantly from dH\(_2\)O and iH\(_2\)O, but precipitation effects did not differ among themselves (Figure 2-4A). In contrast, the effect size of the CO\(_2\) influence on BG biomass was positive and significant while the effect sizes of precipitation changes were not significant (Figure 2-4B). However, their between-factors heterogeneity was equivalent (Table 2-2).

Nitrogen content was reported as a measure of plant nutritional quality in 41.86% of the studies reviewed. When considering all species, CO\(_2\) had a negative effect on N content. This effect was significantly different than the ones from precipitation changes, which had positive and significant effects on N content but did not differ in magnitude (Table 2-2; Figure 2-4C). Moreover, CO\(_2\) had a positive effect on C:N ratio while precipitation changes had no effect on C:N ratio (Figure 2-4D). In addition, CO\(_2\) significantly increased the concentration of sugar and carbohydrates in leaves (Figure 2-4E and 2-4F).
Our analyses also indicate that, across all species, the heterogeneity of CO₂ effects on C-based defenses was significantly different from those of other factors (Table 2-2). Across species, as well as within the majority of species groups, increases in CO₂ significantly increase the concentrations of C-based defenses (Figure 2-4G). CO₂ also increased concentrations of physical defenses across all species (Figure 2-4I), but had no significant effect on N-based ones (Figure 2-4H).

There was no significant difference in the effect of different climate change factors on herbivore body mass or on the rate of plant consumption (Table 2-2; Figure 2-5A and 2-5B). Herbivore body mass generally decreased with increasing CO₂ and reduced precipitation (Figure 2-5A), while herbivore consumption rates significantly increased under elevated CO₂. There was no effect of increases in temperature on both body mass and consumption (Figure 2-5A and 2-5B).

**Support for Predictions: Effects on Tropical vs. Temperate Plant Species**

Testing Coley’s (1998) predictions for tropical plant-herbivore interactions was a challenge due to the small number of studies, the limited number of environmental factors manipulated in these studies, and the few response variables measured. In contrast, our predictions for the responses of temperate species were generally upheld (Figure 2-1). For example, temperate species had a significant decrease in N and an increase in the C:N ratio. In few cases where comparison across biomes was possible, some responses were qualitatively similar – for example, there was a significant increase in the concentration of carbohydrates and C-based defenses under elevated CO₂ (Figure 2-4C and 2-4G). However, other responses differed notably among regions, e.g., tropical species had a greater increase in leaf carbohydrates than
temperate species ($Q_b = 40.31, df=1, P<0.001$). A lack of experiments or failure to measure plant responses and proxies for plant-herbivore interactions in the tropical experiments made other temperate-tropical comparisons impossible.

**Effects of experimental climate change belowground**

Most studies measured only aboveground responses and interactions (Figure 2-4 and 2-5), and there are no predictions for how climate change will influence belowground interactions and plant responses. The belowground experiments that were conducted were all manipulations of CO$_2$ using temperate and crops species; few measured root nutritional quality or defense levels in roots. None of the effect sizes measured for BG studies differ significantly in magnitude from their AG counterparts ($Q_b=0.58$, df=1, $P>0.05$; $Q_b=0.74$, df=1, $P>0.05$; for temperate and crops species at C based defenses respectively). The CO$_2$ effect sizes for N or carbohydrate contents in roots were not significant but was positively significant for C:N ratio. Finally, the CO$_2$ effect size of carbon-based defenses in roots was not significant for temperate species but was positively significant for crop species (Figure 2-4G).

**Discussion**

Although scientists have been predicting and documenting the potential effects of climate change on ecosystems for decades, experimental evaluations of how climate change influences plant-herbivore interactions remain rare. Our meta-analysis uses quantitative data from experiments across the globe to evaluate the magnitude of responses by plants, herbivores, and their interactions to climate change under two predictive frameworks: Coley’s framework for tropical species (Coley 1998) and our framework drawing together predictions for temperate ones. We found evidence to
support most of the predictions for temperate species. However, despite it being 20 years since Coley published her comprehensive predictions, the lack of studies from the region makes it impossible to test them. Other gaps in knowledge revealed by our meta-analysis include how climate-change factors influence belowground processes and the links between above- and below-ground responses. Below we elaborate on these results and propose how to move forward to better understand the mechanisms by which climate change will influence plant-herbivore interactions.

In our study, predicted changed in factors such as precipitation, temperature and ozone levels did not have significant effects on plant-herbivore interactions – though this may well be because there simply have been so few experiments considering the impacts of these changes, the limited number of species in which they have been evaluated, and a failure to consider the impact of linked interactions above- and belowground. However, the best studied factor – increased atmospheric CO₂ – has clear and large effects. Under elevated CO₂ plants had augmented leaf and root production, with outcomes consistent with the “fertilization effect” (LaMarche et al. 1984) predicted to occur under a combination of increased CO₂, temperature, and water stress. Although it is thought to affect C3 and C4 plants in different ways (Lincoln et al. 1984), we detected similar effects across plant groups in studies manipulating CO₂. Although we documented species-specific response intensity, the CO₂ fertilization effect begins with increased photosynthetic CO₂ fixation (Poorter et al. 1988). During the plant acclimation to elevated CO₂ levels an increase in carbohydrates and sugars and decrease in protein content ensues, ultimately leading to increased C:N ratio in leaves (Yelle et al. 1989, Kozlowski and Pallardy 2002). Plants then decrease their
photosynthetic capacity, ultimately leading to lower nitrogen content (Poorter et al. 1988, Yelle et al. 1989). In addition, higher CO$_2$ levels indirectly deprive insect herbivores of necessary nutrients for development. Since insect feeding depends strongly on nutrient availability and quality (Hunter 2001, Awmack and Leather 2002, Bale et al. 2002), the low nitrogen availability causes an increase in consumption despite the decrease in herbivore body mass (Figure 2-5). The CO$_2$ effects were evident for temperate, grass and crop species (Figure 2-4) but may be more pronounced in agricultural systems due to the faster decrease of key nutrients (i.e., N, P, K; Tan et al. 2005) and the potential for insect pest outbreaks to occur more frequently (see Logan et al. 2003).

The increase of greenhouse gas emissions causes increases in temperature, ultraviolet radiation and atmospheric ozone levels, as well as changes in precipitation patterns (IPCC 2014). However, our results reveal that most studies have tested only for the effects of changes in CO$_2$. Addressing single climate change variables in isolation is problematic – recent studies indicate that these factors can act in synergy to directly or indirectly induce changes the responses of plants and insect herbivores? For example, several studies in which only CO$_2$, T, or O$_3$ are increased or precipitation alone is decreased have all shown a decrease in herbivore body mass (Figure 2-5A). However, decreases in body mass are greater in magnitude when CO$_2$ and T are manipulated in combination than when they are manipulated independently (Johns and Hughes 2002). Similarly drought, combined with changes in CO$_2$, T, or both can also decrease body mass, with the magnitude of changes increasing the more factors are considered (Scherber et al. 2013). In contrast, CO$_2$ and O$_3$ can act synergistically to
increase body mass (Kopper and Lindroth 2003). Although more work is needed to consider how interspecific differences in life-history and food availability will alter the generality of these conclusions, the findings are consistent with those of other studies investigating how multiple anthropogenic disturbances alter ecological systems (Cochrane and Laurance 2008, Brodie et al. 2012).

Our review also highlights important gaps in our understanding of how climate change might differentially influence tropical and temperate plant-herbivore interactions. Although few studies in the temperate zone investigated the impacts of increasing temperature and the diversity of species studied was relatively low, the volume of work available provides robust quantitative support to many of our predictions for how these interactions will be altered. In contrast, data for the tropics were so sparse we were unable to test most of the predictions in Coley’s framework. Interestingly, the limited work that was available suggests congruence in the results for these different regions. For example, most predictions regarding the increase in CO₂ levels show that although the magnitude of the effects differs across tropical and temperate species, the direction of the effects was similar. There is clearly a need for more comprehensive, comparative studies considering the responses of a diverse array of species from multiple latitudes.

It is also important to remember that as our understanding of how climate change will impact different regions, it may be necessary to revise our predictions for how these changes influence interspecific interactions. For instance, we suggest that Coley’s framework, which centered on the effects of changes in CO₂, underestimates the impacts on herbivory associated with the decreased precipitation at lower latitudes predicted by more recent models (IPCC 2014). Although many tropical species –
including in lowland forests – are exposed to extended dry seasons, severe or prolonged decreases in rainfall can decrease plant growth and increase rates of mortality (Engelbrecht and Kursar 2003, Breshears et al. 2005). This could change the relative abundance of plant species and hence the local pressure and impact of herbivores. In addition, the decrease in soil moisture could reduce decomposition rates and decrease the absorption of nutrients (Kozlowski and Pallardy 2002), resulting in lower concentrations of these limited nutrients in leaves and roots and favoring nitrogen-fixing species. With the decrease in water availability plants would have to physiologically balance root/shoot growth, production of secondary metabolites, and reproduction. In the short-term the physiological stress and changes in the allocation of assimilated nutrients influenced by hormones (Kozlowski and Pallardy 2002) should favor growth and production of secondary metabolites. However, in the long-term defenses should decrease. Belowground herbivores would be less favored due to the low soil moisture, but leaf herbivory is expected to increase and be more severe because some herbivore populations may increase (Ayres and Lombardero 2000, Jactel et al. 2012) causing more frequent herbivore outbreaks. These predictions are based only on decreases in precipitation; if we consider the potential for additive or even synergistic effects of other factors the effects could be even more severe. However, it is important to highlight that plants are known for their capacity to acclimate to changing conditions (see Valladares and Pearcy 1997, Morison and Lawlor 1999), so the speed at which the climate changes will play a critical role in mediating these responses.
Future Directions in a Changing World

Our meta-analysis has shown that climate change has significant effects on plants, herbivores and their interactions. Here, we highlight five broad research priorities that we feel will greatly improve our ability to understand the potential effects of climate change on these critical interactions, whose effects ultimately cascade through communities and influence ecosystem services.

First, future studies must attempt to evaluate how multiple climate change factors interact to influence plants and their herbivores. Such experiments may be logistically challenging, but they more accurately reflect the reality of simultaneous changes in e.g., temperature, precipitation, and CO$_2$. Second, study length remains a critical weakness in studies of climate change and herbivory – most are of limited duration and conducted plants from a single life-history stage. However, plant physiology and growth in response to environmental conditions vary ontogenetically (Bazzaz and Williams 1991, Kozlowski and Pallardy 2002); focusing on only a single life-history stage could mean failing to detect physiological acclimation to the changing climates. Finally, there is the potential for maternal- and other multi-generational effects; all of these possibilities can only be detected with studies of much longer duration than most of those we reviewed.

Third, there is an urgent need for more studies on tropical species. Although there is a growing literature addressing how climate change will affect tropical species, some major questions regarding plant physiological responses, interaction networks across multiple trophic levels, and changes in biodiversity remain unanswered. In addition, it has been proposed that climate change and human land use interact synergistically (Brodie et al. 2012) and that biodiversity and trophic interactions are
strongly affected by local as well as landscape scale changes (Tscharntke et al. 2005). Thus, climate change associated with external perturbations could have a stronger impact on tropical species and their interactions. Further studies with tropical species would allow more robust predictions of these effects and also provide a more realistic approximation of future conditions and changes. A corollary to this is that while many plant and herbivore species have geographic ranges that transcend biomes, but we know little about how climate change will influence their distribution or interactions with other species (but see Doak and Morris 2010). Studies manipulating climate change variables across latitudes therefore have the potential to greatly improve our existing predictive frameworks for studying these interactions.

