South Dakota State University
Open PRAIRIE: Open Public Research Access Institutional Repository and Information Exchange

Electronic Theses and Dissertations

2018

Impacts of Altered Precipitation Frequency, Clipping, and Competition on Perennial Grasses Mediated Through Belowground Bud Bank in Northern Mixed-Grass Prairie

Surendra Bam
South Dakota State University

Recommended Citation

Bam, Surendra, "Impacts of Altered Precipitation Frequency, Clipping, and Competition on Perennial Grasses Mediated Through Belowground Bud Bank in Northern Mixed-Grass Prairie" (2018). *Electronic Theses and Dissertations*. 2702.
https://openprairie.sdstate.edu/etd/2702

IMPACTS OF ALTERED PRECIPITATION FREQUENCY, CLIPPING, AND COMPETITION ON PERENNIAL GRASSES MEDIATED THROUGH BELOWGROUND BUD BANK IN NORTHERN MIXED-GRASS PRAIRIE

BY

SURENDRA BAM

A thesis submitted in partial fulfillment of the requirements for the

Master of Science

Major in Biological Sciences

South Dakota State University

2018

IMPACTS OF ALTERED PRECIPITATION FREQUENCY, CLIPPING, AND COMPETITION ON PERENNIAL GRASSES MEDIATED THROUGH BELOWGROUND BUD BANK IN NORTHERN MIXED-GRASS PRAIRIE

This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science in Biological Sciences and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Lan Xu, Ph.D. Date
Thesis Advisor

Michele R. Dudash, Ph.D. Date
Head, Department of
Natural Resource Management

Kinchel Doerner, Ph.D. Date
Dean, Graduate School

ACKNOWLEDGEMENTS

I would like to start by thanking my major advisor Dr. Lan Xu for her great support and guidance throughout my MS. It would be hard to travel without her through the bumpy road of abroad studies and advance my academic and research career in the field of plant ecology. I am thankful to her for providing me many great opportunities in field and greenhouse research, encouragement, and avenues to share and learn. I really appreciate her great zeal of mentorship, commitment, and detail orientation of conducting research.

I would also like to thank my committee members Dr. Lora Perkins, Dr. Graham Wrightson (graduate faculty representative), and especially Dr. Jacqueline P. Ott from the U.S. Forest Service for her guidance and unlimited support during the research process. I benefited a lot from her expertise in research design, statistical analysis, and strong research experience on bud bank and plant ecology. I am greatly indebted to both Dr. Ott and Dr. Lan Xu for allowing me to be part of this emerging field of bud bank ecology.

I am also thankful to Dr. Brent Turnipseed from the South Dakota State University Seed Testing Laboratory for providing the necessary greenhouse facilities and to all my wonderful undergraduate technicians for working by my side. Thanks to the Department of Natural Resource Management for providing all the necessary support for completing my MS studies.

I am greatly indebted to my family and especially my late mother, Mahesori Bam, and my elder brother, Suvash Bam, for always understanding and supporting my dreams.

This research has been supported through the South Dakota Agriculture Experiment Station Hatch Project and a joint venture agreement (14-JV-11221632-048) between South Dakota State University and the U.S. Forest Service Rocky Mountain Research Station.

TABLE OF CONTENTS

ABBREVIATIONS....vii

LIST OF TABLES....viii

LIST OF FIGURES....xi

LIST OF PHOTOS....xiii

ABSTRACT....xiv

CHAPTER 1 1

1. Northern Great Plains Grasslands 1
2. Seed versus Vegetative Reproduction.... 2
3. Major Environmental Disturbances and Their Impacts on Belowground Bud Bank 4
   - 3.1 Impacts of Climate Change on Belowground Bud Bank 6
   - 3.2 Impacts of Grazing on Belowground Bud Bank 10
   - 3.3 Impacts of Plant Invasion on Belowground Bud Banks 12

SYNTHESIS 15

LITERATURE CITED 18

CHAPTER 2 30

IMPACTS OF ALTERED PRECIPITATION FREQUENCY AND CLIPPING ON PERENNIAL GRASSES MEDIATED THROUGH BELOWGROUND BUD BANK . 30

ABSTRACT.... 30

INTRODUCTION 32

- 1.1 Research Questions.... 35
- 1.2 Hypotheses.... 35

METHODS 36

- 2.1 Experiment Design.... 36
- 2.2 Treatment.... 37
  - 2.2.1 Precipitation Frequency 37
  - 2.2.2 Clipping Treatment 39
  - 2.2.3 Study Species.... 39
- 2.3 Seedling Establishment and Transplant 40
- 2.4 Growth Condition 40
- 2.5 Data Collection 40
  - 2.5.1 Harvesting Plants 40
  - 2.5.2 Tiller and Rhizome Generation Mapping 41

2.5.3 Bud, Rhizome, and Tiller Development Stage Classification......42
2.6 Data Analysis......44
2.6.1 Data Organization......44
2.6.2 Statistical Analysis......45
RESULTS......47
3.1 Overall Plant Establishment......47
3.2 Number of New Tillers Established per Tiller......52
3.3 Bud Production and Propagule Development......54
3.4 Live Propagules per Tiller by Generation......57
3.5 Investment in Phalanx and Guerilla Growth......59
DISCUSSION......62
CONCLUSION......70
LITERATURE CITED......72
CHAPTER 3......78
GREATER COMPETITVE ABILITY OF *BROMUS INERMIS* THAN *PASCOPYRUM SMITHII* IN TERMS OF BELOWGROUND BUD BANK AND TILLER DEMOGRAPHY......78
ABSTRACT......78
INTRODUCTION......80
1.1 Research Question......82
1.2 Hypotheses......82
METHODS......83
2.1 Experiment Design and Treatment......83
2.1.1 Study Species......84
2.2 Seedling Establishment and Transplant......85
2.3 Growth Condition......85
2.4 Data Collection......86
Newly initiated tilers were carefully marked by Individual Tiller Identifier daily.......86
2.4.1 Harvesting Plants......86
2.4.2 Mapping, Classification and Biomass Measurements......86
2.5 Data Analysis......86
2.5.1 Data Organization......86
2.5.2 Statistical Analysis......88
RESULTS......90

3.1 Bud Production, and Propagule Development 90
3.2 Live Propagules per Tiller by Generation 92
3.3 Number of New Tillers Established per Tiller 93
3.4 Overall Plant Establishment 94
3.5 Investment in Phalanx and Guerilla Growth 97
3.6 Biomass 99
3.7 Relative Interaction Index (RII) 102
DISCUSSION 106
CONCLUSION 110
LITERATURE CITED 112
CHAPTER 4 118
CONCLUSION 118
APPENDICES 121

ABBREVIATIONS

ANPP	Annual Net Primary Productivity
BI	*Bromus inermis*
C	Clipping
C3	Cool-Season
C4	Warm-Season
CON	Containment
GLIMMIX	Generalized Linear Mixed Model
HT	Height
KR	Kenward-Roger’s
LgJT	Large Juvenile Tiller
NC	Non-clipping
NGP	Northern Great Plains
NPK	Nitrogen Phosphorus Potassium
NRCS	National Resources Conservation Service
PROC	Procedure
PS	*Pascopyrum smithii*
RII	Relative Interaction Index
SAS	Statistical Analysis System
SD	South Dakota
SE	Standard Error
SmJT	Small Juvenile Tiller
USDA	United States Department of Agriculture
VWC	Volumetric Water Content

LIST OF TABLES

Chapter 1

Appendix-Table 1.1 Summary of the estimated past area, current area, and percent decline of mixed-grass prairies since 1830 (Adopted from Mac et al. 1998)................ 121

Chapter 2

Table 2.1 The main factors and levels of the experiment 37
Table 2.2 Bud, rhizome and tiller development stages (Adopted from Ott et al. 2017) .. 43
Appendix-Table 2.3 Effects of change in precipitation frequency and clipping on the plant establishment traits of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects) 129
Appendix-Table 2.4 Effects of change in precipitation frequency and clipping on the plant establishment traits of *B. inermis*, and *P. smithii* (Mean ± SE) 130
Appendix-Table 2.5 Effects of change in precipitation frequency and clipping on the number of new tiller established per tiller in each generation of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects) 131
Appendix-Table 2.6 Effects of change in precipitation frequency and clipping on the number of new tiller established per tiller in each generation of *B. inermis*, and *P. smithii* (Mean ± SE) 132
Appendix-Table 2.7 Effects of change in precipitation frequency and clipping on the number of live propagules per plant of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects) 133
Appendix-Table 2.8 Effects of change in precipitation frequency and clipping on the number of live propagules per plant of *B. inermis*, and *P. smithii* (Mean ± SE)............ 133
Appendix-Table 2.9 Effects of change in precipitation frequency and clipping on the proportion of live propagules at development stage per plant of *B. inermis* and *P. smithii* (Type III Tests of Fixed Effects) 134
Appendix-Table 2.10 Effects of change in precipitation frequency and clipping on the proportion of live propagules at development stage per plant of *B. inermis* and *P. smithii* (Mean ± SE) 135
Appendix-Table 2.11 Effects of change in precipitation frequency and clipping on the number of live propagules per tiller by generation of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects) 136
Appendix-Table 2.12 Effects of change in precipitation frequency and clipping on the number of live propagules per tiller by generation of *B. inermis*, and *P. smithii* (Mean ± SE).. 137
Appendix-Table 2.13 Effects of change in precipitation frequency and clipping on the proportion of live propagules from tiller per plant of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects) 138

Appendix-Table 2.14 Effects of change in precipitation frequency and clipping on the proportion of live propagules from tiller per plant of *B. inermis*, and *P. smithii* (Mean ± SE).. 138
Appendix-Table 2.15 Effects of change in precipitation frequency, clipping, and location on the proportion of total tillers at each location per plant of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects) 139
Appendix-Table 2.16 Effects of change in precipitation frequency, clipping, and location on the proportion of total tillers at each location per plant of *B. inermis*, and *P. smithii* (Mean ± SE) 140

Chapter 3

Table 3.1 Competition treatment combination applied at individual species level. 84
Appendix-Table 3.2 Effects of competition on the number of live propagules per plant of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects) 142
Appendix-Table 3.3 Effects of competition on the number of live propagules per plant of *B. inermis*, and *P. smithii* (Mean ± SE) 142
Appendix-Table 3.4 Effects of competition on the proportion of live propagules at each development stage per plant of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects) 143
Appendix-Table 3.5 Effects of competition on the proportion of live propagules at each development stage per plant of *B. inermis*, and *P. smithii* (Mean ± SE) 143
Appendix-Table 3.6 Effects of competition on the number of live propagules per tiller by generation of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects)............... 144
Appendix-Table 3.7 Effects of competition on the number of live propagules per tiller by generation of *B. inermis*, and *P. smithii* (Mean ± SE) 144
Appendix-Table 3.8 Contrast of effects between intra-and interspecific competition, and species on the bud production, and propagule development of *B. inermis*, and *P. smithii*. 145
Appendix-Table 3.9 Effects of competition on the number of new tiller established per tiller in each generation of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects) 146
Appendix-Table 3.10 Effects of competition on the number of new tiller established per tiller in each generation of B. inermis, and P. smithii (Mean ± SE)............................. 146
Appendix-Table 3.11 Contrast of effects between intra-and interspecific competition, and species the on number of new tiller established per tiller in each generation of *B. inermis*, and *P. smithii*. 147
Appendix-Table 3.12 Effects of competition on the overall plant establishment traits of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects) 148
Appendix-Table 3.13 Effects of competition on the overall plant establishment traits of *B. inermis*, and *P. smithii* (Mean ± SE) 148

Appendix-Table 3.14 Contrast of effects between intra-and interspecific competition, and species on the overall plant establishment traits of *B. inermis* and *P. smithii* 149
Appendix-Table 3.15 Effects of competition on the proportion of live propagules from tiller per plant of *B. inermis* and *P. smithii* (Type III Tests of Fixed Effects) 150
Appendix-Table 3.16 Effects of competition on the proportion of live propagules from tiller per plant of *B. inermis* and *P. smithii* (Mean ± SE) 150
Appendix-Table 3.17 Effects of competition on the proportion of total tillers at each location per plant of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects).......... 151
Appendix-Table 3.18 Effects of competition on the proportion of total tillers at each location per plant of *B. inermis*, and *P. smithii* (Mean ± SE) 151
Appendix-Table 3.19 Contrast of effects between intra-and interspecific competition, and species on the investment in phalanx and guerilla growth of *B. inermis*, and *P. smithii* 152
Appendix-Table 3.20 Effects of competition on the aboveground, rhizome, and total biomass plant of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects) 153
Appendix-Table 3.21 Effects of competition on the aboveground, rhizome, and total biomass plant of *B. inermis*, and *P. smithii* (Mean ± SE)... 153
Appendix-Table 3.22 Contrast of effects between intra-and interspecific competition, and species on the aboveground, rhizome, and total biomass plant of *B. inermis*, and *P. smithii*.. 154
Appendix-Table 3.23 Effects of competition on the RII values (with respect to number of total tillers, number of total rhizomes, rhizome length, and number of live propagules per plant) of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects) 155
Appendix-Table 3.24 Effects of competition on the RII values (with respect to number of total tillers, number of total rhizomes, rhizome length, and number of live propagules per plant) of *B. inermis*, and *P. smithii* (Mean ± SE).. 155
Appendix-Table 3.25 Effects of competition on the RII values (with respect to aboveground, rhizome, and total biomass per plant) of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects) 156
Appendix-Table 3.26 Effects of competition on the RII values (with respect to aboveground, rhizome, and total biomass per plant) of *B. inermis*, and *P. smithii* (Mean ± SE).. 156

LIST OF FIGURES

Chapter 1

Appendix-Figure 1.1 Northern Great Plains Ecoregions and Sub-ecoregions (Adopted from Forrest et al. 2004) 122
Appendix-Figure 1.2 Major threats to northern Great Plain Ecological Integrity (Adopted from Schrag 2011) 123
Appendix-Figure 1.3 Cummulative publications related to Bud banks versus Seed banks (Source: Web of Science, February 2018; Keywords: Bud bank and Seed Bank) 123
Appendix-Figure 1.4 Geographical contribution of bud bank studies over the period of 1997-2017 (Source: Web of Science, February 2018) 124
Appendix-Figure 1.5 Grass phytomer and tiller (i.e. stem) organization. (Adopted from Briske 1991 as adapted from Etter 1951). 124
Appendix-Figure 1.6 Cross-section of a grass tiller base. Axillary buds sit between each leaf and have the potential to transtion into emerging tillers. (Adopted from Briske 1991 as adapted from Jewis 1972). 125
Appendix-Figure 1.7 Diagram of a grass showing clonal growth with respect to rhizome (Adopted from Cornelissen et al. 2014). 125
Appendix-Figure 1.8 Conceptual model of bud bank demography and its potential population, community and ecosystem consequences (Adopted from Dalgleish 2007). 126
Appendix-Figure 1.9 Projected change in the number of daily zero ("No-Precip") and non-zero precipitation days (by percentile bins) for late-21st century under a higher emission scenario for contiguous United States (Adopted from Wuebbles et al. 2017). 126
Appendix-Figure 1.10 Relative contribution of various meristematic sources to the rate and duration of biomass production in grass plants (Adopted from Briske 1991). 127
Appendix-Figure 1.11 Illustration of the major processes contributing to population persistence in perennial grasses (Adopted from Briske & Noy-Meir 1998). 127
Appendix-Figure 1.12 Hypothesized relationship between bud bank density, invisibility, and community stability (Adopted from Sprinkle 2010). 128

Chapter 2

Figure 2.1 Conceptual diagram of one replication of an experimental plot in greenhouse experiment 37
Figure 2.2 Conceptual diagram of tiller and rhizome classification according to generation 41
Figure 2.3 Effect of (A) precipitation frequency and (B) clipping on the number of total tillers per plant of *B. inermis* and *P. smithii* 48
Figure 2.4 Effect of (A) precipitation frequency and (B) clipping on number of total rhizomes per plant of *B. inermis* and *P. smithii* 50
Figure 2.5 Effect of (A) precipitation frequency and (B) clipping on total length of rhizome (cm) per plant of *B. inermis* and *P. smithii* 52

Figure 2.6 Effect of (A) precipitation frequency and (B) clipping on number of new tiller established per tiller of *B. inermis* and *P. smithii* 32
Figure 2.7 Effect of (A) precipitation frequency and (B) clipping on number of live propagules per plant of *B. inermis* and *P. smithii* 55
Figure 2.8 Effect of (A) precipitation frequency and (B) clipping on proportion of live propagules at each development stage per plant of *B. inermis* and *P. smithii* 57
Figure 2.9 Effect of (A) precipitation frequency and (B) clipping on number of live propagules per tiller by generation of *B. inermis* and *P. smithii*.................................... 58
Figure 2.10 Effect of (A) precipitation frequency and (B) clipping on proportion of live propagules per plant of *B. inermis* and *P. smithii* 60
Figure 2.11 Effect of (A) precipitation frequency and (B) clipping on proportion of tillers per plant of *B. inermis* and *P. smithii*.. 61
Appendix-Figure 2.12 Conceptual diagram of bud, rhizome and tiller development stages (From Ott and Hartnett 2015b) 141

Chapter 3

Figure 3.1 Conceptual diagram of experimental design 83
Figure 3.2 Effect of competition treatment on number of live propagules per plant of *B. inermis* and *P. smithii* 91
Figure 3.3 Effect of competition treatment on proportion of live propagules at each development stage per plant of *B. inermis* and *P. smithii*... 92
Figure 3.4 Effect of competition treatment on number of live propagules per tiller by generation of *B. inermis* and *P. smithii*.. 93
Figure 3.5 Effect of competition treatment on number of new tiller established per tiller along generation of *B. inermis* and *P. smithii* 94
Figure 3.6 Effect of competition treatment on number of total tillers per plant of *B. inermis* and *P. smithii*. 95
Figure 3.7 Effect of competition treatment on number of total rhizomes per plant of *B. inermis* and *P. smithii* 96
Figure 3.8 Effect of competition treatment on total length of rhizome (cm) per plant of *B. inermis* and *P. smithii* 97
Figure 3.9 Effect of competition treatment on proportion of live propagules per plant of *B. inermis* and *P. smithii* 98
Figure 3.10 Effect of competition treatment on proportion of total tillers based on location per plant of *B. inermis* and *P. smithii*... 99
Figure 3.11 Effect of competition treatment on aboveground biomass (g) per plant of *B. inermis* and *P. smithii* 100
Figure 3.12 Effect of competition treatment on rhizome biomass (g) per plant of *B. inermis* and *P. smithii* 101
Figure 3.13 Effect of competition treatment on total biomass (g) per plant of *B. inermis* and *P. smithii*. 102

Figure 3.14 Effect of competition treatment on RII values with respect to # of tiller per plant, # of rhizomes per plant, and rhizome length (cm) per plant of *B. inermis* and *P. smithii*.. 103
Figure 3.15 Effect of competition treatment on RII values with respect to # of live propagules per plant of *B. inermis* and *P. smithii*.. 104
Figure 3.16 Effect of competition treatment on RII values with respect to aboveground biomass (g) per plant, rhizome biomass (g) per plant, and total biomass (g) per plant of *B. inermis* and *P. smithii* 106

LIST OF PHOTOS

Photo 2.1 Sample processing and segregation of individual plant into first, second, and third generation crown daughter tillers (row first) and rhizome daughter tillers (row second), and first, second, and third generation rhizomes (row third). 42
Photo 2.2 Live vegetative propagules: live bud (22.5x) of *B. inermis*, small juvenile tiller (8x), large juvenile tiller (6.7x), and adult tiller of *P. smithii*.. 43

ABSTRACT

IMPACTS OF ALTERED PRECIPITATION FREQUENCY, CLIPPING, AND COMPETITION ON PERENNIAL GRASSES MEDIATED THROUGH BELOWGROUND BUD BANK IN NORTHERN MIXED-GRASS PRAIRIE

SURENDRA BAM

2018

Perennial grasslands are remarkably resilient to severe natural and anthropogenic disturbances. Such resiliency largely depends on successful tiller recruitment and establishment from belowground bud banks. In the northern Great Plains, introduced perennial smooth bromegrass (*Bromus inermis*) has been rapidly invading and transforming larger tracts of native prairies by replacing native perennial species, such as western wheatgrass (*Pascopyrum smithii*), reducing biodiversity and quality of habitats, and increasing vulnerability of grasslands to other environmental disturbances. In this study, we evaluated the response of belowground bud production, tiller and rhizome recruitment, and plant establishment between the native *P. smithii* and the non-native *B. inermis* to altered precipitation frequency, clipping, and competition with two different controlled greenhouse experiments over two growing seasons.

In the first experiment, the treatments consisted of combinations of three precipitation frequencies (every 2d, 8d, and 16d) representing high, medium, and low, two levels of clipping (clipping *vs.* no-clipping), and two species with 40 replicates for each treatment. One single-leaf seedling of each species was transplanted into individual potting-soil filled pots in mid-June. We initiated precipitation frequency treatments and applied a clipping treatment two weeks after transplanting. Plants were harvested 20 weeks after the treatments had been initiated. The number of tillers and rhizomes based on generation, number of tillers based on location (crown *vs.* rhizome), and rhizome length were recorded. Three randomly sub-sampled tillers and rhizomes from each generation were dissected to record the number of buds and propagule development. We found *B. inermis* significantly decreased their number of tillers, rhizomes, rhizome length, and live propagules at the low precipitation frequency, but increased propagule development at medium precipitation frequency. However, *P. smithii* significantly increased the traits described above under medium precipitation frequency, except for the number of tillers and propagule development, which were not affected at medium and low precipitation frequency. The clipping significantly reduced tiller production for both species and the number of rhizomes for *B. inermis*. The results indicate that non-native *B. inermis* may be more susceptible to the altered precipitation frequency and clipping compared to native *P. smithii*. Native *P. smithii* may be able to resist the soil moisture variability and clipping effects mediated via the belowground bud banks.

The second competition experiment consisted of five treatments including single *B. smithii*, single *P. smithii*, pairwise monoculture of *B. inermis*, pairwise monoculture of *P. smithii*, and pairwise mixed-culture of *B. inermis* and *P. smithii* with 30 replicates for each treatment under every 2d precipitation frequency regime. Double-leaf seedlings of each species were transplanted into individual potting soil-filled pots based on designated treatments. Plants were harvested 12 weeks after the treatments had been initiated. The data collection followed the same protocol as the first experiment. In addition, biomass and relative interaction index (RII) were calculated to determine intra- and inter-specific competition between *P. smithii* and *B. inermis*. We found that the presence of *B. inermis* as a neighbor significantly decreased the number of live propagules, tillers, and aboveground biomass of the native *P. smithii*. However, the presence of *P. smithii* as a neighbor significantly increased the number of live propagules and had significantly less negative effect on tiller production and aboveground biomass of *B. inermis*. Also, investment in dual phalanx and guerilla growth by *B. inermis* while competing with *P. smithii* indicates possible phenotypic plasticity trait. All results demonstrated a strong competitive ability of the non-native *B. inermis* against *P. smithii* during the establishment phase when environmental conditions are favorable (i.e. lack of water stress and grazing).

Overall, we can conclude that species establishment and interaction between these two key perennial grasses in northern mixed-grass prairies is environmentally dependent and species specific. The outcomes are mediated by the response of the belowground bud bank. The findings from this study can help us to better understanding the mechanisms of bud banks in maintaining tiller population, regulating vegetation dynamics, productivity, and response to climate change in the context of grazing practices and invasion by non-native perennial grasses. They could form the basis for a long-term effective grassland management plan.

CHAPTER 1

LITERATURE REVIEW

1. Northern Great Plains Grasslands

The northern Great Plains (NGP) grasslands are North America's largest grassland ecoregion, spanning five states of the United States and two Canadian provinces (Appendix-Figure 1.1), and covering approximately 722,600 square kilometers, or about 25 percent of the entire Great Plains (Ricketts 1999). The northern Great Plains supports a high level of species richness (Forrest et al. 2004). It is one of the 238 most biologically significant places on Earth (Olson and Dinerstein 1998). Out of the thirty-nine endemic North American grassland vertebrates in the Northern Great Plains, 15 percent are listed as endangered or threatened by the U.S.and/or Canada (Samson and Knopf 1996).

These grasslands are dominated by grasses and grass-like plants (Weaver 1968). They evolved under the influence of broad-scale environmental gradients, which significantly impacted the composition and distribution of plant communities (Steinauer and Collins 1996). Thus, the Great Plains grassland vegetation can be abstracted into discrete communities such as tall-grass, mixed-grass, and short-grass prairies based on the east-west precipitation gradient of central North America, overlain by a north-south temperature gradient.

However, there are growing concerns surrounding the conservation and management of these prairies. Since 1830, there has been an estimated decline of 20 to 99.9% in native tallgrass, mixed-grass, and short-grass prairies because of habitat fragmentation, conversion to cropland, inappropriate land use practices, such as fire exclusion and grazing, use and spread of non-native and invasive plants, and drought (Mac et al. 1998, Glaser 2012). The estimated decline in native mixed-grass prairies ranges from 30.5% in Texas to over 99.9% in Manitoba (Appendix-Table 1.1).

A conservation assessment for the North American grasslands identified nine major threats affecting the ecological integrity of the northern Great Plains, such as grazing by livestock and native herbivores, strong inter-annual climate variability, and invasive species (Appendix-Figure 1.2, Schrag 2011). Studies have shown, because of climate variability, the growing season precipitation regimes will become more variable (Koerner et al. 2014). An increase in larger rainfall events and longer dry periods results in more dramatic, temporally soil moisture dynamic regimes (Koerner et al. 2014, Wuebbles et al. 2017). Such increase in climate variability will likely interact with other disturbances, such as grazing, which may profoundly impact the grassland community structure and function by affecting competitive dynamics between native and invasive plant species, and potentially undermining the effectiveness of restoration activities (Schrag 2011).

2. Seed versus Vegetative Reproduction

In the northern Great Plains, many grassland ecosystem processes and functions are defined by their primary perennial grass vegetation. Regeneration, growth, and sustainability of perennial grass populations and regulation of annual net primary productivity are limited by their reproductive strategies and other life history traits (Ott 2014). Thus, it is imperative to delineate the major reproductive strategies these grasses rely on in their life histories. It will certainly be useful in understanding the underlying mechanisms by which management practices and other environmental disturbances affect perennial grasslands.

Many seed plants – grasses included can reproduce sexually (by means of seeds) or asexually (by means of vegetative organs). A plant may reproduce exclusively by seeds (as in the case of most annuals), primarily by vegetative means (as in the case of many water plants), or it may employ both methods (as in the case of most herbaceous perennials) (Fenner 1985). Studies have shown the establishment and productivity of perennial grasses rely not only on successful tiller recruitment from seed, but also from a population of belowground meristems (the bud bank sensu Harper 1977, Benson et al. 2004, Dalgleish and Hartnett 2009, Ott and Hartnett 2015).

These two ways of reproduction differ in their adaptive value in different circumstances and surroundings. Although seeds are important for new genet recruitment, both short- and long-term dispersal, and maintenance of genetic diversity; seed production is variable in perennial grasslands, especially due to herbivory and interannual variability in precipitation (Briske and Derner 1998). In addition, seedling recruitment of perennial grasses is rare. For instance, two studies in tallgrass prairies showed that in undisturbed sites, tiller recruitment from seeds was only 0.6 %, and nearly 99.4% were recruited from belowground buds (Benson and Hartnett 2006). Likewise, in disturbed sites, tiller recruitment from seeds was low and nearly 80% occurred from belowground buds (Rogers and Hartnett 2001).

The bud bank was defined as the belowground population of meristems associated with rhizomes or other perennating organs, which may accumulate over time, and plays a fundamental role in local plant population persistence, structure, and dynamics (Harper 1977). Maintenance of an appropriate bud bank size is critical for tiller population survival, especially during disturbances, and is critical for population persistence and community stability (Benson et al. 2004, Dalgleish and Hartnett 2009, Ott and Hartnett 2015).

The bud bank is more important than the seed bank as a source for plant recruitment in many grasslands (Hartnett and Fay 1998, Benson et al. 2004). For instance, seed banks in tallgrass prairies may be large (> 6000 seeds m^{-2}) (Weaver and Mueller 1942), but the establishment of seedlings of dominant grasses from seed is rare and episodic (Christiansen and Landers 1966). Even in both burned and unburned communities in the tallgrass prairie, >99% of all established stems were recruited from the bud bank (Benson and Hartnett 2004).

Despite their ubiquity, relatively few empirical studies have directly investigated the role of the bud bank in the dynamics of populations, communities, ecosystems or landscapes (Hendrickson and Briske 1997, Chen et al. 2011, VanderWeide et al. 2014, Ott et al. 2017). In contrast, the ecology of seed banks has been well studied (Baskin and Baskin 1998). Over the last two decades (1997-2017), there were almost 6,000 papers on seed banks, compared to only about 300 papers on bud bank-related studies (Appendix-Figure 1.3). he majority of those bud bank studies are limited to a few places around the world, including grasslands of inner Mongolia, steppe and temperate deciduous forests of the Czech Republic, savanna of South Africa, grasslands of southern Brazil, and tallgrass prairies of eastern Kansas of North America (Appendix-Figure 1.4). Within the Great Plains of North America, most of those studies (~70%) occurred in the southern Great Plains and only few (~30%) of the studies were carried out in the northern Great Plains (Appendix-Figure 1.4). All of this implies that bud bank studies are on an infancy level, and there is a great necessity for the roles of bud bank studies in ecological and managerial implications for perennial grassland ecosystems.

3. Major Environmental Disturbances and Their Impacts on Belowground Bud Bank

Bud production is closely tied to tiller growth. Grass tillers are modular units comprised of multiple phytomers (Appendix-Figure 1.5). Each phytomer consists of an internode, leaf sheath, leaf blade, and potentially an axillary bud (Appendix-Figure 1.6). As a tiller grows, its apical meristem continually adds phytomers and thus axillary buds. Grasses condense their internodes at the base of the tiller, only exposing their leaves aboveground during vegetative growth, with axillary buds accumulating belowground (Hyder 1972, Jewiss 1972). The basal accumulation of axillary buds is permanently stopped when a tiller flowers or the apical meristem senesces (Ott and Hartnett 2011). New cohorts of tillers are recruited from these axillary buds during the regular annual tiller recruitment period or following injury to the plant. Because bud and tiller production are dependent on one another, bud activation (i.e. tiller initiation) is critical to new tiller production and tiller establishment is critical to new bud production (Ott and Hartnett 2011). Therefore, bud banks are the source for future tillers and play a decisive role in species population, community composition and structure, and ecosystem functions.

Prairies of the North American Great Plains are dominated by clonal rhizomatous perennial grasses that vary in architecture along the "phalanx-guerilla" rhizomatous growth form continuum (Harper 1977, Doust 1981). Despite the abundance of these rhizomatous grasses, little is known about their bud banks compared with caespitose grasses (Dalgleish et al. 2008, Ott and Hartnett 2015). The rhizomatous growth form is an adaptive plant strategy by which plants can reproduce and spread vegetatively (Appendix-Figure 1.7) and where clonal structures can also serve as storage organs (Klimesova and Klimes 2007, Dong et al. 2010). Based on the spatial arrangement of tillers, such clonal plants can have two types of growth forms: phalanx and guerilla (Doust 1981, Oborny 1997). Clonal plants with the phalanx growth strategy produce a compact structure of closely spaced tillers, whereas those with guerilla growth form produced a loosely arranged group of widely spaced tillers (Doust 1981, Bernard 1990). These two types of growth forms have ecological and evolutionary significance to clonal plant populations. For example, the guerilla growth form is very common in early successional stages, as well as in disturbed habitats, whereas the phalanx form is more common in late successional stages and in relatively less disturbed habitats (Schmid and Harper 1985).

The guerilla growth form enables rhizomatous plants to spread quickly in horizontal space. In the disturbed habitats, rhizomatous plants can more readily escape from stressful microsites and find favorable ones (Doust 1981, Sutherland and Stillman 1988, Humphrey and Pyke 1998). The phalanx growth form, by contrast, may enable clonal plants to tolerate more stressful conditions, make better use of locally abundant resources (monopolization strategy) and outcompete other species in a favorable microsite (Doust 1981, Schmid and Harper 1985, Humphrey and Pyke, 1998). Some species can shift between these two-growth patterns, showing architectural plasticity by the combination of both guerilla and phalanx traits in response to habitat and nutrient conditions (Doust 1981, Ye et al. 2006, Chen et al. 2011).

Studies have shown that different growth forms of perennial grasses depend on environmental conditions (Doust 1981, Garnier and Roy 1988). In *Leymus secalinus*, the plants are more phalanx-like under high nutrient supply and more guerilla-like in low nutrient conditions (Ye et al. 2006). In tidal wetlands, *Elymus repens*, guerilla growth form was changed to phalanx growth form when grazing pressure was released and submitted to competitive stress (Amiaud et al. 2008). *Pascopyrum smithii* substantially invested in both phalanx and guerilla tiller production in natural conditions of western

South Dakota (Ott and Hartnett 2015). However, research on clonal growth forms of perennial grasses in response to disturbance and environmental fluctuations are limited in northern Great Plains compare to the southern Great Plains (Ott and Hartnett 2015) with limited applicability to the drier, more expansive mixed grass prairies of that area. Similarly, there are only few studies examined the changes of clonal growth forms in response to resource availability or biotic competition (Navas and Garnier 1990, Ye et al. 2006).

Therefore, it is imperative to understand the impacts of these environmental stressors on belowground bud bank traits, including bud production, bud viability, bud outgrowth, tiller establishment, and clonal growth form contributing to population persistence in perennial grasses and utimatley structure and function of perennial grasslands (Appendix-Figure 1.8).

3.1 Impacts of Climate Change on Belowground Bud Bank

The annual average temperature over the contiguous United States is projected to rise (Wuebbles et al. 2017). An increase of about 2.5°F (1.4°C) is projected for the period 2021-2050, relative to 1976-2005 in all carbon emission scenarios, implying recent record-setting years (such as 2014- 2016) may be “common” in the next few decades (Solomon et al. 2007, Wuebbles et al. 2017). Projected changes in annual average temperature for northern regions of the contiguous United States are slightly warmer than other regions, roughly 9.0°F (5.5°C) in the Northeast, Midwest, and northern Great Plains by late-century under the high emissions scenario. The frequency and intensity of heavy precipitation events are projected to continue to increase over the contiguous United States, including the northern Great Plains, with larger events and longer dry periods during both mid- and late-century at both low and high emission scenarios (Wuebbles et al. 2017).

However, projections of daily precipitation amounts indicate an overall more extreme climate (Schrag 2011, Wuebbles et al. 2017). Essentially, an increase in dry days or heavy precipitation events (creating longer intervals between events and increased drought length) will increase all over the contagious United States (Appendix-Figure 1.9).

There is evidence that effects of an extreme precipitation climate will be manifested primarily by altered soil moisture availability. Such alterations in precipitation regimes during the growing season will have significant ecological consequences for grassland structure and function (Craine et al. 2011, Jones et al. 2016). For example, the increased rainfall variability in mesic grasslands can reduce annual net primary productivity (ANPP) over the short term and alter the genotypic diversity of the grasses over longer time frames. Together, these results support predictions that grassland ecosystems will be highly responsive to future changes in precipitation variability (Jones et al. 2016).

Bud bank demography, including bud production, longevity and outgrowth, is influenced by current and past precipitation, which can create a legacy effect on grassland aboveground net primary production (ANPP, Ott and Hartnett 2012). This indicates that bud bank density would be high if there was high precipitation in previous years with high ANPP in the subsequent wet year (Knapp and Smith 2001). Elevated CO_2, temperatures and altered moisture regimes not only affect the physiological and phenological traits of plants, but also the demographic plant response *via* the bud bank (Morgan et al. 1994, Zelikova et al. 2014), and especially tiller production in C3 grasses (Wand et al. 1999).

Studies have shown that climate change has the potential to differentially affect reproduction and growth of native and non-native C3 perennial grasses, such as lower seedling establishment and survival of non-native *Bromus inermis* in comparison to native *Pascopyrum smithii* when ambient temperature was elevated by 0.3˚C (Sheppard et al. 2012). However, a recent study by Ott et al. (2017) on northern mixed-grass prairies showed that non-native *B. inermis* maintained a greater number of live buds per tiller and initiated a greater proportion of bud outgrowth than native *P. smithii* under short-term drought and a range of temperatures.

The potential of belowground bud banks to strongly influence patterns of ANPP in ecosystems under different environmental conditions has been explained in terms of the meristem limitation hypothesis (Knapp and Smith 2001). Knapp and Smith (2001) found that ANPP was more variable in grassland biomes which were intermediate in mean annual precipitation, whereas ANPP was less variable in desert and arid grassland biomes. They hypothesized that this could be due to meristem limitation, which constrains their production potential and their ability to respond to pulses of high resource availability.

Dalgleish and Hartnett (2006) used the natural precipitation gradient and productivity across the Great Plains grasslands of the central United States to test the meristem limitaiton hypothesis. They found that along a precipitation gradient in the Great Plains, extending from desert grassland to tallgrass prairie, bud bank density increased with an increase in mean annual precipitation. Their study also found that in arid grasslands, perennial grasses have a very small bud bank and only a small proportion of the bud broke dormancy for tiller recruitment. In addition, they found that mesic grasslands maintained a much larger bud bank and retained a greater ability to break dormancy and recruit into aboveground tillers. The lower ability of tiller recruitment of native perennial grasses in arid grasslands in the Great Plains has been supported by other studies as well. In addition, Hendrickson and Briske (1997) found that tiller recruitments only initiated from younger buds of *Bouteloua curtipendula* and *Helaria belangeri* in the arid grasslands of Texas, as the mature buds were dormant for over two years.

The response of the belowground bud bank of perennial grasses to climatic variability seems to have some significant community- and ecosystem-level consequences. The overall tiller density in restored grasslands seems to be resilient, such that drought effects on belowground bud banks may have longer-term impacts on plant community structure (VanderWeide 2013). The response of perennial grasslands to drought may be mediated by the stable belowground bud bank, and may be insensitive to multi-year, growing season drought (VanderWeide et al. 2014, VanderWeide and Hartnett 2015).

Several studies showed the effects of climatic variability in soil moisture regimes and its interaction with other factors, including competition, clipping and plant invasion, as well as the effects on the structure and function of grasslands at the population, community, and ecosystem levels. One of these studies showed the importance of soil moisture and its interaction with competition and clipping for two montane meadow grasses (Kluse and Diaz 2005). At low (19%) soil moisture, *Deschampsia cespitosa* competitive ability decreases, while the competitive ability of *Poa pratensis* increases. However, at more mesic conditions (50%), each species' aboveground biomass and tillering were adherent to soil moisture conditions. Another study showed the effect of soil moisture and plant invasion, where the short-term increase in water availability facilitated the long-term establishment of alien plant species such as *Kochia scoparia*, *Salsola iberica*, *Sisymbrium altissimum*, and *Cirsium arvense* (Milchunas and Lauenroth 1995).

The Donker et al. (2002) study of *Bromus inermis* and *Poa pratensis* showed that dry matter yield decreased under defoliation but increased with increasing soil moisture availability. Similarly, root: shoot ratio increased significantly with decreasing moisture availability.

There were greenhouse studies that showed *B. inermis* is more tolerant to soil moisture stress than the native green needlegrass (*Nassella viridula*) and *Agropyron dasystacyum* in-terms of leaf demography (Reekie and Redmann 1990). However, prolonged drought is also shown to decrease shoot dry weight, induce dormancy (Dibbern 1947, Donkor et al. 2002) and limit the establishment of *B. inermis* in southern Alberta and central British Columbia (Otfinowski 2008).