Fourth, it is essential to consider belowground subsystems when assessing responses of pant-herbivore interactions to changing climates. Studies with crops have shown that increased allocation of C to roots caused by increased levels of CO$_2$ enhance the proliferation and depth of roots (Prior et al. 1994, Suter et al. 2002, Madhu and Hatfield 2013), which may enhance nutrient acquisition in places where climate change will result in reduced precipitation (Madhu and Hatfield 2013). These climate-change driven shifts in plant physiology and growth, coupled with changes in rhizosphere dynamics, could all have important consequences for plant and herbivore demography and the outcome of plant-herbivore interactions, but data on these belowground changes are extremely limited. Experiments with a more holistic perspective are clearly necessary – not only because this subsystem has been ignored, but because this belowground plant-herbivore interactions are inextricably linked to those aboveground.
Fifth, comprehensively elucidating how plant-herbivore interactions will respond to climate change requires expanding experiments to include such groups as predators, competitors, and mutualists – all of which can influence the outcome of these interactions (Herrera et al. 2002, Trager and Bruna 2006, Poelman et al. 2011) and which may themselves be susceptible to climate change (e.g., Jevanandam et al. 2013). Moving beyond the "pairwise-perspective" (sensu Stanton 2003) has been essential to advancing our understanding of other types of plant-animal interactions, and we anticipate doing so in this context will provide exciting theoretical and empirical advances.

**Concluding Remarks**

The majority of studies we analyzed found dramatic changes in plants, herbivores, and their interactions in response to experimentally simulated climate change. However, our meta-analysis also highlights profound gaps in our knowledge and a major bias towards research in the temperate zone. In light of the potential ecological and economic impacts that changes in these interactions could bring, it is clear that there is an urgent need for more geographically, taxonomically, and ecologically diverse research on this fundamentally important topic.
Table 2-1. Spearman’s rank correlations between effect sizes (d) and sample sizes.

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plant growth</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AG biomass</td>
<td>-0.068</td>
<td>0.633</td>
</tr>
<tr>
<td>BG biomass</td>
<td>-0.014</td>
<td>0.940</td>
</tr>
<tr>
<td><strong>Plant nutritional quality</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrogen</td>
<td>0.089</td>
<td>0.338</td>
</tr>
<tr>
<td>C:N ratio</td>
<td>-0.126</td>
<td>0.326</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>0.314</td>
<td><strong>0.004</strong></td>
</tr>
<tr>
<td>Sugar</td>
<td>-0.316</td>
<td><strong>0.028</strong></td>
</tr>
<tr>
<td><strong>Plant defenses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbon-based</td>
<td>0.136</td>
<td>0.074</td>
</tr>
<tr>
<td>Nitrogen-based</td>
<td>-0.386</td>
<td>0.113</td>
</tr>
<tr>
<td>Physical</td>
<td>0.264</td>
<td>0.095</td>
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<tr>
<td><strong>Plant-Herbivore Interaction</strong></td>
<td></td>
<td></td>
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<tr>
<td>Herbivore body mass</td>
<td>-0.099</td>
<td>0.278</td>
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<tr>
<td>Herbivore consumption</td>
<td>0.004</td>
<td>0.964</td>
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Table 2-2. Heterogeneity ($Q_b$) for the overall plant species between CO$_2$ and the other climate change factors.

<table>
<thead>
<tr>
<th></th>
<th>$Q_b$</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plant growth</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AG Biomass</td>
<td>140.139</td>
<td>2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BG Biomass</td>
<td>14.013</td>
<td>2</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td><strong>Plant nutritional quality</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrogen</td>
<td>130.937</td>
<td>4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>25.095</td>
<td>2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sugars</td>
<td>5.008</td>
<td>2</td>
<td>&gt;0.05</td>
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<tr>
<td>C:N ratio</td>
<td>19.165</td>
<td>1</td>
<td>&lt;0.001</td>
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<tr>
<td><strong>Plant Defenses</strong></td>
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<td></td>
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<tr>
<td>C-based</td>
<td>14.842</td>
<td>4</td>
<td>&lt;0.01</td>
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<tr>
<td><strong>Plant-herbivore interactions</strong></td>
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<td></td>
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<tr>
<td>Body mass</td>
<td>9.250</td>
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<td>&gt;0.05</td>
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<tr>
<td>Consumption</td>
<td>3.914</td>
<td>4</td>
<td>&gt;0.1</td>
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Figure 2.1. Framework for predicted trends and results of how climate change will affect plants and plant-herbivore interactions for (A) tropical and (B) temperate species and support for these predictions based on meta-analyses. In both frameworks plant growth is quantified as AG biomass, nutritional quality is the concentration of N in leaves, and total herbivory is the amount of biomass consumed by herbivores. The environmental changes whose impact we evaluated are increases in carbon dioxide (CO$_2$), temperature (T), and ozone levels (O$_3$), and decreases in precipitation (H$_2$O). Symbols represent increased production (↑) or decreased production (↓); not enough data to test a prediction (ND), and non-significant trends (trend). Grey indicates there are no predictions to test.
Plant growth

Plant nutritional quality
Plant defenses

Figure 2-2. Funnel plots of effect sizes ($d$) and sample sizes. Each point represent the mean effect size for a single experimental case. The climate change factors comprise increase levels of carbon dioxide ($CO_2$) and ozone ($O_3$), increase air temperature ($T$), precipitation decrease ($dH_2O$) and increase ($iH_2O$) levels. Data underlying Figure 2-2 are deposited in the Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.dn048 (Mundim and Bruna 2016).
Figure 2-3. Climate change effect on plant and plant-herbivore interaction general results. A) Percentage of experimental cases in each climate change factor. Precipitation include increase and decrease levels. B) Percentage of publications per subsystem. AG = aboveground herbivory and measurements, AGBG = herbivory and measurements in both subsystems or in opposite subsystems, BG = belowground herbivory and measurements. C) Percentage of publications in each group of species. Number inside the parenthesis represent the absolute number of cases considered. D) Number of publications per year. Dashed line represents the total number of publications (all four group of species), grey line represents the number of publications with temperate species, and the black line represent the number of publications with tropical species.
Figure 2-4. Influence of climate change on plant traits for different groups of plant species. Experimental manipulations include elevated carbon dioxide (CO$_2$) and ozone (O$_3$), increased air temperature (T), and precipitation decreases (dH$_2$O) or increases (iH$_2$O). Numbers in parentheses represent the number of studies considered. "Overall" is the analysis for all plant species combined. BG denotes belowground measurements; all others are aboveground measurements. Mean effect size is shown with 95% CI. Effects are considered significant if their associated CIs do not overlap zero (i.e., the dashed line). Solid circles indicate statistically significant effects. Data underlying Figure 2-4 are deposited in the Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.dn048 (Mundim and Bruna 2016).
Figure 2-5. Influence of climate change on plant-herbivore interactions for different groups of plant species. Details are as described in Figure 2-4.
Overview

Tradeoffs between plant growth and defense are central to theoretical frameworks used to study the ecology and evolution of plant defense against herbivores. However, these frameworks, as well as the experiments designed to test them, rarely include the remarkable diversity of belowground herbivores. We experimentally challenged seedlings of the tropical shrub *Solanum lycocarpum* (Solanaceae) with either aboveground foliar herbivores (*Spodoptera* caterpillars) or belowground root herbivores (the nematode *Meloidogyne incognita*) and measured the resulting changes in plant growth rates, biomass allocation, and the concentration of defensive terpenoids in roots and leaves. We found that plants that suffered aboveground herbivory responded with aboveground growth but belowground defense. Similarly, belowground herbivory resulted in root growth but elevated defenses of leaf defenses. These results underscore the importance of belowground plant-herbivore interactions, and suggest that – in contrast to theoretical predictions – plants can simultaneously invest in both growth and defense. Finally, they emphasize the need for a ‘whole-plant’ perspective in theoretical and empirical evaluations of plant-herbivore interactions.

Background

Herbivory is a selective force that can fundamentally change plant morphology and physiology (*Karban and Baldwin* 1997, *Strauss and Agrawal* 1999, *Ohgushi* 2005) and has resulted in a vast diversity of plant defenses (*Ehrlich and Raven* 1964,
Thompson 1988). Because the resources allocated to the regrowth of tissues consumed by herbivores and the defense of remaining ones are costly, most theoretical frameworks used to study plant-herbivore interactions emphasize the optimal allocation of these resources or potential tradeoffs between growth and defense (McKey 1974, Coley et al. 1985). In addition almost all experimental tests of these frameworks focus on aboveground (AG) herbivory and plant responses (see VanDam et al. 1996, Karban and Baldwin 1997, Strauss and Agrawal 1999, Fine et al. 2006, Mundim and Bruna 2016), which ignores a vast diversity of belowground (BG) herbivores and plant defenses of roots and other belowground structures (e.g., Bardgett et al. 1999, Bais et al. 2006).

Root-feeding herbivores can enhance the production of root exudates (Denton et al. 1999, Bais et al. 2006), reduce plant growth (Bardgett et al. 1999, Bais et al. 2006) and elevate defenses against other belowground herbivores (Bais et al. 2006). It has therefore been suggested that conclusions regarding plant-herbivore theory derived from experimental tests conducted with aboveground herbivores can be readily extended to BG subsystems (van Dam 2009). For instance, it has been hypothesized that severing of roots by BG herbivores should drive plants to invest in root growth to compensate for the loss of absorptive surface, in much the same way foliar herbivory results in the rapid production of new leaves necessary for photosynthesis (van Dam 2009). Recent work, however, suggests it is premature to assume that belowground responses should parallel those aboveground. Under abiotic stress, for example, more energy should be allocated to protection of roots given that plant performance can be severely reduced in limiting soil conditions (Erb and Lu 2013).
Despite the fact that the above and belowground plant-herbivore subsystems are often independently conceptualized and evaluated, they are not physiologically independent. For instance, defenses could be optimally allocated across the above and belowground plant compartments (Huang et al. 2014) such that a belowground herbivore could induce aboveground defenses and vice-versa (Huang et al. 2013, 2014). Such “asymmetrically induced systemic responses” (sensu Huang et al. 2012) could alter the outcome of interactions with other herbivores (Bais et al. 2006, Barber et al. 2015) and plant performance (Bardgett and Wardle 2010, Barber et al. 2011, Zvereva and Kozlov 2012). Accounting for such asymmetries, however, requires a whole-plant perspective — experimentally manipulating herbivory in one plant compartment and measuring plant responses in the other. Studies to have taken this whole-plant approach are rare (e.g., van Dam et al. 2005, Soler et al. 2005, Erb et al. 2009). Even fewer have measured responses in both plant compartments, and those to have done so have only measured the defensive chemistry of plants following herbivory (see Huang et al. 2013, 2014). Without measuring both growth and defense — and doing so both above and belowground — it is impossible to determine plant responses are limited to within one compartment or extend across the entire plant. Such information is essential to development of more precise and comprehensive plant defense theory, especially in light of ongoing global change (Mundim and Bruna 2016).

We experimentally manipulated AG and BG herbivory of seedlings of the tropical shrub *Solanum lycocarpum* (Solanaceae) to address the following questions: (1) Does AG herbivory result in the expression of BG defenses? (2) Does BG herbivory result in the expression of AG defenses? (3) Are patterns of root growth and root defense
following belowground herbivory similar to those in leaves following herbivory aboveground? (4) Do above and belowground herbivory result in similar patterns of plant growth and biomass allocation? Our results indicate that evaluating both above and belowground responses to herbivory is necessary to accurately interpret patterns of growth, defense, and their implications for theoretical predictions.

**Methods**

*Solanum lycocarpum* (Solanaceae) is a perennial, deciduous pioneer shrub common in the more open physiognomies and disturbed areas of South America’s savanna woodlands, known as the *Cerrado* (Lorenzi 2002). This species is commonly known as *lobeira* or wolf’s apple because its large fruits are central to the diet of the maned-wolf, *Chrysocyon brachyurus* (Dietz 1984). The leaves of *S. lycocarpum* are protected by trichomes and thorns (Lorenzi 2002), while roots, leaves and fruits are protected by terpenoids, alkaloids, and other chemical compounds (e.g., Dall’Agnol and von Poser 2000, Sá et al. 2000).

As in many other *Solanum* species, the aboveground parts of *S. lycocarpum* are frequently attacked by generalist herbivores such as Armyworm larvae (*Spodoptera* spp.). The roots of *S. lycocarpum* are damaged by the root-knot nematode *Meloidogyne incognita*, an endogenous root-feeder that infects species from a broad spectrum of plant families (e.g., *S. lycopersicum* (Solanaceae), *Gossypium* spp. (Malvaceae), *Daucus carota* (Apiaceae); Shurtleff and Averre 2005). While the infective stage in many phytophagous nematodes are adults (e.g., *Pratylenchus, Radopholus*), in *M. incognita* they are the second-stage juveniles (J2) (Shurtleff and Averre 2005). They
migrate through the soil and burrow into roots in which they feed, develop, and reproduce; adult females form large galls on roots (Shurtleff and Averre 2005).

On August 2013 we collected fruits from 10 *Solanum lycocarpum* shrubs and removed all pulp from seeds under running water with a strainer. The seeds were then placed in a shadehouse in germination trays filled with a 1:3 ratio of sand and soil; the soil was collected in an area of Cerrado at the Universidade Federal de Uberlândia’s (UFU) Panga Ecological Station (19°10'S, 48°24'W). In November 2013, n=45 seedlings with fully expanded cotyledons (mean total leaf area ± SE: 2.27 ± 0.069 cm²) were transplanted into 12 cm diameter x 20 cm tall transparent bags containing the same ratio of sand and soil from Panga Station. We randomly assigned n=15 plants to each of three treatments – AG herbivory, BG herbivory, or no-herbivory controls – then arranged them at random in the shadehouse. Several plants died in the three months between this assignment and application of experimental herbivory (final sample sizes: n=10 AG, n=9 BG, n=14 Control); there was no significant difference between treatments in the leaf area of plants at the time experimental herbivory was initiated ($F_{2,32}=0.014; P=0.986$).

On 20 February 2014 the plants in the BG herbivory treatment were inoculated with 10 ml of water in which we diluted ca. 5000 *M. incognita* eggs; this solution was delivered to three points around the base of the plant with a pipette (see Bonetti and Ferraz 1981). The eggs were obtained from a colony maintained at the UFU’s Department of Agronomy Nematology Research Lab. We estimate the number of eggs with which we inoculated plants was ~50% of the amount present in the soil surrounding
naturally occurring Solanum lycocarpum seedlings (1.6 Meloidogyne adult males and juveniles per cm$^3$ of soil; F.Mundim, unpubl. data).

It takes approximately 10 days for nematodes to emerge from eggs, develop into J2 stage juveniles, migrate, and infect roots (Shurtleff and Averre 2005). We therefore began the AG herbivory treatment on 2 March 2014 to ensure plants in both AG and BG treatments were exposed to herbivores for the same amount of time. The AG herbivory treatment was applied by placing one Spodoptera larva (second instar or later) on a leaf until it consumed 10% of total leaf area, which typically took about two hours. Induced defensive responses in leaves of Solanum lycopersicum can last at least 20 days (Underwood 2012). We therefore challenged plants with Spodoptera every 10 days to ensure a continuous responsive comparable to one resulting from the continuous BG infection. Plants in the control treatment were not exposed to any leaf or root herbivores. All plants received approx. 200 ml of water every 2 days and were rotated to random positions in the shadehouse weekly. They continued to grow throughout the duration of the experiment (see Results), indicating the observed results are not due to inter-treatment differences in resource utilization or plants becoming pot-bound. After approximately 125 days of exposure to herbivores all surviving plants were removed from the soil and washed. For each plant we measured stem length and diameter, the final number of leaves, leaf area, total root length, and root complexity. We then separated leaves, stems and roots, dried them at 60ºC for 2 days, weighed them, and stored them separately in paper bags for subsequent chemical analyses.

Measuring root length and complexity immediately prior to challenging plants with herbivores would require uprooting them; which could damage them, induce plant
defenses, or result in plant mortality. We therefore measured total root length, root complexity, and initial dry weight of leaves and roots in n=15 plants set aside at the time experimental seedlings were transplanted. These values were then used as the initial ones for all experimental plants. We used the software package ImageJ (Rasband 1997) to calculate leaf areas and total root lengths. We used the centripetal link based ordering system to quantify the topological complexity of the root system (see Fitter 1986, 1987, Berntson 1997).

We calculated the relative growth rates (RGR) of total leaf area and total root length as $RGR = \frac{\ln(L_2) - \ln(L_1)}{t_2 - t_1}$, where $L_1$ is the initial total leaf area or total root length, $L_2$ is the final total leaf area or root length, and $t_2 - t_1$ is the length of the experiment in days. We also used the dry biomass of leaves, and roots at the end of the experiment to calculate the relative allocation of biomass below and aboveground (i.e., the Root:Shoot ratio).

*Solanum* species are known to use a broad profile of terpenoids for defense (War et al. 2012), and our preliminary analyses with gas chromatography / mass spectrometry (GC/MS) found most treatment-dependent variation was in an area of the chromatogram consisting primarily of sesquiterpenes. We therefore used the concentration of root and leaf sesquiterpenes as a metric of plant chemical responses to AG and BG herbivory. The roots and leaves from each experimental seedling were weighed, frozen with liquid nitrogen, and ground. The pulverized tissue was then diluted in a 1:2 volume of dichloromethane (CH$_2$Cl$_2$) and incubated in an ultrasonic bath for 30 min. The tubes were centrifuged and 100µl of supernatants from the final extracted volume were pipetted for analyses. We used an Agilent 7890A gas chromatogram (GC)
combined with a G7001B mass spectrometry (MS) in electron impact and single quad mode for the GC/MS analyses. We injected a one µl sample, using cool on column, on a 30m x 0.25mm ID Agilent DB5 column with 0.25 µl film thickness with Helium carrier at a constant flow of 35cm/min. The column was initially held at 30°C for 1 min and then temperature programmed to increase 10°C/min to 280°C and held at that temperature for 4 minutes (total time = 30min).

We focused on peaks in the 15-21 min retention time (RT). To estimate sesquiterpenes quantities based on peak area, known amounts of e-b-caryophyllene were injected to give an ng/au factor. For each sample all peaks within the retention time range were integrated to give their area after values similar to the blank-solvent and values from background compounds were eliminated. We then eliminated values caused by instrument background fluctuations (i.e., ‘noise’, here those less than 55,000 µmol). We eliminated solitary RT values and those appearing in less than 3 samples per treatment to avoid any biases related to the sample size of seedlings per treatment, then normalized the values for each RT peak by dividing the area by the dry weight (dw) of the plant sample. Finally, we calculated total sesquiterpenes concentration by summing all the normalized peak values for each plant sample.

To determine if the different types of influenced growth and terpenoid concentrations we used Linear Mixed Models. The response variables were relative growth rate (RGR), terpenoid concentration, and biomass. The main effects were herbivore treatment (i.e., AG herbivory, BG herbivory, control), plant part (i.e., leaves, roots), and their interaction. The random effects attributable to variation among individual seedlings were included by treating each plant as a split-plot, with herbivory
type as the whole-plot treatment and the location of measurements (i.e., roots, leaves) as the within-plot treatment (B. Bolker, pers. com.). To test for the effects of herbivore type on Root:Shoot Ratio, total biomass, total terpenoid concentration, final stem length, final stem diameter, final number of leaves, final length of the primary root, and root complexity we used ANOVA after confirming the residuals met the assumptions of the test with graphs and the Lilliefors test. To determine if there was a difference among treatments we computed post-hoc Tukey HSD tests. We conducted all analyses using the R statistical programming language (v3.2.4; R Core Team 2016) with package lme4 (Bates et al. 2015), nlme (Pinheiro et al. 2016), and the glht function in package multcomp. Data are available from the Dryad Digital Repository (see Data Availability).

**Results**

No plants died as a result of the experimental treatments. Across treatments final stem length ranged from 5.5cm to 31.5cm and final diameter ranged from 1.3mm to 3.8mm (Table 3-1), with no significant effect of herbivore type on either (Length: $F_{2, 31}=1.851, P= 0.174$; Diameter: $F_{2, 31}= 0.953, P= 0.396$; Table 3-1). However, there was a significant effect of herbivore type on both final leaf number and the relative growth rate of leaves. The final number of leaves exposed to AG herbivores was 1.6 times greater than that of plants in the other treatments ($F_{2, 31}=13.4, P<0.001$; Table 3-1), while the RGR of leaves in the AG herbivory treatment was 1.3 times greater (Table 3-2; Figure 3-1A).

All plants in the BG herbivory treatment were infected with nematodes (Table 3-3). Across treatments the final length of the primary root ranged from 4cm to 58.5cm and there was no inter-treatment difference ($F_{2, 31}=2.301, P= 0.117$). The growth rate of
roots in plants exposed to BG herbivory was 1.2 times than of control plants (see Table 3-2; Figure 3-1B), however, and root complexity was 2.7 times greater in plants exposed to BG than in those challenged with AG herbivory ($F_{2,31}=6.504, P=0.004$; Table 3-1).

Although there was a trend towards higher total terpenoid concentrations in plants exposed to herbivory, none of the differences between treatments were significant (Figure 3-2; $F_{2,34}=2.91, P=0.05$; Table 3-1). Instead, the responses to herbivory appear to be in the subsystem opposite to the location of herbivore activity. For instance, the leaves of plants exposed to AG herbivory had terpenoid concentrations similar to those observed in control plants. However, the concentration of terpenoids in their roots was 2.4 times greater than in the roots of both control plants and those exposed to belowground herbivores (see Table 3-2; Figure 3-1D). Similarly, plants inoculated with nematodes had similar root terpenoid concentrations to control plants, but their leaves had 2.4 times more terpenoids than both control plants and those exposed to AG herbivores (see Table 3-2; Figure 3-1C).

At the end of the experiment control plants had greater total biomass than plants exposed to herbivores, although only the difference in biomass between control and AG plants was significant (Figure 3-3A; $F_{2,30}=3.958, P=0.03$). There are differences in the allocation of this biomass, however. Across all treatments, the proportional investment by *Solanum lycocarpum* seedlings in roots is 1.75 times greater than that aboveground parts ($F_{1,66}=8.441, P=0.004$). While plants in the BG herbivory and control treatments had similar Root:Shoot ratios, BG herbivory resulted in significantly greater biomass allocation to roots than AG herbivory (see Table 3-1; Figure 3-3B).
Discussion

Although plants are always exposed to both above and belowground herbivores, to date the experiments used to study plant-herbivore interactions have mostly focused on aboveground herbivory and plant responses (e.g., Karban and Baldwin 1997, Fine et al. 2006). Furthermore despite extensive study on belowground herbivory in agricultural systems, only recently have ecologists begun to incorporate it in theoretical frameworks (Masters et al. 1993) and empirically elucidate its breadth in natural systems (e.g., Bezemer et al. 2004, Erb et al. 2011, Barber et al. 2015). Our results underscore the importance of experimentally investigating the potential impact of belowground plant-herbivore interactions, despite their complexity and the challenges to doing so. More importantly, we show that belowground herbivory strongly influences a plant’s aboveground defensive responses and vice-versa. This novel result highlights a need for a whole-plant perspective in the theoretical frameworks used to study herbivory; it also adds to an emerging body of literature (see Huang et al. 2013, 2014) arguing for a need to both reevaluate the results of prior experiments and ensure future ones test for a broader diversity of responses.