In contrast, studies with perennial grasses, conducted by Eneboe et al. (2002) on rangelands of the northern Great Plains, demonstrated that a one-year growing season drought combined with grazing (both during and after drought) did not decrease the relative growth rates of tillers and tiller densities of both *Bouteloua gracilis* and *P. smithii*. Likewise, the effect of a 1-year drought on active axillary buds was insignificant and only after 3 consecutive years of drought there was a reduction in numbers of metabolically active axillary buds in two bunchgrasses, *Agropyron desertorum* and *Agropyron spicatum* (Busso et al. 1989). Similarly, repeated late grazing of both crested wheatgrass (*Agropyron desrtorum*) and bluebunch wheatgrass (*Pseudoroegneria spicate*) under simultaneous influence of drought required more than two years to limit the tiller numbers and herbage accumulation (Busso and Richards 1995).

Native perennial grasses like *P. smithii* are also found to be tolerant to drought stress with the help of different physiological mechanisms. For instance, a study by Frank (1994) showed that *P. smithii* had higher drought tolerance than *Agropyron cristatum* by maintaining 1.7 times higher abscisic acid and proline concentration in its leaf tissue. The increase in proline during the later stages of plant development may have supported *P. smithii* to better tolerate drought and continue slow growth. Other studies have also shown how osmotic adjustment enhanced with proline concentration in the leaves, to assist cool season grasses to tolerant drought (Frank 1994).

3.2 Impacts of Grazing on Belowground Bud Bank

Evaluation of the grazing resistance literature for perennial grasses indicates that architectural attributes and demographic processes are of greater importance than physiological processes (Briske and Richards 1995, Hendrickson and Briske 1997). Long-term selective grazing can differentially affect population persistence mediated by belowground meristems among various species and thereby modify community composition and structure (Briske and Noy-Meir 1998).

The relative contribution of these meristematic sources for plant growth varies among species and is influenced by environmental variables and stage of phenological development (Appendix-Figure 1.10). The ability of grasses to regrow following defoliation depends upon the basal locations of meristematic sources. Culm elongation makes a portion of these meristems, especially intercalary and apical meristems, much more vulnerable to removal by grazing. Several studies have illustrated that persistent grazing over the long-term can result in depletion of the bud bank (Dalgleish and Hartnett 2009, Hendrickson and Briske 1997).

Tiller recruitment from buds is generally more consistent than plant establishment from seeds because juvenile tillers import resources from parent tillers to enhance establishment (Welker and Briske 1992). Tiller recruitment may occur throughout the growing season in both cool-season (C3) and warm-season (C4) perennial grasses, but maximum adult recruitment frequently occurs in the spring (Briske and Richards 1995). Tiller replacement from axillary buds is required for population persistence in perennial grasses (Appendix-Figure 1.11, Briske & Noy-Meir 1998). Grazing can induce a reduction in axillary bud production and activation, thereby affecting tiller recruitment and plant establishment (Hendrickson and Briske 1997).

The compensatory growth, usually defined as a positive response of plants to injury, has been applied to describe plant responses ranging from a partial replacement of lost tissue to a net productivity exceeding that of uninjured control plants (Belsky 1986). Studies have shown the compensatory growth (i.e. the re-establishment of a photosynthetic canopy) of perennial grasses depends on the production of new tillers through activation of buds (Hyder 1972, Busso et al. 1989).

Furthermore, when plants cannot avoid herbivores by defense, herbivory tolerance is an important trait for plant survival and future performance (Lehtila 2000). For instance, *P. smithii* can employ both conservative and foraging growth strategies which will facilitate its persistence under local neighborhood variability and changing resource availability associated with various environmental stressors (Ott and Hartnett 2015). Upholding its reputation as a good space colonizer and local disperser *via* rhizomes*,* species like *P. smithii* invest substantially in both phalanx and guerilla tiller production in undisturbed conditions (Ott and Hartnett 2015). However, simulated grazing or clipping increased *P. smithii* bud mortality and reduced its bud development in a 2-week period of the study (Ott et al. 2017). This may indicate *P. smithii* might need longer time for recovery and it may be further affected by the competition with non-native species.

Likewise, the response of bud banks of perennial grasses to grazing intensities can be species-specific, as shown by the study done on the steppe of Inner Mongolia, with increasing grazing intensity, bud density decreased in *Leymus chinensis*, increased in

Agropyron cristatum, and had no significant effects on *Carex duriuscula* (Qian et al. 2014). However, the effect of grazing frequency or defoliation can be different than grazing intensity on belowground bud bank traits. Increased defoliation frequency increased the percentage of dead and dormant buds when the frequency of defoliation of *Poa ligularis* was increased to third and fourth times annually (Busso 2011).

Studies have also shown that the regrowth potential of *B. inermis* is affected by grazing frequency. For instance, following eight years of annual sheep grazing, plants became shorter and more vigorous (Falkner and Casler 2000). However, aboveground biomass increased at the expense of the roots in *B. inermis* (Dibbern 1947, Reynolds and Smith 1962). On the contrary, *P. smithii* tends to be tolerant to different intensities of grazing. Painter and Detling (1981) found that there was little variation in tiller numbers among clipping treatments and unclipped plant at the end of their 10-day clipping treatment study.

3.3 Impacts of Plant Invasion on Belowground Bud Banks

Biological invasions are global phenomena that threaten terrestrial, marine and freshwater biodiversity (Dukes and Mooney 1999, Pimentel et al. 2005, Vitousek et al. 1996). Biological invasions are regarded as one of the biggest global threats to biodiversity, second only to habitat destruction (Wilcove et al. 1998). Invasions have also altered global biodiversity, reducing at the local scale, increasing at the regional scale and tending towards homogenization at global scales, with widespread ecological and evolutionary implications. Many non-native species have been deliberately introduced for economic purposes such as land rehabilitation, forage, and ornamental use. Although non-native species create economic benefits, they are detrimental to ecosystem services and functions when they escape from cultivation (Reichard and White 2001).

The extent of the northern Great Plains grasslands is declining due to conversion of these grasslands to croplands, resulting in habitat fragmentation and increased disturbance (DeKeyser et al. 2013). Increased disturbance and fragmentation has caused remnant native prairies to become susceptible to invasion by cool-season non-native species (Hobbs and Huenneke 1992, DeKeyser et al. 2013), such as *Bromus inermis* and

Poa pratensis, which account for 62% of exotic species cover in the northern Great Plains (Cully et al. 2003).

In 2004, mixed-grass prairie was estimated to span only 29.1% of its historical range (Samson et al. 2004). Disturbance from invaders and fragmentation of prairie from intense agricultural use have been driving forces in causing this decrease (Cully et al. 2003). Restoration of these invaded prairies seems to need extra resources and time. For instance, analyses of soils and vegetation in southern mixed-grass prairie reseeded with native plants showed that sites may require external inputs and a 30- to 50-year period to recover from established non-native species sites (Fuhlendorf et al. 2002). Because of the long recovery period, preventing exotic invasions is far more crucial than restoring them, for the conservation of remaining tall- and mixed-grass prairie (DeKeyser et al. 2013).

Bromus inermis and *P. pratensis* are highly invasive in North American grasslands. In a 2002-2006 survey of mixed-grass and tallgrass prairie vegetation, *B. inermis* comprised 45%-49% of plant cover in some areas, and *P. pratensis* occupied 27% to 36% of vegetation in other locations (Grant et al. 2009). Comparison of vegetative cover at sites in the northern Great Plains between 1984 and 2007 they found that species composition changed from containing a high percentage of native species to containing a high percentage of invasive species, including *B. inermis* and *P. pratensis* (DeKeyser et al. 2013). Due to the detrimental effects of these two major non-native species on northern prairies, researchers have started to address why these two invaders are so successful. The competitive ability of *B. inermis* has been examined in both mixed and tallgrass prairie. When competing against native species of the mixed-grass prairie, *B. inermis* had a high competitive ability across several moisture regimes (Nernberg and Dale 1997). The non-native *B. inermis* has a significant negative effect on the patch dynamics of a dominant native grass species, *Spartina pectinata* (Dillemuth et al. 2009). For example, the cordgrass patch growth was two times larger in counties not invaded by *B. inermis* versus the areas heavily infested with *B. inermis*. The probability of establishment of a new patch of cordgrass averaged 1.3 times higher in areas of low *B. inermis* coverage (<25%) than areas of high *B. inermis* coverage (>75%). In a 4-year competition field experiment in California grasslands between native and non-native perennial grasses that share similar species traits, native perennial grass biomass was significantly lower in plots with exotic perennial grasses as compared to plots without exotic perennial grasses (Corbin and D'Antonio 2010).

Several management techniques have been developed to control and manage C3 introduced grasses, like *B. inermis*, including herbicide application, prescribed burning, and mowing or grazing (Wilson 1992, Bahm et al. 2011, Harrison and Romo 1994, Willson and Stubbendieck 1996, Donkor et al. 2002). The effects of these management techniques have been mostly addressed from the perspectives of (i) physiological, morphological and ecological traits of plants (Klimesova and Klimes 2007, Lamas et al. 2013), (ii) plant stoichiometry responses (Bai et al. 2012) (iii) spatio-temporal patterns of soil seed bank (Dreber and Esler 2011) and (iv) species composition, structure and function of plant communities (Hoshino et al. 2009, Collins and Calabrese 2012). However, few of these management approaches have been effective due to a lack of understanding the underlying demographic mechanisms responsible.

The success of a plant invading new habitat appears to depend on at least three factors: propagule pressure, plant traits, and habitat invisibility (Barney and Whitlow 2008). Understanding patterns and mechanisms of biological invasions requires consideration of each of these factors. Disturbances such as global environmental change may create "windows of opportunity" for biological invasions to occur, and the frequent disturbances grasslands experience may provide ample opportunities for exotic species establishment and spread. The susceptibility of grasslands to invasion by exotic plants can be related to invader demographic attributes such as bud bank densities in these habitats (Sprinkle 2010). Sprinkle (2010) tested the hypothesis that maintaining a large bud bank enables resident vegetation to rapidly preempt resources following a disturbance (Appendix-Figure 1.12) and make them less susceptible to invasion (Davis et al. 2000).

Some species-based studies have indicated rhizomatous growth form of grasses as an important factor of species invasive attributes. For instance, perennial weeds such as *Agropyron repens*, commonly known as quack grass, are famous for bearing invasive qualities, where studies showed are primarily due to tough rhizomes which are produced abundantly and help to rapidly recruit new plants. Each primary shoot typically bears three tillers and form 3-4 rhizomes that have high tiller and rhizome replacement rates (Palmer 1958). Likewise, invasiveness of non-native *B. inermis* was due to the proliferation of its rhizomes (Dibbern 1947, Romo and Grilz 1990). *B. inermis* continued vegetative growth increases the density of older stands, intensifying both above- and belowground competition (Engel et al. 1987, Gerry and Wilson 1995), and outcompeted alfalfa in pastures (Groya and Sheaffer 1981).

In a recent study by Ott et al. (2017) on mixed-grass prairies of northern Great Plains, belowground bud outgrowth responses of native *P. smithii* and non-native *B. inermis* to grazing and environmental conditions were tested in a growth chamber. They found under short-term drought and a range of temperatures, *B. inermis* maintained a greater number of live buds per stem and initiated a greater proportion bud outgrowth than *P. smithii,* indicating greater competitive ability of non-native *B. inermis* against native *P. smithii*. Nevertheless, whether these outgrowth buds develop into new tillers and established tillers produce new buds under various environmental conditions has not been assessed.

Both native and non-native perennial grasses depend on the belowground bud bank in response to changing environmental conditions. It is important to evaluate these vegetative life history traits, which have been long overlooked (Klimesova and Klimes 2007, Dalgleish and Hartnett 2009, Qian et al. 2014, Ott et al. 2017), and can be important species attributes (Perkins et al. 2011) to assess the invasive characteristics of these grasses. It may help us to understand the underlying mechanisms of plant invasion (Ott et al. 2017) and provide information that has both ecological and management implications.

SYNTHESIS

Semi-arid grasslands of the northern Great Plains (NGP) are experiencing a complex disturbance regime including fire, grazing by livestock and native herbivores and strong interannual climate variability. Increased climate variability is likely to interact with other disturbances, such as grazing in the grassland ecosystem, which may profoundly impact grassland community structure and function by affecting competitive dynamics between native and non-native species, and potentially undermining the effectiveness of restoration activities.

In the NGP, non-native *Bromus inermis* is rapidly invading larger areas of remnant native prairie and replacing the native species, such as *Pascopyrum smithii*, and decreasing biodiversity. Both *B. inermis* and *P. smithii* are perennial, cool-season (C3), sod-forming, rhizomatous grasses where population establishment and persistence of these grasses prominently depends on stem recruitment from the belowground bud bank. However, little is known how the bud bank and its associated tiller establishment of perennial grasses such as non-native *B. inermis* and native *P. smithii* will contribute to the resilience of semi-arid ecosystem in a changing climate and under grazing disturbances.

Much of the research on vegetative regeneration *via* bud banks in response to disturbance and environmental fluctuations has been conducted in mesic tallgrass prairies in the southern Great Plains with limited applicability to the drier, more expansive mixed-grass prairies of the northern Great Plains. In comparison with existing tallgrass prairies, northern mixedgrass prairies are more extensive, have greater topographic variation, contain a complex mixture of cool- and warm-season species, and have a long history of grazing by small and large animals, in conjunction with wider fluctuations in precipitation and temperature. Therefore, the northern Great Plains grasslands provide an ideal environment for examining the possible role of bud banks in providing resilience to climate change in semi-arid ecosystems.

By using the native grass *P. smithii* and non-native grass *B. inermis* as model plant species, the greenhouse microcosm experiments presented in following chapters attempted to evaluate the potential role of belowground bud banks in providing resistance and resilience of the C3 perennial rhizomatous grass to altered environmental conditions in the northern Great Plains perennial grasslands. The overarching aims of this research were: 1) to compare and contrast belowground bud banks and tiller recruitment between native *P. smithii* and non-native *B. inermis* under altered precipitation frequencies and clipping, and 2) to compare and contrast the effects of intra- and inter-specific competition between native *P. smithii* and non-native *B. inermis* in-terms of belowground bud banks, tiller recruitments, and biomass under frequent watering and constant temperature condition.

LITERATURE CITED

1. Amiaud, B., B. Touzard, A. Bonis and J.-B. Bouzillé (2008). "After grazing exclusion, is there any modification of strategy for two guerilla species: Elymus repens (L.) Gould and Agrostis stolonifera (L.)?" Plant Ecology **197**(1): 107-117.
2. Bahm, M. A., T. G. Barnes and K. C. Jensen (2011). "Herbicide and Fire Effects on Smooth Brome (Bromus inermis) and Kentucky Bluegrass (Poa pratensis) in Invaded Prairie Remnants." Invasive Plant Science and Management **4**(2): 189-197.
3. Bai, Y., J. Wu, C. M. Clark, Q. Pan, L. Zhang, S. Chen, Q. Wang and X. Han (2012). "Grazing alters ecosystem functioning and C: N: P stoichiometry of grasslands along a regional precipitation gradient." Journal of Applied Ecology **49**(6): 1204-1215.
4. Barney, J. N. and T. H. Whitlow (2008). "A unifying framework for biological invasions: the state factor model." Biological Invasions **10**(3): 259-272.
5. Baskin, C. C. and J. M. Baskin (1998). Seeds: ecology, biogeography, and evolution of dormancy and germination, Elsevier.
6. Belsky, A. (1986). "Does herbivory benefit plants? A review of the evidence." The American Naturalist **127**(6): 870-892.
7. Benson, E. J. and D. C. Hartnett (2006). "The Role of Seed and Vegetative Reproduction in Plant Recruitment and Demography in Tallgrass Prairie." Plant Ecology **187**(2): 163-177.
8. Benson, E. J., D. C. Hartnett and K. H. Mann (2004). "Belowground bud banks and meristem limitation in tallgrass prairieplant populations." American Journal of Botany **91**(3): 416-421.
9. Bernard, J. M. (1990). "Life history and vegetative reproduction in Carex." Canadian Journal of Botany **68**(7): 1441-1448.
10. Briske, D. D. (1998). "Clonal biology of caespitose grasses” . Population biology of grasses: 106.

11. Briske, D. (1991). "Developmental morphology and physiology of grasses." Grazing management: an ecological perspective. Timber Press, Portland, OR: 85-108.
12. Briske, D. and I. Noy-Meir (1998). "Plant responses to grazing: a comparative evaluation of annual and perennial grasses." Ecological basis of livestock grazing in Mediterranean ecosystems **13**: 26.
13. Briske, D. D. and J. H. Richards (1995). "Plant responses to defoliation: a physiological, morphological and demographic evaluation." Wildland plants: physiological ecology and developmental morphology. Society for Range Management, Denver, CO: 635-710.
14. Busso, C., C. Gittins, G. Becker and L. Ghermandi (2011). "Tiller hierarchy and defoliation frequency determine bud viability in the grass Poa ligularis." Ecological research **26**(5): 985-997.
15. Busso, C. and J. Richards (1995). "Drought and clipping effects on tiller demography andgrowth of two tussock grasses in Utah." Journal of Arid Environments **29**(2): 239-251.
16. Busso, C., R. Mueller and J. Richards (1989). "Effects of drought and defoliation on bud viability in two caespitose grasses." Annals of Botany **63**(4): 477-485.
17. Chen, X.-S., Y.-H. Xie, Z.-M. Deng, F. Li and Z.-Y. Hou (2011). "A change from phalanx to guerilla growth form is an effective strategy to acclimate to sedimentation in a wetland sedge species Carex brevicuspis (Cyperaceae)." Flora-Morphology, Distribution, Functional Ecology of Plants **206**(4): 347-350.
18. Christiansen, P. A. and R. Q. Landers (1966). "Notes on prairie species in Iowa. I. Germination and establishment of several species." Trans. Iowa Acad. Sci **73**: 51-59.
19. Collins, S. L. and L. B. Calabrese (2012). "Effects of fire, grazing and topographic variation on vegetation structure in tallgrass prairie." Journal of Vegetation Science **23**(3): 563-575.

20. Corbin, J. D. and C. M. D’Antonio (2010). "Not novel, just better: competition between native and non-native plants in California grasslands that share species traits." Plant Ecology **209**(1): 71-81.
21. Cornelissen, J. H. C., Y. B. Song, F. H. Yu and M. Dong (2014). "Plant traits and ecosystem effects of clonality: a new research agenda." Annals of Botany **114**(2): 369-376.
22. Craine, J. M., J. B. Nippert, E. G. Towne, S. Tucker, S. W. Kembel, A. Skibbe and K. K. McLauchlan (2011). "Functional consequences of climate change-induced plant species loss in a tallgrass prairie." Oecologia **165**(4): 1109-1117.
23. Cully, A. C., J. F. Cully and R. D. Hiebert (2003). "Invasion of exotic plant species in tallgrass prairie fragments." Conservation Biology **17**(4): 990-998.
24. Dalgleish, H. J. (2007). Belowground bud banks as regulators of grassland dynamics, Kansas State University.
25. Dalgleish, H. J. and D. C. Hartnett (2006). "Belowground bud banks increase along a precipitation gradient of the North American Great Plains: a test of the meristem limitation hypothesis." New Phytologist **171**(1): 81-89.
26. Dalgleish, H. J. and D. C. Hartnett (2009). "The effects of fire frequency and grazing on tallgrass prairie productivity and plant composition are mediated through bud bank demography." Plant Ecology **201**(2): 411-420.
27. DeKeyser, E. S., M. Meehan, G. Clambey and K. Krabbenhoft (2013). "Cool season invasive grasses in northern Great Plains natural areas." Natural Areas Journal **33**(1): 81-90.
28. Dibbern, J. C. (1947). "Vegetative responses of Bromus inermis to certain variations in environment." Botanical Gazette **109**(1): 44-58.
29. Dillemuth, F. P., E. A. Rietschier and J. T. Cronin (2009). "Patch dynamics of a native grass in relation to the spread of invasive smooth brome (Bromus inermis)." Biological invasions **11**(6): 1381-1391.
30. Dong, B.-C., G.-L. Yu, W. Guo, M.-X. Zhang, M. Dong and F.-H. Yu (2010). "How internode length, position and presence of leaves affect survival and growth

of Alternanthera philoxeroides after fragmentation?" Evolutionary Ecology **24**(6): 1447-1461.

31. Donkor, N., E. Bork and R. Hudson (2002). "Bromus-Poa response to defoliation intensity and frequency under three soil moisture levels." Canadian Journal of Plant Science **82**(2): 365-370.
32. Doust, L. L. (1981). "Population Dynamics and Local Specialization in a Clonal Perennial (Ranunculus Repens): I. The Dynamics of Ramets in Contrasting Habitats." Journal of Ecology **69**(3): 743-755.
33. Dreber, N. and K. Esler (2011). "Spatio-temporal variation in soil seed banks under contrasting grazing regimes following low and high seasonal rainfall in arid Namibia." Journal of Arid Environments **75**(2): 174-184.
34. Dukes, J. S. and H. A. Mooney (1999). "Does global change increase the success of biological invaders?" Trends in Ecology & Evolution **14**(4): 135-139.
35. Eneboe, E., B. Sowell, R. Heitschmidt, M. Karl and M. Haferkamp (2002). "Drought and grazing: IV. Blue grama and western wheatgrass." Journal of Range Management: 73-79.
36. Engel, R., L. E. Moser, J. Stubbendieck and S. Lowry (1987). "Yield Accumulation, Leaf Area Index, and Light Interception of Smooth Bromegrass 1." Crop science **27**(2): 316-321.
37. Falkner, L. and M. Casler (2000). "Genetic shifts in smooth bromegrass under grazing: changes in nutritional value and preference for surviving vs. original genotypes." Grass and Forage Science **55**(4): 351-360.
38. Fenner, M. (1985). "Seed Ecology" Chapman and Hall. New York **145**.
39. Fidelis, A., B. Appezzato-da-Gloria, V. D. Pillar and J. Pfadenhauer (2014). "Does disturbance affect bud bank size and belowground structures diversity in Brazilian subtropical grasslands?" Flora **209**(2): 110-116.
40. Forrest, S., H. Strand, W. Haskins, C. Freese, J. Proctor and E. Dinerstein (2004). "Ocean of grass: a conservation assessment for the Northern Great Plains." Northern Plains Conservation Network and Northern Great Plains Ecoregion, WWF-US, Bozeman, MT.

41. Frank, A. (1994). "Physiological comparisons of crested wheatgrass and western wheatgrass to water." Journal of Range Management: 460-466.

42. Fuhlendorf, S. D., H. Zhang, T. Tunnell, D. M. Engle and A. F. Cross (2002). "Effects of grazing on restoration of southern mixed prairie soils." Restoration Ecology **10**(2): 401-407.

43. Funk, J. L. and P. M. Vitousek (2007). "Resource-use efficiency and plant invasion in low-resource systems." Nature **446**(7139): 1079-1081.

44. Garnier, E. and J. Roy (1988). "Modular and demographic analysis of plant leaf area in sward and woodland populations of Dactylis glomerata and Bromus erectus." The Journal of Ecology: 729-743.

45. Gerry, A. K. and S. D. Wilson (1995). "The influence of initial size on the competitive responses of six plant species." Ecology **76**(1): 272-279.

46. Glaser, A., ed. (2012). “America’s Grasslands Conference: Status, Threats, and Opportunities”. Proceedings of the 1st Biennial Conference on the Conservation of America’s Grasslands. August 15- 17, 2011, Sioux Falls, SD. Washington, DC and Brookings, SD: National Wildlife Federation and South Dakota State University.

47. Grant, T. A., B. Flanders-Wanner, T. L. Shaffer, R. K. Murphy and G. A. Knutsen (2009). "An emerging crisis across northern prairie refuges: prevalence of invasive plants and a plan for adaptive management." Ecological Restoration **27**(1): 58-65.

48. Groya, F. and C. Sheaffer (1981). "Establishment of Sod-seeded Alfalfa at Various Levels of Soil Moisture and Grass Competition 1." Agronomy journal **73**(3): 560-565.

49. Harper, J. L. (1977). "Population biology of plants." Population biology of plants.

50. Harrison, T. and J. T. Romo (1994). "Regrowth of smooth bromegrass (Bromus inermis Leyss.) following defoliation." Canadian Journal of Plant Science **74**(3): 531-537.

51. Hartnett, D. and P. Fay (1998). "Plant populations: patterns and processes." Grassland dynamics: long-term ecological research in tallgrass prairie. Oxford University Press, New York: 81-100.
52. Hellström, K., M. M. Kytovitta, J. Tuomi and P. Rautio (2006). "Plasticity of clonal integration in the perennial herb Linaria vulgaris after damage." Functional Ecology **20**(3): 413-420.
53. Hendrickson, J. and D. Briske (1997). "Axillary bud banks of two semiarid perennial grasses: occurrence, longevity, and contribution to population persistence." Oecologia **110**(4): 584-591.
54. Hobbs, R. J. and L. F. Huenneke (1992). "Disturbance, diversity, and invasion: implications for conservation." Conservation biology **6**(3): 324-337.
55. Hoshino, A., Y. Yoshihara, T. Sasaki, T. Okayasu, U. Jamsran, T. Okuro and K. Takeuchi (2009). "Comparison of vegetation changes along grazing gradients with different numbers of livestock." Journal of Arid Environments **73**(6): 687-690.
56. Humphrey, L. D. and D. A. Pyke (1998). "Demographic and growth responses of a guerilla and a phalanx perennial grass in competitive mixtures." Journal of Ecology **86**(5): 854-865.
57. Hyder, D. (1972). "Defoliation in relation to vegetative growth." The biology and utilization of grasses: 302-317.
58. Jewiss, O. (1972). "Tillering in Grasses—Its Significane And Control*." Grass and Forage Science **27**(2): 65-82.
59. Jones, S. K., S. L. Collins, J. M. Blair, M. D. Smith and A. K. Knapp (2016). "Altered rainfall patterns increase forb abundance and richness in native tallgrass prairie." Scientific reports **6**.
60. Klimesova, J. and L. Klimes (2007). "Bud banks and their role in vegetative regeneration - A literature review and proposal for simple classification and assessment." Perspectives in Plant Ecology Evolution and Systematics **8**(3): 115-129.

61. Kluse, J. S. and B. H. A. Diaz (2005). "Importance of soil moisture and its interaction with competition and clipping for two montane meadow grasses." Plant Ecology **176**(1): 87-99.
62. Knapp, A. K. and M. D. Smith (2001). "Variation among biomes in temporal dynamics of aboveground primary production." Science **291**(5503): 481-484.
63. Koerner, S. E., S. L. Collins, J. M. Blair, A. K. Knapp and M. D. Smith (2014). "Rainfall variability has minimal effects on grassland recovery from repeated grazing." Journal of Vegetation Science **25**(1): 36-44.
64. Lamas, M. B., C. Larreguy, A. Carrera and M. Bertiller (2013). "Changes in plant cover and functional traits induced by grazing in the arid Patagonian Monte." Acta oecologica **51**: 66-73.
65. Lehtila, K. (2000). "Modelling compensatory regrowth with bud dormancy and gradual activation of buds." Evolutionary Ecology **14**(4-6): 315-330.
66. Liu, H.-D., F.-H. Yu, W.-M. He, Y. Chu and M. Dong (2007). "Are clonal plants more tolerant to grazing than co-occurring non-clonal plants in inland dunes?" Ecological research **22**(3): 502-506.
67. Mac, M. J., P. A. Opler, C. P. Haecker and P. D. Doran (1998). "Status and trends of the nation’s biological resources." United States Department of the Interior, United States Geological Survey, Reston, Virginia **2**: 437-964.
68. Milchunas, D. t. and W. Lauenroth (1995). "Inertia in Plant Community Structure: State Changes After Cessation of Nutrient□Enrichment Stress." Ecological Applications **5**(2): 452-458.
69. Morgan, J., W. Knight, L. Dudley and H. Hunt (1994). Enhanced root system C-sink activity, water relations and aspects of nutrient acquisition in mycotrophic Bouteloua gracilis subjected to CO 2 enrichment. Belowground Responses to Rising Atmospheric CO2: Implications for Plants, Soil Biota, and Ecosystem Processes, Springer**:** 139-146.
70. Mullahey, J. J., S. S. Waller and L. E. Moser (1991). "Defoliation effects on yield and bud and tiller numbers of two Sandhills grasses." Journal of Range Management: 241-245.

71. Navas, M.-L. and E. Garnier (1990). "Demography and growth forms of the clonal perennial Rubia peregrina in Mediterranean vineyard and unmanaged habitats." The Journal of Ecology: 691-712.

72. Nernberg, D. and M. R. Dale (1997). "Competition of five native prairie grasses with Bromus inermis under three moisture regimes." Canadian Journal of Botany **75**(12): 2140-2145.

73. N'Guessan, M. (2007). Effects of grazing on growth and morphology of rhizomatous and caespitose grasses in tallgrass prairie, Kansas State University.

74. Oborny, B. (1997). "Models of spatial spread and foraging in clonal plants." The ecology and evolution of clonal plants: 155-183.

75. Olson, D. M. and E. Dinerstein (1998). "The Global 200: a representation approach to conserving the Earth's most biologically valuable ecoregions." Conservation Biology **12**(3): 502-515.

76. Otfinowski, R. (2008). "Patterns and processes of exotic plant invasions in Riding Mountain National Park, Manitoba, Canada."

77. Ott, J. P. (2014). Ecological implications of grass bud bank and tiller dynamics in mixed-grass prairie, PhD Dissertation, Kansas State University.

78. Ott, J. P., J. L. Butler, Y. Rong and L. Xu (2017). "Greater bud outgrowth of Bromus inermis than Pascopyrum smithii under multiple environmental conditions." Journal of Plant Ecology **10**(3): 518-527.

79. Ott, J. P. and D. C. Hartnett (2011). "Bud Production and Dynamics of Flowering and Vegetative Tillers in Andropogon Gerardii (Poaceae): The Role of Developmental Constraints." American Journal of Botany **98**(8): 1293-1298.

80. Ott, J. P. and D. C. Hartnett (2012). "Higher-order bud production increases tillering capacity in the perennial caespitose grass Scribner's Panicum (Dichanthelium oligosanthes)." Botany **90**(9): 884-890.

81. Ott, J. P. and D. C. Hartnett (2015). "Bud bank dynamics and clonal growth strategy in the rhizomatous grass, Pascopyrum smithii." Plant Ecology **216**(3): 395-405.

82. Palmer, J. (1958). "Studies In The Behavior of The Rhizome of Agroptron Repens (L.) Beauv." New Phytologist **57**(2): 145-159.
83. Painter, E. and J. Detling (1981). "Effects of defoliation on net photosynthesis and regrowth of western wheatgrass." Journal of range management: 68-71.
84. Perkins, L. B., E. A. Leger and R. S. Nowak (2011). "Invasion triangle: an organizational framework for species invasion." Ecology and evolution **1**(4): 610-625.
85. Pimentel, D., R. Zuniga and D. Morrison (2005). "Update on the environmental and economic costs associated with alien-invasive species in the United States." Ecological Economics **52**(3): 273-288.
86. Qian, J., Z. Wang, Z. Liu and C. A. Busso (2014). "Belowground Bud Bank Responses to Grazing Intensity in the Inner-Mongolia Steppe, China." Land Degradation & Development.
87. Reekie, E. and R. Redmann (1991). "Effects of water stress on the leaf demography of Agropyron desertorum, Agropyron dasystachyum, Bromus inermis, and Stipa viridula." Canadian journal of botany **69**(8): 1647-1654.
88. Reichard, S. H. and P. White (2001). "Horticulture as a Pathway of Invasive Plant Introductions in the United States Most invasive plants have been introduced for horticultural use by nurseries, botanical gardens, and individuals." BioScience **51**(2): 103-113.
89. Reynolds, J. H. and D. Smith (1962). "Trend of Carbohydrate Reserves in Alfalfa, Smooth Bromegrass, and Timothy Grown Under Various Cutting Schedules 1." Crop science **2**(4): 333-336.
90. Ricketts, T. H. (1999). Terrestrial ecoregions of North America: a conservation assessment, Island Press.
91. Rogers, W. E. and D. C. Hartnett (2001). "Temporal vegetation dynamics and recolonization mechanisms on different-sized soil disturbances in tallgrass prairie." American Journal of Botany **88**(9): 1634-1642.
92. Romo, J., P. Grilz and E. Driver (1990). "Invasion of the Canadian prairies by an exotic perennial." Blue Jay **48**(3): 130-135.

93. Samson, F. B. and F. L. Knopf (1996). Prairie conservation: preserving North America's most endangered ecosystem, Island Press.
94. Samson, F. B., F. L. Knopf and W. R. Ostlie (2004). "Great Plains ecosystems: past, present, and future." Wildlife Society Bulletin **32**(1): 6-15.
95. Schmid, B. and J. L. Harper (1985). "Clonal growth in grassland perennials: I. Density and pattern-dependent competition between plants with different growth forms." The Journal of Ecology: 793-808.
96. Schmid, B. and J. L. Harper (1985). "Clonal growth in grassland perennials: I. Density and pattern-dependent competition between plants with different growth forms." The Journal of Ecology: 793-808.
97. Schrag, A. M. (2011). Addendum: climate chnage impacts and adaptation strategies. In: Ocean of grass: a conservation assessment for the Northern Great Plains Bozeman, MT, World Wildlife Fund-US**:** 36.
98. Sheppard, C. S., J. M. Alexander and R. Billeter (2012). "The invasion of plant communities following extreme weather events under ambient and elevated temperature." Plant ecology **213**(8): 1289-1301.
99. Simms, P. L. (1988). Grasslands. Pages 265–286 *in* M. G. Barbour and W. D. Billings, editors. North American terrestrial vegetation. Cambridge University Press, New York.
100. Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor and H. L. Miller (2007). Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change, 2007, Cambridge University Press, Cambridge.
101. Sprinkle, J. W. (2010). Bud bank density regulates invasion by exotic plants, Oklahoma State University.
102. Steinauer, E. M., and S. L. Collins. (1996). Disturbance as a management component in tall-grass prairie. Pages 39–52 *in* F. B. Samson and F. L. Knopf, editors. Prairie conservation: preserving North America’s most endangered ecosystem. Island Press, Covelo, Calif.

103. Sutherland, W. J. and R. A. Stillman (1988). "The foraging tactics of plants." Oikos: 239-244.

104. VanderWeide, B. L. (2013). Grazing and drought in tallgrass prairie: the role of belowground bud banks in vegetation dynamics, Kansas State University.

105. VanderWeide, B. L. and D. C. Hartnett (2015). "Belowground bud bank response to grazing under severe, short-term drought." Oecologia **178**(3): 795-806.

106. VanderWeide, B. L., D. C. Hartnett and D. L. Carter (2014). "Belowground bud banks of tallgrass prairie are insensitive to multi-year, growing-season drought." Ecosphere **5**(8).

107. Violle, C., D. R. Nemergut, Z. Pu and L. Jiang (2011). "Phylogenetic limiting similarity and competitive exclusion." Ecology letters **14**(8): 782-787.

108. Vitousek, P. M., C. M. Antonio, L. L. Loope and R. Westbrooks (1996). "Biological invasions as global environmental change." American Scientist **84**(5): 468.

109. Wand, S. J., G. Midgley, M. H. Jones and P. S. Curtis (1999). "Responses of wild C4 and C3 grass (Poaceae) species to elevated atmospheric CO2 concentration: a meta□analytic test of current theories and perceptions." Global Change Biology **5**(6): 723-741.

110. Weaver, J. E. (1968). "Prairie plants and their environment. A fifty-year study in the midwest." Prairie plants and their environment. A fifty-year study in the midwest.

111. Weaver, T., E. M. Payson, and D. L. Gustafson (1996). Adequacy for conservation for short-grass prairie. Pages 67–76 *in* F. B. Samson and K. L. Knopf, editors. Prairie conservation: preserving North America’s most endangered ecosystem. Island Press, Covelo, Calif.

112. Weaver, J. E. and I. M. Mueller (1942). “Role of seedlings in recovery of midwestern ranges from drought.” Ecology **23**(3): 275-294.

113. Welker, J. M. and D. D. Briske (1992). "Clonal Biology of the Temperate, Caespitose, Graminoid Schizachyrium scoparium: A Synthesis with Reference to Climate Change." Oikos **63**(3): 357-365.

114. Wilcove, D. S., D. Rothstein, J. Dubow, A. Phillips and E. Losos (1998). "Quantifying threats to imperiled species in the United States." BioScience **48**(8): 607-615.
115. Willson, G. D. (1992). Morphological characteristics of smooth brome used to determine a prescribed burn date. Proceedings, 12th North American Prairie Conference: Recapturing a Vanishing Heritage. Cedar Falls, IA: University of Northern Iowa.
116. Willson, G. D. and J. L. Stubbendieck (1996). "Suppression of smooth brome by atrazine, mowing, and fire."
117. Wuebbles, D., D. Fahey, K. Hibbard, D. Dokken, B. Stewart and T. Maycock (2017). "Climate Science Special Report: Fourth National Climate Assessment." US Global Change Research Program: Washington, DC, USA.
118. Ye, X.-H., F.-H. Yu and M. Dong (2006). "A trade-off between guerilla and phalanx growth forms in Leymus secalinus under different nutrient supplies." Annals of Botany **98**(1): 187-191.
119. Zelikova, T. J., D. M. Blumenthal, D. G. Williams, L. Souza, D. R. LeCain, J. Morgan and E. Pendall (2014). "Long-term exposure to elevated CO2 enhances plant community stability by suppressing dominant plant species in a mixed-grass prairie." Proceedings of the National Academy of Sciences **111**(43): 15456-15461.

CHAPTER 2

IMPACTS OF ALTERED PRECIPITATION FREQUENCY AND CLIPPING ON PERENNIAL GRASSES MEDIATED THROUGH BELOWGROUND BUD BANK

ABSTRACT

In perennial grasses, the belowground population of meristems (i.e. the bud bank) plays a fundamental role in plant population persistence, community stability, and grassland response to disturbances. In this study, we evaluated the response of belowground bud production, tiller and rhizome recruitment, and plant establishment between two perennial grass species, the native *Pascopyrum smithii* and the non-native *Bromus inermis,* to altered precipitation frequency and clipping under controlled temperature conditions. A greenhouse experiment consisted of the combinations of three precipitation frequencies (every 2d, 8d, and 16d) representing high, medium, and low, two levels of clipping (clipping *vs.* no-clipping), and two species with 40 replicates for each treatment combination. Individual plants from seedlings were grown in potting soil. We initiated precipitation frequency treatments two weeks after transplanting and applied the clipping treatment at 3-collared leaf stage. Plants were harvested 20 weeks after the treatments had been initiated. The number of tillers and rhizomes based on generation, number of tillers based on location (crown *vs.* rhizome), and rhizome length were recorded. Three randomly sub-sampled crown tillers and rhizome tillers from each generation were dissected to record the number of buds, and propagule development. We found *B. inermis* significantly decreased its number of tillers, rhizomes, rhizome length, and live propagules at the low precipitation frequency, but advanced propagule development at medium precipitation frequency. However, *P. smithii* significantly increased the traits described above under medium precipitation frequency except for number of tillers and propagule development, which were not affected at medium and low precipitation frequency. Clipping treatment significantly reduced tiller production for both species and number of rhizomes for *B. inermis*. The results indicate that the non-

native *B. inermis* may be more susceptible to the low precipitation frequency and clipping compared to the native *P. smithii*. Native *P. smithii* may be able to resist the water stress and clipping effects mediated *via* the belowground bud bank.

INTRODUCTION

Grasslands are estimated to cover 40.5% (52,544,000 km^2) of the global land area, and provide valuable ecosystem goods and services, such as food, carbon storage, and recreation (Murray et al. 2000). However, over the recent decades, semi-arid grasslands of the northern Great Plains (NGP) are experiencing a complex disturbance regime, including fire, grazing by livestock and native herbivores and strong inter-annual climate variability (Schrag 2011). Because of climate change, the growing season precipitation regimes are predicted to become more variable, with an increase in larger precipitation events and longer dry periods, resulting in more soil moisture temporally dynamic (Koerner et al. 2014). Increased climate variability is likely to interact with other disturbances, such as grazing, in the grassland ecosystem, and may profoundly impact grassland community structure and function by affecting competitive dynamics between native and invasive species, and potentially undermining the effectiveness of restoration activities. In the NGP, non-native smooth bromegrass (*Bromus inermis*) is rapidly invading larger areas of remnant native prairie and replacing the native species, such as western wheatgrass (*Pascopyrum smithii*), and decreasing biodiversity (Cully et al. 2003).