There is growing evidence of the potential feedbacks between above and belowground subsystems and how herbivory in one could influence the other (see Soler et al. 2007, Erb et al. 2008, 2011, Rasmann et al. 2009, Barber et al. 2015). For instance, because the main function of roots is nutrient uptake, changes in plant physiology and allocation in response to root herbivory could ultimately have negative effects on aboveground herbivores (Erb et al. 2011, Rasmann et al. 2011b). These potential changes have been often attributed to distributive source-sink defensive
allocation – plants respond to herbivory by allocating defenses produced elsewhere to
the part being attacked (van Dam and Heil 2011). However, we found the opposite to be true, as on average plants experimentally infected by nematodes had significantly
greater terpenoid concentrations in leaves, not roots (Figure 3-1). This cross-subsystem
response was even more dramatic in plants where herbivory was aboveground, which
resulted in root terpenoid concentrations that were 2.4-fold higher than in the other
treatments. This suggests BG herbivory leads plants to protect the tissue needed for
photosynthesis while the attacked parts recover – a conclusion consistent with the lower
growth and greater defense of their leaves. Similarly, AG herbivory likely leads plants to
allocate primary metabolites to regrowth while preemptively protecting the undamaged
roots or sequestering terpenoids in roots for reallocation to regrown leaves. While it has
been suggested that defensive responses could be organ-specific and
compartmentalized (Rasmann and Agrawal 2008), our study demonstrates that AG and
BG herbivory can simultaneously trigger regrowth and allocation to defense, and in a
different subsystem than that attacked by herbivores.

A fundamental concept underlying plant responses to herbivory is that because
resources cannot be allocated simultaneously to defense and growth, plants invest in
defending tissues with the greatest fitness value (McKey 1974, Rhoades 1979). The
basis for this trade-off has been shown to depend on such factors as the duration or
severity of herbivore and pathogen attacks, the availability of resources, and plant
species identity (e.g., Bryant et al. 1983, Coley et al. 1985, Fine et al. 2006). While our
study was designed to test for growth-defense tradeoffs, the results do suggest properly
interpreting patterns of plant-defense tradeoffs likely requires a more comprehensive
evaluation of where herbivores are feeding and how plants respond to them. Simultaneously evaluating growth and defense responses above and belowground also suggests that in contrast to predictions from theory, plants facing herbivore pressure can concurrently grow and defend – plants in the nematode inoculation treatment both defended leaves and produced longer and more complex roots (Figures 3-2 and 3-3; Table 3-1). This observation was only evident with the benefit of a whole-plant perspective, however, as measuring plant responses solely above or belowground would have led to different conclusions about growth-defense tradeoffs.

Our results also have implications for another important framework used to study plant-herbivore interactions. Plants are often identified as either resistant or tolerant to herbivores (van der Meijden et al. 1988, Agrawal and Fishbein 2006), with the former having traits that decrease the feeding of herbivores and the latter responding to herbivory with rapid regrowth (van der Meijden et al. 1988, Karban and Baldwin 1997, Agrawal and Fishbein 2006). These alternative adaptive responses have been primarily assessed with AG herbivores (e.g., Bryant et al. 1983, Fine et al. 2006), so it is unclear if they extend to BG plant structures and herbivores. While the finding that S. lycocarpum’s AG subsystem is tolerant of foliar herbivory is not unexpected based on prior work with other Solanum species, it reassuring to see that this can be extended to the BG subsystem as well. Most notable, however, is the intriguing possibility that plants can actually shift between resistance and tolerance as a function of where herbivory is occurring and the subsequent responses of above and belowground subsystems. If so this suggests the “tolerant vs. resistant” dichotomy may also be overly simplistic, and
that – as with growth-defense tradeoffs – a more holistic perspective would also advance this body of theory and empiricism.

Our study is the first to isolate simultaneous above and belowground responses to both above and belowground herbivores. Although it was conducted with a single plant species, we are nevertheless cautiously optimistic the results will be broadly applicable across plant taxa. First, our results are consistent with those showing plants under attack by AG herbivores can allocate chemical defenses to their roots (e.g., Bezemer et al. 2004, Huang et al. 2014);. Second, both herbivores with which we challenged plants are widespread generalists, so the observed changes in plant chemistry and growth are not simply the result of how S. lycocarpum’s responds to specialized behavior or feeding physiology (Karban and Agrawal 2002). Finally, while the type and quantity of defensive secondary metabolites varies with plant and herbivore identity (see Adler and Wink 2001, Bezemer et al. 2004, Chen et al. 2004, Walls et al. 2005), terpenoids are the largest class of secondary defensive compounds and are found in all plants.

Our results suggest four directions for future empirical and theoretical studies of plant-herbivore interactions. First, heterogeneity in water, soil nutrients, and other resources can have a major influence plant growth, plant defense, and herbivore activity. Experimental manipulations of resources superimposed on manipulations of the location of herbivory are therefore an important next step in gaining a more comprehensive understanding of asymmetries in plant responses to herbivores. Second, future studies should consider how the whole-plant responds when challenged with sequential or simultaneous above and belowground herbivory, since there can be
important priority and additive effects of herbivores on plants (Erb et al. 2011). Third, better elucidating the physiological mechanisms underpinning the patterns we observed, such as the hormonal pathways responsible for defenses or environmental conditions limiting root or leaf growth, can help determine under what circumstances asymmetric responses are more or less likely to be observed. Finally, the outcome of interactions between plants and herbivores can alter their respective population and community dynamics, other interspecific interactions, and even ecosystem processes (Huntly 1991). A whole-plant perspective can provide new insights into the nature of these cascading effects, for instance on above or belowground herbivore communities (e.g., Huang et al. 2014) and how they are influenced by heterogeneity in environmental conditions (Mundim and Bruna 2016).
Table 3-1. Measurements of the aboveground (AG) and belowground (BG) plant parameters.

<table>
<thead>
<tr>
<th></th>
<th>Overall Range</th>
<th>Overall mean ± SE</th>
<th>Experimental Treatments (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>AG Herbivory</td>
</tr>
<tr>
<td><strong>AG parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stem length (cm)</td>
<td>5.5 – 31.5</td>
<td>14.63 ± 1.25</td>
<td>11.15 ± 2.47</td>
</tr>
<tr>
<td>Stem diameter (mm)</td>
<td>1.3 – 3.8</td>
<td>2.412 ± 0.115</td>
<td>2.19 ± 0.252</td>
</tr>
<tr>
<td>Total final leaf area (cm²)</td>
<td>8.85 – 71.38</td>
<td>37.325 ± 3.17</td>
<td>52.852 ± 4.268</td>
</tr>
<tr>
<td>Total dry biomass (g)</td>
<td>0.095 – 0.788</td>
<td>0.288 ± 0.031</td>
<td>0.212 ± 0.049</td>
</tr>
<tr>
<td>Number of leaves</td>
<td>2 – 11</td>
<td>5.36 ± 0.334</td>
<td>7.3 ± 0.578</td>
</tr>
<tr>
<td>Leaf terpenoids (µmol/g dw)</td>
<td>0 – 13230.97</td>
<td>3596.07 ± 643.3</td>
<td>2782.1 ± 570.68</td>
</tr>
<tr>
<td><strong>BG parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total final root length (cm)</td>
<td>61.2 – 583.1</td>
<td>262.436 ± 19.29</td>
<td>231.44 ± 35.874</td>
</tr>
<tr>
<td>Root complexity*</td>
<td>3.5 – 99</td>
<td>34.53 ± 3.82</td>
<td>18.1 ± 4.11</td>
</tr>
<tr>
<td>Total dry biomass (g)</td>
<td>0.039 – 1.44</td>
<td>0.512 ± 0.065</td>
<td>0.239 ± 0.088</td>
</tr>
<tr>
<td>Root terpenoids (µmol/g dw)</td>
<td>0 – 46514.24</td>
<td>7183.5 ± 2124.8</td>
<td>14686.5 ± 6152.5</td>
</tr>
</tbody>
</table>

* For Root complexity we used the centripetal link based ordering system (see Berntson 1997). In this system each root segment is assigned an order of one. The complexity of the root system is equal to the number of segments in the system.
Table 3-2. Results of the analyses from linear mixed models for the effects of the herbivory treatments on growth and terpenoid concentration. Significant effects are in bold.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Herbivory treatment</th>
<th>Plant part</th>
<th>Herbivory treatment x plant part</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>RGR</td>
<td>2,17</td>
<td>4.778</td>
<td>0.023</td>
</tr>
<tr>
<td>Terpenoid conc.</td>
<td>2,17</td>
<td>3.191</td>
<td>0.066</td>
</tr>
</tbody>
</table>
Table 3-3. Abundance of *Meloidogyne incognita* eggs, J2 juveniles, and females in root egg masses of different sizes. Data were collected at the end of the experiment from plants in the belowground herbivory treatment by visualizing under a microscope.

<table>
<thead>
<tr>
<th>Egg mass</th>
<th>Mean (± SE) Length (mm) x Mean (± SE) Width (mm)</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Eggs</td>
</tr>
<tr>
<td>Small</td>
<td>1.07 ± 0.05 x 1.8 ± 0.056</td>
<td>72</td>
</tr>
<tr>
<td>Median</td>
<td>2.33 ± 0.056 x 2.74 ± 0.08</td>
<td>51</td>
</tr>
<tr>
<td>Large</td>
<td>4.51 ± 0.26 x 4.98 ± 0.226</td>
<td>126</td>
</tr>
</tbody>
</table>
Figure 3-1. Results of experimental treatments showing A) Relative growth rate of leaf area, B) relative growth rate of total roots length, C) terpenoid concentration in leaves and D) terpenoid concentration in roots for plants exposed to aboveground herbivory (AG), belowground herbivory (BG), and no-herbivory controls. AG herbivory was by a generalist caterpillar (*Spodoptera* sp.) and BG herbivory was by a generalist endoparasitic nematode (*Meloidogyne incognita*). The boxes represent the interquartile range, the horizontal black lines indicate the medians, whiskers extent to 25% and 75% quartiles, and outliers are indicated by dots. Different letters indicate significant differences among herbivory treatments (P<0.05) based on post hoc Tukey’s tests.
Figure 3-2. Whole-plant terpenoid concentration of n=6 seedlings harvested at the time herbivory treatments were initiated (“Initial”) as well of experimental plants exposed to aboveground herbivory (AG), belowground herbivory (BG), no-herbivory controls. The boxes represent the interquartile range, the horizontal black lines indicate the medians, whiskers extent to 25% and 75% quartiles, and outliers are indicated by dots. Different letters indicate significant differences among herbivory treatments (P<0.05) based on post hoc Tukey’s tests.
Figure 3-3. Results of experimental treatments showing A) Total plant biomass and B) Root:Shoot ratio of *Solanum lycocarpum* in the three different herbivory treatments. Aboveground herbivory was by *Spodoptera* sp. and belowground herbivory was by the nematode *Meloidogyne incognita*. The boxes represent the interquartile range, the horizontal black lines indicate the medians, whiskers extent to 25% and 75% quartiles, and outliers are indicated by dots. Different letters indicate significant differences among herbivory treatments (P<0.05) based on post hoc Tukey’s tests.
Overview

Stressful environmental conditions and herbivores have major impacts on plant survivorship, physiology, growth, and reproduction. Variation in plant responses is a result of the timing, nature, and severity of each abiotic and biotic stress. Most studies to date have evaluated plant responses to herbivory and water variation in either leaves or roots, ignoring that these biotic and abiotic stressors can occur simultaneously. Furthermore, these stressors can be enhanced by anthropogenic climate change. Here, we experimentally simulated water variation and manipulated above and belowground herbivory in seedlings of the tropical shrub *Solanum lycocarpum* (Solanaceae) to test how their isolated and simultaneous effects would change plant survival and growth, biomass allocation, and the concentration of defensive terpenoids in roots and leaves. We found that 1) drought can severely affect plant survivorship and the effect is exacerbated by BG herbivory, 2) herbivory has a greater effect on plant growth than variation in water, and the responses caused by AG and BG herbivores were distinct, making the impacts of belowground herbivores unique and context-dependent and 3) while water and herbivory can significantly alter plant defensive chemistry, the changes are not consistent in direction or magnitude. These results emphasize that aboveground and belowground responses to biotic and abiotic stressors can differ strongly. Since some of these abiotic and biotic stressors are expected to vary more with climate change, understanding how some mechanisms in isolation or in combination affect the
plant is essential for predicting how plant-herbivore interactions may affect community
dynamics in the future.