Both *B. inermis* and *P. smithii* are perennial, C3 (cool-season), sod forming, dominant rhizomatous grasses in the mixed-grass prairies of the NGP (the PLANTS database, USDA-NRCS 2006). The population establishment and persistence of these perennial grasses depends on stem recruitment from the belowground bud bank (Benson and Hartnett 2006, Dalgleish and Hartnett 2009, Ott et al. 2017). For example, in undisturbed tallgrass prairie, recruitment from seed is extremely rare and >99% of tiller recruitment occurs from belowground buds rather than seeds (Benson and Hartnett 2006). The response of these belowground bud banks to grazing disturbances (Dalgleish and Hartnett 2009) and climatic variability (Dalgleish and Hartnett 2006) have demonstrated great potential for shaping the resilience of grassland plant communities (Klimesova and Klimes 2007, Ott and Hartnett 2011, Ott and Hartnett 2012).

Not only this, these two rhizomatous grasses can vary in architecture along the "phalanx-guerilla" clonal growth form continuum (Harper 1977, Doust 1981), which may determine the structure and fate of the belowground bud bank (Ott and Hartnett 2015a). Studies have shown that investment in different growth forms of rhizomatous perennial grasses depends on environmental conditions (Doust 1981, Garnier and Roy 1988). However, little is known how the belowground bud bank, tiller recruitments, and clonal growth form of non-native perennial grasses, such as *B. inermis* interacting with native *P. smithii,* will influence the resilience of semi-arid ecosystems in a changing climate under grazing disturbances.

According to the recent Fourth National Climate Assessment Report 2017, there are projections of an increase in dry days or heavy precipitation events, creating longer intervals between events and repeated droughts all over the contiguous United States (Wuebbles et al. 2017). There is evidence that these effects of an extreme precipitation climate will be manifested primarily in altered soil moisture availability. Such alterations in precipitation regimes during the growing season will have significant ecological consequences for grassland structure and function (Craine et al. 2011, Jones et al. 2016). Jones et al. (2016) reported that increase rainfall variability resulted in decreased soil respiration, leaf level photosynthesis, and scaled up to annual net primary productivity. Similar studies have shown that the importance of soil moisture and its interaction with competition and clipping on the productivity and phenological traits of grasses at the population level (Kluse and Diaz 2005) to community levels, and overall grassland ecosystem function and services (Knapp et al. 2001).

Therefore, it is reasonable to expect that climate change, along with decreased soil moisture availability, could alter these bud processes of bud natality and dormancy. Bud dormancy can inhibit the number of buds that can be activated and become emerging tillers (Hendrickson and Briske 1997). Similarly, bud activation (i.e. tiller initiation) could be reduced under low water availabilty. Beacuse new tiller establishment produces the next generations of buds, previous year tiller production can have a strong influence, mediated by the bud bank, on the next year's aboveground net primary production (Ott and Hartnett 2012, Reichmann et al. 2013). Studies have shown that soil moisture variability could greatly alter the success of tiller recruits and the number of buds, tillers are able to produce, with the ultimate effects on ANPP (Knapp and Smith 2001, Dalgleish and Hartnett 2006).

Much of the research on vegetative regeneration *via* the bud bank in response to disturbance and environmental fluctuations has been conducted in mesic tallgrass prairies in the southern Great Plains with limited applicability to the drier, more expansive mixed grass prairies of the northern Great Plains. In comparison with existing tallgrass prairie, northern mixed grass prairies are more extensive, have greater topographic variation, contain a complex mixture of cool- and warm-season species, and have a long history of grazing by small and large animals, in conjunction with wider fluctuations in precipitation and temperature (Russell et al. 2015). Thus, the northern Great Plains grasslands provide an ideal environment for studying the potential role of the bud bank in providing resilience to climate change.

According to the recent study by Ott et al. (2017) on mixed-grass prairies of the northern Great Plains, it was clear that under short-term drought and a range of temperatures, *B. inermis* maintained a greater number of live buds per stem and initiated a greater proportion bud outgrowth than *P. smithii*. However, whether this bud outgrowth established into tillers that will produce new buds under various environmental conditions has not been evaluated. Therefore, this study by using the native C3 perennial grass *P. smithii* and non-native C3 perennial grass *B. inermis* as model plants is expected to extend the work of Ott et al. (2017), by providing the additional information on population demography under various environmental conditions. Evaluating the belowground bud bank and tiller demography of the two species would provide considerable insight into how these two species might respond to climate change and other environmental disturbances, individually and under competition.

We have selected *P. smithii* as a model plant for the study because of its native status, along with its widespread distribution and is often the dominant species in many grassland communities. Similarly, *B. inermis* has been selected because, like *P. smithii* , it is strongly rhizomatous with widespread distribution, and invading areas in both tallgrass and mixed-grass prairies makes it a problematic non-native. The outcome of competition between *P. smithii* and *B. inermis* may depend on differential expression of their respective bud banks under a scenario of climate change.

1.1 Research Questions

1. Do *P. smithii* and *B. inermis* establish differently under all precipitation frequencies and simulated grazing? (RQ1)

2. Does *B. inermis* produce more tillers than *P. smithii* in each tiller generation under all precipitation frequencies and simulated grazing? (RQ2)

3. Do the propagule development differ between *P. smithii* and *B. inermis* under all precipitation frequencies and simulated grazing? (RQ3)

4. Does live propagule production differ for each tiller generation between *P. smithii* and *B. inermis* under all precipitation frequencies and simulated grazing? (RQ4)

5. Does the investment in phalanx and guerilla growth differ between *P. smithii* and *B. inermis* under all precipitation frequencies and simulated grazing? (RQ5)

1.2 Hypotheses

H_a1: Overall plant establishment in terms of total tiller, total rhizomes, and total rhizome length of the non-native C3 perennial grass (*B. inermis*) will be greater than the native C3 perennial grass (*P. smithii*) under all precipitation frequencies and clipping conditions. (RQ1)

H_a2: The non-native C3 perennial grass (*B. inermis*) will produce a greater number of tillers than the native C3 perennial grass (*P. smithii*) in each tiller generation under all precipitation frequencies and clipping conditions. (RQ2)

H_a3: Propagule development of the non-native C3 perennial grass (*B. inermis*) will exceed that of the native C3 perennial grass (*P. smithii*) under all precipitation frequencies and clipping conditions. (RQ3)

H_a4: The non-native C3 perennial grass (*B. inermis*) will have a greater number of live propagules than the native C3 perennial grass (*P. smithii*) for each tiller generation under all precipitation frequencies and clipping conditions. (RQ4)

H_a5: Investment in guerilla growth *via* total live propagule and total tillers will be greater than phalanx growth in the non-native C3 perennial grass *B. inermis* than the native C3 perennial grass *P. smithii* under all precipitation frequencies and clipping conditions. (RQ5)

METHODS

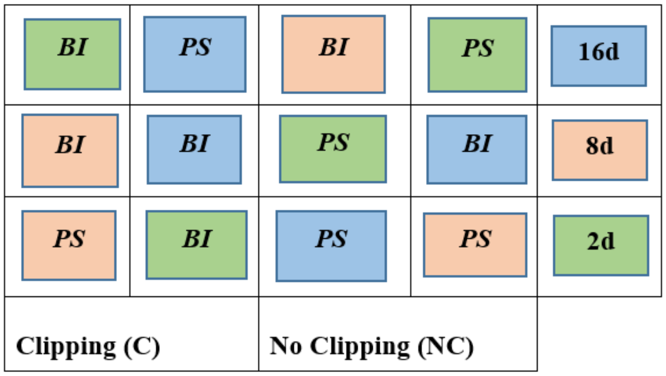
2.1 Experiment Design

A temperature-controlled greenhouse experiment was carried out in the South Dakota Seed Testing Laboratory (44.324764, -96.767247) over a growing season of 2016 (June-November). A three-way factorial (3x2x2) experimental design consisted of the combination of three precipitation frequencies and two clipping levels (clipping and no-clipping). This included two species with 40 replicates for each treatment which were randomly placed in two chambers of the greenhouse (Table 2.1).

Table 2.1 The main factors and levels of the experiment

Factors	**Precipitation Frequency**	**Clipping**	**Species**
Levels	Every 2d (72ml) VWC ~ 24% - 28% High	Clipping(C)	*B. inermis*
	Every 8d (288ml) VWC ~ 13% - 14% Medium	No-Clipping(NC)	*P. smithii*
	Every 16d (576ml) VWC at 8% Low		

We had two greenhouse chambers with the same temperature conditions containing a total of 480 pots (i.e. 240 pots in each chamber). In each chamber, these pots were randomized weekly within the matrix of clipping treatment and precipitation frequency treatment as shown in Figure 2.1.



BI	***PS***	***BI***	***PS***	**16d**
BI	***BI***	***PS***	***BI***	**8d**
PS	***BI***	***PS***	***PS***	**2d**
Clipping (C)		**No Clipping (NC)**		

Figure 2.1 Conceptual diagram of one replication of an experimental plot in greenhouse experiment. Where, BI = *B. inermis* plant, PS = *P. smithii* plant, and 2d, 8d, and 16d are precipitation frequencies as shown in Table 2.1 and color coded.

2.2 Treatment

2.2.1 Precipitation Frequency

Before the start of the watering frequency treatments, each pot (16.5-cm dia. X 16.5-cm depth) with 600g of potting soil (PRO-MIX® BX) was saturated with 500ml of water. Then another 600ml of water was added after seedling transplanted (for 1 week) to reach water saturation of 44%-45% volumetric water content (VWC) (Decagon Devices, Soil Moisture Sensor: Model EC-5 factory calibrated to the potting soil).

Under this water treatment, we only manipulated the precipitation frequency with high, medium, and low (2 days, 8 days, and 16 days, respectively) between precipitation events, by maintaining the same total average monthly growing season precipitation of 51.43 mm/month. This is the monthly average of the growing season (March, April, May, June, July and August) precipitation amount from Rapid City Regional AP station, South Dakota (1981-2010) (https://climate.sdstate.edu/), which represents the spring growing season precipitation characteristics of mixed-grass prairies of the northern Great Plains (Schrag 2011). The watering frequency treatment was applied for 20 weeks (from July 1, 2016 to November 20, 2016). The mean length of dry periods (watering interval) such as every eighth day (8d) and sixteenth day (16d) was derived based upon a drop-in soil moisture content and was consistent with the predicted climate change scenario of the U.S. Great Plains (Jones et al. 2016). We had three levels of precipitation frequency as explained below:

Every 2d watering frequency: This was started on July 1, 2016 (18 days after the seedling transplant), when VWC dropped ~ 24% - 28%. The 160 randomly assigned pots were watered every other day (2d) with 72ml to maintain the soil moisture level at ~ 24% - 28%. This precipitation amount of 72ml every other day represented high precipitation frequency over the mixed-grass prairie region and was calculated based on average spring season monthly precipitation and the surface area of the pot.

Every 8d watering frequency: This was started on July 7, 2016 (24 days later the seedling transplant) when VWC dropped ~ 13% - 14%. Another set of 160 randomly assigned pots were watered every eighth day (8d) with 288ml to maintain the soil moisture to ~ 13% - 14%. This precipitation amount of 288ml every eighth day represented medium precipitation frequency over the mixed grass prairie region and was calculated based on average spring season monthly precipitation and the surface area of the pot.

Every 16d watering frequency: This was started on July 15, 2016 (31 days after seedling transplant), when VWC dropped ~ 8%. The remaining set of 160 randomly assigned pots irrespective of clipping condition were watered every sixteenth day (16d) with 576ml to maintain the soil moisture to this value. This precipitation amount of 576ml every sixteenth day represented low precipitation frequency over this region and was calculated based on average spring season monthly precipitation and the surface area of the pot.

*Note: We lost 42 pots combined of all the water treatments during 2nd and 3rd week of July due to roof leakage and sudden water outburst from greenhouse chamber pump.

2.2.2 Clipping Treatment

Clipping treatments consisted of clipped or unclipped. A one-time clipping treatment was randomly assigned to half of the pots for each precipitation frequency treatment and species. The clipping treatment was applied when each species reached the 3 collared-leaf stage and was clipped to the 4-cm subtle height to simulate early grazing by ungulates (Pfeiffer and Harnett 1995).

2.2.3 Study Species

Pascopyrum smithii (western wheatgrass), and *B. inermis* (smooth bromegrass) are both strongly rhizomatous, perennial C3 grasses that begin flowering in late May (the PLANTS database, USDA-NRCS 2006). *Pascopyrum smithii* is native to North America and most abundant in the areas receiving 254 to 508-mm precipitation. It is an important component of many native plant communities. In contrast, *B. inermis* was introduced from Eurasia in the late 1880s for forage productivity and has made an extensive impact on the grasslands of North America. *B. inermis* establishes by invading disturbed prairies and through repeated introductions for soil retention and livestock grazing (Otfinowski et al. 2007). These two species produce both phalanx and guerilla tillers *via* its clonal growth strategy and quickly spreads out into open habitat (Asay and Jensen 1996, Judziewicz et al. 1999, Ott and Hartnett 2015a).

Seed Sources

The seeds of *P. smithii* were from Golden Willow Seeds, INC. (Midland, SD) and the *B. inermis* seeds were from Dakota's Best Seed LLC (Platte, SD). These seeds were provided by the South Dakota State University Seed Testing Laboratory (Brookings, SD).

2.3 Seedling Establishment and Transplant

Seeds were screened for intactness under a magnifying glass and were germinated in Miracle-Gro® potting mix soil in the greenhouse with a temperature regime of 16°C night/ 22°C day. Seeds of *P. smithii* were sown five days earlier than *B. inermis* to obtain the same growth stage for transplant. Two hundred-forty single-leaf seedlings with similar size for each species were transplanted simultaneously into each non-fertilizer potting-soil (PRO-MIX® BX) filled pot (16.5-cm dia. X 16.5-cm depth) in the 3rd week of June 2016.

2.4 Growth Condition

Photoperiods and temperature regimes of greenhouse chambers were set up similar to mixed-grass prairie field conditions during the growing season with the constant averaged monthly photoperiod and temperature regime based on the ten years of climate data from Rapid city, South Dakota. To ensure the survival and growth of transplanted seedlings, a 100ml solution of 1.5% NPK (15-30-15) was added to all 480 pots one week after seedling transplants and before applying any precipitation frequency or clipping treatment.

2.5 Data Collection

2.5.1 Harvesting Plants

Plants were harvested 20 weeks after the treatments had been initiated, and underground structures were then washed free of soil and sorted. Harvested plants were mapped out to record number of tillers and rhizomes based on generation, number of tillers based on location (crown *vs.* rhizome), and total rhizome length (Photo 2.1).

The guidelines of data collection were developed, and data were collected under the following headings:

2.5.2 Tiller and Rhizome Generation Mapping

Daughter tillers and rhizomes were classified into different generations based on the guidelines (Figure 2.2) developed following the work of Ott and Harnett (2015a). The rhizomes were considered belowground stems that had elongated internodes and were at least 0.5-cm long.

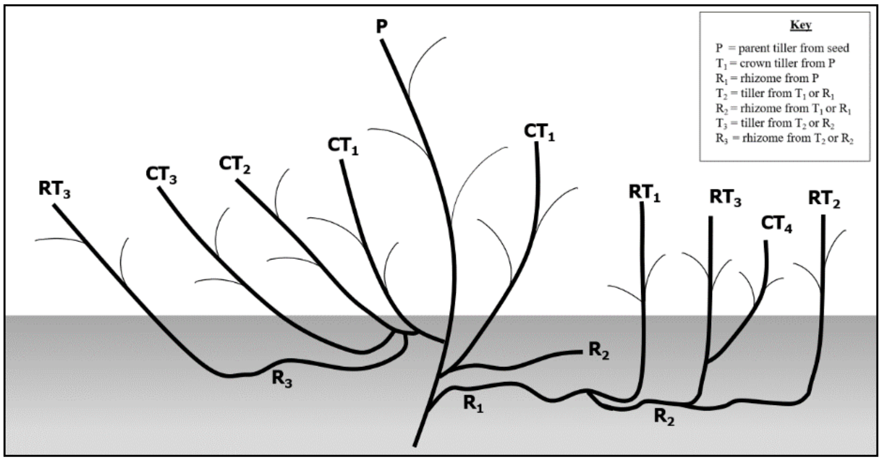


Figure 2.2 Conceptual diagram of tiller and rhizome classification according to generation (1= primary, 2= secondary, 3= tertiary).

As shown in Figure 2.2, the parent tiller is from the seed, the primary generation tiller/rhizome (T1/R1) are the ones directly come from the parent tiller, the secondary generation tiller/rhizome (T2/R2) are those come from the primary generation tiller/rhizome (T1/R1), and tertiary generation tiller/rhizome (T3/R3) are those come from secondary generation tiller/rhizome (T2/R2). When a tiller comes from the tip of a rhizome it would be of the same generation of that rhizome on which tip it is growing.

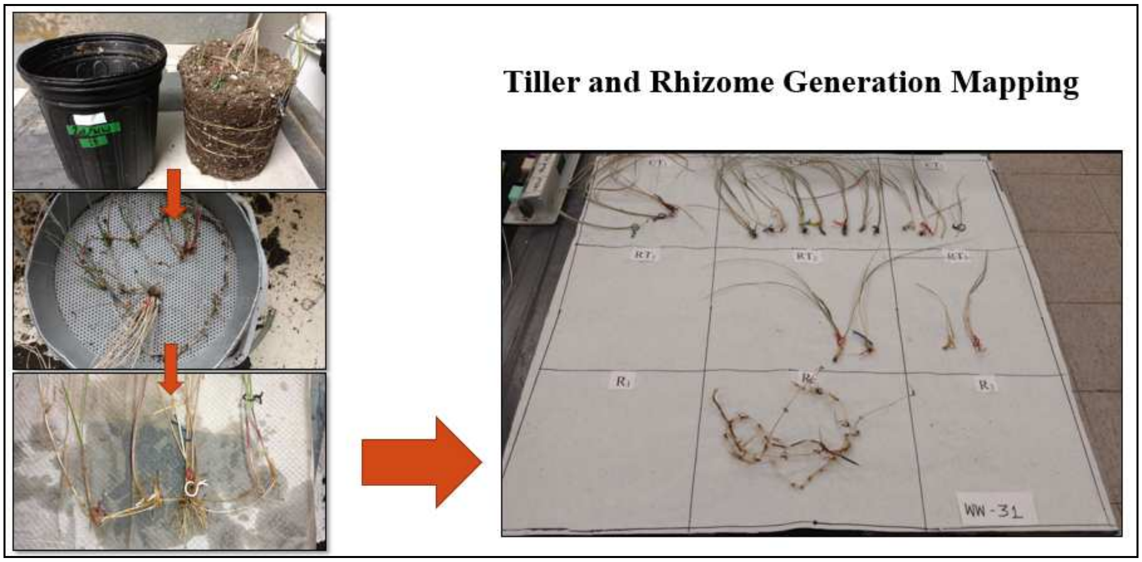


Photo 2.1 Sample processing and segregation of individual plant into first, second, and third generation crown daughter tillers (top row) and rhizome daughter tillers (middle row), and first, second, and third generation rhizomes (bottom row).

2.5.3 Bud, Rhizome, and Tiller Development Stage Classification

A random sub-sample of 3 tillers from each generation of tillers (T1, T2, & T3) and 3 rhizomes from each generation of rhizome (R1, R2, & R3) per individual plant was selected to assess bud production and bud development stages. Each tiller/rhizome was examined using a dissecting scope (Olympus® Stereo Microscope) with magnification between 6.7x and 45x. Rhizomes, belowground buds, and new tillers borne on tillers were counted and assessed to be living or dead and classified by their size (Table 2.2, Photo 2.2). Buds were contained within the prophyll, whereas tillers and rhizomes had elongated past the prophyll. Dead buds were identified by their soft, spongy or mealy brown interiors and easily distinguished from live buds (Ott et al. 2017). For each sub-sampled tiller/rhizome, we recorded the number of live and dead buds, small juvenile tillers, large juvenile tillers, adult tillers, and rhizomes coming off from the tiller or rhizome.

Table 2.2 Bud, rhizome and tiller development stages (Adopted from Ott et al. 2017)

Development Stage	**Description**
Buds	Contained within prophyll
Small juvenile tillers/rhizomes	Apex elongated <3.0mm past prophyll
Large juvenile tillers/rhizomes	Vertically elongated >3.0mm past prophyll AND <4.5cm in total height (*P. smithii*) <3.6cm in total height (*B. inermis*) OR Horizontally elongated >3.0mm past prophyll
Adult tillers	>4.5cm vertical height (*P. smithii*) >3.6cm vertical height (*B. inermis*)

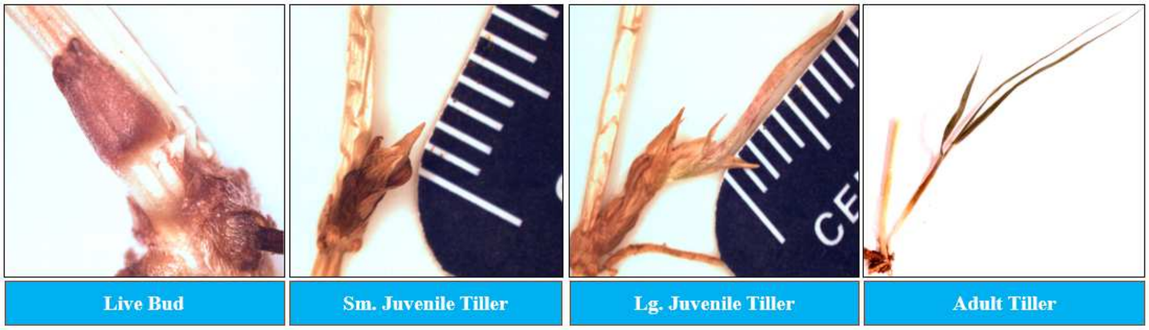

Live Bud
Sm. Juvenile Tiller
Lg. Juvenile Tiller
Adult Tiller

Photo 2.2 Live vegetative propagules: live bud (22.5x) of *B. inermis*, small juvenile tiller (8x), large juvenile tiller (6.7x), and adult tiller of *P. smithii*.

2.6 Data Analysis

2.6.1 Data Organization

Live propagules included live buds, small juvenile, large juvenile tillers, and excluded adult tillers. Based on the five research questions of this study, the following response variables were calculated.

Overall plant establishment (RQ1)

(i) Number of total tillers per plant = Sum of the number of all the tillers recruited from the crown, rhizome nodes, and rhizome tips of a plant.

(ii) Number of total rhizomes per plant = Sum of the number of all the primary, secondary, and tertiary generation rhizomes recruited from a plant.

(iii) Total rhizome length (cm) per plant = Sum of length of all the primary, secondary, and tertiary generation rhizomes recruited from a plant.

Number of new tillers established per tiller (RQ 2)

Number of new tillers established per tiller = Number of tillers recruited at that generation divided by the number of tillers recruited by preceding generation. The daughter tiller generations were named as primary tillers (recruited from parent tiller), secondary tillers (recruited from primary tiller), and tertiary tillers (recruited from secondary tiller) as shown in Figure 2.2.

Bud production and Propagule development (RQ 3)

(i) Number of live propagules per plant = Sum of all live buds, small juvenile, and large juvenile tillers growing from a plant (i.e. combined all generation tillers and rhizomes). First, we counted the average number of live propagules per tiller, and then it was multiplied with the total number of tillers per plant to get the number of live propagules per plant.

(ii) Number of live propagules per plant belonging to each development stage = Number of live propagules of each development stage out of total live propagules per plant (i.e. number of live bud, small juvenile tiller, and large juvenile tiller out of total live propagules per plant).

Live propagules per tiller by generation (RQ 4)

Live propagules per tiller by generation = Sum of all live buds, small juvenile, and large juvenile tillers from each tiller generation (i.e. from each primary, secondary, and tertiary generation tiller).

Investment in phalanx and guerilla growth (RQ 5)

To assess and compare investment of resources between *P. smithii* and *B. inermis* in terms of live propagule availability, tiller production from two locations of plant-crowns versus rhizomes may reveal how a plant prioritizes phalanx and guerilla growth.

(i) Proportion of live propagules from tiller per plant = Number of live propagules from tillers (i.e. from crown) divided by the total live propagules per plant. This helped us to compare live propagule investment from the crown (prioritizing phalanx growth) versus live propagules investment from the rhizome (prioritizing guerilla growth).

(ii) Proportion of total tillers per plant belonging to each location = Proportion of each tiller type based on location: from crown, rhizome, and rhizome tip out of total tillers per plant. This helped us to compare tiller recruitment from the crown (prioritizing phalanx growth) versus tiller recruitment from the nodes and tip of the rhizome (prioritizing guerilla growth).

2.6.2 Statistical Analysis

The effect of altered precipitation frequency, and clipping on species belowground bud bank, tiller recruitment and establishment were analyzed using linear mixed models through PROC GLIMMIX in SAS$^{®}$ Studio 3.6 University Edition (SAS Institute 2017). All treatments were applied at plant level, except generation which was applied at the tiller level. Kenward-Roger’s (KR) method was used to approximate the denominator degrees of freedom, except in the case of total rhizomes per plant and number of live propagules per tiller by generation, where the containment (CON) method was used. Model goodness-of-fit was checked by ensuring the deviance was at or near 1. Potential outliers were identified if studentized residual values were smaller than -3 and larger than +3. The multiple pairwise comparison between treatments was significant at $P < 0.05$ (Kendall 1993). The nine response variables above were analyzed in accordance with the following four research questions:

Overall Plant Establishment (RQ1)

Both the number of total tillers per plant and the number of total rhizomes per plant were analyzed using a negative binomial distribution. Total rhizome length per plant was analyzed using a gamma distribution in a three-way factorial treatment structure with the factors of precipitation frequency (three levels), clipping (two levels), and species (two levels) in a randomized complete block design with chamber as the block effect.

Number of new tillers established per tiller (RQ2)

The number of new tillers established per tiller in each generation was analyzed using a negative binomial distribution in a four-way factorial treatment structure with the fixed factors of precipitation frequency (three levels), clipping (two levels), species (two levels), and generation (three levels) in a randomized complete block design, with the chamber as the block effect.

Bud production and Propagule development (RQ3)

The number of live propagules per plant was analyzed using a gamma distribution in a three-way factorial treatment structure with the fixed factors of precipitation frequency (three levels), clipping (two levels), and species (two levels) in a randomized complete block design, with the chamber as the block effect. The number of live propagules per plant belonging to each development stage was analyzed using the same distribution in a four-way factorial treatment structure with the fixed factors of precipitation frequency (three levels), clipping (two levels), species (two levels), and development stage (three levels) in a randomized compete block design, with the chamber as the block effect.

Live propagules per tiller by generation (RQ4)

The number of live propagules per tiller in each generation was analyzed using a negative binomial distribution in a four-way factorial treatment structure with the fixed factors of precipitation frequency (three levels), clipping (two levels), species (two levels), and generation (three levels) in a split-plot randomized complete block design, with the chamber as the block effect. The factor of generation was applied at the tiller level (or sub-plot level).

Investment in phalanx and guerilla growth (RQ5)

The proportion of total live propagules from all tillers per plant was analyzed using a beta distribution in a three-way factorial treatment structure with the fixed factors of precipitation frequency (three levels), clipping (two levels), and species (two levels) in a randomized complete block design, with the chamber as the block effect. Proportion of total tillers per plant belonging to each location (crown, rhizome nodes, and rhizome tips) was analyzed using negative binomial distribution in a four-way factorial treatment structure with the fixed factors of precipitation frequency (three levels), clipping (two levels), species (two levels), and location (three levels) in a randomized complete block design, with the chamber as the block effect.

RESULTS

3.1 Overall Plant Establishment

The number of total tillers per plant was significantly affected by species, precipitation frequency, clipping, and species x precipitation frequency (Appendix-Table 2.3). The total tiller production of *B. inermis* was significantly lower than native *P. smithii* at the low precipitation frequency (16d). The mean tiller production of *B. inermis* (13.07 ± 0.66) was significantly lower than native *P. smithii* (18.85 ± 0.92) at the low precipitation frequency. The total tiller production of *Bromus inermis* remarkably decreased as precipitation frequency decreased from high to medium to low. In comparison, *P. smithii* total tiller production was unaffected by precipitation frequency (Figure 2.3-A). Clipping significantly reduced tiller production (~3 tillers per plant) compared to no-clipping for both species (Figure 2.3-B).

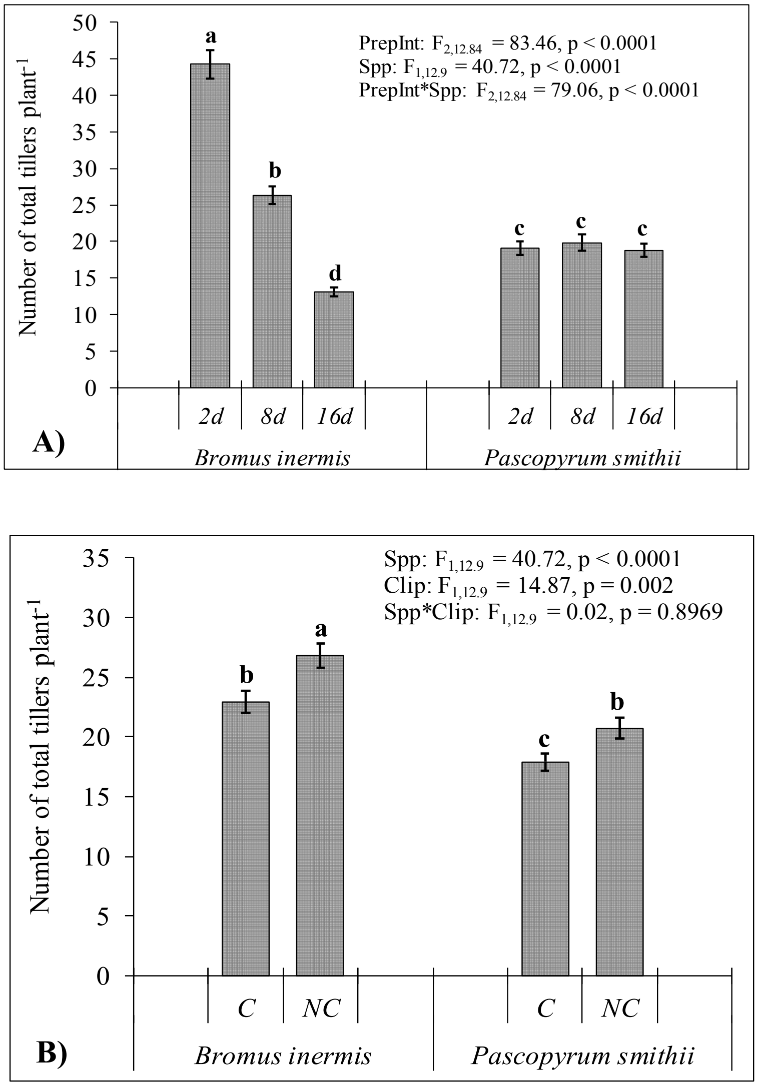


Figure 2.3 Effect of (A) precipitation frequency and (B) clipping on the number of total tillers per plant of *B. inermis* and *P. smithii*. Values are mean ± SE based upon the statistical model. Different letters above bars indicate significant difference across treatments at p-value < 0.05.

Similarly, the number of total rhizomes per plant was significantly affected by precipitation frequency, clipping, and species x precipitation frequency (Appendix-Table

2.3). The mean number of rhizomes per plant significantly increased in *P. smithii* (10.43 ± 1.40) at the medium precipitation frequency (8d) and decreased in *B. inermis* (3.83 ± 0.52) at the low precipitation frequency (16d). The low precipitation frequency lowered *B. inermis* rhizome production by 50% (Figure 2.4-A). Clipping lowered *B. inermis* rhizome production by ~27% compared to non-clipping but had no significant difference on *P. smithii* (Figure 2.4-B).

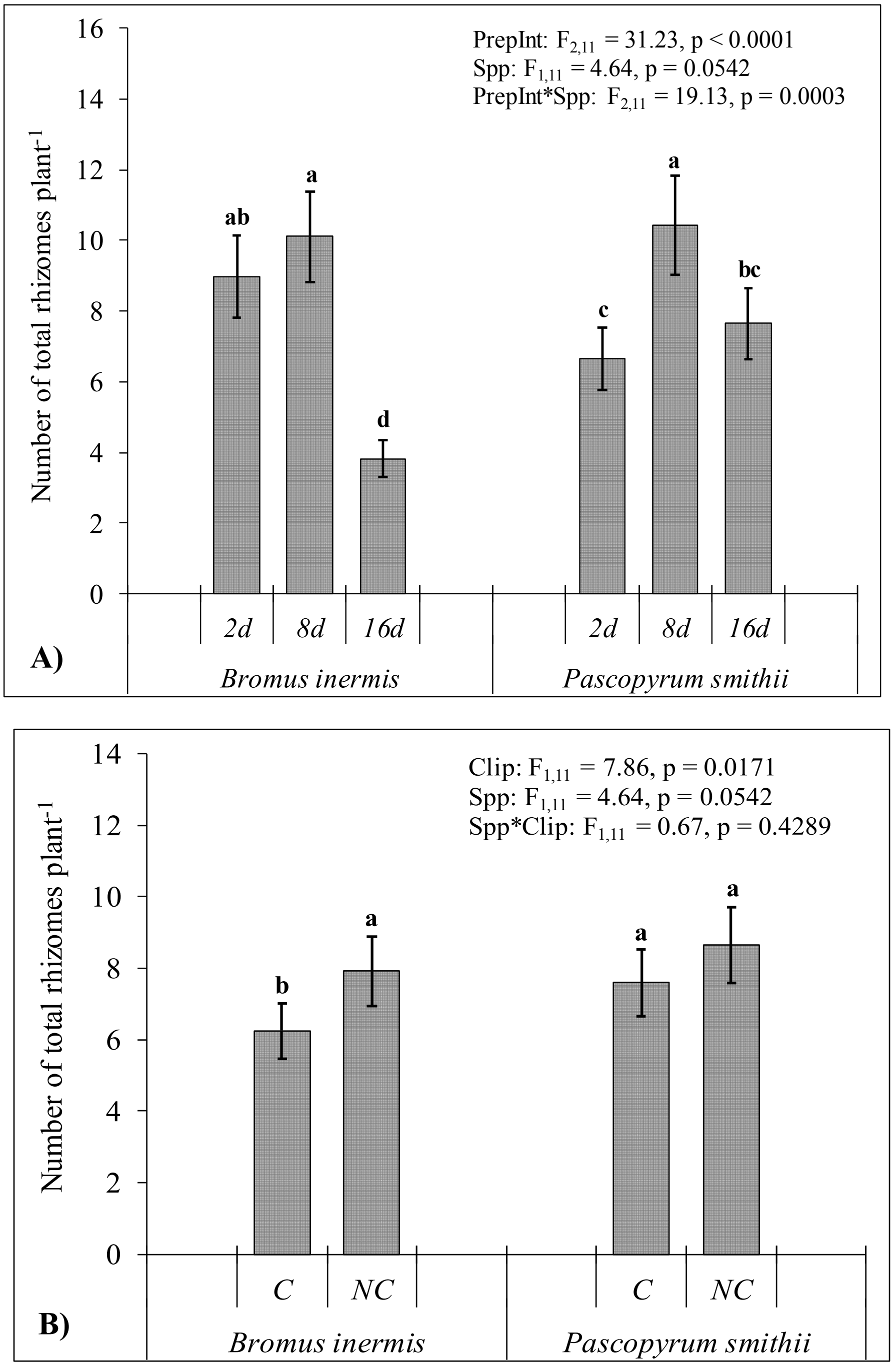
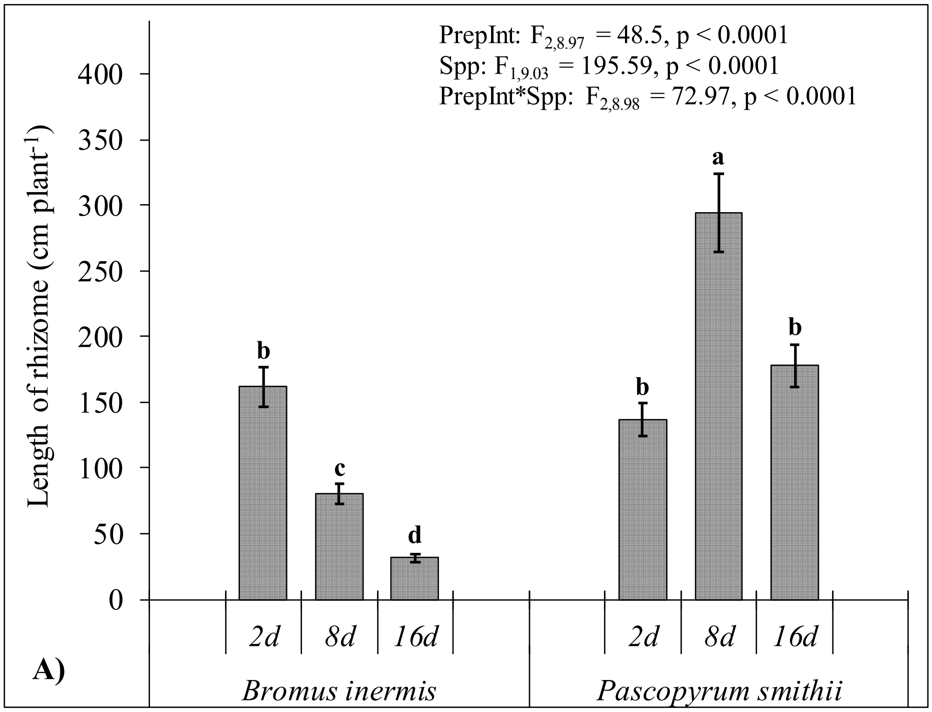


Figure 2.4 Effect of (A) precipitation frequency and (B) clipping on number of total rhizomes per plant of *B. inermis* and *P. smithii*. Values are mean ± SE based upon the statistical model. Different letters above bars indicate significant difference across treatments at p-value < 0.05.

The total rhizome length per plant was significantly affected by species, precipitation frequency, and species x precipitation frequency (Appendix-Table 2.3). The rhizome length of *B. inermis* significantly decreased at both the medium (8d) (~50%) and the low precipitation frequency (16d) (~81%) compared to the high precipitation frequency. On the contrary, native *P. smithii* rhizome length doubled at the medium precipitation frequency (8d) and was unaffected at the low precipitation frequency (16d) (Figure 2.5-A). Clipping had no effect on total rhizome length for both species (Figure 2.5-B).