**Background**

During their life cycle, plants face and adjust to different abiotic and biotic challenges. Most of the deviation from optimal environmental conditions are sustained for a period causing the plant to be stressed (White 1984). Prolonged exposition to variation in resource availability associated with intrinsic changes caused by herbivory that influence nutrient acquisition and allocation can modify plant responses (Zangerl and Bazzaz 1992), resulting in some biotic and abiotic factors being considered stressors and not only episodic events (White 1984). Importantly, plants responses to stress are costly (McKey 1974, Rhoades 1979) and variation in biotic and abiotic stressors during plant development can affect the growth and defense tradeoff and also shift the balance between cost and benefits of expressing resistance and/or tolerance (e.g., Kleine and Mueller 2014). Consequently, heterogeneous abiotic and biotic conditions could cause changes in plant developmental strategies resulting in root or shoot plastic responses that best maximize their fitness. In this sense, while it is important to consider the independent effect of herbivores and environmental conditions on root and shoot growth and defense traits, understanding their interactive effects is crucial to predicting how plants perform under these changing conditions.

Herbivores can strongly affect plant survivorship, physiology, growth, and reproduction (see Trlica and Rittenhouse 1993, Karban and Agrawal 2002), as can stressful environmental conditions such as excess heat, cold, or drought (Chapin et al. 1987, Kozlowski and Pallardy 2002, Niinemets 2010). For instance, plants exposed to
herbivores may respond with rapid growth (Mundim et al. 2016), shift biomass to belowground structures, or increase production of defensive chemistry (Karban and Myers 1989, Karban and Agrawal 2002); similarly, drought or cold-stressed plants may shed shoots and leaves (Bruna et al. 2002, Breda et al. 2006) or produce lateral roots, close stomata and reduce turgor pressure to decrease stress and prevent further damage (Meier et al. 1992, Chaves et al. 2003, Kempel et al. 2011). Despite the fact that plants are simultaneously exposed to both biotic and abiotic stress, however, experiments assessing the responses of plants to both biotic and abiotic stress are surprisingly rare. Consequently, it remains unclear if biotic and abiotic stresses on plants act independently, additively, or synergistically.

However, the few experimental studies quantifying plant responses to both herbivory and abiotic stress have shown that plant responses can vary among species and as a result of the timing, nature, and severity of each stress (see Niinemets 2010, Bansal et al. 2013, Trowbridge et al. 2014, Ryalls et al. 2016). Most studies to date have evaluated plant responses in either leaves (e.g., Niinemets 2010, Bansal et al. 2013) or roots and other belowground structures (see Zvereva and Kozlov 2012, Erb and Lu 2013, Kleine and Mueller 2014), despite the fact that the above and belowground parts of a plant do not respond to abiotic and biotic stress in isolation. In fact, the signaling pathways responsible for plant responses to different stresses can actually interfere with each other, resulting in distinct responses in leaves and roots (Lu et al. 2016, Ryalls et al. 2016, Johnson et al. 2016). For example, infection by nematodes can exacerbate the effects of abiotic stress on plants, with root parasitism severely disrupting plant water relations that affect whole-plant growth, leaf water
potential, stomatal conductance, and root development (e.g., Haverkort et al. 1991, Smit and Vamerali 1998, Audebert et al. 2000). Results such as these underscore the need for experiments elucidating both above and belowground responses as a necessary element of building a more robust theory for how plants respond to environmental stress.

In addition to the natural environmental variation, plants also face physiological stress resulting from anthropogenic climate change. Although multiple components of climate change agents are likely to have substantial effects on plants and their interactions with herbivores, to date most experimental studies addressing this issue have focused on the potential effects of increases in CO$_2$ (Mundim and Bruna 2016). Indeed, few studies have integrated abiotic factors associated with climate change (i.e., precipitation, temperature, CO$_2$) and biotic factors (i.e., above and belowground herbivores) to investigate their combined effects on plant responses in each plant compartment and as a whole (but see, Ryalls et al. 2013, 2016). Based on that, the increased level of defensive compounds in plant tissues predicted to occur under different climate change scenarios (Robinson et al. 2012) are expected to have a disproportionate effect on herbivores feeding above or belowground (Scherber et al. 2013), as defense compounds may be at higher concentrations in leaves or roots. However, to our knowledge only one study has manipulated precipitation patterns to test how the interaction of above and belowground herbivory altered plant biomass and herbivore abundance and performance under environmental stress (Ryalls et al. 2016). Linking herbivore and environmental stress variation is necessary to provide a more
holistic understanding of the mechanisms underpinning plant responses to combined stress factors.

Our study was designed to investigate if roots and shoots are equally valuable to the plant and if their investment in growth and defense depends on abiotic and biotic drivers of plant responses within a resource availability and optimal-defense context. Building upon a previous study of seedlings of the tropical shrub *Solanum lycocarpum* (Solanaceae) revealing a cross-response pattern of plant growth and chemical defenses to above (AG) and belowground (BG) herbivory (Mundim et al. 2016), we measure seedling responses to experimental manipulations of a key form of environmental stress – variation in water availability – in addition to AG and BG herbivory. To do so we addressed the following questions: (1) How changes in water availability and herbivory affect plant survival? (2) How changes in water availability and herbivory affect plant growth and defenses? (3) Are *Solanum lycocarpum* seedling responses to changes in water availability and herbivory specific to a plant-part or similar between above and belowground? Overall, our goal is to reveal the underlying mechanisms of how two of the most common and detrimental stressors to tropical species impact performance and development of establishing seedlings.

**Methods**

**Study System**

*Solanum lycocarpum* (Solanaceae) is a perennial, deciduous, pioneer shrub commonly found in more open physiognomies and disturbed areas of the South American savanna woodlands known as the Cerrado (Lorenzi 2002). It is commonly known as “lobeira” or wolf’s apple because its large fruits are central to the diet of the
maned-wolf, *Chrysocyon brachyurus* (Dietz 1984). As in many other *Solanum* species, the aboveground parts of *S. lycocarpum* are frequently attacked by generalist herbivores such as Armyworm larvae (*Spodoptera* spp.). The roots of *S. lycocarpum* are damaged by the root-knot nematode *Meloidogyne incognita*, an endogenous phytophagous nematode that attacks a broad spectrum of plant species (Shurtleff and Averre 2005). The second-stage juveniles (J2) of *M. incognita* are the infective stage. They migrate through the soil and burrow into roots in which they feed, develop, and reproduce; the adult females ultimately form large galls on roots (Shurtleff and Averre 2005). The leaves of *S. lycocarpum* are protected by trichomes and thorns (Lorenzi 2002), while roots, leaves and fruits are protected by terpenoids, alkaloids, and other chemical compounds (e.g., Dall’Agnol and von Poser 2000, Sá et al. 2000).

**Experimental Design**

On August 2013 we collected fruits from 10 *Solanum lycocarpum* and separated the seeds from the fruit pulp under running water. The seeds were then placed in a shadehouse in germination trays filled with a 1:3 ratio of sand and soil collected from an area of Cerrado habitat at the Universidade Federal de Uberlândia’s Panga Ecological Station (PES, 19°10’S, 48°24’W). Before germination, soil cores were inspected for the presence of phytophagous nematodes, and only samples without such type of nematodes were used in the experiment. In September 2013, n = 328 seedlings with fully expanded cotyledons (mean total leaf area ± SE: 2.27 ± 0.069 cm²) were transplanted into 12 cm diameter x 20 cm tall seedling propagation bags containing the same ratio of sand and soil from PES. We then randomly assigned n = 45 seedlings to each of three precipitation treatments and arranged them at random in the shadehouse.
The water treatments were based on the wet season rainfall for the past 10 years in the region (Table 4-1; Appendix A) and two alternative IPCC projections for the Cerrado: either an increase or decrease of ~30% (Boulanger et al. 2007, Christensen et al. 2007). The three treatments were (1): historical average levels of precipitation, ca. 200 ml of water 48 h⁻¹ (Average), (2) increased precipitation, ca. 130 ml of water 24 h⁻¹ (iH₂O), and (3) decreased precipitation, ca. 200 ml of water 96 h⁻¹ (dH₂O) (see the calculation in Appendix A). Since the shadehouse allowed natural ambient rain, the treatments were daily readjusted so the plants did not receive more water than the treatments allowed (see further explanations in Appendix A). Seedlings were grown under the water treatment for approximately 170 days, at which time we randomly assigned n = 15-20 plants from each water treatment to one of four herbivory treatments and again rearranged them at random in the shadehouse. The four herbivory treatments were aboveground herbivory (AG), belowground herbivory (BG), both above and belowground herbivory (AGBG), or no herbivory (control).

In the beginning of February 2014 we measured the number of leaves and total leaf area of all surviving plants. On 20 February 2014 we then inoculated the plants receiving BG herbivory (i.e., plants in the BG and AGBG treatments) with 10 ml of water in which we had diluted ca. 5000 *M. incognita* eggs; this solution was delivered to three points around the base of the plant with a pipette (see Bonetti and Ferraz 1981). This number of eggs was selected because it would result in a nematode density comparable to that in field (Mundim et al. 2016). Because inoculation of roots by nematodes takes ~ 10 days, we waited until 2 March 2014 to apply the AG herbivory to plants in the AG and AGBG treatments; doing so ensured plants were exposed to
above and belowground herbivory for the same amount of time (Mundim et al. 2016). In order to inflict damage capable to provoke a plant response and constant production of defense without causing major loss of aboveground biomass, the AG herbivory treatment was applied every 10 days. It consisted of placing one *Spodoptera* larvae (second instar or later) on a leaf until it consumed 10% of total leaf area. Plants in the control treatment were not exposed to any leaf or root herbivores. For the next 130 days plants continued to receive their respective precipitation treatments and were rotated weekly to random positions in the shadehouse; we surveyed plants daily to record the mortality of any individuals.

After 300 days of exposure to precipitation variation and herbivore treatments we re-measured the length and diameter of each surviving plant’s stem and the number of leaves it had. We then removed all surviving plants from the soil, washed them, and measured the total length of roots and root complexity. We did not measure root length and complexity of plants prior to applying treatments because this would require uprooting them, which could damage them, induce plant defenses, or result in plant death. To estimate pre-treatment root length and complexity we used *n* = 15 plants set aside at the time experimental seedlings were selected for the precipitation treatments and used the average of their values as the initial ones for all experimental plants. We used the software package ImageJ (Rasband 1997) to calculate the area of individual leaves and total root lengths, and the centripetal link based ordering system to quantify the topological complexity of the root system (see Fitter 1986, 1987, Berntson 1997). Finally, we separated the leaves, stems, and roots and dried them at 60ºC for 2 days,
after which we weighed, and stored them separately in paper bags for subsequent chemical analyses.

We calculated the relative growth rates (RGR) for all plants based on total leaf area and total root length using the formula $RGR = (\ln L2 - \ln L1)/(t2-t1)$, where $L1$ is the initial total leaf area or total root length, $L2$ is the final total leaf area or root length, and $t2-t1$ is the length of the treatment or experiment in days. We also used the dry biomass of leaves, and roots at the end of the experiment to calculate the relative allocation of biomass (i.e., the Root:Leaf ratio).

Our preliminary analyses with gas chromatography/mass spectrometry (GC/MS) found most treatment-dependent variation was in an area of the chromatogram consisting primarily of sesquiterpenes. We therefore used the concentration of root and leaf sesquiterpenes as a metric of plant chemical responses to herbivory in the different parts of the plant. We used an Agilent 7890A gas chromatogram (GC) combined with a G7001B mass spectrometry (MS) in electron impact and single quad mode for the GC/MS analyses. For each sample all peaks within the retention time range of 15-21 min were integrated to give their area after values similar to the blank-solvent and values from background compounds were eliminated. Finally, we calculated total sesquiterpenes concentration by summing all the normalized peak values for each plant sample. For further details regarding the chemical analysis see Mundim et al. (2016).

**Statistical Analyses**

We investigated the effects of water, herbivory type and their interaction on the survivorship of *Solanum lycocarpum* seedlings using a Weibull regression model. For each treatment the survivorship (0 or 1) at N observation days was calculated using the
survreg function from the survival package. To determine how the experimental treatments influenced total biomass and terpenoid concentrations in above and belowground plant parts we used Linear Mixed Models (LMMs). The response variables were terpenoid concentration, and biomass (dry weight). The main effects were water treatment (i.e., average, iH$_2$O, dH$_2$O), herbivore treatment (i.e., AG herbivory, BG herbivory, AGBG herbivory, control), plant part (i.e., leaves, roots), and their interaction. The random effects attributable to variation among individual seedlings were included by treating each plant as a split-plot, with herbivory type as the whole-plot treatment and the location of measurements (i.e., roots, leaves) and precipitation treatments as the within-plot treatment. We also used LMMs with this split-plot structure to test the effects of water, herbivory and their interaction on stem length and diameter, leaf number, total leaf area, $\text{RGR}_{\text{leaf area}}$, $\text{RGR}_{\text{root length}}$, root complexity, plant total biomass, and plant total terpenoids. We again treated each plant as a split-plot, with herbivory type as the whole-plot treatment and the water treatments as the within-plot treatment. For all Linear Mixed Models we used the nlme (Pinheiro et al. 2016) and lme4 (Bates et al. 2015) packages. We used the glht function in package multcomp (Hothorn et al. 2008) for post-hoc Tukey’s tests. We conducted all analyses using the R statistical programming language (v3.2.4; R Core Team 2016). Data are available from the Dryad Digital Repository (see Data Availability).

**Results**

**Survival**

A total of 50.6% of the *Solanum lycocarpum* seedlings died during the experiments 300 days. Mortality differed significantly among water treatments, however,
with plants in the treatment decreased in water having significantly lower survivorship than those in the ones simulating average and increased water ($\chi^2 = 67.02$, $df = 2$, $P < 0.001$), with most mortality in the first 35 days of the experiment (Figure 4-1). There was also a significant interaction between herbivory and water ($\chi^2 = 962.87$, $df = 14$, $P < 0.001$). Furthermore, for plants that survive the first 170 days at the water treatment, the co-occurrence between dH$_2$O and BG herbivory resulted in a 70% decrease in survival.

**Growth**

After growing under simulated variation in water but prior to the application of herbivory treatments (170 days), seedlings exposed to simulated decreased water had no change in growth compared to the average treatment, while those under the increased water treatment had significantly more leaves, greater total leaf area, and greater RGR$_{leaf}$ area than plants in the other treatments (leaf number 1.6 times greater: deviance $\chi^2 = 49.627$, $df = 2$, $P < 0.0001$; leaf area 1.7 times greater, deviance $\chi^2 = 11808$, $df = 2$, $P < 0.0001$; RGR$_{leaf}$ area 1.13 times greater, deviance $\chi^2 = 13.909 e^{-5}$, $df = 2$, $P = 0.005$). After 130 days of herbivory there were also significant differences in growth between plants in the different treatments. However, these differences were no longer attributable solely to the water treatments (Table 4-2). In some cases, herbivory was the sole factor driving these differences – seedlings in treatment AG herbivory had lower stem diameter but greater number of leaves than those in the other three herbivory treatments (see Table 4-3). In the case of stem length, final leaf area, and RGR$_{leaf}$ area, however, herbivory acted in concert with changes in water (Table 4-2 and Table 4-3). The interactive effects of water and herbivory on growth were more complex than those on survivorship. Decreased water and simultaneous AG & BG herbivory
resulted in greater final leaf area and RGR\textsubscript{leaf area}. However, leaf growth was significantly lower in seedlings exposed to decreased water and belowground herbivory than for seedlings in other treatments (Table 4-2 and Table 4-3).

In contrast to the effects on leaf growth, there were no significant effects of water, herbivory or the co-occurrence effect between water and herbivory on final root length (range: 52.6-736.1 cm) or RGR\textsubscript{root length} (0.0049-0.015 cm/day; Table 4-2 and Table 4-3). However, root complexity – which represents the investment in secondary roots for absorption – was 1.37 times greater in plants exposed to BG herbivory or simultaneous AG & BG herbivory than in seedlings challenged with only AG herbivores (Table 4-3).

Although the co-occurrence between water and herbivory had a marginally significant effect at total biomass (see Table 4-2), only herbivory had an effect on final plant biomass (Table 4-2), with seedlings exposed to simultaneous AG & BG herbivory having the highest final biomass (Figure 4-2A-C). Belowground herbivory resulted in significantly greater allocation of biomass to roots than AG herbivory, both in isolation and in combination with decreased water (Table 4-3 and Figure 4-2B). In general, however, the allocation of biomass investment to above vs. belowground plant parts was idiosyncratic, showing no pattern (see Table 4-4).

**Defense**

At the end of the experiment (300 days) the total terpenoid concentration in *Solannum lycocarpum* seedlings ranged from 221.03 to 48,495.17 µmol/g(dw). Water and herbivory had no significant main effects on total terpenoid concentration (Table 4-2), although there were some significant differences in some treatment combinations (e.g., decreases in total terpenoids in the dH\textsubscript{2}O*AG and iH\textsubscript{2}O*BG combinations; Figure
4-3A and 4-3C). However, terpenoids concentrations did differ significantly among roots and leaves. Roots contained twice as many sesquiterpenoids as leaves did (mean ± SE: 5490.5 ± 717.3 µmol/g(dw) vs. 2459.7 ± 304.04 µmol/g(dw); Figure 4-3D-F; Table 4-4; Table 4-3), which the non-significant correlation between an individual seedling’s leaf and root concentrations (df = 122, R = 0.24, P = 0.008) suggests is due to differential allocation rather than inter-seedling variation. Furthermore, there was a significant increase in root and leaf terpenoids resulting from the co-occurrence between water and herbivory (Table 4-2), yet the inter-treatment difference in the water*herbivory*plant.part term suggests that this effect may be different between leaves and roots. For instance, seedlings in the increase water and aboveground herbivory combined treatment had a leaf terpenoid concentration 2.07 times greater than those with only iH₂O or AG herbivory. Similarly, a decrease in water coupled with belowground herbivory resulted in root terpenoid concentrations 5.8 times lower than due to the main effects of water or herbivory (Table 4-3; Figure 4-4D-F).

**Discussion**

Despite extensive research on the potential effects of herbivory and/or abiotic resources availability on plant responses, most studies have focused on one driver/response variable, which unfortunately limits our understanding of how multiple factors simultaneously affect these plant characteristics. Few studies have tested plant responses to simultaneous biotic and abiotic stressors (Niinemets 2010, Bansal et al. 2013, Trowbridge et al. 2014, Ryalls et al. 2016), but to our knowledge this is the first experimental manipulation attempting to disentangle plant responses to predicted changes in water availability and aboveground and belowground herbivory to show their
isolated and simultaneous effects on plant survival and allocation to biomass and terpenoid defenses. We also evaluated both leaf and root responses to aboveground (AG) herbivory, belowground (BG) herbivory and their simultaneous attacks (AGBG). Our results demonstrate that drought can severely affect plant survivorship and the effect is exacerbated by BG herbivory. Further, herbivory has a greater effect on plant growth than variation in water, and the responses caused by AG and BG herbivores were distinct, making the impacts of belowground herbivores unique and context-dependent. Finally, while water and herbivory can significantly alter plant defensive chemistry, the changes are not consistent in direction or magnitude. Thus, our results underscore that although the impacts of water, herbivory and their co-occurrence caused different and unpredicted responses to survival, growth and defense for a tropical species, the responses also depend on the attacked plant part and herbivory type.

**Decreased Water Drastically Reduces Seedling Survivorship; This Is Further Exacerbated by Belowground Herbivory**

Our experimental decrease in water of 30% reduced seedling survival by more than 50%, with most individuals that survived reaching the wilting point. Changes in rainfall are frequently associated with changes in temperature (Boulanger et al. 2007, IPCC 2014), and we indeed observed an association between higher temperature and changes in precipitation during our study period (2013-2014; see Table 4-1). Such association can result in higher evapotranspiration rate, which could have been the cause of mortality of seedlings in the average and increased water treatments. These results emphasize how variable plant survival and growth may be in face of variability in
precipitation regimes and temperature due to climate changes. Nevertheless, after the beginning of the herbivory treatments, seedling survivorship was further impaired by BG herbivory. Although nematode foraging, like many other belowground herbivores, is compromised by decreased soil water availability (Kardol et al. 2010), our results suggest that the nematodes attacking roots promoted further decreases in plant water and nutrient uptake which are vital for seedling survivorship and growth. Thus, changes in precipitation can interact with changes in temperature causing seedling mortality. More importantly, lower soil water availability can lead to a significant negative effect of BG herbivory on plants.

**Herbivory Has a Greater Effect on Plant Growth Responses than Changes in Precipitation; However, Responses to AG and BG Herbivores Were Highly Idiosyncratic**

Once herbivory treatments started (after 170 days) there were no detectable effects of water treatments as there had been before herbivory treatments. Although, water and herbivory co-occurring together have a significant effect on some growth parameters, the effects caused by AG or BG herbivory overcome that of the variation in water (see Table 4-2). Despite previous studies suggesting that leaf growth parameters are sharply reduced when both stressors co-occurred (see Bansal et al. 2013), our experiment shows that herbivory has a dominant role in affecting leaf growth parameters but not root growth (see Table 4-2). Since herbivory AG and BG have a positive effect on leaf and root growth (Mundim et al. 2016), it is safe to assume that although water did not have an effect on root growth parameters it caused a reduction of the herbivory effect. This reduction was expected since soil water availability is very
important for the establishment, growth and survivorship of roots and BG herbivores (van Dam 2009, Kardol et al. 2010). In fact, most Cerrado plant species use the growing stage to produce as many and deep roots to avoid future soil drought caused by the strong seasonality (Oliveira and Marquis 2002). Furthermore, although the effects of water variation are not directly shown in leaf and root growth responses, they affected AG and BG herbivores in different ways causing a distinctive response from each plant-part.

**While Precipitation and Herbivory Can Significantly Alter Plant Defensive Chemistry, These Changes Are Not Consistent in Direction or Magnitude**

Interestingly, some treatment combinations (e.g., dH$_2$O$*$AG herbivory, iH$_2$O $*$BG herbivory; Table 4-3) acted in synergy to result in lower leaf terpenoid concentrations while others (e.g., dH$_2$O $*$BG herbivory, iH$_2$O $*$AG herbivory; Table 4-3) increased it. Such results suggest that changes in plant defensive chemistry caused by water and herbivory co-occurrence are not consistent in direction or magnitude, but dependent on the specific combination of stressors and in which part of the plant they occur. In this sense, seedling roots and leaves could cope with the impacts of multiple stressors. To date very few studies have empirically tested the co-occurrence of water and herbivory stress on plant performance and secondary chemical responses (see Niinemets 2010, Bansal et al. 2013, Trowbridge et al. 2014, Ryalls et al. 2016). Contrary to the results for growth parameters, the production of terpenoid compounds in our study was more strongly influenced by the water acting in concert with herbivory than each of these stressors independently (see Table 4-2). This synergy was unexpected; drought and herbivory have opposite effects on plant carbon budgets – herbivory tends to increase
plant carbon demands while drought stress decreases carbon gain – that should decrease carbon-based defenses (see Bansal et al. 2013, Trowbridge et al. 2014). Instead these results may be explained by the previous exposure of plants to the water treatments; water and herbivory trigger very similar hormone cascades in plants as a response to prevent further stresses (Fujita et al. 2006). If so, the exposure of seedlings to experimental variation in water could have initiated or facilitated the hormone cascade, essentially priming the process for increases in terpenoids once experimental herbivory began. This would allow the seedling to be protected against further attacks while still responding and adjusting to the variation in water. These unpredictable outcomes of the production of terpenoid compounds demonstrate that the effects of multiple environmental stressors on plant defenses cannot be predicted based on studies in which stressors are evaluated independently.