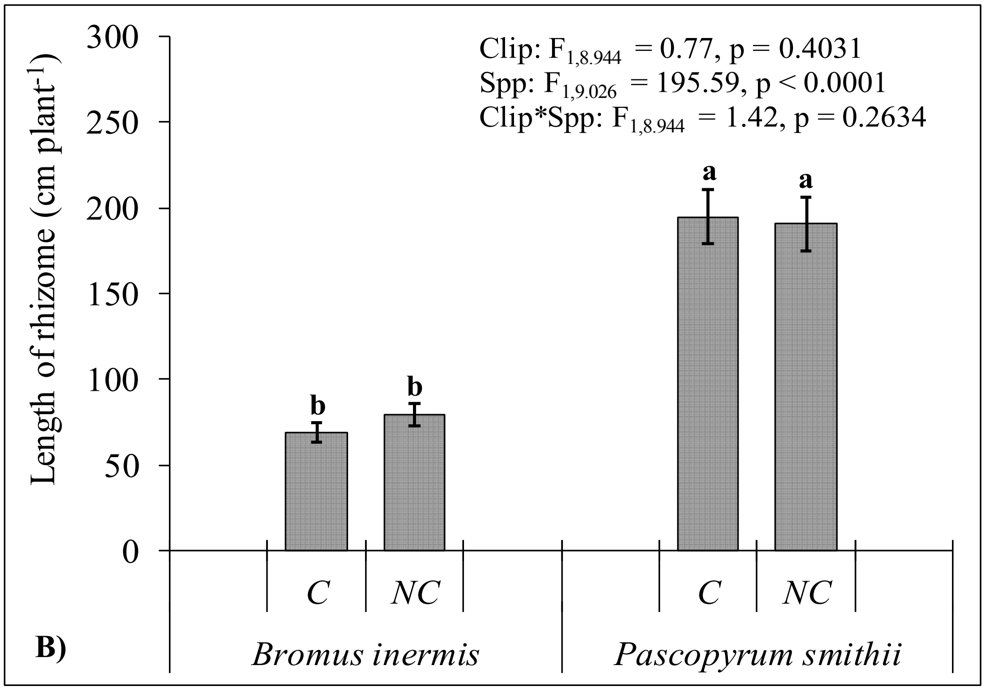


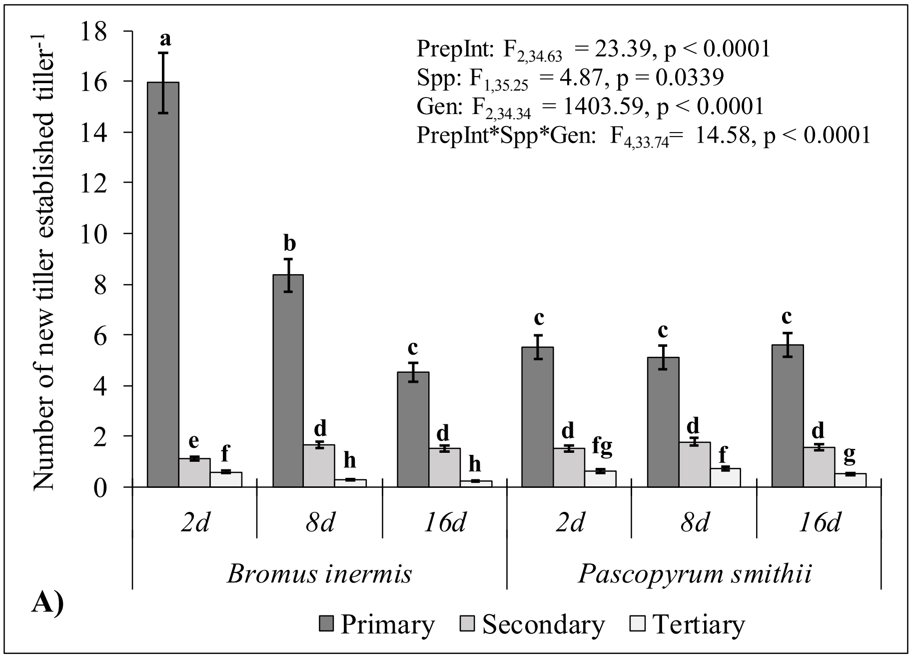
Figure 2.5 Effect of (A) precipitation frequency and (B) clipping on total length of rhizome (cm) per plant of *B. inermis* and *P. smithii*. Values are mean ± SE based upon the statistical model. Different letters above bars indicate significant difference across treatments at p-value < 0.05.

3.2 Number of New Tillers Established per Tiller

The number of new tillers established per tiller in each generation was significantly affected by species, precipitation frequency, clipping, generation, species x precipitation frequency, species x generation, precipitation frequency x generation, species x precipitation frequency x generation, and species x clip x generation (Appendix-Table 2.5).

As the precipitation frequency decreased, new tillers established from the parent tiller were significantly lowered for *B. inermis*, but *P. smithii* was not affected. The percentage of new established primary tillers from the parent tiller decreased from 90%, to 80%, and 70% as the precipitation frequency decreased in *B. inermis* but stayed relatively constant at 67 to 73% for *P. smithii,* regardless of precipitation frequency regimes. Also, the tertiary tiller production of *B. inermis* was significantly lower than *P.*

smithii at both medium (0.31 ± 0.03 *vs* 0.74 ± 0.07) and low (0.23 ± 0.03 *vs* 0.53 ± 0.05) frequency of precipitation (Figure 2.6-B). Clipping had no effect on new established tillers from each generation for both species. Although clipped *B. inermis* produced significantly fewer tertiary tillers compared to non-clipped *B. inermis,* it might not be biologically significant due to only occasional tertiary tiller production (<0.3 new established tiller per tiller) (Figure 2.6-B). Overall, the graph shows both species were driven by the number of primary tillers.



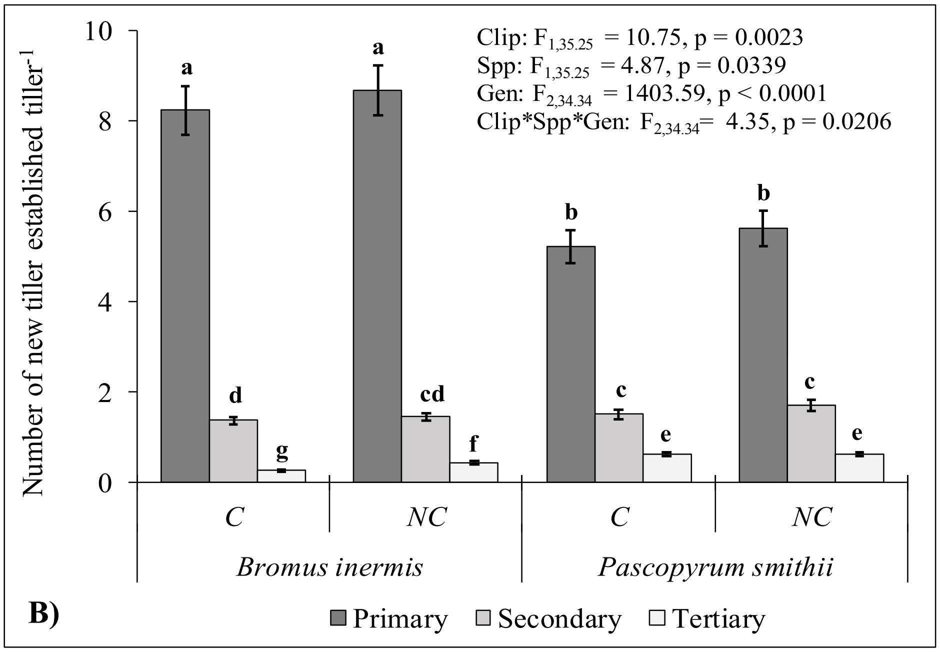
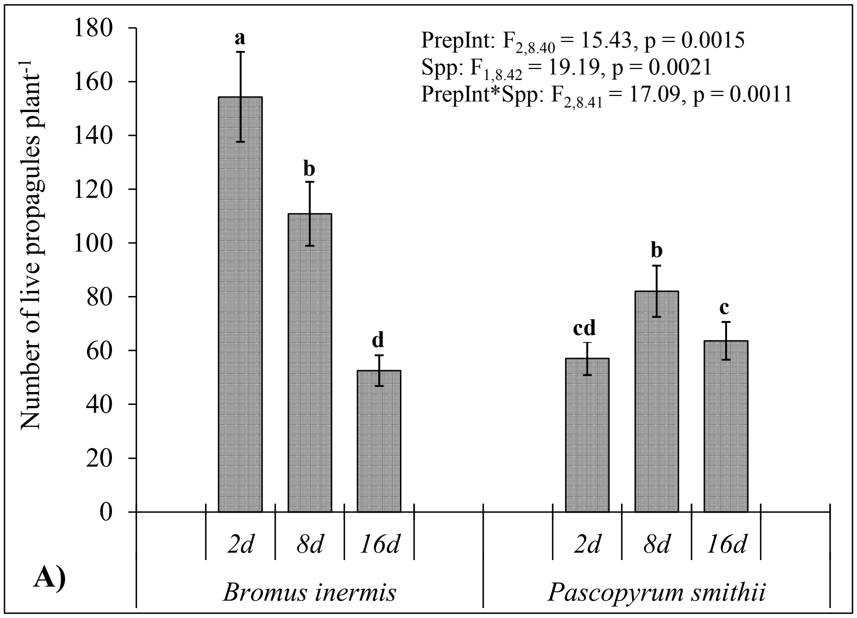


Figure 2.6 Effect of (A) precipitation frequency and (B) clipping on number of new tiller established per tiller of *B. inermis* and *P. smithii*. We had three daughter tiller generation cohorts including primary tiller generation, secondary tiller generation, and tertiary tiller generation (see Figure 2.2 for more detailed descriptions). Values are mean ± SE based upon the statistical model. Different letters above bars indicate significant difference across treatments at p-value < 0.05.

3.3 Bud Production and Propagule Development

The number of live propagules per plant was significantly affected by species precipitation frequency, and species x precipitation frequency (Appendix-Table 2.7). The live propagules of *B. inermis* significantly decreased at both medium (28%) and low precipitation frequency (66%) compared to high precipitation frequency. Whereas, native *P. smithii* significantly increased its live propagules production at medium precipitation frequency (by ~44% compare to high precipitation frequency) and remained unaffected at low precipitation frequency (Figure 2.7-A). Clipping had no effect on live propagules production for both species (Figure 2.7-B).



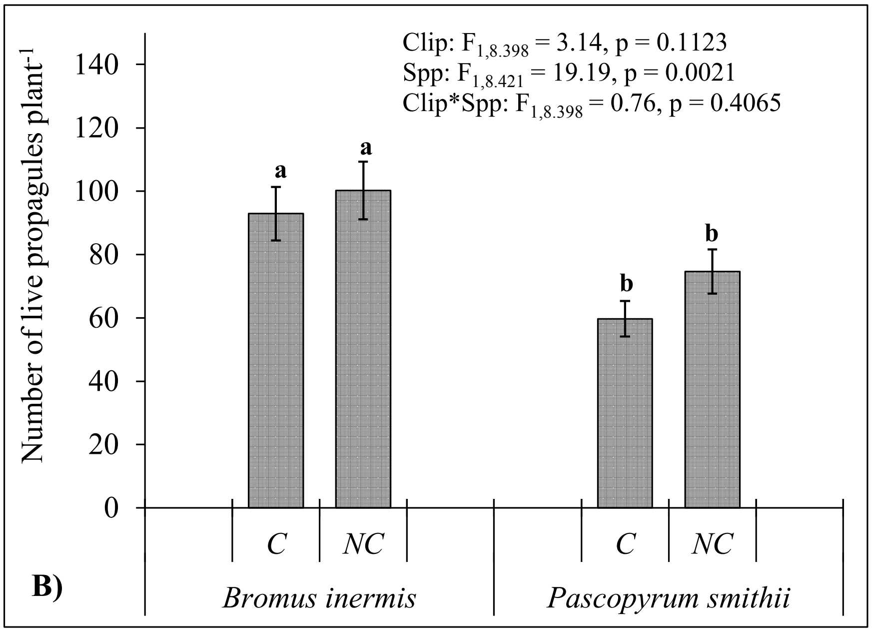
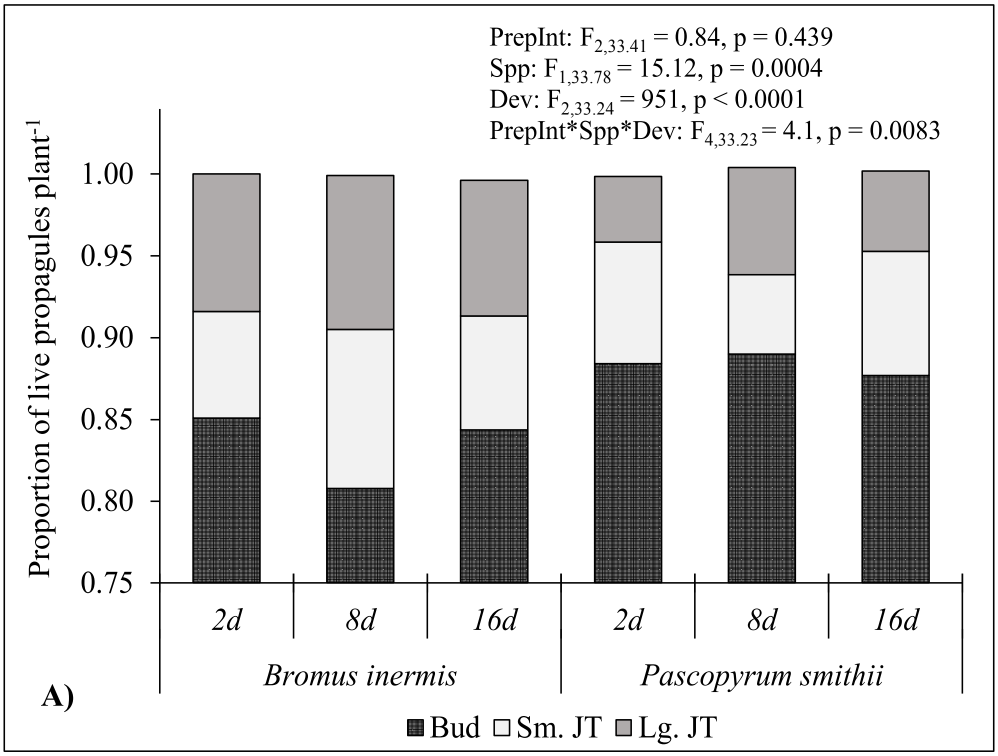


Figure 2.7 Effect of (A) precipitation frequency and (B) clipping on number of live propagules per plant of *B. inermis* and *P. smithii*. Values are mean ± SE based upon the statistical model. Different letters above bars indicate significant difference across treatments at p-value < 0.05.

Similarly, the proportion of live propagules at each development stage (i.e. number of bud, small juvenile tiller, and large juvenile tiller) per plant was significantly affected by species, development stage, species x clipping, species x development stage, and species x precipitation frequency x development stage (Appendix-Table 2.9). There was significantly greater propagule development in *B. inermis* than *P. smithii* at all level of precipitation frequency. *Bromus inermis* chances of propagules being at higher development stage was ~2x higher than *P. smithii* at medium precipitation frequency. Whereas, *P. smithii* remained comparatively unaffected by medium and low precipitation frequency (Figure 2.8-A). Although not statistically significant clipping reduces propagule development in *P. smithii* (Figure 2.8-B).



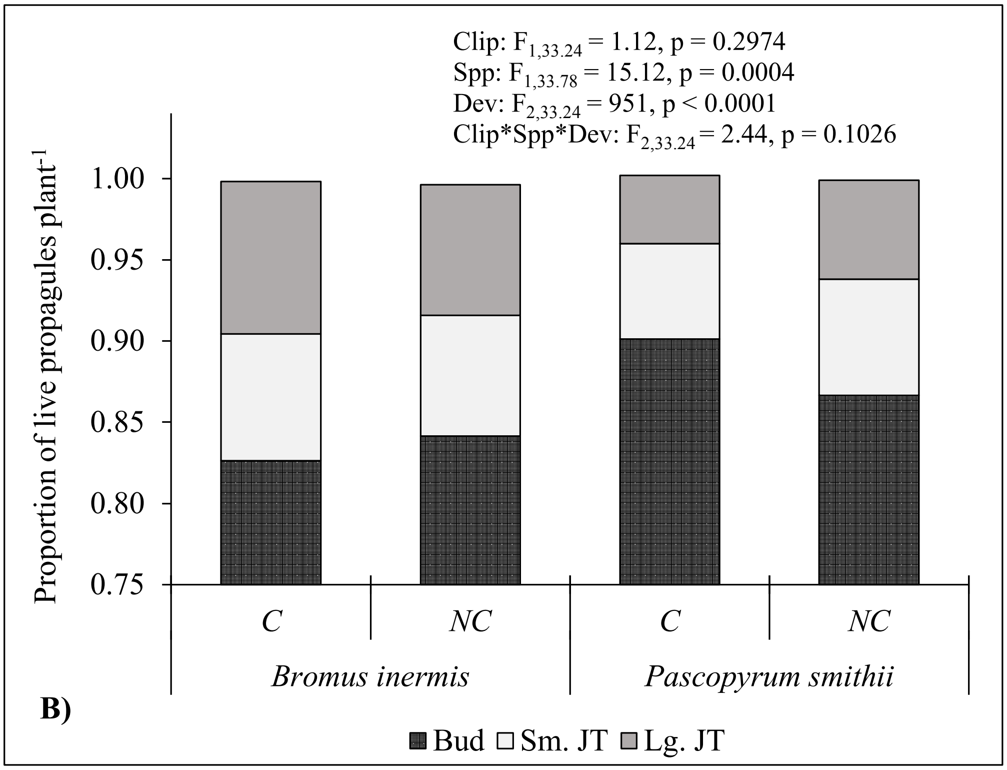


Figure 2.8 Effect of (A) precipitation frequency and (B) clipping on proportion of live propagules at each development stage per plant of *B. inermis* and *P. smithii*. The proportions of live propagules were classified into three development/size classes including buds, small juvenile tillers/rhizomes (Sm. JT) and large juvenile tillers/rhizome (Lg. JT) (see Table 2.2 for more detailed descriptions). The number of live propagules per plant belonging to each development stage was analyzed to get this proportion of live propagules at each development stage per plant. Values are the means of the proportion of live propagules per plant.

3.4 Live Propagules per Tiller by Generation

The number of live propagules per tiller in each generation was significantly affected by species, clipping, generation, species x clipping, and species x generation (Appendix-Table 2.11). *Bromus inermis* significantly produced higher number of live propagules per tiller at each generation than *P. smithii*. And the live propagules production was significantly greater for secondary tillers for both the species (Figure 2.9-A). *Pascopyrum smithii* live propagules production by primary and secondary generation tiller significantly decreased under clipping condition whereas, *B. inermis* live propagules production per tiller remain unaffected (Figure 2.9-B).

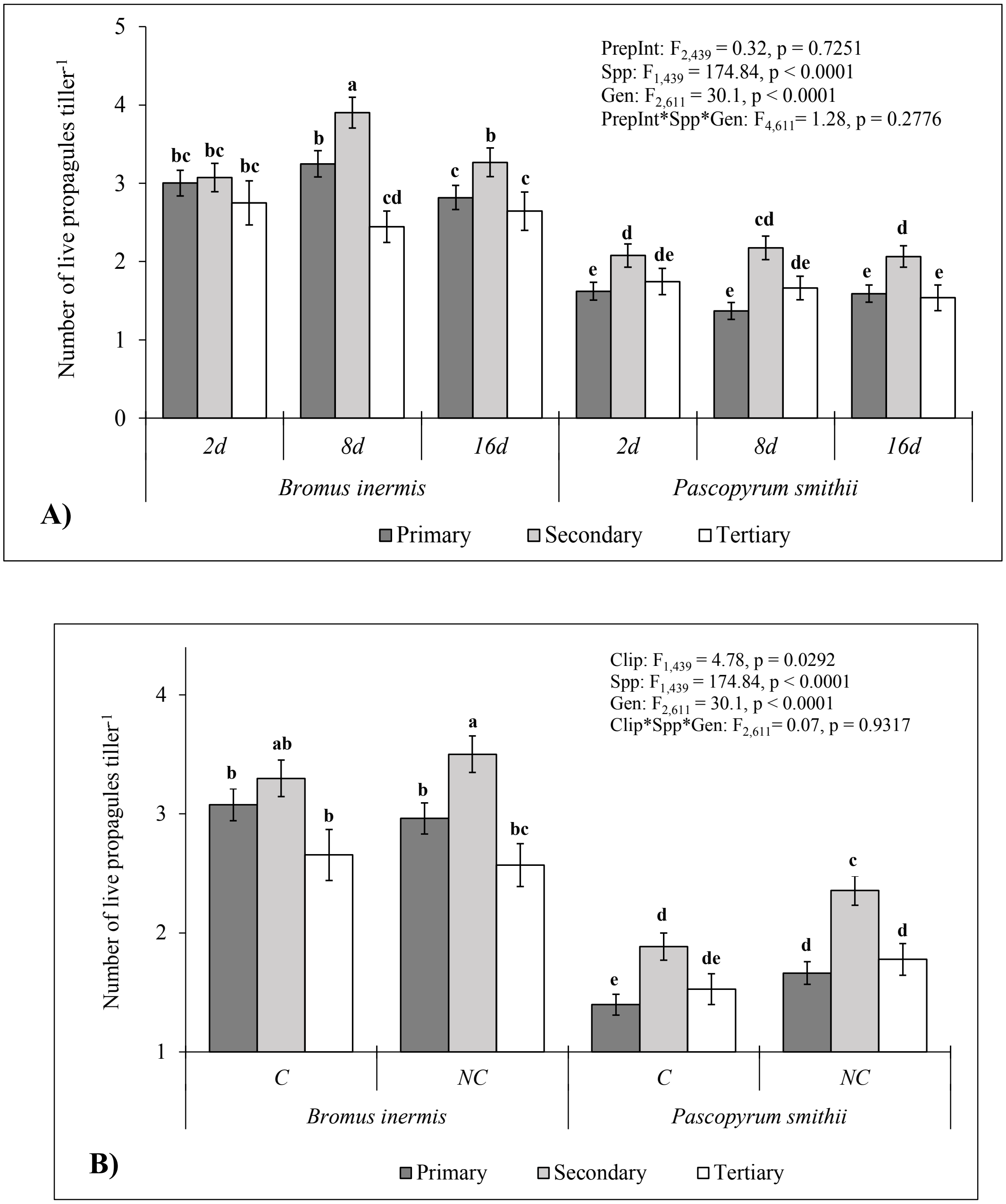
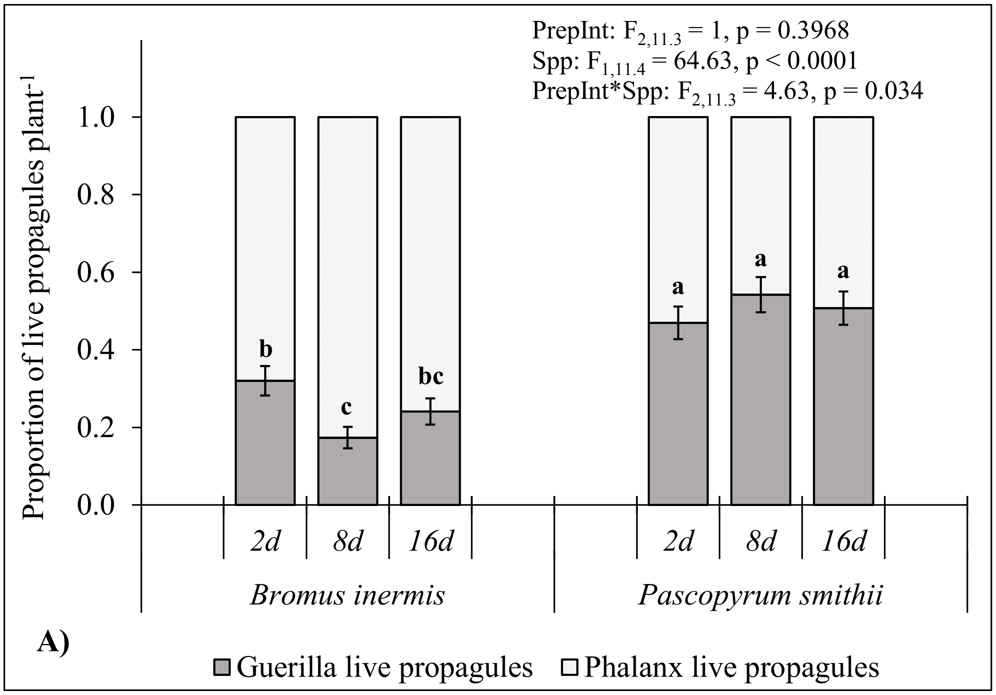


Figure 2.9 Effect of (A) precipitation frequency and (B) clipping on number of live propagules per tiller by generation of *B. inermis* and *P. smithii*. We had three daughter tillers generation cohort including primary tiller generation (F1), secondary tiller generation (F2), and tertiary tiller generation (F3) (see Figure 2.2 for more detailed

descriptions). Values are mean ± SE based on the statistical model. Different letters above bars indicate significant difference across treatments at p-value < 0.05.

3.5 Investment in Phalanx and Guerilla Growth

The proportion of live propagules from tiller per plant was significantly affected by species, species x precipitation frequency (Appendix-Table 2.13). *Bromus inermis* primarily maintained phalanx growth form as 67 to 83% live propagules were produced from tillers and <30% of live propagules were produced from rhizome. Whereas, *P. smithii* maintained dual phalanx and guerilla growth form as ~50% each of live propagules were produced from both tiller and rhizome irrespective of change in precipitation frequency. At medium precipitation frequency, *B. inermis* invested by 15% higher in phalanx growth form compare to high precipitation frequency (Figure 2.10-A). Clipping did not alter either species investment in phalanx and guerilla growth (Figure 2.10-B).



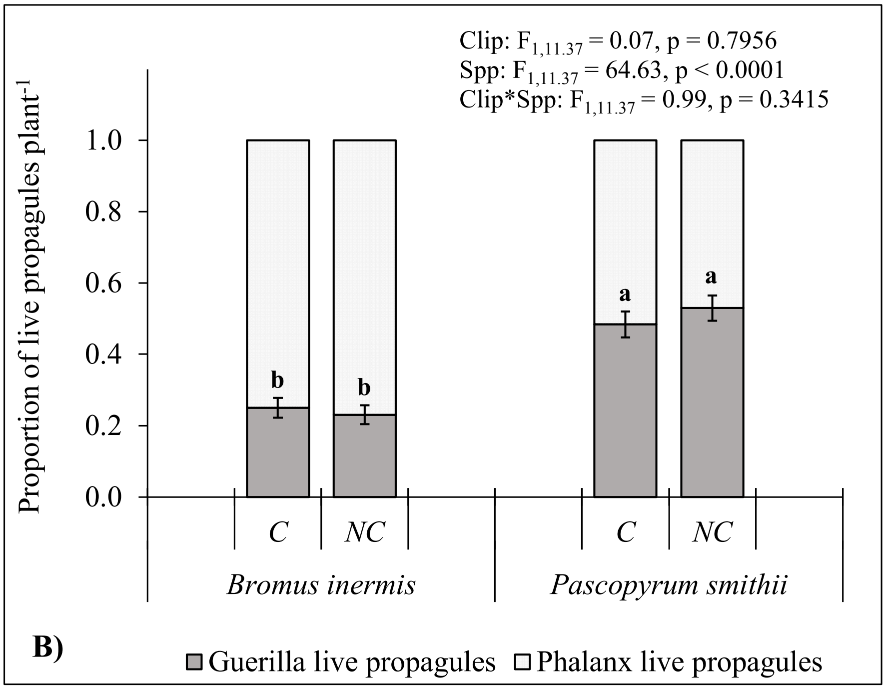


Figure 2.10 Effect of (A) precipitation frequency and (B) clipping on proportion of live propagules per plant of *B. inermis* and *P. smithii*. Values are mean ± SE based upon the statistical model. Different letters above bars indicate significant difference across treatments at p-value < 0.05. Live propagules from rhizome and crown were called guerilla live propagules and phalanx live propagules respectively.

Similarly, the proportion of total tillers at each location (crown, rhizome nodes, and rhizome tips) per plant was significantly affected by species, precipitation frequency, location, species x precipitation frequency, species x location, precipitation frequency x location, species x precipitation frequency x location, and species x clipping x location (Appendix-Table 2.15). *Bromus inermis* predominantly invested in phalanx growth form as 65 to 80% of tillers were borne from crown and 20 to 30% were recruited from rhizome tip, and less than 3% recruited from rhizome nodes with some exception in medium precipitation frequency where tiller recruited from tip of rhizome (apical buds) increased by ~37% than high precipitation frequency and by ~29% than low precipitation frequency. However, *P. smithii* maintained dual phalanx and guerilla growth form as ~40% of tillers recruited from crown and ~60% of tillers recruited from nodes and tip of rhizome irrespective of change in precipitation frequency (Figure 2.11-A). The clipping significantly increased tiller recruitment from nodes of rhizome by ~15% of *P. smithii* but had no significant on *B. inermis* (Figure 2.11-B).

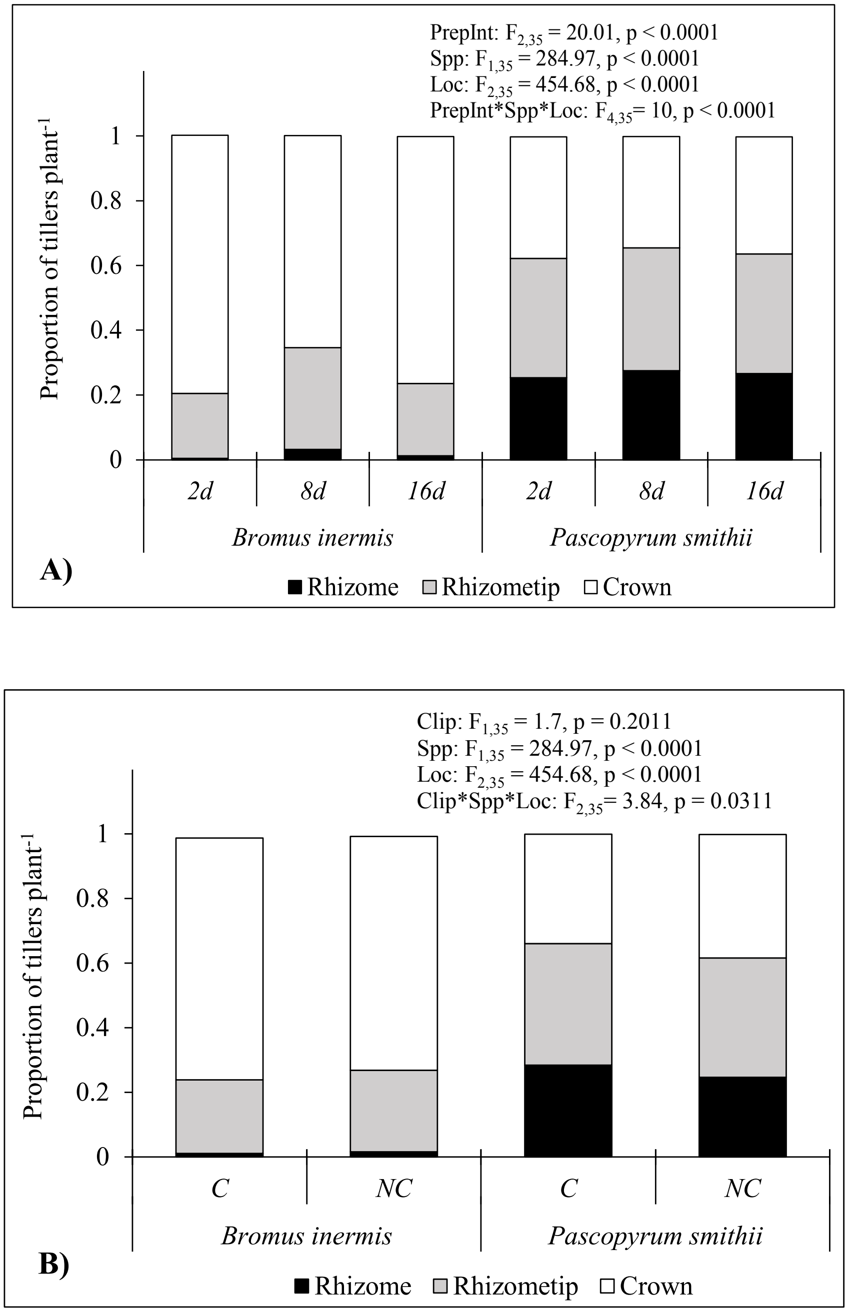


Figure 2.11 Effect of (A) precipitation frequency and (B) clipping on proportion of tillers per plant of *B. inermis* and *P. smithii*. Values are mean ± SE based upon the statistical

model. Different letters above bars indicate significant difference across treatment at p-value < 0.05. Tillers recruited from crown contributed to phalanx growth and tillers recruited from nodes and tips of rhizomes contributed to guerilla growth. Values were mean of proportion of tillers per plant.

DISCUSSION

Overall Plant Establishment

Different to our hypothesis, overall plant establishment in terms of number of total tillers, total rhizomes, and total rhizome length of the non-native *B. inermis* was not greater than native *P. smithii* under altered precipitation frequency. Non-native *B. inermis* plant establishment traits were susceptible to soil moisture variability created by medium and low precipitation frequencies compared to native *P. smithii*. *Bromus inermis* plant establishment was negatively affected with decrease in the number of tillers and rhizome length at both medium and low precipitation frequency and rhizome number at low precipitation frequency indicating vulnerability to less frequent precipitation. Whereas, native *P. smithii* plant establishment traits remain unaffected and seems to be enhanced with increased rhizome number and rhizome length at medium precipitation frequency.

There was a similar pattern of response in plant establishment traits between species to the one-time earlier clipping. Where, as hypothesized, although clipping reduces tiller number of native *P. smithii* greater than non-native *B. inermis*. But clipping only decreased rhizome number of non-native *B. inermis*, whereas native *P. smithii* rhizome number and rhizome length remain unaffected.

In this experiment, the overall tiller production by both the species was high within the single growing season of 2016 because we started our experiment from seeds, and treatments were applied at the seedling phase of the species. We were interested to see how treatments affect the establishment traits of these two perennial grasses. The greater vulnerability of non-native *B. inermis* establishment to altered precipitation frequency or soil moisture availability and clipping has been supported by several studies. Prolonged drought has been shown to decrease shoot dry weight (Dibbern 1947, Donkor et al. 2002) and limit the establishment of *B. inermis* in southern Alberta and central British Columbia (Otfinowski 2008). Even a study by Dong et al. (2014) has shown that non-native *B. inermis* tends to produce less rhizomes in first season's growth regardless of the water stress level.

Bromus inermis is affected by grazing or clipping treatment in other studies. There was reduction of number of tillers, above-ground biomass, and regrowth occurred at the expense of rhizomes and roots in *B. inermis* with increase in frequency of clipping (Dibbern 1947, Reynolds and Smith 1962). Also, competition among tillers of *B. inermis* for available resources may have reduced recovery following clipping.

The native perennial grass *P. smithii* tiller and rhizome production remained unaffected by soil moisture variability and clipping only decreased tiller production. The greater recruitment of rhizomes and rhizome length at medium precipitation frequency may indicate stress tolerance attributes of *P. smithii*. The relative growth rates of tillers and tiller densities of *P. smithii* were unaffected by 1-year growing season drought and grazing (Eneboe et al. 2002). However, the clipping or grazing can decrease these plant establishment traits if they are applied frequently (multiple clipping) unlike one time clipping in our study. For instance, 2-years of repeated grazing and drought could limit the tiller numbers and herbage accumulation of both crested wheatgrass (*Agropyron desrtorum*) and bluebunch wheatgrass (*Pseudoroegneria spicate*) (Busso and Richards 1995). In contrast, study have also shown that *P. smithii* may be tolerant to multiple grazing intensities applied for short time as shown by a short-term study, where at the end of 10-day treatment period, there was little variation in tiller numbers between clipping (both moderate- 50%, and heavy 75%) and unclipped plants (Painter and Detling 1981).

Native perennial grass *P. smithii* tolerance to water stress may be related to different physiological traits of this species as shown by a study. Frank (1994) showed western wheatgrass had higher water stress tolerance than crested wheatgrass by maintaining 1.7 times higher abscisic acid and proline concentration in its leaf tissue. The increase in proline during the later stages of plant development may have supported western wheatgrass to better tolerate water stress and continue slow growth. Other studies have also shown how osmotic adjustment which is enhanced with proline concentration in leaf was important for drought tolerance in cool season grasses such as *P. smithii* (Frank 1994).

Similarly, the capability to establish with a dual phalanx-guerilla growth form with higher number of rhizome tillers by native *P. smithii* in mixed-grass prairies of the northern Great Plains (Ott and Hartnett 2015a) may be mechanism through which they are able to survive in resource-heterogeneous and/or disturbed habitats (Schmid and Harper 1985).

Number of new tillers established per tiller

The difference in total tiller production between species was further elucidated by the response of both species to changes in precipitation frequency and clipping on the number of new tillers established per tiller at each tiller generation. Although its rejects our hypothesis, that the non-native C3 perennial grass (*B. inermis*) will produce greater number of tillers than the native C3 perennial grass (*P. smithii*) in each generation of tillers under altered precipitation frequency and clipping conditions but clearly supported above result of different tiller production between species by giving in depth understanding on how new tiller production at each generation of daughter tiller play their role (sum up) for overall plant establishment and are prone to variability in environmental conditions such as medium and low precipitation frequency and grazing.

Percentage decline of primary generation tiller from 90%, to 80%, and 70% as the precipitation frequency decreased in *B. inermis*. Lowest production of tertiary generation tillers at medium and low precipitation frequency and clipping conditions explains the negative effect of treatments on overall *B. inermis* tiller establishment. Whereas relative insignificant effect on the number of new tillers established per tiller at each generation for native *P. smithii* was consistent with the result of insignificant effect to total tiller production of this species and may indicate resistance ability of this species against the effects of altered precipitation and clipping.

Also, this differential response to clipping of daughter tiller generation cohorts between native *P. smithii* and non-native *B. inermis* might be species-specific traits as suggested by some studies (Olson and Richards 1988, Vinton and Hartnett 1992). The grazing tolerance/avoidance of native *P. smithii* with respect to tiller recruitment and establishment has been seen in some perennial grass like *Poa ligularis,* which can be defoliated twice a year without affecting its tiller growth (Busso et al. 2011). Several studies have supported this response and explains that maintaining optimum tiller growth and size is an important mechanism of compensatory regrowth following grazing in prairie grassland (Harrison and Romo 1994, Dalgleish and Hartnett 2009). Likewise, when plants cannot avoid herbivores by defense or escape, herbivore tolerance is an important trait for plant survival and future performance (Lehtila 2000). In contrast, a decrease in tiller recruitment and establishment with grazing has also seen in perennial grasses, including little bluestem (N'Guessan 2007), bunch grass species (Busso et al. 1989), and rhizomatous *B. inermis* (Dibbern 1947, Reynolds and Smith 1962).

Tiller establishment at each generation is necessary to produce the next generation of buds and subsequent tillers for overall plant establishment (Ott and Hartnett 2012). Native *P. smithii* tiller recruitment being unaffected or less response to drought and grazing and non-native *B. inermis* showing the opposite response, will sure to enhance competative ability of native perennial grasses against non-native perennial grass and might help to increase the stability of native mixed-grass prairies grassland ecosystem under such disturbance scenarios.

Bud production, and propagule development at tiller and plant level

As hypothesized, propagule development of the non-native C3 perennial grass (*B. inermis*) was greater than the native C3 perennial grass (*P. smithii*) irrespective of change in precipitation frequency and clipping conditions. *Bromus inermis* produce greater number of live propagules per tiller at each tiller generation than *P. smithii.*

Although the live propagule production of *B. inermis* remained comparatively unaffected at the tiller generation level, but at plant level, the total live propagules production was negatively affected with decrease in frequency of precipitation. Whereas, live propagule production of the native *P. smithii* at tiller level were unaffected under medium and low precipitation frequency and increased at plant level by both medium and low precipitation frequency.

We could see the non-native *B. inermis* propagules development was significantly higher at medium precipitation frequency also implies sensitivity to the change in precipitation frequency or soil moisture availability. Whereas, irrespective of the change in frequency of precipitation and clipping the native *P. smithii* seems to maintain a stable number of buds, and juvenile tillers and becoming resilient to change in environmental conditions. This result has been supported by a growth-chamber study by Ott et al. (2017) that showed under short-term drought and a range of temperatures, *B. inermis* maintained a greater number of live buds per stem and initiates a greater proportion of bud at higher development stages than *P. smithii*.

However, as Ott et al. (2017) study was focus on regrowth from established tillers from field and not from seedlings, where there was an absence of treatment effect of wider range of environmental conditions such as longer precipitation intervals as in case of our study and didn’t assess the long-term growth and survival of both the species. We propose that under short term drought, the non-native grasses like *B. inermis* might have the capability to outcompete native perennial grasses like *P. smithii* with higher bud supply and outgrowths but as these buds and outgrowths transit into tillers and its establishment in necessary for overall plant persistent and resilient to environmental disturbances for longer period of time (Dagleish and Hartnett 2009, Klimesˇová and Klimeš 2007, Rusch et al. 2011, VanderWeide and Hartnett 2015), the non-native perennial grass like *B. inermis* may not be able to withstand, established and outcompete native perennial grass like *P. smithii* under the environmental condition applied by this study.

This proposition has been supported by the finding of our study where, the non-native *B. inermis* tiller recruitment and overall plant establishment was negatively affected with decrease in precipitation frequency and clipping condition. The number of live propagules per plant for the non-native *B. inermis* seems to be driven by the number of primary tillers as seen in Figure 2.6-A, where primary generation tillers are significantly higher than other generation and decreased significantly with decreased in precipitation frequency. It showed that after primary generation, all the secondary and tertiary tillers are equal tiller producers between the two species. So, the precipitation conditions seem to determine whether non-native *B. inermis* gets a quick start (lots of primary generation tillers) or a slow start (not so many primary generation tillers).

The native *P. smithii* remained insensitive to change in environmental conditions with consistent response of tiller recruitment at each generation and enhanced overall plant establishment. So, although the non-native perennial grasses may have greater bud availability and development but tiller growth and survival for overall plant establishment will be higher in native *P. smithii* by maintaining a higher number of dormant buds, lesser outgrowths and transition to tillers and may be a mechanism through native perennial grasses like *P. smithii* may respond to the change in environmental conditions such as drought and heavy grazing in grasslands of northern Great Plains.

Differential expression of non-native *B. inermis* and native *P. smithii* in terms of bud production, outgrowth/juvenile tillers production are supported by some studies where the range of these belowground bud bank traits response depended on environmental systems exposed and may be species-specific. This lower ability of tiller recruitment of native perennial grasses in arid grasslands in the Great Plains has been supported by some findings. Hendrickson and Briske (1997) found that less than 10% of *Bouteloua curtipendula* and *Helaria belangeri* tillers in the arid grasslands of Texas were recruited from younger belowground buds, and many of the mature buds were dormant over two years. Likewise, effect of 1-year drought on numbers of active axillary buds of native perennial grasses was insignificant and only after 3 consecutive years of drought there was reduction in numbers of metabolically active axillary buds in two bunchgrass *Agropyron desertorum* and *Agropyron spicatum* (Busso et al. 1989).

Species-specific response of bud banks to grazing as shown by the study done in the steppe of Inner Mongolia, where with increasing grazing intensity, bud density decreased in *Leymus Chinensis*, increased in *Agropyron cristatum*, and had no significant effects on

Carex duriuscula (Qian et al. 2014). Also, when grazing frequency was increased to third and fourth times annually, there was only then increase in the percentage of dead and dormant buds of *Poa ligularis* (Busso 2011).

Investment in Phalanx and Guerilla growth

Prairies of the North American Great Plains are dominated by clonal rhizomatous perennial grasses that vary in architecture along the "phalanx - guerilla" clonal growth form continuum (Harper 1977, Doust 1981). Despite the abundance of these clonal rhizomatous grasses, little is known about their bud banks compared with caespitose grasses (Dalgleish et al. 2008, Ott and Hartnett 2015). The clonal growth form is an adaptive plant strategy by which plants can reproduce and spread vegetatively and where clonal structures can also serve as storage organs (Dong et al. 2010). Based on the spatial arrangement of tillers, such clonal plants have two classes of growth form: phalanx and guerilla (Doust 1981, Oborny 1997). Clonal plants with the phalanx growth strategy produce a compact structure of closely spaced tillers, whereas those with guerilla growth form produced loosely arranged group of widely spaced tillers (Doust 1981, Bernard 1990). This two-growth form/strategy has ecological and evolutionary significance to clonal plant populations (Doust 1981).

Prioritization in phalanx and guerilla growth in this study was assessed between species in terms of investment of live propagules from crown versus rhizome and investment of tiller recruitment from crown, nodes of rhizome and tip of rhizome. As hypothesized, the *P. smithii* maintained dual phalanx and guerilla growth form as ~50% of live propagules or ~40% of the tillers were produced from crown and ~50% of live propagules or ~60% of the tillers were produced from rhizome irrespective of change in precipitation frequency and clipping. Whereas, the *B. inermis* primarily maintained phalanx growth form as >70% of live propagules or tillers were produced from crown and <30% of live propagules or tillers were produced from rhizome (i.e. from nodes and tip of rhizomes). Prioritization of phalanx growth form by non-native *B. inermis* irrespective of altered precipitation frequency and clipping conditions may be affiliated to overall less rhizome production in *B. inermis*. This has been supported by a study by

Dong et al. (2014) where non-native *B. inermis* tends to produce less rhizomes in first season's growth regardless of the soil moisture variability. This predominantly phalanx growth form by *B. inermis* might have enabled them to tolerate this stressful condition, make better use of locally abundant resources (monopolization strategy) (Doust 1981, Schmid and Harper 1985, Humphrey and Pyke 1998).

Investment in dual phalanx and guerilla clonal growth form in *P. smithii* may has enabled them to employ both conservative (phalanx) and foraging (guerilla) growth strategies which may facilitate its persistence under fluctuating resource availability associated with environmental change (Ott and Hartnett 2015a). This also provides opportunity to show architectural plasticity in *P. smithii* by the combination of both guerilla and phalanx traits in response to habitat and nutrient conditions (Doust 1981, Ye et al. 2006, Chen et al. 2011).

This result has been supported by a study done in the mixed-grass prairie of western South Dakota were *P. smithii* tends to recruit tillers from both rhizome and crown buds which made them capable of immediate regrowth following plant injury from rhizome buds or persist from environmental alteration over the wider grassland area (Ott and Hartnett 2015a). We could also see the higher proportion of tillers were recruited from tip than nodes of rhizomes may indicate the significant contribution of apical meristems to guerilla tillers (Briske and Richards 1995). As, study has shown that apical meristems plays a major role in the growth and biomass production of perennial grasses and are also the source of phytomer production including, axillary buds, thereby contributing to the persistence and sustainable productivity of perennial grasses (Briske and Richards 1995).

The higher density of rhizomes, greater rhizome length, greater bud supply and outgrowth also from rhizomes (guerilla growth form) in native *P. smithii* in compare to non-native *B. inermis* may indicate the disturbance avoidance mechanism by maintaining a greater number of dormant rhizome buds that may take longer time to be viable and initiate tillers (Briske 1991, Hyder 1972) or may have greater requirements to break bud dormancy as seen in wheatgrass species such as *P. smithii* and *Pseudoroegneria spicata* (Caldwell et al. 1981).

CONCLUSION

We conclude that *B. inermis* showed greater sensitivity of propagule development at medium precipitation frequency. The total live propagules, tiller replacement rate, total tillers, total rhizomes, rhizome length decreased under low precipitation frequency. Clipping also decreased the total tillers and total rhizomes. All of this may indicate that establishment of non-native *B. inermis* may be vulnerable to change in precipitation regime and grazing conditions and may not be able to easily establish during drought years and heavy grazing.

The number of live propagules per plant for the non-native *B. inermis* seems to be driven by the number of primary tillers, because primary tillers are significantly higher than other generations and decreased significantly with decreased in precipitation frequency. It showed that after primary generation, all the secondary and tertiary tillers are equal tiller producers between the two species. Therefore, the precipitation conditions seem to determine whether non-native *B. inermis* gets a quick start (lots of primary generation tillers) or a slow start (not so many primary generation tillers) and effects its establishment and survival. It is reasonable to expect that wet year could help *B. inermis* for establishment. Likewise, it implies, control/management of non-native *B. inermis* seems to be effective in early stages of grasses when they are not expanding and during dry year. Lack of precipitation during dry years can decrease soil moisture, where we might possibly more severe effect on plant growth of *B. inermis* if there are combination other treatments such as haying/grazing and fire.

In contrast, for native *P. smithii,* no-effect on propagule development, tiller replacement rate, total tillers by medium and low precipitation frequency, while significant increase in total rhizomes and rhizome length at medium precipitation frequency and no-effect of clipping on all above traits expect total tiller indicate positive effect and comparatively insensitive to change precipitation frequency and grazing. In addition, prioritizing of dual phalanx-guerilla growth form irrespective to altered precipitation frequency and clipping may indicate that they may be able to establish or persist at drought year and heavy early grazing conditions. This uniform insensitive response of demographic and clonal traits of *P. smithii* against different level of soil moisture availability and grazing were primarily mediated through belowground bud bank and can be useful information to develop guidelines for effective land management. This study provides new approach to global change research by provide valuable insight into the factors influencing belowground vegetation dynamics and population persistence of two important northern Great Plains grass species and expected to support the development of adaptive grazing management plans under predicted scenarios of climate change.

LITERATURE CITED

1. Asay, K. and K. Jensen (1996). "Wheatgrasses." Cool-season forage grasses (coolseasonforag): 691-724.
2. Benson, E. J. and D. C. Hartnett (2006). "The Role of Seed and Vegetative Reproduction in Plant Recruitment and Demography in Tallgrass Prairie." Plant Ecology **187**(2): 163-177.
3. Bernard, J. M. (1990). "Life history and vegetative reproduction in Carex." Canadian Journal of Botany **68**(7): 1441-1448.
4. Briske, D. (1991). "Developmental morphology and physiology of grasses." Grazing management: an ecological perspective. Timber Press, Portland, OR: 85-108.
5. Briske, D. D. and J. H. Richards (1995). "Plant responses to defoliation: a physiological, morphological and demographic evaluation." Wildland plants: physiological ecology and developmental morphology. Society for Range Management, Denver, CO: 635-710.
6. Busso, C., C. Gittins, G. Becker and L. Ghermandi (2011). "Tiller hierarchy and defoliation frequency determine bud viability in the grass Poa ligularis." Ecological research **26**(5): 985-997.
7. Busso, C. and J. Richards (1995). "Drought and clipping effects on tiller demography andgrowth of two tussock grasses in Utah." Journal of Arid Environments **29**(2): 239-251.
8. Busso, C., R. Mueller and J. Richards (1989). "Effects of drought and defoliation on bud viability in two caespitose grasses." Annals of Botany **63**(4): 477-485.
9. Chen, X.-S., Y.-H. Xie, Z.-M. Deng, F. Li and Z.-Y. Hou (2011). "A change from phalanx to guerilla growth form is an effective strategy to acclimate to sedimentation in a wetland sedge species Carex brevicuspis (Cyperaceae)." Flora-Morphology, Distribution, Functional Ecology of Plants **206**(4): 347-350.
10. Coffin, D. and W. Lauenroth (1990). "A gap dynamics simulation model of succession in a semiarid grassland." Ecological Modelling **49**(3): 229-266.

11. Cully, A. C., J. F. Cully and R. D. Hiebert (2003). "Invasion of exotic plant species in tallgrass prairie fragments." Conservation Biology **17**(4): 990-998.
12. Dalgleish, H. J. and D. C. Hartnett (2006). "Below□ground bud banks increase along a precipitation gradient of the North American Great Plains: a test of the meristem limitation hypothesis." New Phytologist **171**(1): 81-89.
13. Dalgleish, H. J. and D. C. Hartnett (2009). "The effects of fire frequency and grazing on tallgrass prairie productivity and plant composition are mediated through bud bank demography." Plant Ecology **201**(2): 411-420.
14. Dibbern, J. C. (1947). "Vegetative responses of Bromus inermis to certain variations in environment." Botanical Gazette **109**(1): 44-58.
15. DiTommaso, A. and L. Aarssen (1991). "Effect of nutrient level on competition intensity in the field for three coexisting grass species." Journal of Vegetation Science **2**(4): 513-522.
16. Dong, B.-C., G.-L. Yu, W. Guo, M.-X. Zhang, M. Dong and F.-H. Yu (2010). "How internode length, position and presence of leaves affect survival and growth of Alternanthera philoxeroides after fragmentation?" Evolutionary Ecology **24**(6): 1447-1461.
17. Dong, X., J. Patton, G. Wang, P. Nyren and P. Peterson (2014). "Effect of drought on biomass allocation in two invasive and two native grass species dominating the mixed□grass prairie." Grass and forage science **69**(1): 160-166.
18. Donkor, N., E. Bork and R. Hudson (2002). "Bromus-Poa response to defoliation intensity and frequency under three soil moisture levels." Canadian Journal of Plant Science **82**(2): 365-370.
19. Doust, L. L. (1981). "Population Dynamics and Local Specialization in a Clonal Perennial (Ranunculus Repens): I. The Dynamics of Ramets in Contrasting Habitats." Journal of Ecology **69**(3): 743-755.
20. Eneboe, E., B. Sowell, R. Heitschmidt, M. Karl and M. Haferkamp (2002). "Drought and grazing: IV. Blue grama and western wheatgrass." Journal of Range Management: 73-79.

21. Frank, A. (1994). "Physiological comparisons of crested wheatgrass and western wheatgrass to water." Journal of Range Management: 460-466.
22. Garnier, E. and J. Roy (1988). "Modular and demographic analysis of plant leaf area in sward and woodland populations of Dactylis glomerata and Bromus erectus." The Journal of Ecology: 729-743.
23. Harper, J. L. (1977). "Population biology of plants." Population biology of plants.
24. Harrison, T. and J. T. Romo (1994). "Regrowth of smooth bromegrass (Bromus inermis Leyss.) following defoliation." Canadian Journal of Plant Science **74**(3): 531-537.
25. Hendrickson, J. and D. Briske (1997). "Axillary bud banks of two semiarid perennial grasses: occurrence, longevity, and contribution to population persistence." Oecologia **110**(4): 584-591.
26. https://climate.sdstate.edu/ (acessed on 05/27/2016)
27. Humphrey, L. D. and D. A. Pyke (1998). "Demographic and growth responses of a guerilla and a phalanx perennial grass in competitive mixtures." Journal of Ecology **86**(5): 854-865.
28. Hyder, D. (1972). "Defoliation in relation to vegetative growth." The biology and utilization of grasses: 302-317.
29. Jones, S. K., S. L. Collins, J. M. Blair, M. D. Smith and A. K. Knapp (2016). "Altered rainfall patterns increase forb abundance and richness in native tallgrass prairie." Scientific reports **6**.
30. Judziewicz, E. J., L. G. Clark, X. Londono and M. J. Stern (1999). American bamboos, Smithsonian Institution Press.
31. Kendall, J. A. (1993). "Strategies for performing multiple comparisons on means." computer **10**: 10.9688.
32. Klimesova, J. and L. Klimes (2007). "Bud banks and their role in vegetative regeneration - A literature review and proposal for simple classification and assessment." Perspectives in Plant Ecology Evolution and Systematics **8**(3): 115-129.

33. Kluse, J. S. and B. H. A. Diaz (2005). "Importance of soil moisture and its interaction with competition and clipping for two montane meadow grasses." Plant Ecology **176**(1): 87-99.
34. Knapp, A., J. Briggs and J. Koelliker (2001). "Frequency and extent of water limitation to primary production in a mesic temperate grassland." Ecosystems **4**(1): 19-28.
35. Knapp, A. K. and M. D. Smith (2001). "Variation among biomes in temporal dynamics of aboveground primary production." Science **291**(5503): 481-484.
36. Koerner, S. E., S. L. Collins, J. M. Blair, A. K. Knapp and M. D. Smith (2014). "Rainfall variability has minimal effects on grassland recovery from repeated grazing." Journal of Vegetation Science **25**(1): 36-44.
37. Lehtila, K. (2000). "Modelling compensatory regrowth with bud dormancy and gradual activation of buds." Evolutionary Ecology **14**(4-6): 315-330.
38. Murray, S., R. White and M. Rohweder (2000). "Pilot Analysis of Global Ecosystems: Grasslands Ecosystems."
39. Navas, M.-L. and E. Garnier (1990). "Demography and growth forms of the clonal perennial Rubia peregrina in Mediterranean vineyard and unmanaged habitats." The Journal of Ecology: 691-712.
40. N'Guessan, M. (2007). Effects of grazing on growth and morphology of rhizomatous and caespitose grasses in tallgrass prairie, Kansas State University.
41. Oborny, B. (1997). "Models of spatial spread and foraging in clonal plants." The ecology and evolution of clonal plants: 155-183.
42. Olson, B. and J. Richards (1988). "Annual replacement of the tillers of Agropyron desertorum following grazing." Oecologia **76**(1): 1-6.
43. Otfinowski, R. (2008). "Patterns and processes of exotic plant invasions in Riding Mountain National Park, Manitoba, Canada."
44. Otfinowski, R., N. Kenkel and P. Catling (2007). "The biology of Canadian weeds. 134. Bromus inermis Leyss." Canadian Journal of Plant Science **87**(1): 183-198.

45. Ott, J. P. and D. C. Hartnett (2011). "Bud Production and Dynamics of Flowering and Vegetative Tillers in Andropogon Gerardii (Poaceae): The Role of Developmental Constraints." American Journal of Botany **98**(8): 1293-1298.
46. Ott, J. P. and D. C. Hartnett (2012). "Higher-order bud production increases tillering capacity in the perennial caespitose grass Scribner's Panicum (Dichanthelium oligosanthes)." Botany **90**(9): 884-890.
47. Ott, J. P. and D. C. Hartnett (2015a). "Bud bank dynamics and clonal growth strategy in the rhizomatous grass, Pascopyrum smithii." Plant Ecology **216**(3): 395-405.
48. Ott, J. P. and D. C. Hartnett (2015b). "Bud-bank and tiller dynamics of co-occurring C-3 caespitose grasses in mixed-grass prairie." American Journal of Botany **102**(9): 1462-1471.
49. Ott, J. P., J. L. Butler, Y. Rong and L. Xu (2017). "Greater bud outgrowth of Bromus inermis than Pascopyrum smithii under multiple environmental conditions." Journal of Plant Ecology **10**(3): 518-527.
50. Ott, J. and D. Hartnett (2012). "Contrasting bud bank dynamics of two co-occurring grasses in tallgrass prairie: implications for grassland dynamics." Plant Ecology **213**(9): 1437-1448.
51. Painter, E. and J. Detling (1981). "Effects of defoliation on net photosynthesis and regrowth of western wheatgrass." Journal of range management: 68-71.
52. Pfeiffer, K. E. and D. C. Hartnett (1995). "Bison selectivity and grazing response of little bluestem in tallgrass prairie." Journal of Range Management: 26-31.
53. Reichmann, L. G., O. E. Sala and D. P. Peters (2013). "Precipitation legacies in desert grassland primary production occur through previous-year tiller density." Ecology **94**(2): 435-443.
54. Reynolds, J. H. and D. Smith (1962). "Trend of Carbohydrate Reserves in Alfalfa, Smooth Bromegrass, and Timothy Grown Under Various Cutting Schedules 1." Crop science **2**(4): 333-336.
55. Rusch, G. M., B. Wilmann, J. Klimesova and M. Evju (2011). "Do Clonal and Bud Bank Traits Vary in Correspondence with Soil Properties and Resource

Acquisition Strategies? Patterns in Alpine Communities in the Scandian Mountains." Folia Geobotanica **46**(2-3): 237-254.

56. Russell, M. L., L. T. Vermeire, A. C. Ganguli and J. R. Hendrickson (2015). "Season of fire manipulates bud bank dynamics in northern mixed-grass prairie." Plant Ecology **216**(6): 835-846.
57. SAS Institute Inc. (2017) Studio 3.6 University Edition. SAS Institute Inc, Gary, NC.
58. Schrag, A. M. (2011). Addendum: climate chnage impacts and adaptation strategies. In: Ocean of grass: a conservation assessment for the Northern Great Plains Bozeman, MT, World Wildlife Fund-US**:** 36.
59. Sutherland, W. J. and R. A. Stillman (1988). "The foraging tactics of plants." Oikos: 239-244.
60. USDA, N. (2006). "The PLANTS Database, 6 March 2006 (http://plants. usda. gov). Data compiled from various sources by Mark W. Skinner." National Plant Data Center, Baton Rouge, LA: 70874-74490.
61. VanderWeide, B. L. and D. C. Hartnett (2015). "Belowground bud bank response to grazing under severe, short-term drought." Oecologia **178**(3): 795-806.
62. VanderWeide, B. L., D. C. Hartnett and D. L. Carter (2014). "Belowground bud banks of tallgrass prairie are insensitive to multi-year, growing-season drought." Ecosphere **5**(8).
63. Vitousek, P. M., C. M. Antonio, L. L. Loope and R. Westbrooks (1996). "Biological invasions as global environmental change." American Scientist **84**(5): 468.
64. Wuebbles, D., D. Fahey, K. Hibbard, D. Dokken, B. Stewart and T. Maycock (2017). "Climate Science Special Report: Fourth National Climate Assessment." US Global Change Research Program: Washington, DC, USA.
65. Ye, X.-H., F.-h. Yu and M. Dong (2006). "A trade-off between guerilla and phalanx growth forms in Leymus secalinus under different nutrient supplies." Annals of Botany **98**(1): 187-191

CHAPTER 3

GREATER COMPETITIVE ABILITY OF *BROMUS INERMIS* THAN *PASCOPYRUM SMITHII* IN TERMS OF BELOWGROUND BUD BANK AND TILLER DEMOGRAPHY

ABSTRACT

In the northern Great Plains, the resilience of perennial grasslands largely depends on successful tiller recruitment and establishment from belowground bud banks. However, over the recent decades, these grasslands are rapidly invaded by introduced perennial grasses like *Bromus inermis* and transforming larger tracts of native prairies by replacing native perennial grasses, such as *Pascopyrum smithii*, reducing biodiversity and quality of habitats, and increasing vulnerability of grasslands to other environmental disturbances. In this study we evaluated the effects of intra-and inter-specific competition on belowground bud production, tiller and rhizome recruitment, plant establishment, and biomass between native *P. smithii* and non-native *B. inermis* under frequent water and constant temperature condition. A greenhouse experiment consisted of five treatments including single *B. smithii*, single *P. smithii*, pairwise monoculture of *B. inermis*, pairwise monoculture of *P. smithii*, and pairwise mixed-culture of *B. inermis* and *P. smithii* with 30 replicates for each treatment under high precipitation frequency regime. Seedlings at the 2-leaf stage of each species were transplanted into individual pots based on designated treatments. Plants were harvested 12 weeks after the treatments had been initiated. The data collection followed the same protocol as the first experiment. In addition, biomass and RII values were calculated to measure intra- and interspecific competition between the native *P. smithii* and the non-native *B. inermis*. We found that the presence of the non-native *B. inermis* as a neighbor significantly decreased the number of live propagules, tillers, and aboveground biomass of the native *P. smithii.* Whereas the presence of the native *P. smithii* as a neighbor significantly increased the number of live propagules and had a significantly less negative effect on tiller production

and aboveground biomass of the non-native *B. inermis*. The results demonstrated strong competitive ability of non-native *B. inermis* against *P. smithii* during the establishment phase when environmental conditions were favorable (i.e. lack water stress and grazing).

INTRODUCTION

Biological invasions are currently global phenomena that threaten terrestrial, marine and freshwater biodiversity (Dukes and Mooney 1999, Pimentel et al. 2005, Vitousek et al. 1996). Biological invasions are regarded as the second biggest global threat to biodiversity after habitat destruction (Wilcove et al. 1998). Invasions have also altered global biodiversity byreducing it at local habitat scale, increasing diversity at the regional scale and tending towards homogenization at global scales, with widespread ecological and evolutionary implications (need citations here). Many non-native species have been deliberately introduced for economic purposes such as land rehabilitation, forage, and ornamental use. Although non-native species create economic benefits, they are detrimental to ecosystem services and functions when they escape from cultivation (Reichard and White 2001).

The extent of the northern Great Plains grasslands is declining due to conversion of these grasslands to croplands, consequently resulting in habitat fragmentation and disturbance (DeKeyser et al. 2013). Increasing disturbance and fragmentation has caused remnant native prairies to become susceptible to invasion by cool-season non-native species (Hobbs and Huenneke 1992, DeKeyser et al. 2013), such as *Bromus inermis* Leyss. and *Poa pratensis* L*.,* accounting for 62% of exotic species cover in the northern Great Plains (Cully et al. 2003). *Bromus inermis* and *P. pratensis* are highly invasive in North American grasslands (need citation here). In a 2002-2006 survey of mixed-grass and tallgrass prairie vegetation, *B. inermis* comprised 45%-49% of plant cover in some areas, and *P. pratensis* occupied 27% to 36% of vegetation in other locations (Grant et al. 2009). Non-native perennial grasses like *Bromus inermis* are rapidly invading larger areas of remnant native prairie and replacing the native species, such as western wheatgrass (*Pascopyrum* smithii), and decreasing biodiversity (Cully et al. 2003).

Due to the negative effects of *B. inermis* and *P. pratensis* on prairies, researchers have tried to understand why these two invaders are so successful. The competitive ability of perennial grasses like *B. inermis* has been examined in both mixed and tallgrass prairie in-terms of physiological, morphological and ecological traits of plants (Lamas et al. 2013), plant stoichiometric responses (Bai et al. 2012), spatiotemporal patterns of soil seed bank (Dreber and Esler 2011) and species composition, structure and function of plant communities (Hoshino et al. 2009, Collins and Calabrese 2012). However, very few of these studies has addressed the lack of understanding the underlying demographic mechanisms.

Studies have shown that in perennial grasslands, seedling recruitment of perennial grasses are rare as most of the tillers are recruited from vegetative belowground buds (Rogers and Hartnett 2001, Benson and Hartnett 2006). For example, in undisturbed tallgrass prairie, seedlings recruitment from seed is extremely rare and >99% of tiller recruitment occurs from belowground buds rather than seed (Benson and Hartnett 2006). Both non-native *B. inermis* and native *P. smithii* are perennial, cool-season, rhizomatous grasses where population establishment and persistence of these grasses, prominently depends on stem recruitment from the belowground bud bank (Benson and Hartnett 2006, Ott et al. 2017). The response of these belowground bud banks to grazing disturbances (Dalgleish and Hartnett 2009) and climatic variability (Dalgleish and Hartnett 2006) has great potential for shaping the resilience of grassland plant communities (Klimesova and Klimes 2007, Ott and Hartnett 2011 & 2012).

Besides, these two-rhizomatous grasses can vary in architecture along the "phalanx - guerilla" clonal growth form continuum (Harper 1977, Doust 1981) and may determine the structure and fate of belowground bud bank (Ott and Hartnett 2015a). Studies have shown that investment in different growth forms of rhizomatous perennial grasses depend on environmental conditions (Doust 1981, Garnier and Roy 1988). Yet, little is known how the bud bank, tiller establishment and clonal growth form of non-native perennial grasses such as *B. inermis* interacting with native *P. smithii* will contribute to the resilience of the semi-arid ecosystem of northern Great Plains (Russell et al. 2015, Ott et al. 2017). It is imperative to evaluate and understand the responses of the belowground bud bank that have potential to explain the effect of non-native plants on the native species, and competitive ability of native species.

Therefore, by using the native perennial grass *P. smithii* and non-native perennial grass *B. inermis* as model species through a competition greenhouse study, we tried to understand, how intra-and interspecific competition affects the belowground bud production, tiller and rhizome recruitment, plant establishment, and biomass of this native *versus* non-native perennial grass.

1.1 Research Question

How do intra- and inter-specific competitions effect on belowground bud production, propagule development, tiller and rhizome recruitment, plant establishment, and biomass between native *P. smithii* and non-native *B. inermis* under high precipitation frequency and constant temperature condition?

1.2 Hypotheses

H_a1: The propagule development, live propagule production per tiller generation, total live propagule production, number of new tillers established per tiller, total tiller and rhizome production, rhizome length, aboveground and rhizome biomass of the native *P. smithii* will be lower under interspecific than intraspecific competition.

H_a2: The propagule development, live propagule production per tiller generation, total live propagule production, number of new tillers established per tiller, total tiller and rhizome production, rhizome length, aboveground and rhizome biomass of the non-native *B. inermis* will be greater under interspecific than intraspecific competition.

H_a3: Investment in guerilla growth *via* total live propagule and total tillers will be greater than phalanx growth in the non-native *B. inermis* than the native *P. smithii* under intraspecific and interspecific competition.

METHODS

2.1 Experiment Design and Treatment

A temperature-controlled competition greenhouse experiment was conducted at South Dakota State University in the Forestry and Horticulture Greenhouse (44.320559, -96.784205) over a growing season of 2017 (May-August). Two-way factorial (3 x 2) complete randomized experimental design. We had three levels of competition [None (Single/without neighbor), Intraspecific (Monostand), and Interspecific (Mixed-stand)] and two level of species [*B. inermis*, and *P. smithii*] as shown in Table 3.1. Therefore, the experiment consisted of five treatment combination: single *B. inermis*, single *P. smithii*, pairwise monostand of *B. inermis*, pairwise monostand of *P. smithii*, and pairwise mixed-stand of *B. inermis* and *P. smithii* with 30 replicates for each treatment and with individual plant as the experimental unit (Figure 3.1).

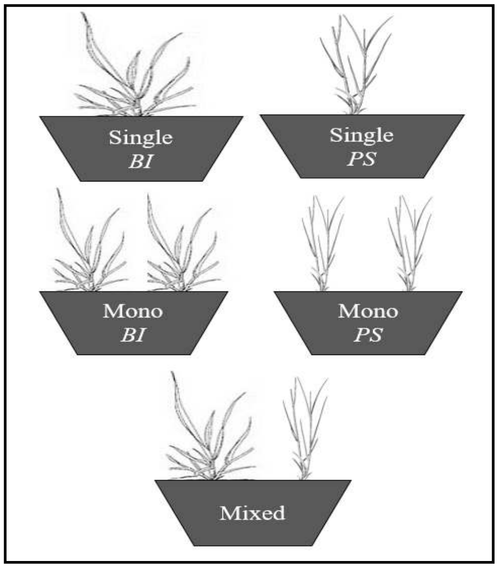
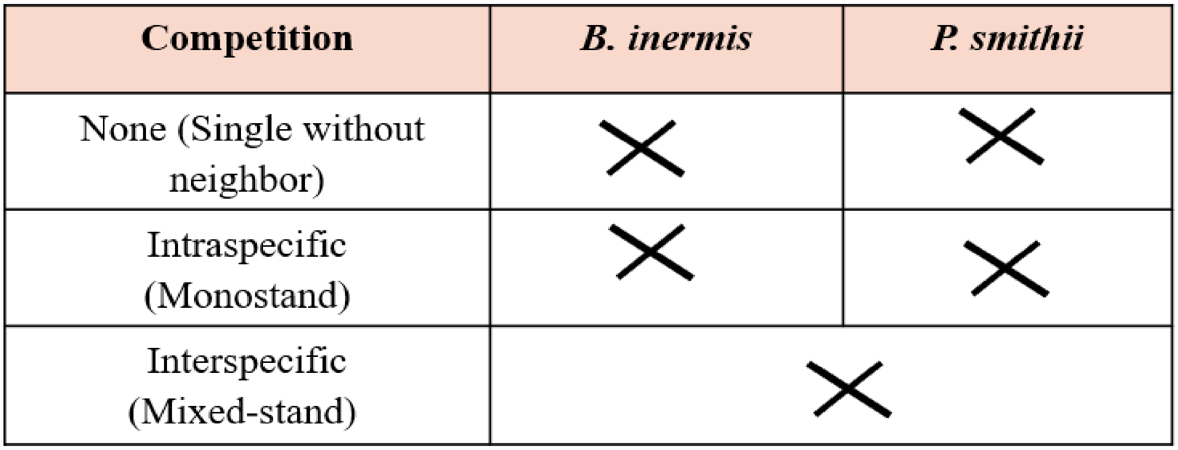


Figure 3.1 Conceptual diagram of experimental design. Treatments include the pots with single stand of *B. inermis*, and *P. smithii* (indicating no neighbor), Pots with Monostand of *B. inermis*, and *P. smithii* (indicating conspecific neighbor), and Mixed-stand of both the species (indicating either of neighbor). Each with 30 replications.

Table 3.1 Competition treatment combination applied at individual species level.



Competition	***B. inermis***	***P. smithii***
None (Single without neighbor)	X	X
Intraspecific (Monostand)	X	X
Interspecific (Mixed-stand)	X	

'X' indicates that treatment combination occurred at the specified species.

2.1.1 Study Species

Pascopyrum smithii (commonly known as western wheatgrass), and *B. inermis* (commonly known as smooth bromegrass) are both strongly rhizomatous perennial C_3 grasses that begin flowering in late May (the PLANTS database, USDA-NRCS 2006). *Pascopyrum smithii* is native to North America, is most abundant in the areas receiving 254 to 508 mm precipitation. It is an important component of many native plant communities. In contrast, *B. inermis* was introduced from Eurasia in late 1880s for improving forage production and control soil erosion and has made an extensive impact on the grasslands of North Americas. *Bromus inermis* establishes by invading disturbed prairies and through repeated introductions for soil retention and livestock graze (Otfinowski et al. 2007). These two species produce both phalanx and guerilla tillers *via* their clonal growth strategy and quickly spreads out into open habitat (Asay and Jensen 1996, Judziewicz et al. 1999, Ott and Hartnett 2015a).

Seed Sources

The seeds of *P. smithii* were from Golden Willow Seeds, INC. (Midland, SD) and *B. inermis* were from Dakota's Best Seed LLC (Platte, SD). These seeds were provided by South Dakota State University Seed Testing Laboratory (Brookings, SD).

2.2 Seedling Establishment and Transplant

Seeds were screened for intactness under a magnifying glass and were germinated in the Miracle-Gro® potting mix soil filled trays in the greenhouse with temperature regime of 16˚C night/ 22˚C day. Seeds of *P. smithii* were sowed five days earlier than *B. inermis* to obtain the same growth stage for transplant. The 120 two-leaf stage seedlings for each species were transplanted simultaneously into each individual non-fertilizer potting-soil (PRO-MIX® BX) filled pot (16.5 cm dia. X 16 cm depth) based on the randomly assigned treatment combination during 3rd week of May 2017.

2.3 Growth Condition

Photoperiods and temperature regimes of greenhouse chambers were set up similar to mixed grass prairie field conditions during the growing season with constant averaged monthly photoperiod and temperature regime based upon pervious 10 years climate data of Rapid City, South Dakota. Before the seedling transplant, each individual pot filled with 600 gm potting soil (PRO-MIX® BX) was saturated with 500 ml [44%-45% volumetric water content (VWC; Decagon Devices; Soil Moisture Sensor: Model 10HS custom calibrated to the potting soil)]. Additionally, 450 ml of water was added after seedling transplant for seedling establishment. Soil moisture level (VWC ~ 25% - 28%) was achieved on the first week of June 2017 (i.e. 17 days after the seedling transplant). Total of 150 pots was watered every other day with 72 ml to maintain the 25-28% VWC soil moisture level. Water regime represent frequent precipitation over mixed-grass prairie region; and was calculated based upon average growing seasonal monthly precipitation and the surface area of the pot. The monthly average of the growing season (March, April, May, June, July & August) precipitation amount from Rapid City Regional AP station, South Dakota (1981-2010) (www.climate.sdstate.edu) which represents the spring growing season precipitation characteristics of mixed-grass prairies of northern Great Plains (Schrag 2011).

2.4 Data Collection

Newly initiated tilers were carefully marked by Individual Tiller Identifier daily.

2.4.1 Harvesting Plants

All plants from the pots were harvested after 12 weeks of treatments, where underground structures were washed free of soil and were air dried and stored in paper sample bags under room temperature.

2.4.2 Mapping, Classification and Biomass Measurements

The lab protocol developed in Experiment 1 were used to collect: Tiller and rhizome generation mapping data (see Chapter 2, 2.2.3 Tiller and rhizome generation mapping, Page 40), and Bud, rhizome and tiller development stage classification data (see Chapter 2, 2.3.4 Bud, rhizome and tiller development stage classification, Page 41).

In addition, we measured the aboveground and rhizome biomass (g) per plant by taking 10 random subsamples from each treatment combination, biomass was oven-dried for at least 72 hours at 60˚C.

2.5 Data Analysis

2.5.1 Data Organization

The data were organized, and some of the response variables created similar to experiment 1(chapter 2) as listed below:

Bud production, and Propagule development

(i) Number of live propagules per plant
(ii) Number of live propagules per plant belonging to each development stage
(iii) Live propagules per tiller by generation

Number of new tillers established per tiller

Number of new tillers established per tiller at each daughter tiller generation was calculated as the number of tillers recruited at that generation divided by the number of tillers recruited by preceding generation.

Overall Plant Establishment

(i) Number of total tillers per plant
(ii) Number of total rhizomes per plant
(iii) Total rhizome length (cm) per plant

Investment in phalanx and guerilla growth

(iv) Proportion of live propagules from tiller per plant
(v) Proportion of total tillers per plant belonging to each location

In addition, biomass and RII values were also calculated as follows:

Biomass

(i) Aboveground biomass (g) per plant
(ii) Rhizome biomass (g) per plant
(iii) Total biomass (g) per plant = Sum of aboveground biomass (g) per plant and rhizome biomass (g) per plant (excluding roots).

Relative interaction index (RII)

The interaction between species at intraspecific and interspecific competition was evaluated with a relative interaction index (RII; Armas et al. 2004, Ulrich and Perkins 2014, Li et al. 2015). RII values were calculated with respect to some of the above response variables, including; (a) number of total tiller per plant, (b) number of total rhizome per plant, (c) total rhizome length (cm) per plant, (d) number of total live propagule per plant, (e) aboveground biomass per plant, (f) rhizome biomass per plant, and (g) total biomass per plant by using following equation (*Note: the below equation shown by taking the number of total tiller per plant as a typical response variable);

$$\text{Relative Interaction Index (Rii)} = (N_{BW} - N_{BO}) / (N_{BW} + N_{BO})$$

N_{BW} = number of total tillers per plant of a species with competition
N_{BO} = number of total tillers per plant of a species without competition

Where, RII value ranges from [-1, +1], the positive value indicates facilitative effect and negative value indicate competitive effect and the magnitude of interaction increases with increase in value. The more the negative number is, the more the intensity of competition and vice-versa.

We calculated the RII values for any competition type by pairing the pots. For instance, the Pot #1 (Single *B. inermis*) was randomly paired with the Pot #61 (Monoculture *B. inermis*), and with the Pot #121 (Mixed-culture *B. inermis*) to calculate RII value for intraspecific (competing itself) and interspecific (with *P. smithii*) competition of *B. inermis* We had 30 RII values for each of four-treatment combination based on target species (i.e. Monoculture *B. inermis*, Monoculture *P. smithii*, Mixed-culture *B. inermis*, and Mixed-culture *P. smithii*).

2.5.2 Statistical Analysis

The effect of competition and species on belowground bud production, tiller and rhizome recruitment, plant establishment, biomass, and RII values were analyzed using linear mixed models through PROC GLIMMIX in SAS® Studio 3.6 University Edition (SAS Institute 2017). All the treatments were applied at the plant level except generation which was applied at the tiller level. Residual method (res) was used to approximate the denominator degrees of freedom except in case of number of live propagules per tiller by generation where the containment (CON) method was used. Model goodness-of-fit was checked by insuring the deviance was at or near 1. Potential outliers were identified if studentized residuals value smaller than -3 and larger than +3. The multiple pairwise comparison between treatments were significant at $P < 0.05$ (Kendall 1993). The data were analyzed under the following response variables as follows:

Bud production, and Propagule development

The number of live propagules per plant analyzed using gamma distribution in a two-way factorial treatment structure with the factor of competition (three levels), and species (two levels) in a randomized complete design, and the number of live propagules per plant belonging to each development stage analyzed using gamma distribution in a three-way factorial treatment structure with the factors of competition (three levels), species (two levels), and development stage (three levels) in a randomized complete design.

Live propagules per tiller by generation

The number of live propagules per tiller in each generation analyzed using negative binomial distribution in a three-way factorial treatment structure with the factors of competition (three levels), species (two levels), and generation (two levels) in a split-plot randomized complete design. The factor of generation was applied at tiller level (sub-plot level). Note: Live propagules production at tertiary generation tiller could not be included in this analysis model because of lack of data of *B. inermis*.

Number of new tillers established per tiller

The number of new tillers established per tiller in each generation analyzed using negative binomial distribution in a three-way factorial treatment structure with the factors of competition (three levels), species (two levels), and generation (three levels) in a randomized complete design.