**The Impacts of Belowground Herbivores Are Unique and Context-Dependent**

Although there is evidence that belowground herbivores can induce more defensive responses in leaves than aboveground herbivores (Mundim et al. 2016), the core predictions about how plants should respond to BG attacks have emerged from studies evaluating responses to foliar herbivory (see van Dam 2009, Johnson et al. 2016). Our results further emphasize that interpreting BG interactions based on theoretical framework derived from AG interactions can lead to incorrect predictions. First, the outcome of BG plant-herbivore interactions was context-dependent in ways that the outcome of AG interactions was not – decreased water and BG herbivory reduced plant survival by 70%, while AG or AGBG herbivory did not alter survival in any water treatment. This reveals that the effects of BG herbivory can be more important for
plant survival than AG herbivory. Second, once nematodes were unable to absorb
nutrients/water by forming galls there was proliferation of lateral roots from the main
root, which increased root complexity. This compensatory response resulted in
additional area for absorption indicating that roots are extremely plastic in their growth
patterns (Jansen et al. 2006). In fact, previous studies have shown that despite roots
and shoots producing the same hormones to regulate growth, roots have different
regulatory networks to control tissue growth (Acosta et al. 2013). Finally, we also found
that terpenoid concentrations after herbivory were greater in roots than in leaves. Roots
and shoots have different allocation patterns, potentially due to the use of different
metabolic cascades for their production (see Heil 2009, Lu et al. 2015). Therefore, the
distinct nature of root herbivory, interactions and metabolism yield contrasting
responses compared to their leaf counterparts. These contrasting responses suggest
that rather than use leaf responses to predict roots responses, it is essential to conduct
experiments on roots – preferably targeting more than one compound or compound
class – to elucidate their differences in physiological mechanisms.

**Plant-Herbivore Interactions in a Changing World**

Global climate changes resulting from greenhouse gas emissions will drastically
affect temperature and precipitation in the tropics (see Parmesan and Yohe 2003,
Parmesan 2006, Boulanger et al. 2007). However, the extent of the effects of increased
CO₂ levels on plants, herbivores and their interactions is still being debated (Mundim
and Bruna 2016). A framework was created with specific predictions regarding the
consequences of drought effects on plant-herbivore interactions in the tropics (see
Coley 1998), but few studies have since tested the effects of changes in the amount or
distribution of precipitation and the linkage between plants and herbivores. Our results corroborate some of these predictions, for example, that lower water availability lead to premature abscission causing a decrease in growth and survival of plants. However, the predictions do not include the aboveground vs. belowground plant parts, and in doing so it fails to determine their relative importance and which plant traits respond to the interaction between herbivory and changing precipitation. For instance, our study shows that BG herbivores act in synergy with drought to reduce plant survivorship. In addition, another prediction states that for tropical species, drought stress is associated with increased leaf susceptibility to insect herbivory without changes in carbon or nitrogen-based defenses (Coley 1998). Although our study did not show an effect of water variation on leaf and root defenses (but see Bansal et al. 2013, Ryalls et al. 2016), the combination of water and herbivory significantly affected leaf and root terpenoid concentration. This indicates that assessing multiple stressors in combination and their relative intensity is fundamental to determine their overall effect on plant performance. It also implies that anthropogenic disturbances such as climate change in combination with other environmental drivers could affect above and belowground herbivore outbreaks, plant establishment and growth, plant-herbivore interactions and community dynamics. Thus, the study of water variation based on predicted climate change models can help advance theoretical and empirical knowledge about above and belowground plant-herbivore interactions and responses to these changes.

Concluding Remarks

Our study is the first experimental manipulation attempting to disentangle plant responses to predicted changes in precipitation, aboveground and belowground
herbivory. Consequently, our conclusions come with the caveat that whether context-dependent responses are the exception or the rule requires similar experiments conducted in other systems. Another caveat is that while we used a single species of AG and BG herbivore in our experiments, under natural conditions plants are often attacked by more than one species of AG and BG herbivore at a time (Strauss 1991, Thompson 1998). Herbivory by one species can induce different plant metabolic pathways than simultaneous herbivory by multiple ones (e.g., Pierre et al. 2011). One herbivore may drive a stronger or faster activation of plant signaling pathways than the other, masking the responses to simultaneous herbivory. Similarly, the order of herbivory can matter as well, as the type and order of herbivory may be relatively more important for plant performance than the degree of damage inflicted. Thus, we suggest future experiments should both investigate the potential for priority effects and how they vary with changes in environmental conditions. Ultimately, our study highlights that future work should investigate the effects of multiple stressors in field conditions to determine the importance of anthropogenic changes in simultaneous abiotic and biotic stressors for plant-herbivore interactions.
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<td>24.08</td>
<td>-</td>
</tr>
<tr>
<td>February</td>
<td>8.07</td>
<td>24.31</td>
<td>-</td>
</tr>
<tr>
<td>March</td>
<td>8.19</td>
<td>23.99</td>
<td>-</td>
</tr>
<tr>
<td>April</td>
<td>4.29</td>
<td>22.98</td>
<td>-</td>
</tr>
<tr>
<td>May</td>
<td>1.72</td>
<td>20.13</td>
<td>-</td>
</tr>
<tr>
<td>June</td>
<td>0.77</td>
<td>19.35</td>
<td>-</td>
</tr>
<tr>
<td>July</td>
<td>0.29</td>
<td>19.41</td>
<td>-</td>
</tr>
<tr>
<td>August</td>
<td>0.35</td>
<td>21.43</td>
<td>0.00</td>
</tr>
<tr>
<td>September</td>
<td>1.48</td>
<td>23.89</td>
<td>2.31</td>
</tr>
<tr>
<td>October</td>
<td>4.56</td>
<td>24.59</td>
<td>4.52</td>
</tr>
<tr>
<td>November</td>
<td>6.55</td>
<td>24.03</td>
<td>6.35</td>
</tr>
<tr>
<td>December</td>
<td>9.75</td>
<td>24.12</td>
<td>7.25</td>
</tr>
</tbody>
</table>
Table 4-2. *Solanum lycocarpum* general growth responses to water and herbivory treatments from linear mixed models. For each response variable three models were tested (water alone, herbivory alone, and then the full model with the interaction water*herbivory), and the table reports the result from the full model for each response variable. The symbol (+) indicates which of those models is the best fit.

<table>
<thead>
<tr>
<th>Response variables</th>
<th>Water</th>
<th>Herbivory</th>
<th>Water*Herbivory</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dF</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Stem length</td>
<td>2,72</td>
<td>0.627</td>
<td>0.537</td>
</tr>
<tr>
<td>Stem diameter</td>
<td>2,72</td>
<td>0.112</td>
<td>0.895</td>
</tr>
<tr>
<td>N of leaves</td>
<td>2,72</td>
<td>0.431</td>
<td>0.651</td>
</tr>
<tr>
<td>Total leaf area</td>
<td>2,72</td>
<td>1.271</td>
<td>0.287</td>
</tr>
<tr>
<td>Leaves RGR</td>
<td>2,72</td>
<td>1.257</td>
<td>0.291</td>
</tr>
<tr>
<td>Total root length</td>
<td>2,72</td>
<td>1.872</td>
<td>0.161</td>
</tr>
<tr>
<td>Root complexity</td>
<td>2,72</td>
<td>2.208</td>
<td>0.117</td>
</tr>
<tr>
<td>Roots RGR</td>
<td>2,72</td>
<td>2.23+</td>
<td>0.115</td>
</tr>
<tr>
<td>Total Biomass</td>
<td>2,72</td>
<td>2.319</td>
<td>0.106</td>
</tr>
<tr>
<td>Total Terpenoids</td>
<td>2,72</td>
<td>0.47</td>
<td>0.627</td>
</tr>
</tbody>
</table>

+ model with the lowest AIC
Table 4-3. Measurements of the stem, leaf and root parameters after 300 days of experimentation.

<table>
<thead>
<tr>
<th>Treatments (mean±SE)</th>
<th>Stem parameters</th>
<th>Leaf parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stem length (cm)</td>
<td>Stem diameter (mm)</td>
</tr>
<tr>
<td><strong>Precipitation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>15.93±1.07</td>
<td>2.53±0.1</td>
</tr>
<tr>
<td>dH₂O</td>
<td>15.57±1.25</td>
<td>2.47±0.09</td>
</tr>
<tr>
<td>iH₂O</td>
<td>14.62±1.19</td>
<td>2.51±0.12</td>
</tr>
<tr>
<td><strong>Herbivory</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>14.74±1.21</td>
<td>2.39±0.11</td>
</tr>
<tr>
<td>AG</td>
<td>10.94±1.24</td>
<td>2.19±0.13</td>
</tr>
<tr>
<td>BG</td>
<td>14.96±1.32</td>
<td>2.54±0.09</td>
</tr>
<tr>
<td>AGBG</td>
<td>20.69±0.97</td>
<td>2.39±0.11</td>
</tr>
<tr>
<td><strong>Interaction</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average *Control</td>
<td>16.93±2.02</td>
<td>2.45±0.19</td>
</tr>
<tr>
<td>Average *AG</td>
<td>11.15±2.47</td>
<td>2.19±0.25</td>
</tr>
<tr>
<td>Average *BG</td>
<td>14.94±1.54</td>
<td>2.6±0.11</td>
</tr>
<tr>
<td>Average *AGBG</td>
<td>20.20±1.41</td>
<td>2.39±0.11</td>
</tr>
<tr>
<td>dH₂O *Control</td>
<td>10.78±1.68</td>
<td>2.11±0.09</td>
</tr>
<tr>
<td>dH₂O *AG</td>
<td>12.73±2.1</td>
<td>2.36±0.18</td>
</tr>
<tr>
<td>dH₂O *BG</td>
<td>17.1±3.66</td>
<td>2.54±0.33</td>
</tr>
<tr>
<td>dH₂O *AGBG</td>
<td>23.54±1.1</td>
<td>3±0.13</td>
</tr>
<tr>
<td>iH₂O *Control</td>
<td>16.65±2.28</td>
<td>2.63±0.27</td>
</tr>
<tr>
<td>iH₂O *AG</td>
<td>8.72±1.74</td>
<td>2±0.26</td>
</tr>
<tr>
<td>iH₂O *BG</td>
<td>13.78±2.39</td>
<td>2.49±0.13</td>
</tr>
<tr>
<td>iH₂O *AGBG</td>
<td>18.05±2.12</td>
<td>2.82±0.14</td>
</tr>
</tbody>
</table>
For Root complexity we used the centripetal link based ordering system (see Berntson 1997). In this system each root segment is assigned an order of one. The complexity of the root system is equal to the number of segments in the system.