Overall Plant Establishment

Both the number of total tillers per plant, and number of total rhizomes per plant was analyzed using a negative binomial distribution, Total rhizome length (cm) per plant was analyzed using gamma distribution in a two-way factorial treatment structure with the factor of competition (three levels), and species (two levels) in a randomized complete design.

Investment in phalanx and guerilla growth

Proportion of total live propagule from tiller per plant analyzed using a beta distribution in a two-way factorial treatment structure with the factor of competition (three levels), and species (two levels) in a randomized complete design, and proportion of total tillers per plant belonging to each location (crown, rhizome nodes, and rhizome tips) was analyzed using a negative binomial distribution in a three-way factorial treatment structure with the factors of competition (three levels), species (two levels), and location (three levels) in a randomized complete design.

Biomass

Total biomass (g) per plant, aboveground biomass (g) per plant, and rhizome biomass (g) per plant were analyzed using normal distribution, in a two-way factorial treatment structure with the factors of competition (three levels), and species (two levels) in a randomized complete design.

Contrasts

The significant tests for effect of: (i) intraspecific versus interspecific competition across species, and (ii) *B. inermis* versus *P. smithii* species effect excluding the none competition treatment for all the response variables except RII was evaluated adding contrast statement in each of above linear model.

Relative interaction index (RII)

RII values were analyzed using normal distribution, in a two-way factorial treatment structure with the factors of competition (two levels), and species (two levels) in a randomized complete design.

RESULTS

3.1 Bud Production, and Propagule Development

The number of live propagules per plant was significantly affected by species, competition, and species x competition (Appendix-Table 3.2). The mean live propagules production was significantly higher for *B. inermis* in interspecific (30.45 ± 2.74) than intraspecific (11.41 ± 1.03) competition, whereas mean live propagules production was significantly lower for *P. smithii* in interspecific (30.60 ± 2.75) than the intraspecific (52.78 ± 4.75) competition (Figure 3.2).

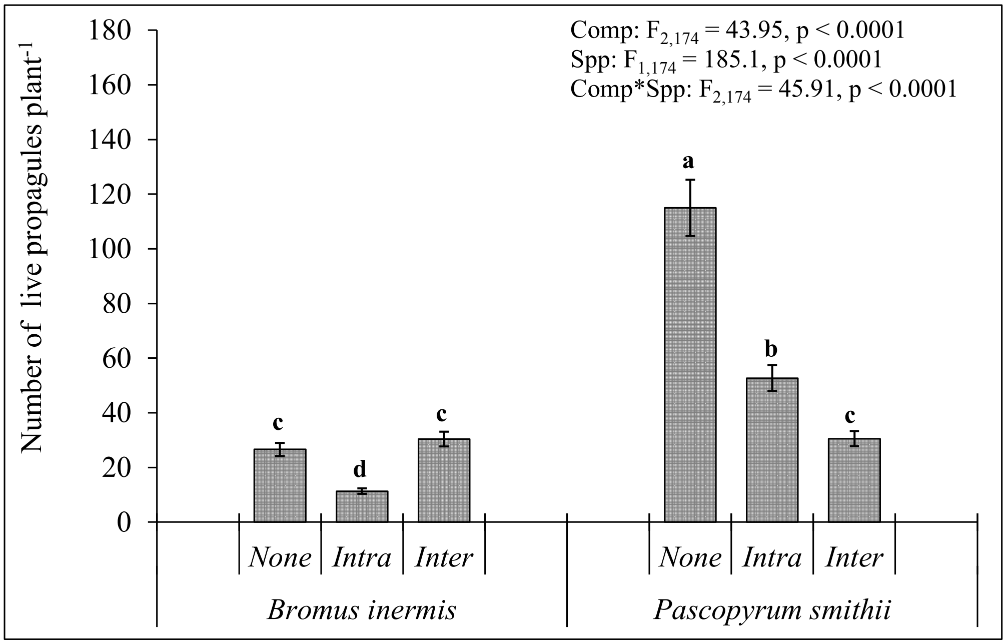


Figure 3.2 Effect of competition treatment on number of live propagules per plant of *B. inermis* and *P. smithii*. Values are mean ± SE based on the statistical model. Different letters above bars indicate significant difference across competition and species treatment combination at p-value < 0.05.

Similarly, the proportion of live propagule at each development stage per plant was significantly affected by species, and development stage (Appendix-Table 3.4). Although not statistically significant, the propagule development (i.e. proportion of small juvenile and large juvenile tillers) of *B. inermis* was greater at interspecific by ~10% than intraspecific competition, whereas *P. smithii* propagule development didn't differ much between interspecific and intraspecific competition (Figure 3.3).

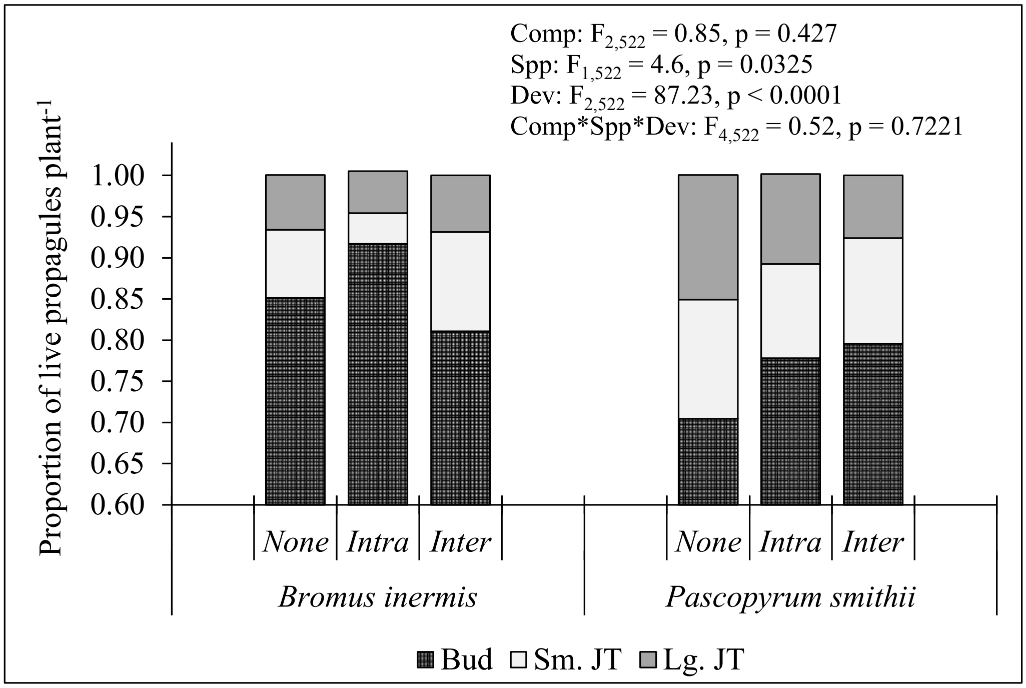


Figure 3.3 Effect of competition treatment on proportion of live propagules at each development stage per plant of *B. inermis* and *P. smithii*. Proportion of live propagules were classified into three development/size classes including buds, small juvenile tillers/rhizomes (Sm. JT) and large juvenile tillers/rhizome (Lg. JT). Here, the number of live propagules belonging to each development stage per plant was analyzed to get this proportion of live propagules at each development stage per plant. Values are the mean of the proportion of live propagules per plant.

3.2 Live Propagules per Tiller by Generation

The number of live propagules per tiller in each generation was significantly affected by species, competition, and competition x generation (Appendix-Table 3.6). *Pascopyrum smithii* significantly produced a higher number of live propagules in both primary and secondary generation than *B. inermis* irrespective of competition level (contrast *P. smithii* versus *B. inermis*: $F_{1,174} = 54.09$, $p < 0.0001$, Appendix-Table 3.8). Intraspecific competition significantly lowered *B. inermis* live propagules production by secondary tillers, whereas interspecific competition significantly lowered *P. smithii* live propagules production of secondary tillers compared no-competition treatment (Figure 3.4).

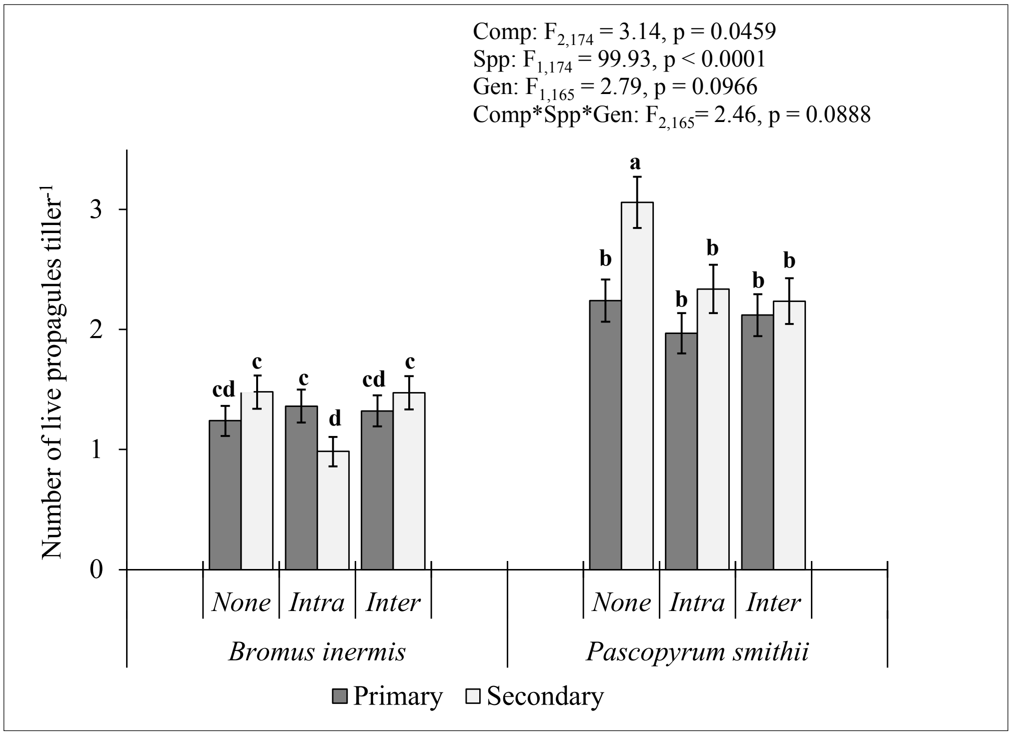


Figure 3.4 Effect of competition treatment on number of live propagules per tiller by generation of *B. inermis* and *P. smithii*. We had only two daughter tiller generation cohorts including primary and secondary in this model because there was no sufficient data available for tertiary tiller generation. Values are mean ± SE based on the statistical model. Different letters above bars indicate significant difference across competition and species treatment combination at p-value < 0.05.

3.3 Number of New Tillers Established per Tiller

The number of new tillers established per tiller in each generation was significantly affected by species, competition, generation, species x competition, species x generation, and species x competition x generation (Appendix-Table 3.9). Intraspecific competition significantly lowered secondary and tertiary tiller production than the interspecific competition in *B. inermis*, whereas there were no significant changes in *P. smithii* due to intra-and interspecific competition (Figure 3.5). Although under intraspecific competition *B. inermis* statistically significant produced fewer numbers of secondary and tertiary tillers compared interspecific competition but it might not be biologically significant due to only occasional secondary (<0.6 new established tiller per tiller) and tertiary (<0.2 new established tiller per tiller) tiller production (Figure 3.5.

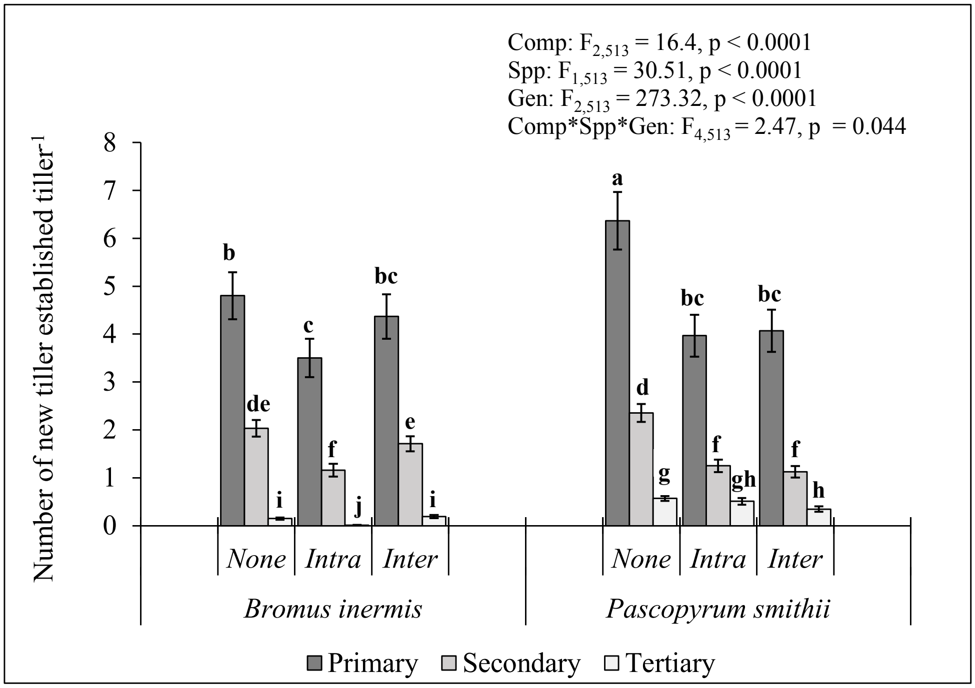


Figure 3.5 Effect of competition treatment on number of new tiller established per tiller along generation of *B. inermis* and *P. smithii*. We had three daughter tillers generation cohort including primary, secondary, and tertiary tiller generations. Values are mean ± SE based on the statistical model. Different letters above bars indicate significant difference across competition and species treatment combination at p-value < 0.05.

3.4 Overall Plant Establishment

The number of total tillers per plant was significantly affected by species, competition, and species x competition (Appendix-Table 3.12). *Bromus inermis* mean tiller production was significantly higher in interspecific (12.87 ± 0.74) than the intraspecific (7.33 ± 0.53) competition whereas, *P. smithii* tiller production was not significantly different between intra-and interspecific competition (11.23 ±0.68 vs. 10.03 ±0.64) Interspecific competition significantly reduced tiller production in *P. smithii* compared to *B. inermis*. (Figure 3.6).

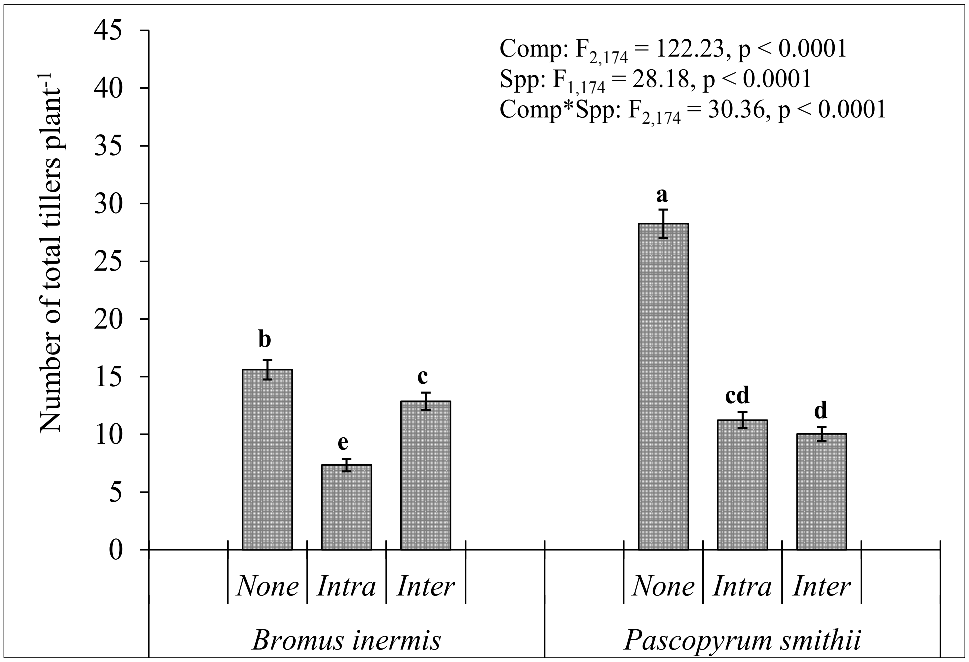


Figure 3.6 Effect of competition treatment on number of total tillers per plant of *B. inermis* and *P. smithii*. Values are mean ± SE based on the statistical model. Different letters above bars indicate significant difference across competition and species treatment combination at p-value < 0.05.

Similarly, the number of total rhizomes per plant was significantly affected by species and competition but no significance of the interaction. The total rhizome number in *P. smithii* was twice of *B. inermis* in intraspecific competition (contrast *P. smithii* versus *B. inermis*: $F_{1,174} = 15.84$, $p < 0.0001$, Appendix-Table 3.14) (Figure 3.7).

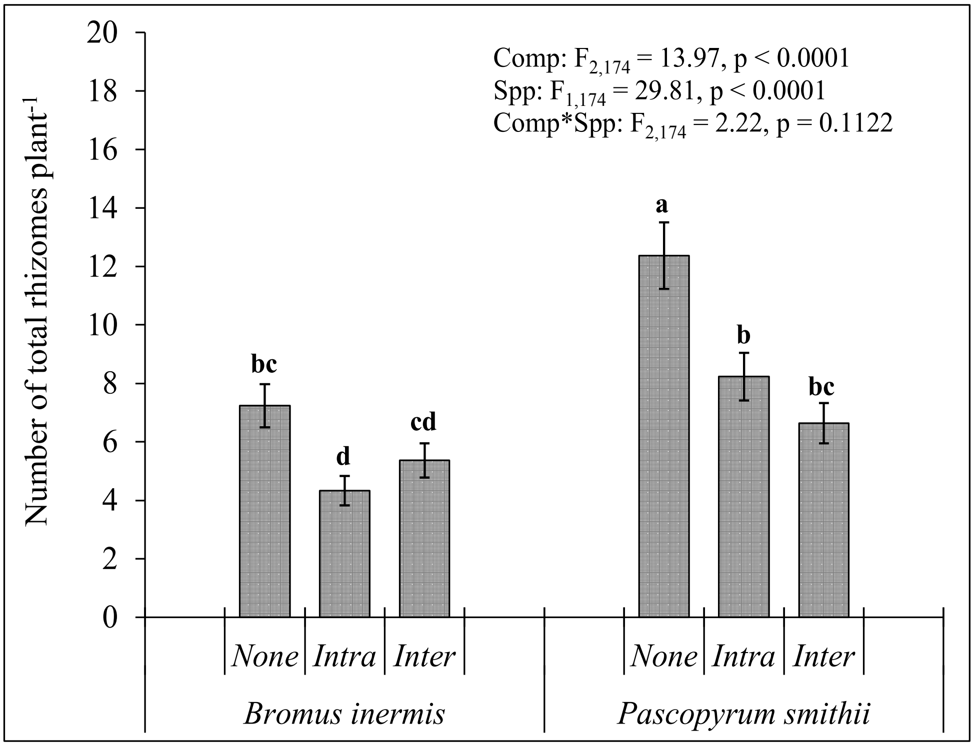


Figure 3.7 Effect of competition treatment on number of total rhizomes per plant of *B. inermis* and *P. smithii*. Values are mean ± SE based on the statistical model. Different letters above bars indicate significant difference across competition and species treatment combination at p-value < 0.05.

The total rhizome length (cm) per plant was significantly affected by species and competition but not the interaction (Appendix-Table 3.12). On average, *P. smithii* total rhizome length (cm) was significantly greater by ~75% than *B. inermis* irrespective of competition level (contrast *P. smithii* versus *B. inermis*: $F_{1,174} = 70.52$, $p < 0.0001$, Appendix-Table 3.14) (Figure 3.8).

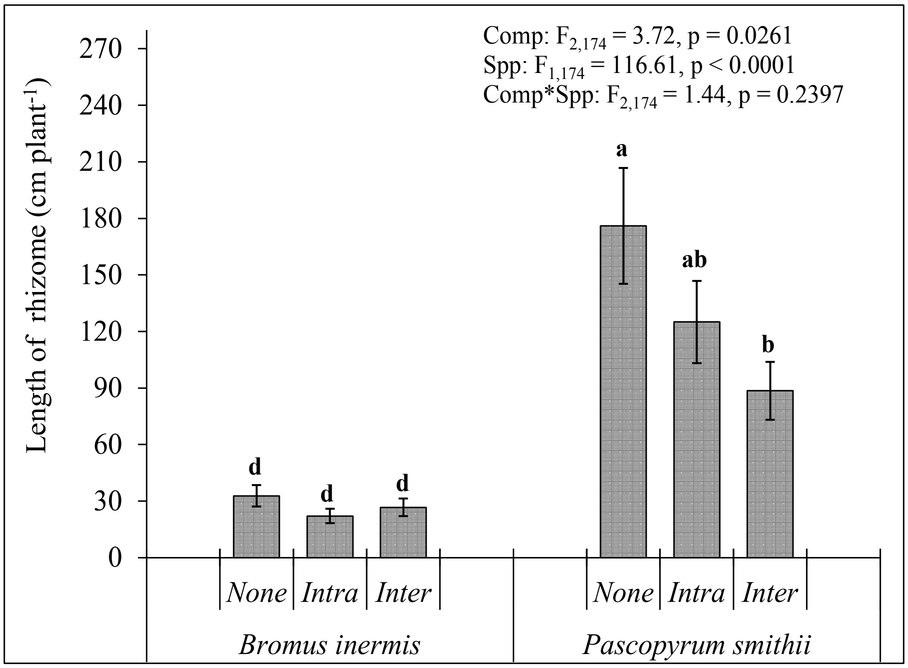


Figure 3.8 Effect of competition treatment on total length of rhizome (cm) per plant of *B. inermis* and *P. smithii*. Values are mean ± SE based on the statistical model. Different letters above bars indicate significant difference across competition and species treatment combination at p-value < 0.05.

3.5 Investment in Phalanx and Guerilla Growth

The proportion of total live propagules from tiller per plant was significantly affected by the interaction of species and competition (Appendix-Table 3.15). Proportion of live propagules has been used to define the two-clonal growth form in perennial grasses (as in experiment 1) - the phalanx growth form (where live propagules are from crown tiller) and guerilla (where live propagules are from rhizomes). *Bromus inermis* investment in clonal growth form shifted from strict phalanx (as ~80% of live propagules were produced from crown tillers and only ~20% of live propagules were produced from rhizome) to dual phalanx and guerilla (as ~ 51% from crown tiller and ~49% from rhizome). In contrast, *P. smithii* investment in growth form shifted from dual phalanx and guerilla (as ~53% crown tiller and ~47% from rhizome) to strict phalanx (as ~78% from crown tiller and only ~22% from rhizome) as changing from intraspecific to interspecific competition (Figure 3.9).

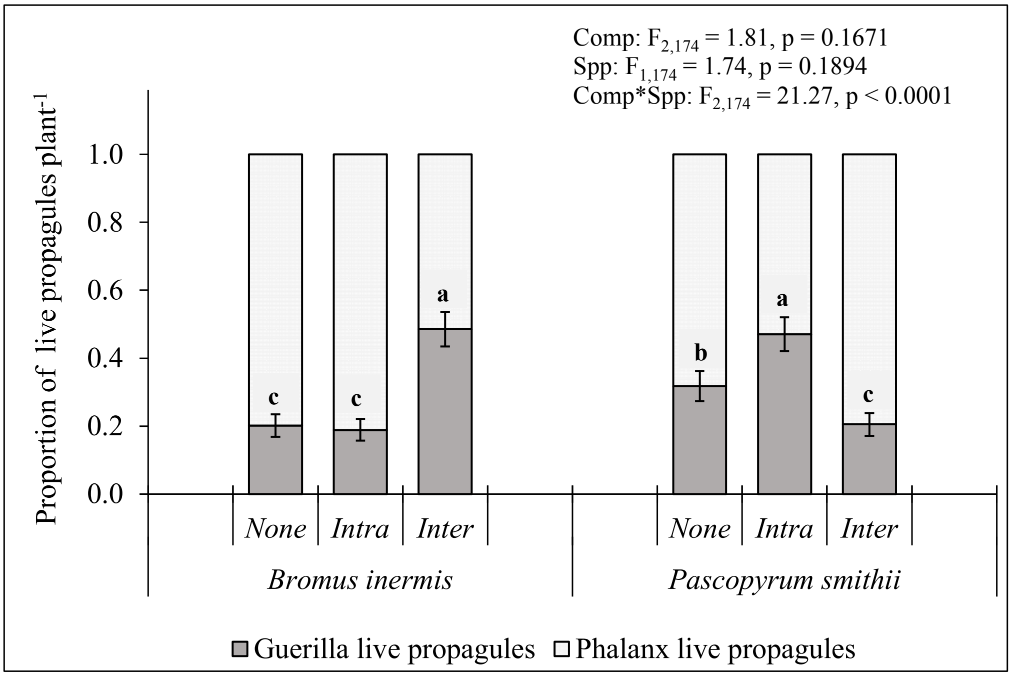


Figure 3.9 Effect of competition treatment on proportion of live propagules per plant of *B. inermis* and *P. smithii*. Values are mean of the proportion of live propagules per plant. Live propagules produced from rhizome and crown were referred to guerilla live propagules and phalanx live propagules, respectively. Values are mean ± SE based on the statistical model. Different letters above bars indicate significant difference across competition and species treatment combination at p-value < 0.05.

Similarly, the proportion of total tillers at each location per plant was significantly affected by species, location, and species x location (Appendix-Table 3.17). The proportion of total tillers at each location per plant has been used to define the two-clonal growth from in perennial grasses (as in experiment 1) - the phalanx growth form (where tillers are recruited from crown tiller of plant) and guerilla growth form (where tillers are recruited either from nodes or/and from tip the rhizomes of plant). *Bromus inermis* invested in dual phalanx and guerilla growth at both intraspecific (as ~52% from crown tiller and ~48% from nodes and tip of rhizome) and interspecific competition (as ~62%

from tiller and ~38% from nodes and tip of rhizome). Whereas *P. smithii* prioritized phalanx growth at both intraspecific (as ~74% from crown tiller and ~26% from nodes and tip of rhizome) and interspecific competition (as ~73% from tiller and ~27% from nodes and tip of rhizome) (Figure 3.10).

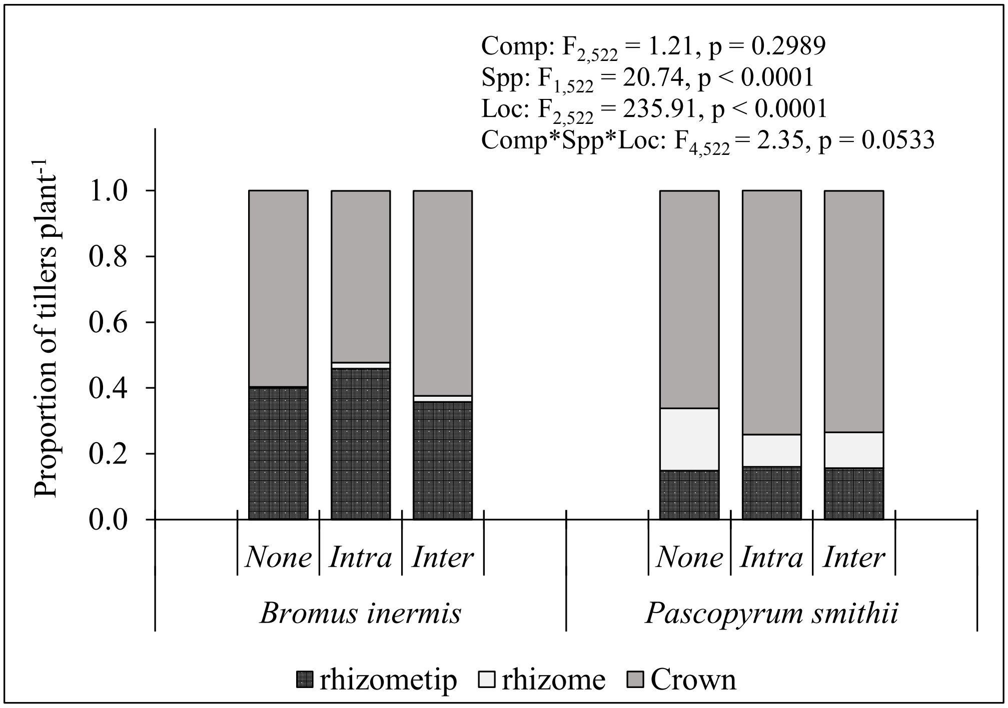


Figure 3.10 Effect of competition treatment on proportion of total tillers based on location per plant of *B. inermis* and *P. smithii*. We had three sources/location of tillers including crown, rhizome nodes, and rhizome tip. Tillers recruited from crown contributed to phalanx growth and the tillers recruited from nodes and tips of rhizomes contributed to guerilla growth. Values were mean proportion of tillers per plant.

3.6 Biomass

The aboveground biomass (g) per plant was significantly affected by competition, and species x competition (Appendix-Table 3.20). Aboveground biomass was significantly greater for *B. inermis* under interspecific (4.12 ± 0.32) rather than intraspecific (2.21 ± 0.32) competition, whereas aboveground biomass was not significantly different for *P.*

smithii. Also, *B. inermis* aboveground biomass production was greater than *P. smithii* in interspecific competition (Figure 3.11).

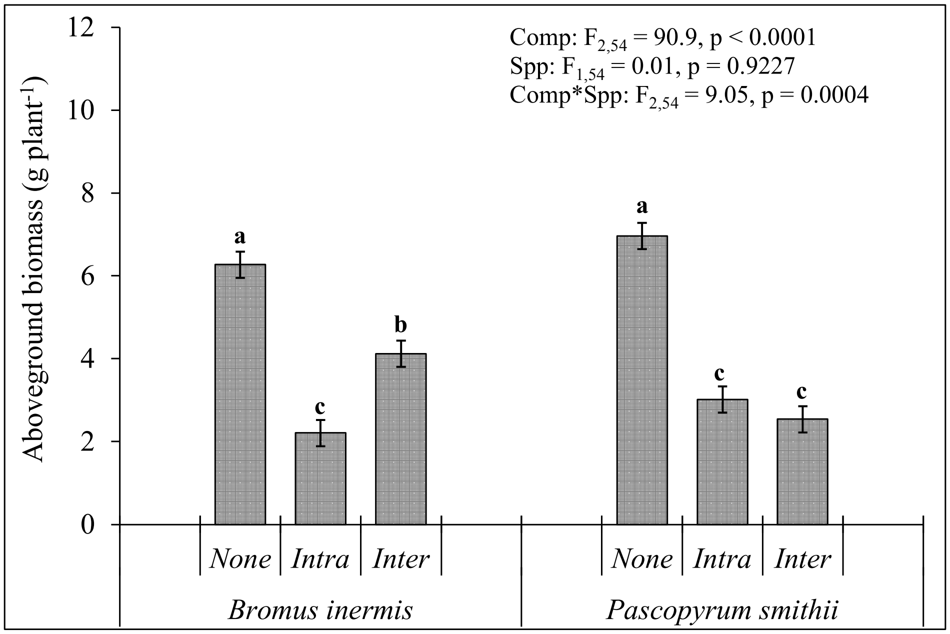


Figure 3.11 Effect of competition treatment on aboveground biomass (g) per plant of *B. inermis* and *P. smithii*. Values are mean ± SE based on the statistical model. Different letters above bars indicate significant difference across competition and species treatment combination at p-value < 0.05.

Similarly, rhizome biomass (g) per plant was significantly affected by species, and competition (Appendix-Table 3.20). On average, *P. smithii* rhizome biomass was significantly greater by ~60% than *B. inermis* irrespective of competition level (contrast *P. smithii* versus *B. inermis*: $F_{1,54}$ = 10.08, *p = 0.0025*, Appendix-Table 3.22) (Figure 3.12).

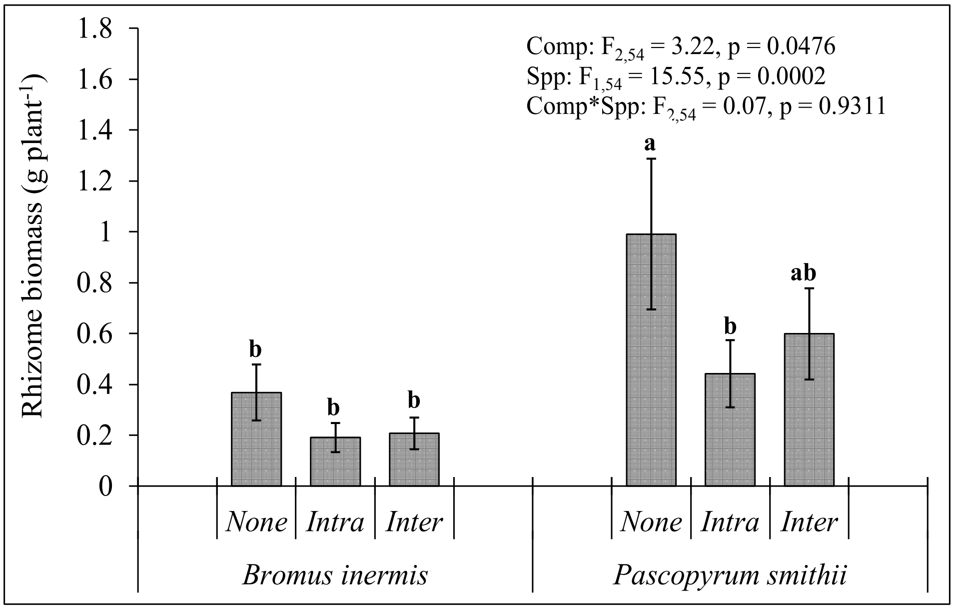


Figure 3.12 Effect of competition treatment on rhizome biomass (g) per plant of *B. inermis* and *P. smithii*. Values are mean ± SE based on the statistical model. Different letters above bars indicate significant difference across competition and species treatment combination at p-value < 0.05.

Total biomass (g) per plant was significantly affected by species, competition, and species x competition (Appendix-Table 3.20). The mean total biomass production was significantly higher for *B. inermis* in interspecific (4.33 ± 0.34) than intraspecific (2.26 ± 0.34) competition, whereas total biomass production was not significantly different for *P. smithii*. Also, *B. inermis* total biomass production was greater than *P. smithii* in interspecific competition (Figure 3.13).

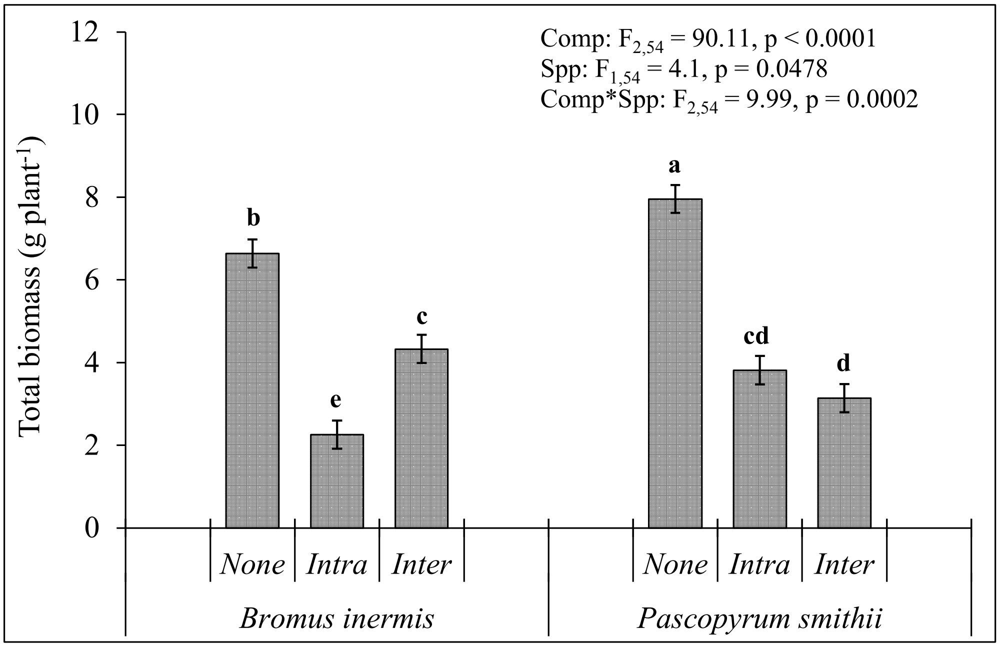


Figure 3.13 Effect of competition treatment on total biomass (g) per plant of *B. inermis* and *P. smithii*. Values are mean ± SE based on the statistical model. Different letters above bars indicate significant difference across competition and species treatment combination at p-value < 0.05. Here total biomass is the sum of aboveground and rhizome biomass and doesn't include roots.

3.7 Relative Interaction Index (RII)

(a) Overall plant establishment

The RII values with respect to the number of total tillers per plant was significantly affected by species, competition and species x competition (Appendix-Table 3.23). RII values in all the treatment combination were significantly different from zero and negative indicating a competitive (or negative) interaction between individuals. The magnitude of negative effect on tiller production of *B. inermis* was significantly larger in interspecific than the intraspecific competition (0.35 > 0.08). Whereas, the magnitude of negative effect on tiller production of *P. smithii* was not significantly different in interspecific and intraspecific competition. Also, the magnitude of negative effect on tiller production of *P. smithii* was significantly larger than on *B. inermis* in interspecific competition (0.46 > 0.35) (Figure 3.14).

Similarly, the RII values with respect to number of total rhizomes per plant was not significantly affected by species, competition and species x competition (Appendix-Table 3.23). RII values in all the treatment combination were significantly different from zero and negative indicating a competitive (or negative) interaction between individuals. The magnitude of negative effect on the number of rhizomes of *P. smithii* was larger than on *B. inermis* in interspecific competition (0.25 > 0.09) (Figure 3.14).

RII values with respect to length(cm) of rhizome per plant was not significantly affected by species, competition and species x competition (Appendix-Table 3.23). RII values under intraspecific, and interspecific competition of *P. smithii* were significantly different from zero and negative indicating a competitive (or negative) interaction between individuals. The magnitude of negative effect on length of rhizome of *P. smithii* was larger than on *B. inermis* in interspecific competition (0.23 > 0.06) (Figure 3.14).

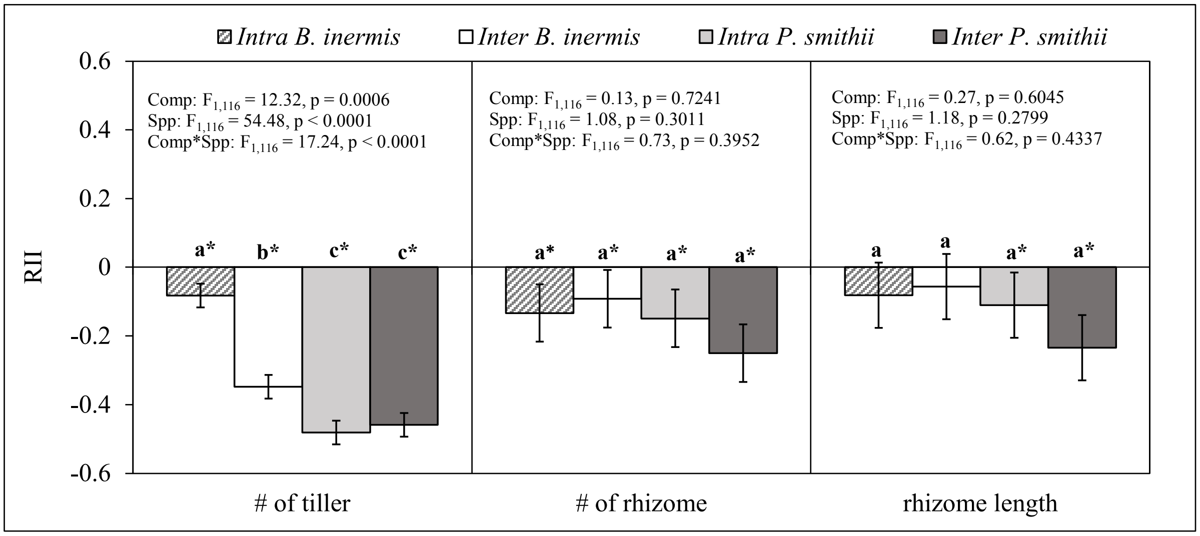


Figure 3.14 Effect of competition treatment on RII values with respect to number of tiller per plant, number of rhizomes per plant, and rhizome length (cm) per plant of *B. inermis* and *P. smithii*. Values are mean ± SE based on the statistical model. Different letters above bars indicate significant difference between competition and species treatment

combination within a response variable at p-value < 0.05. RII values that are significantly different from zero are indicated with an asterix (*).

(b) Live Propagules

The RII values with respect to the number of live propagules per plant was significantly affected by species, competition and species x competition (Appendix-Table 3.23). RII values in all the treatment combination were significantly different from zero. There was a significant facilitative effect (RII = +0.1109) on live propagule production of *B. inermis* in interspecific, whereas, significant competitive or negative effect on live propagule production of *B. inermis* in intraspecific competition (RII = -3.1551). Conversely, both the Intra-and Interspecific interaction was competitive (or negative) in case of *P. smithii* but the magnitude of negative effect on live propagule production of *P. smithii* was significantly higher in interspecific than the intraspecific competition (0.57 > 0.37). Also, there was significant difference on live propagule production of *P. smithii* than *B. inermis* with interspecific competition. Where, the interspecific competition had a negative effect (RII = -0.57) on live propagule production of *P. smithii* whereas, facilitative effect (RII = +0.11) on live propagule production of *B. inermis* (Figure 3.15).

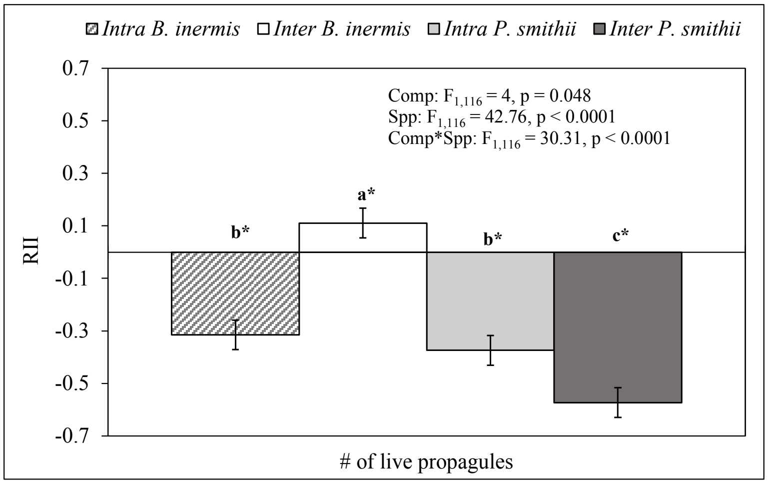


Figure 3.15 Effect of competition treatment on RII values with respect to # of live propagules per plant of *B. inermis* and *P. smithii*. Values are mean ± SE based on the

statistical model. Different letters above bars indicate significant difference between competition and species treatment combination at P-value < 0.05. RII values that are significantly different from zero are indicated with an asterix (*).

(c) Biomass

The RII values with respect to aboveground biomass (g) per plant was significantly affected by species, competition and species x competition (Appendix-Table 3.25). RII values in all the treatment combination were significantly different from zero and negative indicating a competitive (or negative) interaction between individuals. The magnitude of negative effect on aboveground biomass of *B. inermis* was significantly larger in intraspecific than the interspecific competition (0.48 > 0.20). Whereas the magnitude of negative effect on aboveground biomass of *P. smithii* was not significantly different in intraspecific and interspecific competition. Also, the magnitude of negative effect on aboveground biomass of *P. smithii* was significantly larger than of *B. inermis* in interspecific competition (0.46> 0.20) (Figure 3.16).

Similarly, the RII values with respect to rhizome biomass (g) per plant were not significantly affected by species, competition and species x competition (Appendix-Table 3.25). RII values under intraspecific, and interspecific competition of *P. smithii* were significantly different from zero and negative indicating a competitive (or negative) interaction between individuals. The magnitude of negative effect on rhizome biomass of *P. smithii* was larger than on *B. inermis* in intraspecific competition (0.34 > 0.05) and interspecific competition (0.21 > 0.13) (Figure 3.16).

The RII values with respect to total biomass (g) per plant was significantly affected by competition and species x competition (Appendix-Table 3.25). RII values in all the treatment combination were significantly different from zero and negative indicating a competitive (or negative) interaction between individuals. The magnitude of negative effect on total biomass of *B. inermis* was significantly larger in intraspecific than the interspecific competition (0.49 > 0.21). Whereas, the magnitude of negative effect on total biomass of *P. smithii* was not significantly different in intraspecific and interspecific competition. The magnitude of negative effect on total biomass of *P. smithii* was significantly larger than of *B. inermis* in interspecific competition (0.43 > 0.21) (Figure 3.16).

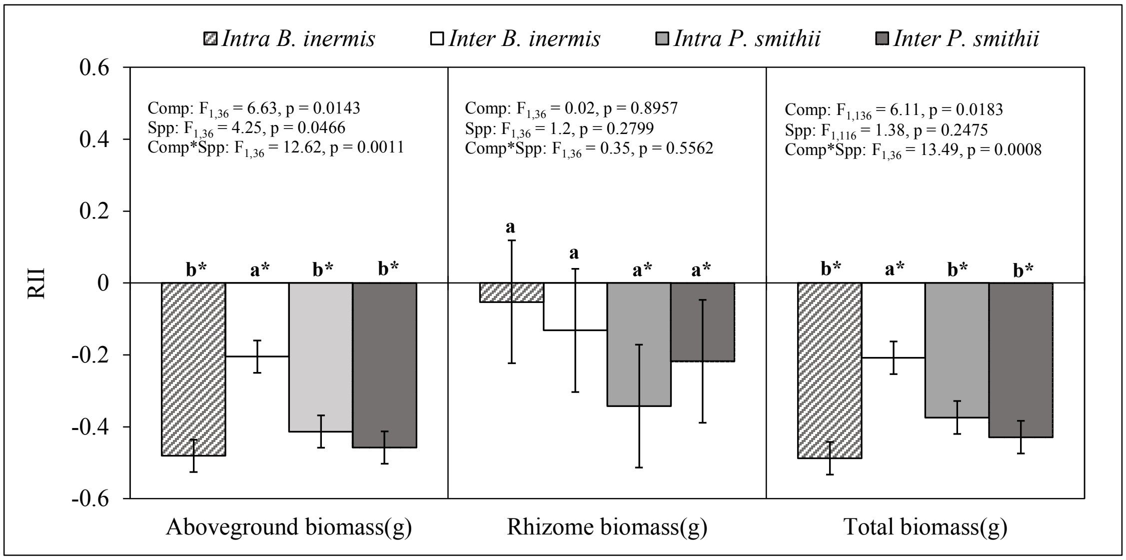


Figure 3.16 Effect of competition treatment on RII values with respect to aboveground biomass (g) per plant, rhizome biomass (g) per plant, and total biomass (g) per plant of *B. inermis* and *P. smithii*. Values are mean ± SE based on the statistical model. Different letters above bars indicate significant difference between competition and species treatment combination within a response variable at P-value < 0.05. RII values that are significantly different from zero are indicated with an asterix (*).

DISCUSSION

As hypothesized, the total live propagule production of native *P. smithii* was lower at interspecific than the intraspecific competition. Whereas, different to the hypothesis, the propagule development, live propagules production per tiller generation, number of new tillers established per tiller, total tiller and rhizome production, rhizome length, aboveground and rhizome biomass of native *P. smithii* was not significantly different between these two competition levels.

The lesser production of live propagules by native *P. smithii* in the presence of non-native *B. inermis* as a neighbor may indicate that non-native *B. inermis* had a significant negative effect on the belowground bud production and live propagule development of the native *P. smithii* (Figure 3.2). This has been supported by RII values, were the non-native *B. inermis* had highest negative or competitive effect on live propagule production (RII = -0.5728) of native *P. smithii* (Figure 3.15) and higher negative RII values in case of total rhizome production, total rhizome length (Figure 3.14), and aboveground biomass (Figure 3.16).

As we know that belowground buds, and vegetative live propagules are the source of tiller recruitment, establishment, and resilience of species against any environmental change or disturbance (Benson et al. 2004, Klimesˇová and Klimeš 2007, Dalgleish and Hartnett 2009, Ott and Hartnett 2015, Ott et al. 2017), the negative effect on these attributes in the presence of non-native *B. inermis* may be a mechanism through which non-native perennial grasses do have long term, legacy, or displacement effect on native perennial grasses (Wilson and Pärtel 2003, Schmidt et al. 2008, Ott et al. 2017).

No effect of on the current tiller and rhizome number, rhizome length and overall biomass of the native *P. smithii* may because of our study limitation, as plant were grown from seeds and were competing at their vegetative and elongation growth phase for only 12 weeks treatment period. We assume that there may be difference in life history of these two grass, where non-native *B. inermis* may have lag phase to activate buds, initiate tillers/rhizomes and establish but compete more effectively after it get established (Theoharides and Dukes 2007) as seen in the field where well established non-native *B. inermis* are very competitive and resilient with networks of tillers and rhizomes (Harrison and Romo 1994, Otfinowski et al. 2007, Biederman et al. 2014). This no effect on number of rhizomes (Figure 3.7) and length of rhizome (Figure 3.8) of the native *P. smithii* irrespective of competition level has increased the number of rhizome generation which has directly played their role to increase the number of live propagules per tiller generation of *P. smithii* in compared to *B. inermis* as seen in Figure 3.4).

This greater competitive ability of the non-native *B. inermis* over native *P. smithii* was also shown by the acceptance of second hypothesis, where the bud production and propagule development, live propagules production per tiller generation, total live propagules production, number of new tillers established per tiller, total tiller production, and aboveground biomass except for total rhizomes, total rhizome length, and rhizome biomass of non-native *B. inermis* was greater at interspecific than intraspecific competition. That is, the presence of native *P. smithii* as a neighbor to non-native *B. inermis* promoted the non-native plant performance implying the greater competitive ability of non-native *B. inermis*. This was also supported by RII values, where the presence of native *P. smithii* as neighbor promoted or facilitated the vegetative live propagules production (Figure 3.15) in non-native *B. inermis*. Also, the total tillers, total rhizomes, total rhizome length (Figure 3. 14), and total biomass (including above ground and rhizome biomass) (Figure 3.16) of non-native *B. inermis* was less negatively affected compared to the neighboring effect of *B. inermis* on native *P. smithii*.

These results have been supported by several studies, for instance, a 4-year competition field experiment on California grasslands between native and non-native perennial grasses (Corbin and D'Antonio 2010) showed that native perennial grass biomass was significantly lower in plots with exotic perennial grasses as compared to plots without exotic perennial grasses. Similarly, based on another target neighbor study to assess both interspecific and intraspecific competition between two introduced Old World Bluestem (OWB) species (*Bothriochloa caucasica*, and *Bothriochloa ischaemum*) with three native grass species (*Andropogon gerardii*, *Schizachyrium scoparium*, and *Bouteloua curtipendula*), Schmidt et al. 2008 found that, *B. bladhii* reduced vegetative tiller height of *S. scoparium* and *A. gerardii* by 47% and 53% respectively and belowground biomass of *B. curtipendula*. Similarly, *B. ischaemum* as a neighbor, significantly reduced height, the above-and belowground biomass of all three-native species. All these results infer the greater competitive ability of non-native perennial grasses like *B. inermis* and may be a mechanism through which they invade an ecosystem (Perkins et al. 2011).

But opposite to the facilitative/less negative effect of native *P. smithii* as a neighbor to non-native *B. inermis*, the conspecific (intraspecific competition) effect of *B. inermis* was highly significant than other types of interactions in our study. The possible mechanism of this significant conspecific effect in *B. inermis* could be auto-allelopathy (Greer et al. 2014) as shown by some perennial grasses like *B. bladhii*, where the intraspecific competition was significant (Schmidt et al. 2008). The self-shading effect may be greater with *B. inermis* because of larger leaf surface area as shown by some of perennial grass including Big bluestem (*Andropogon gerardii*) (Jurik and Kliebenstein 2000). Additionally, the intensity of competition between species depends on the degree to which their ecological niches overlap (Hutchinson 1957), with greater intensity of competition is expected between/among closely related species (Hardin 1960, Violle et al. 2011).

As hypothesized, the investment in phalanx and guerilla growth differed between native *P. smithii* and non-native *B. inermis* under both intraspecific and interspecific competition. Prioritization in these two-clonal growth forms was assessed between species in terms of investment of live propagules from crown versus rhizome and investment of tiller recruitment from crown, nodes of rhizome and tip of rhizome. Based on live propagules investment, the non-native *B. inermis* shifted from primarily prioritizing phalanx growth to a combination of the phalanx and guerilla growth whereas, native *P. smithii* shifted from combination of phalanx and guerilla growth form to strict phalanx when there was a change in competition form (intraspecific to interspecific competition). Similar pattern was found in investment of tiller recruitment of two species.

The shift in the growth form (as seen with number of live propagules investment) may help us to understand the mechanism behind the higher competitive ability of non-native *B. inermis* as discussed earlier. Non-native *B. inermis* allocated equal resource at both aboveground tissue (tillers) and belowground tissue (rhizomes) to outcompete its native neighbor *P. smithii* and forced its native neighbor *P. smithii* to remain confined to small areas (strict phalanx growth) by limiting the available resources. As prairies of the North American Great Plains are dominated by clonal perennial grasses that vary in architecture along the "phalanx - guerilla" clonal growth form continuum (Doust 1981, Harper 1985). This shift in growth strategy has ecological and evolutionary significance to clonal plant populations. The investment in the dual phalanx and guerilla clonal growth form by non-native *B. inermis* may have enabled them to employ both conservative (phalanx) and foraging (guerilla) growth strategies which may facilitate its competitive and persistence traits under resource availability associated with environmental change (Ott and Hartnett 2015). Similarly, in a study of the clonal perennial grass *Panicum virgatum*, neighborhood competition greatly influenced clonal architecture and expansion rates, where removal of neighbors resulted in a >95% increase in radial clone expansion, intraconal tiller densities, and tiller population growth rates (Hartnett 1993). The strict phalanx growth form by native *P. smithii* under the influence of non-native *B. inermis* as neighbor may be the mechanism to tolerate more stressful conditions, make better use of locally abundant resources (monopolization strategy) and out-compete other species in a favorable microsite (Doust 1981, Schmid and Harper 1985, Humphrey and Pyke 1998).

CONCLUSION

We conclude that the presence of non-native *B. inermis* as neighbor significantly decreased number of live propagules, tillers, and aboveground biomass of the native *P. smithii.* Whereas, the presence of native *P. smithii* as neighbor significantly increased number of live propagules and had significantly less negative effect on tiller production and aboveground biomass of non-native B. *inermis*. The results demonstrated strong competitive ability of non-native *B. inermis* against *P. smithii* during the establishment phase when environmental conditions are favorable, such as, higher soil moisture availability, and absence of grazing disturbances.

The shift from primarily prioritizing phalanx growth under intraspecific competition to a combination of the phalanx and guerilla growth under interspecific competition by non-native *B. inermis* may indicate the phenotypic plasticity of non-native species like *B. inermis* which may have contributed to higher competitive ability and invasiveness in grassland dominated by native perennial grasses like *P. smithii*. This study has evaluated the competitive ability between two dominant perennial grasses native *P. smithii* and non-native *B. inermis* in terms of species reproductive and demographic traits that has shown potential the explanation of the invasiveness of non-native species and competitive ability of native species.

We expect this study will enhance our understanding of the potential utilizing reproduction and demography traits as important attributes of a plant in response to disturbance and will provide significant insights for developing strategies for sustainably manage non-native invaded perennial grasslands in remnant prairies of northern Great Plains.

LITERATURE CITED

1. Armas, C., R. Ordiales and F. I. Pugnaire (2004). "Measuring plant interactions: a new comparative index." Ecology **85**(10): 2682-2686.
2. Asay, K. and K. Jensen (1996). "Wheatgrasses." Cool-season forage grasses(coolseasonforag): 691-724.
3. Bai, Y., J. Wu, C. M. Clark, Q. Pan, L. Zhang, S. Chen, Q. Wang and X. Han (2012). "Grazing alters ecosystem functioning and C: N: P stoichiometry of grasslands along a regional precipitation gradient." Journal of Applied Ecology **49**(6): 1204-1215.
4. Benson, E. J. and D. C. Hartnett (2006). "The Role of Seed and Vegetative Reproduction in Plant Recruitment and Demography in Tallgrass Prairie." Plant Ecology **187**(2): 163-177.
5. Benson, E. J., D. C. Hartnett and K. H. Mann (2004). "Belowground bud banks and meristem limitation in tallgrass prairieplant populations." American Journal of Botany **91**(3): 416-421.
6. Biederman, L. A., J. Beckman, J. Prekker, D. Anderson, N. P. Sather and R. Dahle (2014). "Phenological monitoring aids habitat management of threatened plant." Natural Areas Journal **34**(1): 105-110.
7. Caldwell, M., J. Richards, D. Johnson, R. Nowak and R. Dzurec (1981). "Coping with herbivory: photosynthetic capacity and resource allocation in two semiarid Agropyron bunchgrasses." Oecologia **50**(1): 14-24.
8. Coffin, D. and W. Lauenroth (1990). "A gap dynamics simulation model of succession in a semiarid grassland." Ecological Modelling **49**(3): 229-266.
9. Collins, S. L. and L. B. Calabrese (2012). "Effects of fire, grazing and topographic variation on vegetation structure in tallgrass prairie." Journal of Vegetation Science **23**(3): 563-575.
10. Corbin, J. D. and C. M. D’Antonio (2010). "Not novel, just better: competition between native and non-native plants in California grasslands that share species traits." Plant Ecology **209**(1): 71-81.

11. Cully, A. C., J. F. Cully and R. D. Hiebert (2003). "Invasion of exotic plant species in tallgrass prairie fragments." Conservation Biology **17**(4): 990-998.
12. Dalgleish, H. J. and D. C. Hartnett (2006). "Below□ground bud banks increase along a precipitation gradient of the North American Great Plains: a test of the meristem limitation hypothesis." New Phytologist **171**(1): 81-89.
13. Dalgleish, H. J. and D. C. Hartnett (2009). "The effects of fire frequency and grazing on tallgrass prairie productivity and plant composition are mediated through bud bank demography." Plant Ecology **201**(2): 411-420.
14. DeKeyser, E. S., M. Meehan, G. Clambey and K. Krabbenhoft (2013). "Cool season invasive grasses in northern Great Plains natural areas." Natural Areas Journal **33**(1): 81-90.
15. Doust, L. L. (1981). "Population Dynamics and Local Specialization in a Clonal Perennial (Ranunculus Repens): I. The Dynamics of Ramets in Contrasting Habitats." Journal of Ecology **69**(3): 743-755.
16. Dreber, N. and K. Esler (2011). "Spatio-temporal variation in soil seed banks under contrasting grazing regimes following low and high seasonal rainfall in arid Namibia." Journal of Arid Environments **75**(2): 174-184.
17. Dukes, J. S. and H. A. Mooney (1999). "Does global change increase the success of biological invaders?" Trends in Ecology & Evolution **14**(4): 135-139.
18. Funk, J. L. and P. M. Vitousek (2007). "Resource-use efficiency and plant invasion in low-resource systems." Nature **446**(7139): 1079-1081.
19. Garnier, E. and J. Roy (1988). "Modular and demographic analysis of plant leaf area in sward and woodland populations of Dactylis glomerata and Bromus erectus." The Journal of Ecology: 729-743.
20. Grant, T. A., B. Flanders-Wanner, T. L. Shaffer, R. K. Murphy and G. A. Knutsen (2009). "An emerging crisis across northern prairie refuges: prevalence of invasive plants and a plan for adaptive management." Ecological Restoration **27**(1): 58-65.
21. Greer, M. J., G. W. Wilson, K. R. Hickman and S. M. Wilson (2014). "Experimental evidence that invasive grasses use allelopathic biochemicals as a

potential mechanism for invasion: chemical warfare in nature." Plant and soil **385**(1-2): 165-179.

22. Hardin, G. (1960). "The competitive exclusion principle." science **131**(3409): 1292-1297.
23. Harper, J. L. (1985). "Modules, branches, and the capture of resources."
24. Harrison, T. and J. T. Romo (1994). "Regrowth of smooth bromegrass (Bromus inermis Leyss.) following defoliation." Canadian Journal of Plant Science **74**(3): 531-537.
25. Hartnett, D. C. (1993). "Regulation of clonal growth and dynamics of Panicum virgatum (Poaceae) in tallgrass prairie: effects of neighbor removal and nutrient addition." American Journal of Botany: 1114-1120.
26. Hobbs, R. J. and L. F. Huenneke (1992). "Disturbance, diversity, and invasion: implications for conservation." Conservation biology **6**(3): 324-337.
27. Hoshino, A., Y. Yoshihara, T. Sasaki, T. Okayasu, U. Jamsran, T. Okuro and K. Takeuchi (2009). "Comparison of vegetation changes along grazing gradients with different numbers of livestock." Journal of Arid Environments **73**(6): 687-690.
28. https://climate.sdstate.edu/ (acessed on 05/02/2017)
29. Humphrey, L. D. and D. A. Pyke (1998). "Demographic and growth responses of a guerilla and a phalanx perennial grass in competitive mixtures." Journal of Ecology **86**(5): 854-865.
30. Hutchinson, G. E. (1957). "Cold spring harbor symposium on quantitative biology." Concluding remarks **22**: 415-427.
31. Jones, S. K., S. L. Collins, J. M. Blair, M. D. Smith and A. K. Knapp (2016). "Altered rainfall patterns increase forb abundance and richness in native tallgrass prairie." Scientific reports **6**.
32. Judziewicz, E. J., L. G. Clark, X. Londono and M. J. Stern (1999). American bamboos, Smithsonian Institution Press.

33. Jurik, T. W. and H. Kliebenstein (2000). "Canopy Architecture, Light Extinction and Self-Shading of a Prairie Grass, Andropogon Gerardii." The American Midland Naturalist **144**(1): 51-65.
34. Klimesˇová, J. and L. Klimeš (2007). "Bud banks and their role in vegetative regeneration – A literature review and proposal for simple classification and assessment." Perspectives in Plant Ecology, Evolution and Systematics **8**(3): 115-129.
35. Klimesova, J. and L. Klimes (2008). "Clonal growth diversity and bud banks of plants in the Czech flora: an evaluation using the CLO-PLA3 database." Preslia **80**(3): 255-275.
36. Kluse, J. S. and B. H. A. Diaz (2005). "Importance of soil moisture and its interaction with competition and clipping for two montane meadow grasses." Plant Ecology **176**(1): 87-99.
37. Koerner, S. E., S. L. Collins, J. M. Blair, A. K. Knapp and M. D. Smith (2014). "Rainfall variability has minimal effects on grassland recovery from repeated grazing." Journal of Vegetation Science **25**(1): 36-44.
38. Lamas, M. B., C. Larreguy, A. Carrera and M. Bertiller (2013). "Changes in plant cover and functional traits induced by grazing in the arid Patagonian Monte." Acta oecologica **51**: 66-73.
39. Li, W., J. Luo, X. Tian, W. S. Chow, Z. Sun, T. Zhang, S. Peng and C. Peng (2015). "A new strategy for controlling invasive weeds: selecting valuable native plants to defeat them." Scientific reports **5**.
40. Murray, S., R. White and M. Rohweder (2000). "Pilot Analysis of Global Ecosystems: Grasslands Ecosystems."
41. Otfinowski, R., N. Kenkel and P. Catling (2007). "The biology of Canadian weeds. 134. Bromus inermis Leyss." Canadian Journal of Plant Science **87**(1): 183-198.
42. Ott, J. P. and D. C. Hartnett (2011). "Bud Production and Dynamics of Flowering and Vegetative Tillers in Andropogon Gerardii (Poaceae): The Role of Developmental Constraints." American Journal of Botany **98**(8): 1293-1298.

43. Ott, J. P. and D. C. Hartnett (2012). "Higher-order bud production increases tillering capacity in the perennial caespitose grass Scribner's Panicum (Dichanthelium oligosanthes)." Botany **90**(9): 884-890.
44. Ott, J. P. and D. C. Hartnett (2015). "Bud bank dynamics and clonal growth strategy in the rhizomatous grass, Pascopyrum smithii." Plant Ecology **216**(3): 395-405.
45. Ott, J. P., J. L. Butler, Y. Rong and L. Xu (2017). "Greater bud outgrowth of Bromus inermis than Pascopyrum smithii under multiple environmental conditions." Journal of Plant Ecology **10**(3): 518-527.
46. Perkins, L. B., E. A. Leger and R. S. Nowak (2011). "Invasion triangle: an organizational framework for species invasion." Ecology and evolution **1**(4): 610-625.
47. Pfeiffer, K. E. and D. C. Hartnett (1995). "Bison selectivity and grazing response of little bluestem in tallgrass prairie." Journal of Range Management: 26-31.
48. Pimentel, D., R. Zuniga and D. Morrison (2005). "Update on the environmental and economic costs associated with alien-invasive species in the United States." Ecological Economics **52**(3): 273-288.
49. Reichard, S. H. and P. White (2001). "Horticulture as a Pathway of Invasive Plant Introductions in the United States Most invasive plants have been introduced for horticultural use by nurseries, botanical gardens, and individuals." BioScience **51**(2): 103-113.
50. Reichmann, L. G., O. E. Sala and D. P. Peters (2013). "Precipitation legacies in desert grassland primary production occur through previous-year tiller density." Ecology **94**(2): 435-443.
51. Rogers, W. E. and D. C. Hartnett (2001). "Temporal vegetation dynamics and recolonization mechanisms on different-sized soil disturbances in tallgrass prairie." American Journal of Botany **88**(9): 1634-1642.
52. Russell, M. L., L. T. Vermeire, A. C. Ganguli and J. R. Hendrickson (2015). "Season of fire manipulates bud bank dynamics in northern mixed-grass prairie." Plant Ecology **216**(6): 835-846.

53. SAS Institute Inc. (2017) Studio 3.6 University Edition. SAS Institute Inc, Gary, NC.
54. Schmid, B. and J. L. Harper (1985). "Clonal growth in grassland perennials: I. Density and pattern-dependent competition between plants with different growth forms." The Journal of Ecology: 793-808.
55. Schmidt, C. D., K. R. Hickman, R. Channell, K. Harmoney and W. Stark (2008). "Competitive abilities of native grasses and non-native (Bothriochloa spp.) grasses." Plant Ecology **197**(1): 69-80.
56. Schrag, A. M. (2011). Addendum: climate chnage impacts and adaptation strategies. In: Ocean of grass: a conservation assessment for the Northern Great Plains Bozeman, MT, World Wildlife Fund-US**:** 36.
57. Theoharides, K. A. and J. S. Dukes (2007). "Plant invasion across space and time: factors affecting nonindigenous species success during four stages of invasion." New Phytologist **176**(2): 256-273.
58. Ulrich, E. and L. Perkins (2014). "Bromus inermis and Elymus canadensis but not Poa pratensis demonstrate strong competitive effects and all benefit from priority." Plant ecology **215**(11): 1269-1275.
59. Violle, C., D. R. Nemergut, Z. Pu and L. Jiang (2011). "Phylogenetic limiting similarity and competitive exclusion." Ecology letters **14**(8): 782-787.
60. Wilcove, D. S., D. Rothstein, J. Dubow, A. Phillips and E. Losos (1998). "Quantifying threats to imperiled species in the United States." BioScience **48**(8): 607-615.
61. Vitousek, P. M., C. M. Antonio, L. L. Loope and R. Westbrooks (1996). "Biological invasions as global environmental change." American Scientist **84**(5): 468.
62. Wilson, S. D. and M. Pärtel (2003). "Extirpation or Coexistence? Management of a Persistent Introduced Grass in a Prairie Restoration." Restoration Ecology **11**(4): 410-416.

CHAPTER 4

CONCLUSION

This research project evaluates the potential role of belowground bud bank in providing resistance and resilience to change in precipitation frequency, grazing, and competition with non-native species in perennial grasslands of the northern Great Plains. We compared the vegetative reproduction and demographic trait response of two dominant cool season perennial grasses of northern mixed-grass prairies including native *Pascopyrum smithii* (western wheatgrass) and non-native *Bromus inermis* (Smooth brome) under various soil moisture, clipping, and competition conditions with two different controlled greenhouse experiments over the growing seasons of 2016 and 2017. The reproductive and demographic traits of interest were belowground bud production, propagule development, tiller and rhizome recruitments according generation cohort, investment in clonal growth form, overall plant establishment, and biomass. The grasses were grown from seeds and treatments were only applied at establishment phase for both experiments.

In our first experiment, the treatments consisted of the combinations of three precipitation frequencies (every 2d, 8d, and 16d) representing high, medium, and low, two levels of clipping (clipping *vs.* no-clipping), and two species with 40 replicates for each treatment. One single-leaf seedling of each species was transplanted into individual potting-soil filled pots in mid-June. During the first week of July 2016, we initiated precipitation frequency treatments and applied a clipping treatment (at subtle HT 4-cm; 3 collar-leaf stage). Plants were harvested 20 weeks after the treatments had been initiated, and underground structures were washed free of soil to record number of tillers and rhizomes based on generation, number of tillers based on location (crown *vs.* rhizome), and measure rhizome length. Three randomly sub-sampled tillers and rhizomes from each generation were dissected to record the number of buds, and propagule development. We found *B. inermis* significantly decreased their number of tillers, rhizomes, rhizome length, and live propagules at the lowest precipitation frequency, however, increased juvenile tiller production at medium precipitation frequency. Whereas, *P. smithii* significantly increased the traits described above under medium precipitation frequency except for the number of tillers and bud outgrowth which were not affected at medium and low precipitation frequency. The clipping treatment significantly reduced tiller production for both species and the number of rhizomes for *B. inermis*. The results indicate that non-native *B. inermis* may be more susceptible to the altered precipitation frequency and clipping compared to native *P. smithii*. Native *P. smithii* may be able to resist these soil moisture variability and clipping effects mediated *via* the belowground bud banks.

The second competition experiment consisted of five treatments including single *B. smithii*, single *P. smithii*, pairwise monoculture of *B. inermis*, pairwise monoculture of *P. smithii*, and pairwise mixed-culture of *B. inermis* and *P. smithii* with 30 replicates for each treatment under every 2d precipitation frequency regime. Double-leaf seedlings of each species were transplanted into individual potting-soil filled pots based on designated treatments. Plants were harvested 12 weeks after the treatments had been initiated. The data collection followed the same protocol as the first experiment. In addition, biomass and relative interaction index (RII) were calculated to determine effect of intra- and inter-specific competition between *P. smithii* and *B. inermis*. We found that the presence of *B. inermis* as a neighbor significantly decreased the number of live propagules, tillers, and aboveground biomass of the native *P. smithii.* Whereas, the presence of *P. smithii* as a neighbor significantly increased the number of live propagules and had significantly less negative effect on tiller production and aboveground biomass of *B. inermis*. Also, investment in dual phalanx and guerilla growth by *B. inermis* while competing with *P. smithii* indicates possible phenotypic plasticity trait. All the results demonstrated a strong competitive ability of the non-native *B. inermis* against *P. smithii* during its establishment phase when environmental conditions were favorable (i.e. lack water stress and grazing).

Overall, we can conclude that species establishment and interaction between these two key perennial grasses in northern mixed-grass prairies is environmentally dependent and species specific. The outcomes are mediated by the response of the belowground bud bank. The decrease in soil moisture content due altered precipitation frequency and grazing might change the competitive dynamics between native and non-native perennial grasses and might probably help to increase the stability of native mixed-grass prairies. Non-native *B. inermis* might not be able to easily establish during drought years and heavy grazing. Whereas, wet year could help *B. inermis* for establishment. Likewise, control/management of non-native *B. inermis* seems to be effective in early stages of grasses when they are not expanding and are applied during dry year. Lack of precipitation during dry years can decrease soil moisture, and where we might possibly more severe effect on plant growth of *B. inermis* if there are combination other treatments such as mowing/grazing and fire. In contrast, uniform insensitive response of demographic and clonal traits of *P. smithii* under such disturbance conditions can be useful information to develop guidelines for effective land management. The findings from this study help us to a greater understanding of the mechanism of bud bank in maintaining tiller population, regulating vegetation dynamics, productivity, and response to climate change in the context of grazing practices and invasion by native and non-native perennial grasses. They could also form the basis for a long-term effective grassland management plan.

Future works could replicate these greenhouse works on field and try to validity the result obtained for better generalization and implications. We also recommend to study bud and tiller dynamics of other major native and non-native perennial species of northern Great Plains grasslands and inclusion of other biological and ecological aspects of bud banks including bud physiology, bud dormancy, bud and live propagules mortality etc. We can understand the bud bank and tiller demography response to others environmental factors such as fire, temperature, diseases, pollutants and other.

APPENDICES

CHAPTER 1

Table 1.1 Summary of the estimated past area, current area, and percent decline of mixed-grass prairies since 1830 (Adopted from Mac et al. 1998)

Location	Past area (hectares)	Current area (hectares)	Decline (percent)
Alberta	8,700,000	3,400,000	60.9
Manitoba	6,00,000	300	99.9
Saskatchewan	13,400,000	2,500,000	81.3
Nebraska	7,700,000	1,900,000	75.3
North Dakota	14,200,000	4,500,000	68.3
South Dakota	1,600,000	480,000	70.0
Texas	14,100,000	9,800,000	30.5

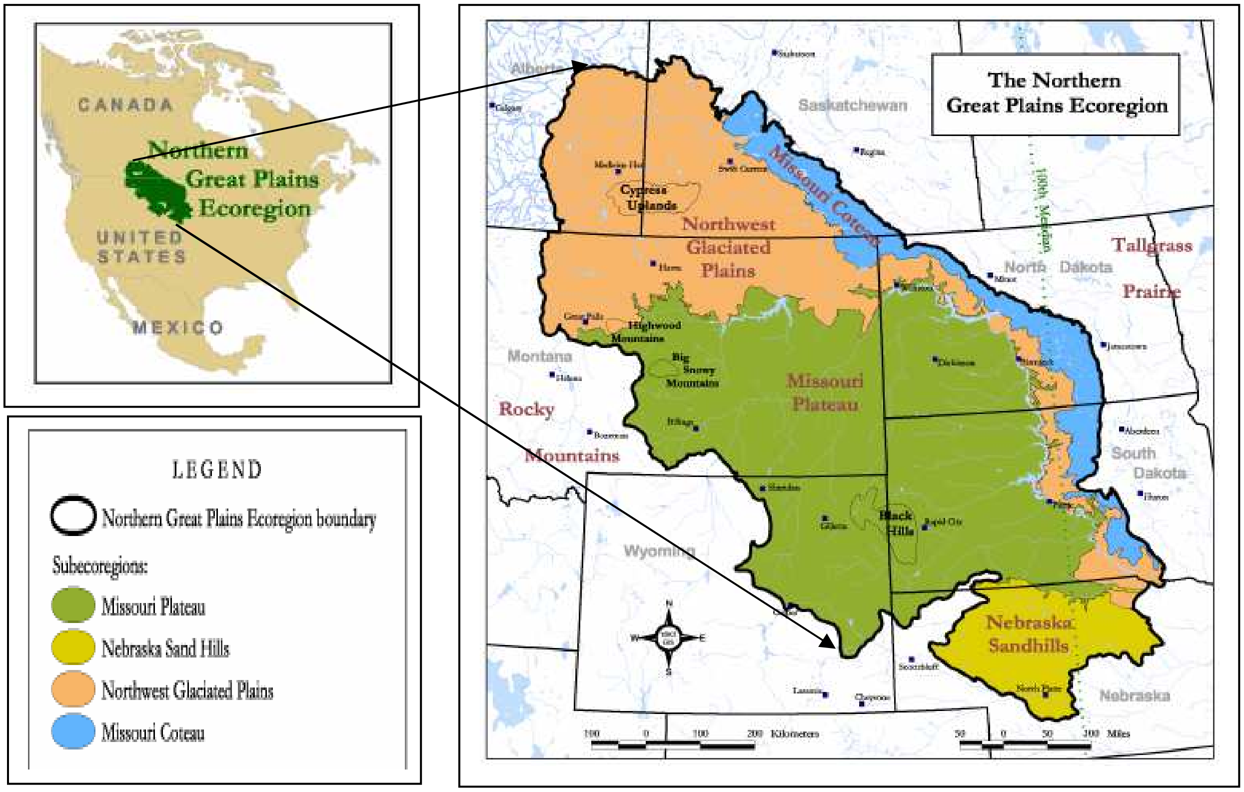


Figure 1.1 Northern Great Plains Ecoregions and Sub-ecoregions (Adopted from Forrest et al. 2004)

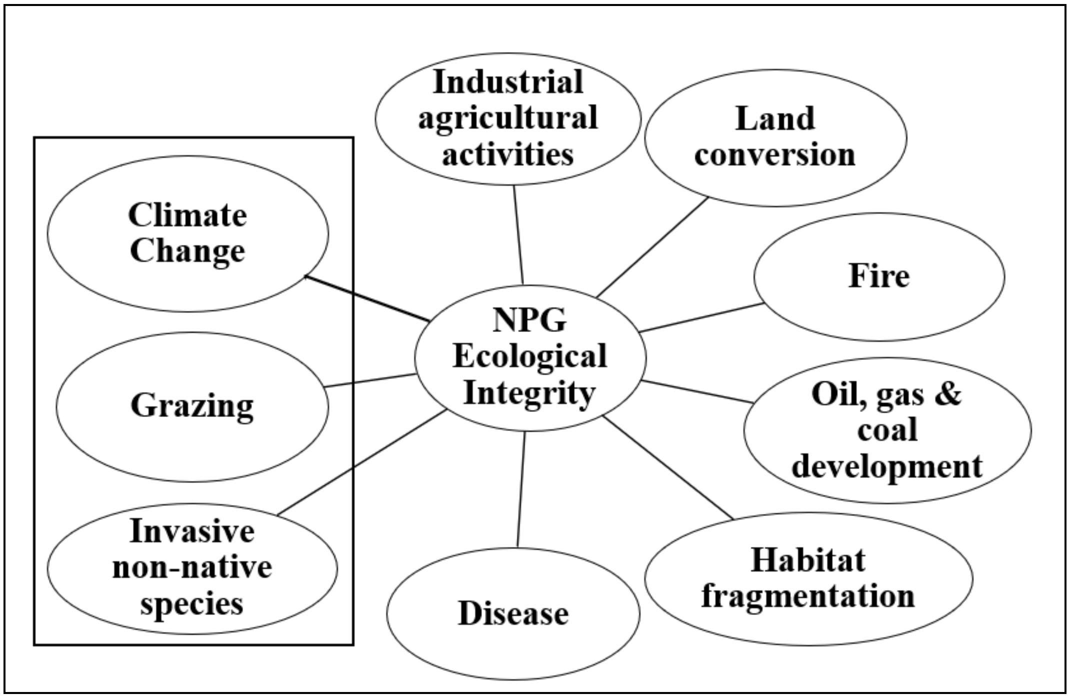


Figure 1.2 Major threats to northern Great Plain Ecological Integrity (Adopted from Schrag 2011)