<table>
<thead>
<tr>
<th>Treatments (mean±SE)</th>
<th>Total final root length (cm)</th>
<th>¹Root complexity</th>
<th>Final Root-RGR (cm/day)</th>
<th>Biomass (g (dw))</th>
<th>Terpenoid concentration (µmol/g(dw))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Precipitation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>287.36±16.72</td>
<td>39.5±3.78</td>
<td>0.0102±0.0003</td>
<td>0.589±0.06</td>
<td>5948.02±1667.1</td>
</tr>
<tr>
<td>dH₂O</td>
<td>239.14±23.05</td>
<td>31.88±3.74</td>
<td>0.0094±0.0004</td>
<td>0.392±0.056</td>
<td>5382.88±922.67</td>
</tr>
<tr>
<td>iH₂O</td>
<td>273.87±19.35</td>
<td>38.74±3.23</td>
<td>0.0099±0.0003</td>
<td>0.529±0.066</td>
<td>5115.76±951.75</td>
</tr>
<tr>
<td><strong>Herbivory</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>248.85±19.11</td>
<td>34.23±2.36</td>
<td>0.0092±0.0002</td>
<td>0.53±0.069</td>
<td>5127.16±894.14</td>
</tr>
<tr>
<td>AG</td>
<td>243.32±20.33</td>
<td>16.16±2.08</td>
<td>0.01±0.0005</td>
<td>0.258±0.058</td>
<td>8346.97±2461.1</td>
</tr>
<tr>
<td>BG</td>
<td>283.97±31.57</td>
<td>47.07±4.91</td>
<td>0.0104±0.0005</td>
<td>0.49±0.051</td>
<td>3008.64±1022</td>
</tr>
<tr>
<td>AGBG</td>
<td>301.95±22.84</td>
<td>51.85±4.54</td>
<td>0.0101±0.0003</td>
<td>0.717±0.069</td>
<td>5140.36±869.1</td>
</tr>
<tr>
<td><strong>Interaction</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average *Control</td>
<td>267.84±21.75</td>
<td>36.75±4.09</td>
<td>0.0094±0.0003</td>
<td>0.645±0.1</td>
<td>2510.49±772.56</td>
</tr>
<tr>
<td>Average *AG</td>
<td>244.14±35.87</td>
<td>18.1±4.11</td>
<td>0.01±0.0007</td>
<td>0.239±0.088</td>
<td>14686.5±6152.5</td>
</tr>
<tr>
<td>Average *BG</td>
<td>320.92±47.39</td>
<td>49.3±9.23</td>
<td>0.0114±0.0006</td>
<td>0.609±0.108</td>
<td>6115.79±2276.3</td>
</tr>
<tr>
<td>Average *AGBG</td>
<td>327.7±31.8</td>
<td>55.9±8.71</td>
<td>0.0106±0.0004</td>
<td>0.844±0.115</td>
<td>1871.03±623.58</td>
</tr>
<tr>
<td>dH₂O *Control</td>
<td>180.22±16.64</td>
<td>26.29±2.49</td>
<td>0.0086±0.0003</td>
<td>0.244±0.032</td>
<td>8035.39±1853.7</td>
</tr>
<tr>
<td>dH₂O *AG</td>
<td>212.47±41.35</td>
<td>14.4±3.83</td>
<td>0.01±0.0011</td>
<td>0.355±0.117</td>
<td>2097.33±575.06</td>
</tr>
<tr>
<td>dH₂O *BG</td>
<td>255.04±93.94</td>
<td>38.6±9.39</td>
<td>0.0094±0.0012</td>
<td>0.388±0.082</td>
<td>516.82±70.71</td>
</tr>
<tr>
<td>dH₂O *AGBG</td>
<td>331.14±52.2</td>
<td>51.82±9.27</td>
<td>0.0102±0.0007</td>
<td>0.616±0.149</td>
<td>7205.65±1682.6</td>
</tr>
<tr>
<td>iH₂O *Control</td>
<td>302.32±47.65</td>
<td>40.08±4.75</td>
<td>0.0097±0.0004</td>
<td>0.713±0.159</td>
<td>4813.19±1522.5</td>
</tr>
<tr>
<td>iH₂O *AG</td>
<td>276.68±25.41</td>
<td>15.94±2.88</td>
<td>0.0105±0.0006</td>
<td>0.171±0.095</td>
<td>8247.06±3093.7</td>
</tr>
<tr>
<td>iH₂O *BG</td>
<td>263.08±45.26</td>
<td>49.5±7.32</td>
<td>0.01±0.0008</td>
<td>0.429±0.041</td>
<td>1285.84±385.3</td>
</tr>
<tr>
<td>iH₂O *AGBG</td>
<td>244.08±22.39</td>
<td>47.85±5.38</td>
<td>0.0095±0.0003</td>
<td>0.7±0.077</td>
<td>6137.86±1461.6</td>
</tr>
</tbody>
</table>

¹For Root complexity we used the centripetal link based ordering system (see Berntson 1997). In this system each root segment is assigned an order of one. The complexity of the root system is equal to the number of segments in the system.
Table 4-4. Biomass (g dw) and terpenoid concentration (µmol/g dw) responses to water, herbivory and the interactive treatments from linear mixed models. Since biomass and terpenoid concentration had measured for leaf and roots independently, the location of measurement was also considered as within-plot treatment. Seven models were tested and the table reports the result from the full model. The symbol (+) indicates which of those models is the best fit.

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biomass</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>1, 184</td>
<td>250.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Water treatments</td>
<td>2, 184</td>
<td>3.256</td>
<td>0.041</td>
</tr>
<tr>
<td>Herbivory treatments</td>
<td>3, 27</td>
<td>6.269</td>
<td>0.002</td>
</tr>
<tr>
<td>Plant.part</td>
<td>1, 184</td>
<td>131.713</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Water*Herbivory</td>
<td>6, 184</td>
<td>2.87</td>
<td>0.011</td>
</tr>
<tr>
<td>Water*Plant.part</td>
<td>2, 184</td>
<td>3.838</td>
<td>0.023</td>
</tr>
<tr>
<td>Herbivory*Plant.part</td>
<td>3, 184</td>
<td>7.049†</td>
<td>0.0002</td>
</tr>
<tr>
<td>Water<em>Herbivory</em>Plant.part</td>
<td>6, 184</td>
<td>1.45</td>
<td>0.198</td>
</tr>
</tbody>
</table>

<table>
<thead>
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<th></th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Terpenoid concentration</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>1, 184</td>
<td>104.648</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Water treatments</td>
<td>2, 184</td>
<td>0.556</td>
<td>0.575</td>
</tr>
<tr>
<td>Herbivory treatments</td>
<td>3, 27</td>
<td>1.765</td>
<td>0.178</td>
</tr>
<tr>
<td>Plant.part</td>
<td>1, 184</td>
<td>18.698</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Water*Herbivory</td>
<td>6, 184</td>
<td>6.334</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Water*Plant.part</td>
<td>2, 184</td>
<td>0.0014</td>
<td>0.998</td>
</tr>
<tr>
<td>Herbivory*Plant.part</td>
<td>3, 184</td>
<td>3.027</td>
<td>0.031</td>
</tr>
<tr>
<td>Water<em>Herbivory</em>Plant.part</td>
<td>6, 184</td>
<td>2.706†</td>
<td>0.015</td>
</tr>
</tbody>
</table>

† model with the lowest AIC
Figure 4-1. Effects of water treatments on the survivorship of *Solanum lycocarpum* seedlings. Shaded areas indicate 95% confidence intervals. The dashed line indicates the start of the herbivory treatments – after 170 days of water treatment.
Figure 4-2. *Solanum lycocarpum* seedlings Total Biomass (g dw) and Root:Leaf Biomass Ratio for plants exposed to (A and D) decreased water, (B and E) average water level and (C and F) increased water and also to aboveground herbivory (AG), belowground herbivory (BG), simultaneous herbivory (AGBG) and no-herbivory controls. The boxes represent the interquartile range, the horizontal black lines indicate the medians, whiskers extent to 25% and 75% quartiles, and outliers are indicated by dots. Different letters indicate significant differences among herbivory treatments (P < 0.05) based on post hoc Tukey’s tests.
Figure 4-3. Plant Total Terpenoid concentration (µmol/g dw) and Root:Leaf Terpenoid Ratio when plants were exposed to (A and D) decreased water, (B and E) average water level and (C and F) increased water and also to aboveground herbivory (AG), belowground herbivory (BG), simultaneous herbivory (AGBG) and no-herbivory controls. The boxes represent the interquartile range, the horizontal black lines indicate the medians, whiskers extent to 25% and 75% quartiles, and outliers are indicated by dots. Different letters indicate significant differences among herbivory treatments (P < 0.05) based on post hoc Tukey's tests.
CHAPTER 5
CONCLUSIONS

Changes in abiotic factors cause dramatic changes in plants, herbivores, and their interactions. In light of the potential ecological and economic impacts that changes in these interactions could bring, it is clear that there is an urgent need for more geographically, taxonomically, and ecologically diverse research on this fundamentally important topic. My study also shows a whole-plant perspective can provide new insights into the nature of these cascading effects, for instance on above or belowground herbivore communities and how they are influenced by heterogeneity in environmental conditions. Thus, the biotic and abiotic factors simultaneous can affect the plant interactions with herbivores, visitors and others and can alter their respective population and community dynamics, other interspecific interactions, and even ecosystem processes.
APPENDIX A
WATER TREATMENTS EXPLANATION FOR CHAPTER 4

The experiment started with the water treatments in September 2013 and was
carried out until June-July 2014, with the herbivory treatments starting in March 2014.
The water rates for the water treatments were based on the growing season of the focal
species *Solanum lycocarpum* (October to March). Thus, the water treatments were
based on the rainfall for the past 10 years (2004-2013) in the region (historical levels;
Table 4-1). The average historical precipitation and temperature values were acquired
at “Instituto Nacional de Meteorologia”, and they were collected at a station in Uberaba-
MG. The station is about 100 km from the Panga Ecological Station (PES, 19º10’S,
48º24’W) where the soils for the experiment were collected.

We calculated the water treatments as:

1mm = 1L/m²
1m² = 10000cm²
1L = 1000ml

The precipitation historical mean average between October to March is 7.97 mm per day:
7.97 mm = 7.97 L/m² = 7970ml/m² = 0.797 ml/cm²

The experimental bags were 12.4 cm of diameter
Circle area of the bags:  \( A = \pi r^2 \)
\[ A = 3.1415 \times 6.22 = 120.8 \text{ cm}^2 \]

0.797 ml – 1cm²
96.3 ml – 120.8 cm²

To do the calculations we use 100ml per day

Thus, the precipitation treatments were: (1) historical average levels of
precipitation, ca. 200 ml of water 48 h⁻¹ (*Average*), (2) Increased 30% in the
precipitation, ca. 130 ml of water 24 h⁻¹ (*iH₂O*), and (3) decreased 30% in the
precipitation, ca. 200 ml of water 96 h⁻¹ (*dH₂O*).
In addition, since the shadehouse allowed natural ambient rain, we daily measured the precipitation inside and outside of the shadehouse (see also Table 4-1 for monthly values during the experiment). If a particular day the precipitation was different from 7.97mm, the treatments were readjusted by increasing or decreasing the water that each treatment received to fulfill their rainfall requirements.
LIST OF REFERENCES


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Rasmann, S., A. C. Erwin, R. Halitschke, and A. A. Agrawal. 2011b. Direct and indirect root defences of milkweed (Asclepias syriaca): trophic cascades, trade-offs and


BIOGRAPHICAL SKETCH

Fabiane M. Mundim was born in Monte Carmelo, Minas Gerais, Brazil in 1982. She grew up on a family neighborhood as the eldest of three sisters. She attended the local middle and high schools like any other kid in their hometown. After high school, Fabiane moved to a bigger city nearby, Uberlândia, to attend the University of Uberlândia, where she received her BA in biology in 2007. Fabiane defended her BA working with phenology of Cerrado’s plants attacked by herbivores. During her Bachelor, she meet Dr. Emilio Bruna, a collaborator of her Bachelor’s advisor Dr. Heraldo Vasconcelos. At the same month of her Bachelor defense, she was accepted to the M.S. program in ecology and conservation of natural resources with a fellowship. Her work with plant-herbivore interactions in the Cerrado Biome continues, but now with a more experimental approach. During her master’s she had the opportunity to take a field work course in the Amazonia and expand her knowledge about tropical biomes. In 2008 she was invited by Dr. Bruna to visit for two months the University of Florida (UF). Her interaction with Dr. Bruna and other members of his lab provided the inspiration for her to return as a Ph.D. student. Fabiane finished her master’s degree in 2009 with Dr. Bruna as a member of her committee, and moved soon after to Gainesville. In the summer of 2010 Fabiane joined the Wildlife Ecology and Conservation Department at UF as Emilio Bruna’s Ph.D. student. She continues working with plant-herbivore interactions, but for her dissertation she expanded the scope of her research. Her dissertation comprehend above and belowground herbivores and how they affect plant growth and defenses. Fabiane finished her Ph.D. in 2016 and soon after moved to Reno, Nevada as a post-doc in chemical ecology.