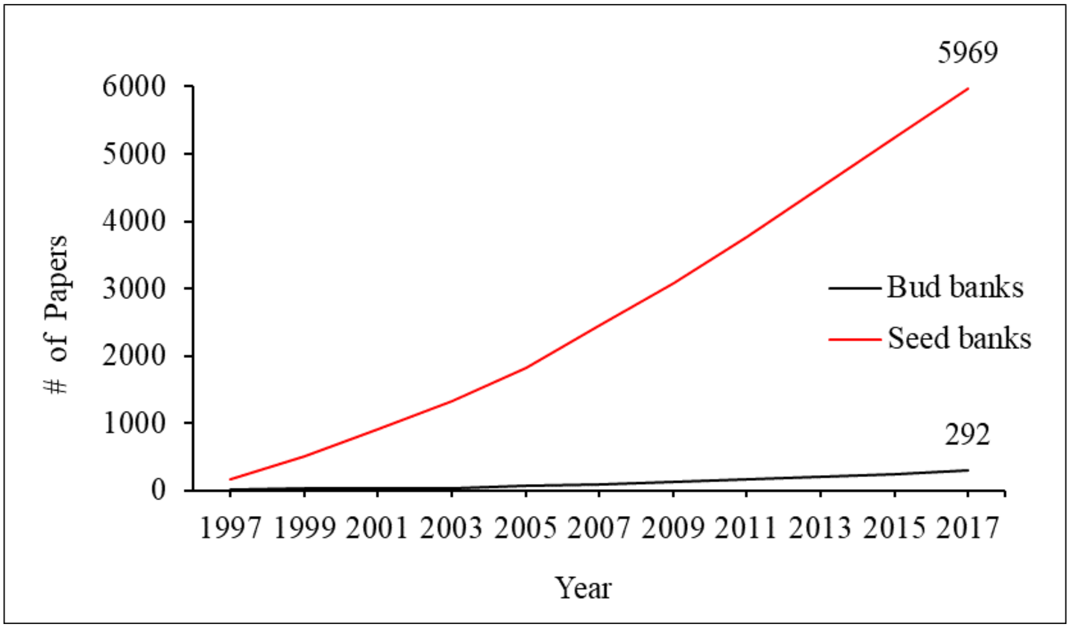


Figure 1.3 Cummulative paper publication related to Bud banks versus Seed banks (Source: Web of Science, February 2018; Keywords: Bud bank and Seed Bank)

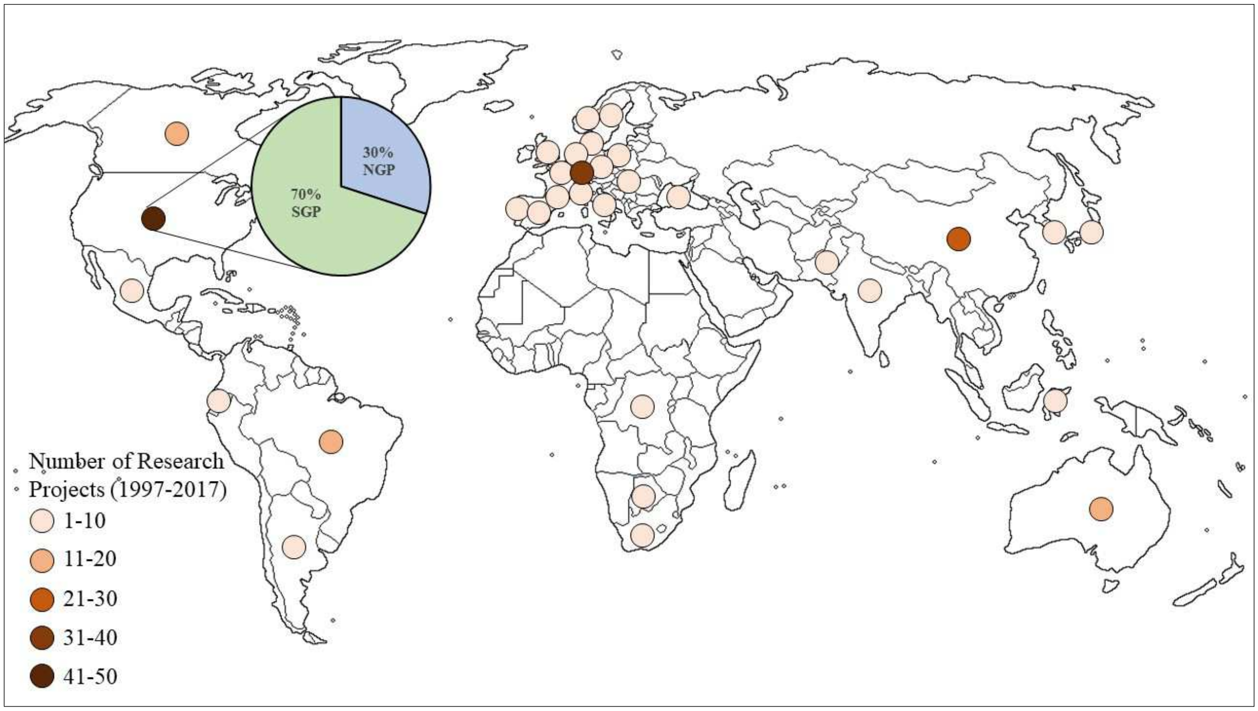


Figure 1.4 Geographical contribution of bud banks studies over the period of 1997-2017 (Source: Web of Science, February 2018)

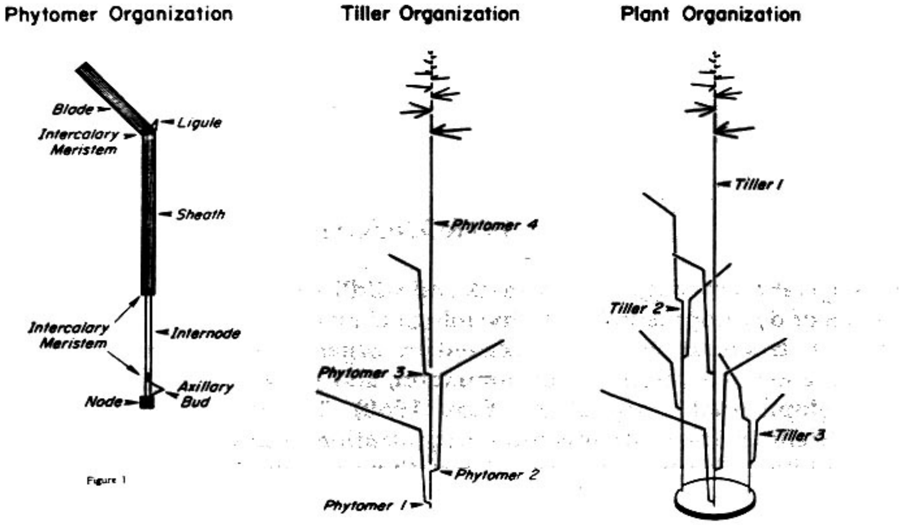


Figure 1.5 Grass phytomer and tiller (i.e. stem) organization. (Adopted from Briske 1991 as adapted from Etter 1951).

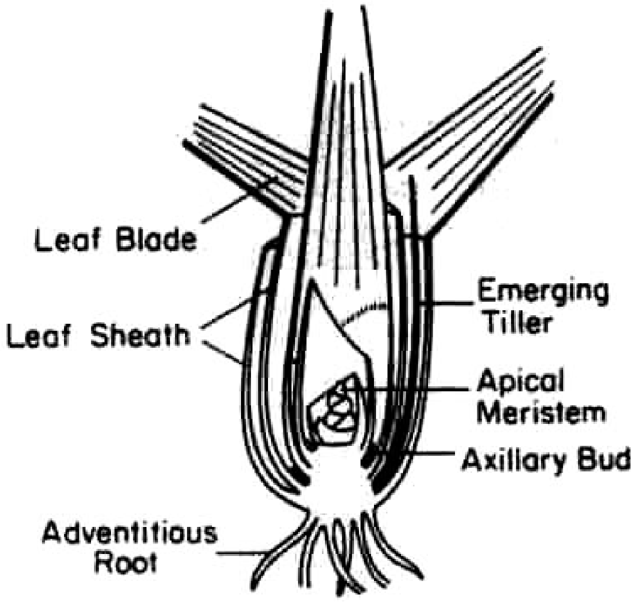


Figure 1.6 Cross-section of a grass tiller base. Axillary buds sit between each leaf and have the potential to transtion into emerging tillers. (Adopted from Briske 1991 as adapted from Jewis 1972).

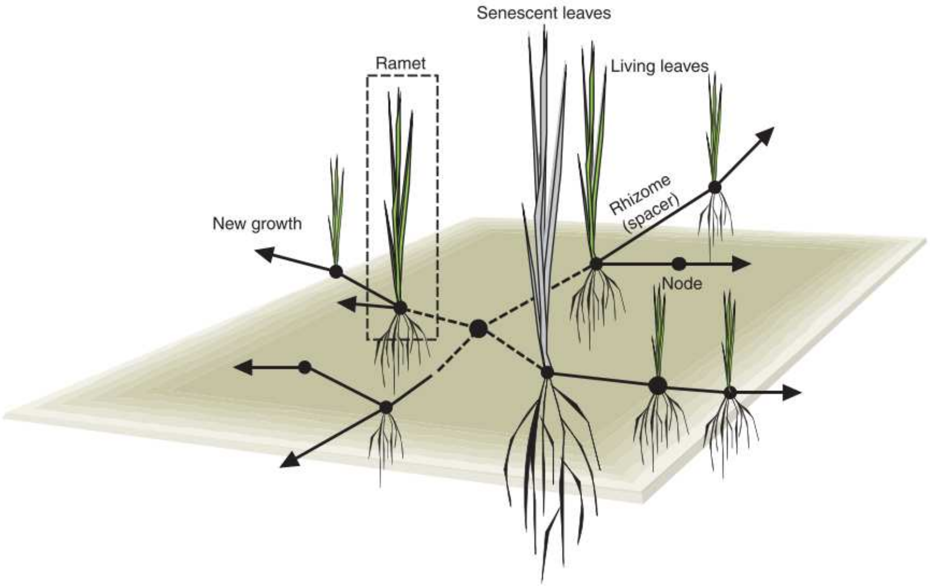


Figure 1.7 Diagram of a grass showing clonal growth with respect to rhizome (Adopted from Cornelissen et al. 2014).

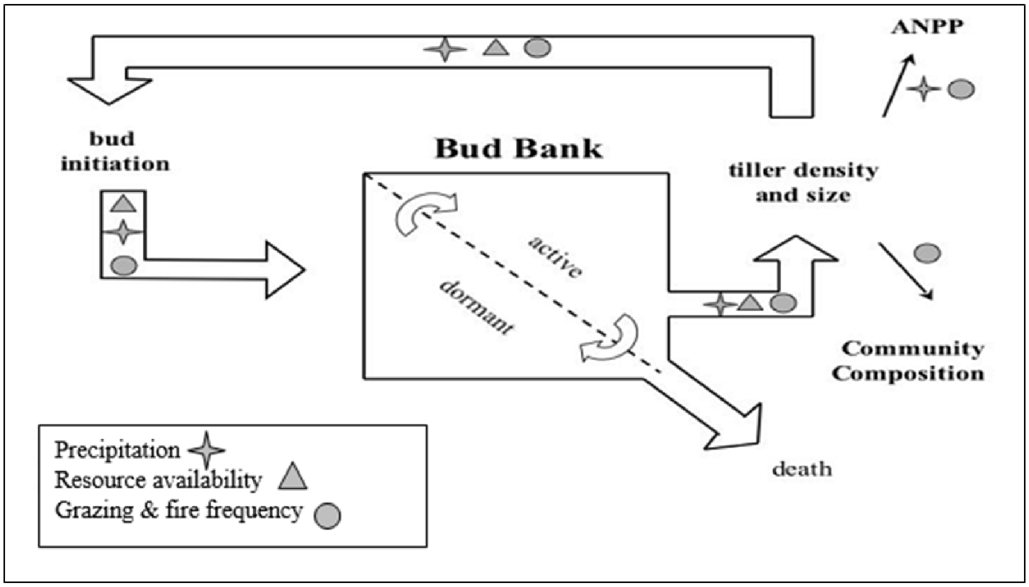


Figure 1.8 Conceptual model of bud bank demography and its potential population, community and ecosystem consequences (Adopted from Dalgleish 2007).

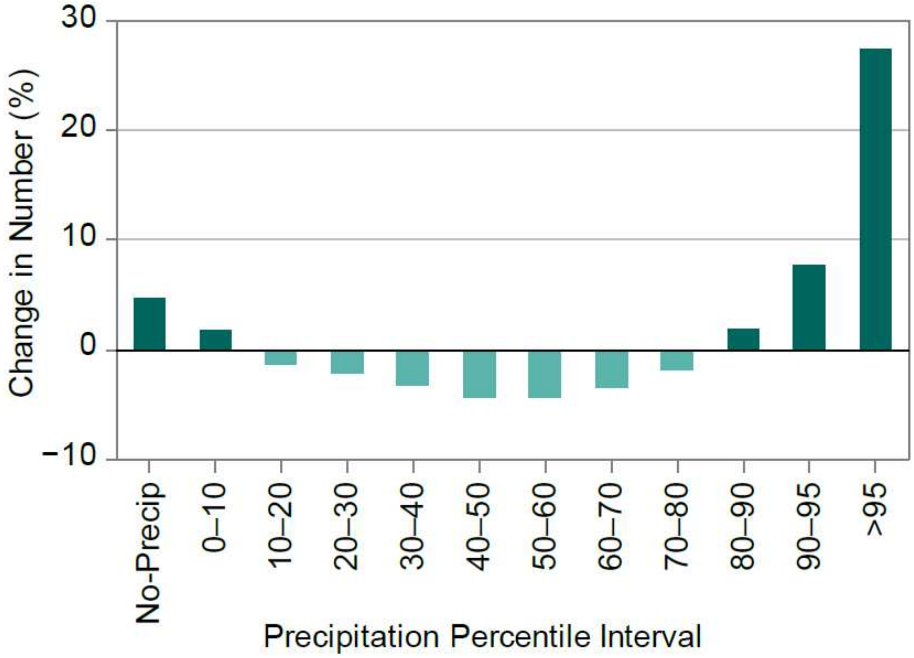


Figure 1.9 Projected change in the number of daily zero ("No-Precip") and non-zero precipitation days (by percentile bins) for late-21st century under a higher emission scenario for contiguous United States (Adopted from Wuebbles 2017).

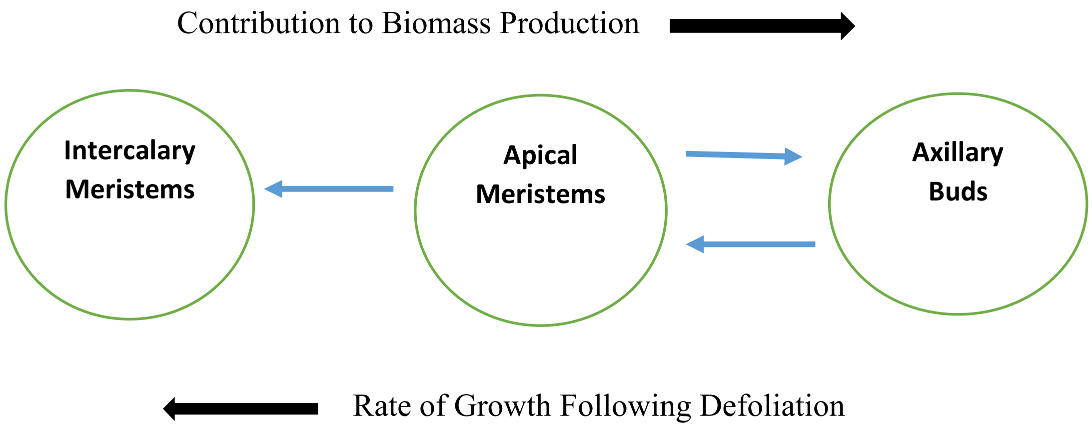


Figure 1.10 Relative contribution of various meristematic sources to the rate and duration of biomass production in grass plants (Adopted from Briske 1991).

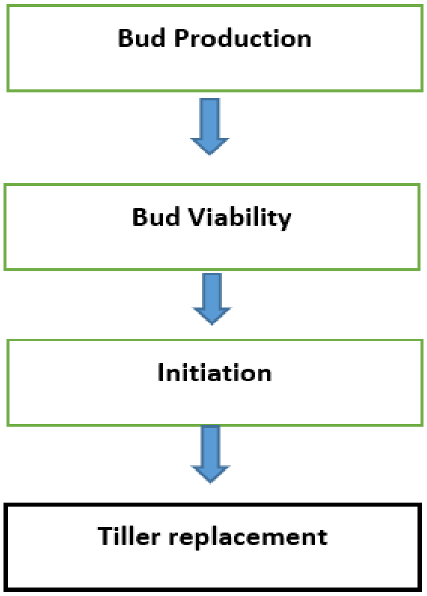


Figure 1.11 Illustration of the major processes contributing to population persistence in perennial grasses (Adopted from Briske & Noy-Meir 1998).

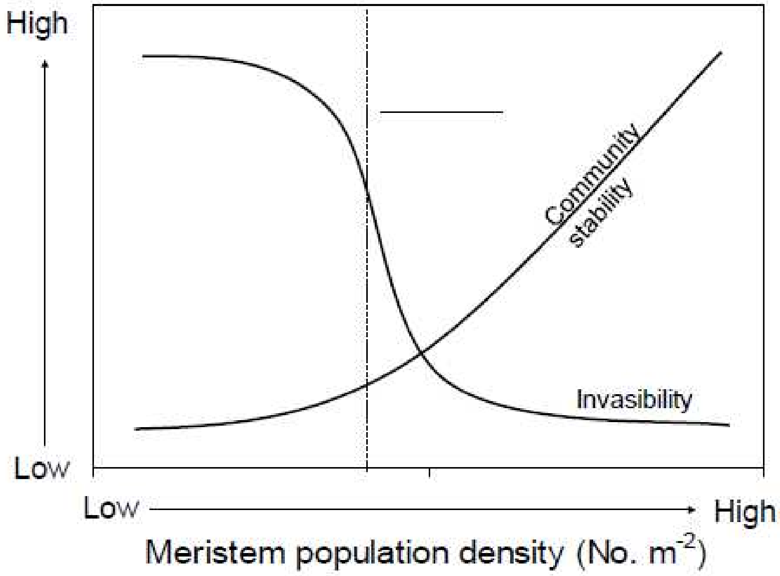


Figure 1.12 Hypothesized relationship between bud bank density, invisibility, and community stability (Adopted from Sprinkle 2010).

CHAPTER 2

Table 2.3 Effects of change in precipitation frequency and clipping on the plant establishment traits of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects)

Effect	Number of total tillers per plant	Number of total rhizomes per plant	Total rhizome length(cm) per plant
Species	**$F_{1,12.9} = 40.72$, $P < 0.0001$**	$F_{1,11} = 4.64$, $P = 0.0542$	**$F_{1,9.026} = 195.59$, $P < 0.0001$**[1]
Precipitation Frequency	**$F_{2,12.84} = 83.46$, $P < 0.0001$**	**$F_{2,11} = 31.23$, $P < 0.0001$**	**$F_{2,8.968} = 48.5$, $P < 0.0001$**
Species*Precipitation Frequency	**$F_{2,12.84} = 79.06$, $P < 0.0001$**	**$F_{2,11} = 19.13$, $P = 0.0003$**	**$F_{2,8.975} = 72.97$, $P < 0.0001$**
Clipping	**$F_{1,12.9} = 14.87$, $P = 0.002$**	**$F_{1,11} = 7.86$, $P = 0.0171$**	$F_{1,8.944} = 0.77$, $P = 0.4031$
Species*Clipping	$F_{1,12.9} = 0.02$, $P = 0.8969$	$F_{1,11} = 0.67$, $P = 0.4289$	$F_{1,8.944} = 1.42$, $P = 0.2634$
Precipitation Frequency *Clipping	$F_{2,12.84} = 1.49$, $P = 0.2611$	$F_{2,11} = 1.31$, $P = 0.3092$	$F_{2,8.939} = 0.59$, $P = 0.5724$
Species*Precipitation Frequency *Clipping	$F_{2,12.84} = 2.26$, $P = 0.1444$	$F_{2,11} = 3.34$, $P = 0.0735$	$F_{2,8.939} = 1.74$, $P = 0.2294$

The values in bold represent significant effects with $P < 0.05$.

Table 2.4 Effects of change in precipitation frequency and clipping on the plant establishment traits of *B. inermis*, and *P. smithii* (Mean ± SE)

Species	Precipitation Frequency	Number of total tillers per plant	Number of total rhizomes per plant	Total rhizome length (cm) per plant
BI	2d	44.29 ± 1.94	8.97 ± 1.16	161.65 ± 14.89
BI	8d	26.31 ± 1.18	10.10 ± 1.29	80.22 ± 7.24
BI	16d	13.07 ± 0.66	3.83 ± 0.52	31.44 ± 2.86
PS	2d	19.12 ± 0.93	6.66 ± 0.88	136.75 ± 12.65
PS	8d	19.85 ± 1.06	10.43 ± 1.40	294.37 ± 29.97
PS	16d	18.85 ± 0.92	7.65 ± 1.00	177.69 ± 16.53

Species	Clipping	Number of total tillers per plant	Number of total rhizomes per plant	Total rhizome length (cm) per plant
BI	C	22.91 ± 0.89	6.24 ± 0.77	69.11 ± 5.42
BI	NC	26.83 ± 0.99	7.91 ± 0.96	79.56 ± 6.21
PS	C	17.90 ± 0.74	7.59 ± 0.94	194.76 ± 15.93
PS	NC	20.74 ± 0.84	8.64 ± 1.07	190.62 ± 15.64

BI = *Bromus inermis*, PS = *Pascopyrum smithii*, 2d = High precipitation frequency, 8d = Medium precipitation frequency, 16d = Low precipitation frequency, C = Clipping, NC = No-Clipping

Table 2.5 Effects of change in precipitation frequency and clipping on the number of new tillers established per tiller in each generation of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects)

Effect	Number of new tillers established per tiller
Species	**$F_{1,35.25} = 4.87, P = 0.0339$**
Precipitation Frequency	**$F_{2,34.63} = 23.39, P < 0.0001$**
Species*Precipitation Frequency	**$F_{2,34.63} = 18.61, P < 0.0001$**
Clipping	**$F_{1,35.25} = 10.75, P = 0.0023$**
Species*Clipping	$F_{1,35.25} = 2.68, P = 0.1103$
Precipitation Frequency*Clipping	$F_{2,34.63} = 2.94, P = 0.0662$
Species*Precipitation Frequency*Clipping	$F_{2,34.63} = 2.32, P = 0.1133$
Generation	**$F_{2,34.34} = 1403.59, P < 0.0001$**
Species*Generation	**$F_{2,34.34} = 52.17, P < 0.0001$**
Precipitation Frequency*Generation	**$F_{4,33.74} = 16.83, P < 0.0001$**
Species*Precipitation Frequency*Generation	**$F_{4,33.74} = 14.58, P < 0.0001$**
Clipping*Generation	$F_{2,34.34} = 1.75, P = 0.1887$
Species*Clipping*Generation	**$F_{2,34.34} = 4.35, P = 0.0206$**
Precipitation Frequency*Clipping*Generation	$F_{4,33.74} = 1.48, P = 0.2312$
Species*Precipitation Frequency*Clipping*Generation	$F_{4,33.74} = 1.01, P = 0.4174$

The values in bold represent significant effects with $P < 0.05$.

Table 2.6 Effects of change in precipitation frequency and clipping on the number of new tillers established per tiller in each generation of *B. inermis*, and *P. smithii* (Mean ± SE)

Species	Precipitation Frequency	Primary tiller	Secondary tiller	Tertiary tiller
BI	2d	15.94 ± 1.17	1.12 ± 0.082	0.6 ± 0.05
BI	8d	8.36 ± 0.65	1.66 ± 0.12	0.31 ± 0.03
BI	16d	4.53 ± 0.39	1.54 ± 0.12	0.23 ± 0.03
PS	2d	5.51 ± 0.46	1.52 ± 0.12	0.64 ± 0.06
PS	8d	5.11 ± 0.47	1.78 ± 0.15	0.74 ± 0.07
PS	16d	5.63 ± 0.47	1.58 ± 0.12	0.53 ± 0.05

Species	Clipping	Primary tiller	Secondary tiller	Tertiary tiller
BI	C	8.23 ± 0.54	1.38 ± 0.09	0.28 ± 0.02
BI	NC	8.68 ± 0.56	1.46 ± 0.09	0.45 ± 0.03
PS	C	5.23 ± 0.37	1.52 ± 0.10	0.63 ± 0.05
PS	NC	5.62 ± 0.39	1.71 ± 0.11	0.63 ± 0.04

BI = *Bromus inermis*, PS = *Pascopyrum smithii*, 2d = High precipitation frequency, 8d = Medium precipitation frequency, 16d = Low precipitation frequency, C = Clipping, NC = No-Clipping

Table 2.7 Effects of change in precipitation frequency and clipping on the number of live propagules per plant of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects)

Effect	Number of live propagules per plant
Species	**$F_{1,8.421}$ = 19.19, P = 0.0021**
Precipitation Frequency	**$F_{2,8.404}$ = 15.43, P = 0.0015**
Species*Precipitation Frequency	**$F_{2,8.405}$ = 17.09, P = 0.0011**
Clipping	$F_{1,8.398}$ = 3.14, P = 0.1123
Species*Clipping	$F_{1,8.398}$ = 0.76, P = 0.4065
Precipitation Frequency*Clipping	$F_{2,8.394}$ = 2.05, P = 0.188
Species*Precipitation Frequency*Clipping	$F_{2,8.394}$ = 0.14, P = 0.8722

The values in bold represent significant effects with $P < 0.05$.

Table 2.8 Effects of change in precipitation frequency and clipping on the number of live propagules per plant of *B. inermis*, and *P. smithii* (Mean ± SE)

Species	Precipitation Frequency	Number of live propagules per plant	Species	Clipping	Number of live propagules per plant
BI	2d	154.33 ± 16.76	BI	C	92.93 ± 8.45
BI	8d	110.84 ± 11.90	BI	NC	100.22 ± 9.09
BI	16d	52.55 ± 5.67	PS	C	59.74 ± 5.58
PS	2d	57.05 ± 6.21	PS	NC	74.62 ± 6.98
PS	8d	82.00 ± 9.50			
PS	16d	63.61 ± 6.95			

BI = *Bromus inermis*, PS = *Pascopyrum smithii*, 2d = High precipitation frequency, 8d = Medium precipitation frequency, 16d = Low precipitation frequency, C = Clipping, NC = No-Clipping

Table 2.9 Effects of change in precipitation frequency and clipping on the proportion of live propagules at development stage per plant of *B. inermis* and *P. smithii* (Type III Tests of Fixed Effects)

Effect	Proportion of live propagules at each development stage per plant
Species	**$F_{1,33.78}$ = 15.12, P = 0.0004**
Precipitation Frequency	$F_{2,33.41}$ = 0.84, P = 0.439
Species*Precipitation Frequency	$F_{2,33.43}$ = 0.98, P = 0.3855
Clipping	$F_{1,33.24}$ = 1.12, P = 0.2974
Species*Clipping	**$F_{1,33.24}$ = 4.8, P = 0.0355**
Precipitation Frequency*Clipping	$F_{2,33.26}$ = 1.6, P = 0.2171
Species*Precipitation Frequency*Clipping	$F_{2,33.28}$ = 0.34, P = 0.712
Development Stage	**$F_{2,33.24}$ = 951, P < 0.0001**
Species*Development Stage	**$F_{2,33.24}$ = 10.29, P = 0.0003**
Precipitation Frequency*Development Stage	$F_{4,33.23}$ = 1.36, P = 0.2694
Species*Precipitation Frequency*Development Stage	**$F_{4,33.23}$ = 4.1, P = 0.0083**
Clipping*Development Stage	$F_{2,33.24}$ = 0.43, P = 0.6513
Species*Clipping*Development Stage	$F_{2,33.24}$ = 2.44, P = 0.1026
Precipitation Frequency*Clipping*Development Stage	$F_{4,33.23}$ = 0.64, P = 0.6405
Species*Precipitation Frequency*Clipping*Development Stage	$F_{4,33.23}$ = 1.25, P = 0.3106

The values in bold represent significant effects with $P < 0.05$.

Table 2.10 Effects of change in precipitation frequency and clipping on the proportion of live propagules at development stage per plant of *B. inermis* and *P. smithii* (Mean ± SE)

Species	Precipitation Frequency	Bud	Small Juvenile Tiller	Large Juvenile Tiller
BI	2d	0.85 ± 0.10	0.07 ± 0.01	0.08 ± 0.01
BI	8d	0.81 ± 0.10	0.10 ± 0.01	0.09 ± 0.01
BI	16d	0.84 ± 0.10	0.07 ± 0.01	0.08 ± 0.01
PS	2d	0.88 ± 0.10	0.07 ± 0.01	0.04 ± 0.005
PS	8d	0.89 ± 0.12	0.05 ± 0.01	0.07 ± 0.01
PS	16d	0.88 ± 0.10	0.08 ± 0.01	0.05 ± 0.01

Species	Clipping	Bud	Small Juvenile Tiller	Large Juvenile Tiller
BI	C	0.83 ± 0.08	0.08 ± 0.01	0.09 ± 0.01
BI	NC	0.84 ± 0.08	0.07 ± 0.01	0.08 ± 0.01
PS	C	0.90 ± 0.09	0.06 ± 0.01	0.04 ± 0.004
PS	NC	0.87 ± 0.09	0.07 ± 0.01	0.06 ± 0.01

BI = *Bromus inermis*, PS = *Pascopyrum smithii*, 2d = High precipitation frequency, 8d = Medium precipitation frequency, 16d = Low precipitation frequency, C = Clipping, NC = No-Clipping

Table 2.11 Effects of change in precipitation frequency and clipping on the number of live propagules per tiller by generation of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects)

Effect	Number of live propagules per tiller by generation
Species	**$F_{1,439}$ = 174.84, P < 0.0001**
Precipitation Frequency	$F_{2,439}$ = 0.32, P = 0.7251
Species*Precipitation Frequency	$F_{2,439}$ = 0.82, P = 0.4426
Clipping	**$F_{1,439}$ = 4.78, P = 0.0292**
Species*Clipping	**$F_{1,439}$ = 5.22, P = 0.0228**
Precipitation Frequency*Clipping	$F_{2,439}$ = 0.5, P = 0.6075
Species*Precipitation Frequency*Clipping	$F_{2,439}$ = 1.93, P = 0.1464
Generation	**$F_{2,661}$ = 30.1, P < 0.0001**
Species*Generation	**$F_{2,661}$ = 6.02, P = 0.0026**
Precipitation Frequency*Generation	$F_{4,611}$ = 1.89, P = 0.1104
Species*Precipitation Frequency*Generation	$F_{4,611}$ = 1.28, P = 0.2776
Clipping*Generation	$F_{2,611}$ = 0.81, P = 0.4445
Species*Clipping*Generation	$F_{2,661}$ = 0.07, P = 0.9317
Precipitation Frequency*Clipping*Generation	$F_{4,661}$ = 0.85, P = 0.4952
Species*Precipitation Frequency*Clipping*Generation	$F_{4,661}$ = 0.32, P = 0.8617

The values in bold represent significant effects with $P < 0.05$.

Table 2.12 Effects of change in precipitation frequency and clipping on the number of live propagules per tiller by generation of *B. inermis*, and *P. smithii* (Mean ± SE)

Species	Precipitation Frequency	Primary tiller	Secondary tiller	Tertiary tiller
BI	2d	3.00 ± 0.16	3.07 ± 0.18	2.75 ± 0.28
BI	8d	3.25 ± 0.17	3.9 ± 0.20	2.45 ± 0.20
BI	16d	2.82 ± 0.15	3.27 ± 0.18	2.65 ± 0.25
PS	2d	1.63 ± 0.12	2.08 ± 0.15	1.75 ± 0.17
PS	8d	1.37 ± 0.11	2.18 ± 0.15	1.66 ± 0.15
PS	16d	1.59 ± 0.11	2.07 ± 0.14	1.54 ± 0.16

Species	Clipping	Primary tiller	Secondary tiller	Tertiary tiller
BI	C	3.08 ± 0.13	3.30 ± 0.15	2.66 ± 0.21
BI	NC	2.96 ± 0.13	3.50 ± 0.15	2.57 ± 0.18
PS	C	1.40 ± 0.09	1.88 ± 0.11	1.53 ± 0.13
PS	NC	1.66 ± 0.09	2.35 ± 0.12	1.78 ± 0.13

BI = *Bromus inermis*, PS = *Pascopyrum smithii*, 2d = High precipitation frequency, 8d = Medium precipitation frequency, 16d = Low precipitation frequency, C = Clipping, NC = No-Clipping

Table 2.13 Effects of change in precipitation frequency and clipping on the proportion of live propagules from tiller per plant of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects)

Effect	Proportion of live propagules from tiller per plant
Species	**$F_{1,11.37} = 64.63$, $P < 0.0001$**
Precipitation Frequency	$F_{2,11.3} = 1$, $P = 0.3968$
Species*Precipitation Frequency	**$F_{2,11.3} = 4.63$, $P = 0.034$**
Clipping	$F_{1,11.37} = 0.07$, $P = 0.7956$
Species*Clipping	$F_{1,11.37} = 0.99$, $P = 0.3415$
Precipitation Frequency*Clipping	$F_{2,11.3} = 0.19$, $P = 0.8312$
Species*Precipitation Frequency*Clipping	$F_{2,11.3} = 1.63$, $P = 0.2387$

The values in bold represent significant effects with $P < 0.05$.

Table 2.14 Effects of change in precipitation frequency and clipping on the proportion of live propagules from tiller per plant of *B. inermis*, and *P. smithii* (Mean ± SE)

Species	Precipitation Frequency	Proportion of live propagules from tiller per plant	Species	Clipping	Proportion of live propagules from tiller per plant
BI	2d	0.68 ± 0.04	BI	C	0.75 ± 0.03
BI	8d	0.83 ± 0.03	BI	NC	0.77 ± 0.03
BI	16d	0.76 ± 0.03	PS	C	0.52 ± 0.04
PS	2d	0.53 ± 0.04	PS	NC	0.47 ± 0.04
PS	8d	0.46 ± 0.05			
PS	16d	0.49 ± 0.04			

BI = *Bromus inermis*, PS = *Pascopyrum smithii*, 2d = High precipitation frequency, 8d = Medium precipitation frequency, 16d = Low precipitation frequency, C = Clipping, NC = No-Clipping

Table 2.15 Effects of change in precipitation frequency, clipping, and location on the proportion of total tillers at each location per plant of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects)

Effect	Proportion of total tillers at each location per plant
Species	**$F_{1,35} = 284.97$, $P < 0.0001$**
Precipitation Frequency	**$F_{2,35} = 20.01$, $P < 0.0001$**
Species*Precipitation Frequency	**$F_{2,35} = 19.09$, $P < 0.0001$**
Clipping	$F_{1,35} = 1.7$, $P = 0.2011$
Species*Clipping	$F_{1,35} = 2.38$, $P = 0.1317$
Precipitation Frequency*Clipping	$F_{2,35} = 1.79$, $P = 0.1813$
Species*Precipitation Frequency*Clipping	$F_{2,35} = 1.94$, $P = 0.1585$
Location	**$F_{2,35} = 454.68$, $P < 0.0001$**
Species*Location	**$F_{2,35} = 372.81$, $P < 0.0001$**
Precipitation Frequency*Location	**$F_{4,35} = 15.32$, $P < 0.0001$**
Species*Precipitation Frequency*Location	**$F_{4,35} = 10$, $P < 0.0001$**
Clipping*Location	$F_{2,35} = 0.17$, $P = 0.847$
Species*Clipping*Location	**$F_{2,35} = 3.84$, $P = 0.0311$**
Precipitation Frequency*Clipping*Location	$F_{4,35} = 0.93$, $P = 0.456$
Species*Precipitation Frequency*Clipping*Location	$F_{4,35} = 1.44$, $P = 0.2411$

The values in bold represent significant effects with $P < 0.05$.

Table 2.16 Effects of change in precipitation frequency, clipping, and location on the proportion of total tillers at each location per plant of *B. inermis*, and *P. smithii* (Mean ± SE)

Species	Precipitation Frequency	Crown	Rhizome	Rhizome tip
BI	2d	0.80 ± 0.03	0.01 ± 0.001	0.20 ± 0.01
BI	8d	0.65 ± 0.03	0.03 ± 0.004	0.31 ± 0.02
BI	16d	0.76 ± 0.04	0.01 ± 0.004	0.22 ± 0.02
PS	2d	0.38 ± 0.02	0.25 ± 0.02	0.37 ± 0.02
PS	8d	0.34 ± 0.02	0.28 ± 0.02	0.38 ± 0.02
PS	16d	0.36 ± 0.02	0.27 ± 0.02	0.37 ± 0.02

Species	Clipping	Crown	Rhizome	Rhizome tip
BI	C	0.75 ± 0.03	0.01 ± 0.002	0.23 ± 0.01
BI	NC	0.72 ± 0.03	0.02 ± 0.002	0.25 ± 0.01
PS	C	0.34 ± 0.02	0.28 ± 0.02	0.38 ± 0.02
PS	NC	0.38 ± 0.02	0.25 ± 0.02	0.37 ± 0.02

BI = *Bromus inermis*, PS = *Pascopyrum smithii*, 2d = High precipitation frequency, 8d = Medium precipitation frequency, 16d = Low precipitation frequency, C = Clipping, NC = No-Clipping

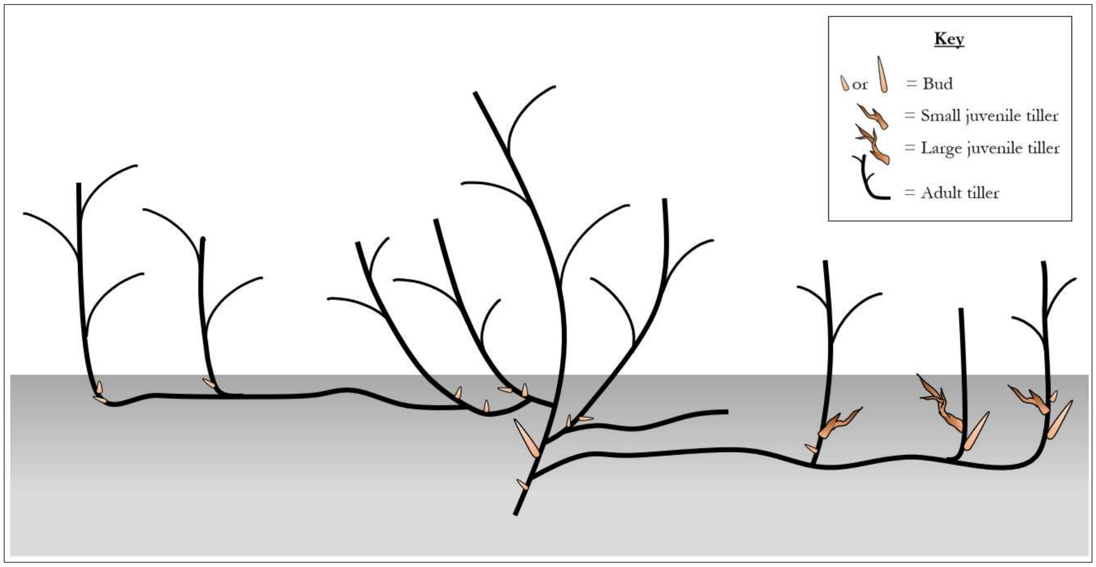


Figure 2.12 Conceptual diagram of bud, rhizome and tiller development stages (From Ott and Hartnett 2015b)

CHAPTER 3

Table 3.2 Effects of competition on the number of live propagules per plant of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects)

Effect	Number of live propagules per plant
Species	**$F_{1,174}$ = 185.1, P < 0.0001**
Competition	**$F_{2,174}$ = 43.95, P < 0.0001**
Species*Competition	**$F_{2,174}$ = 45.91, P < 0.0001**

The values in bold represent significant effects with $P < 0.05$.

Table 3.3 Effects of competition on the number of live propagules per plant of *B. inermis*, and *P. smithii* (Mean ± SE)

Species	Competition	Number of live propagules per plant
BI	None	26.69 ± 2.40
BI	Intra	11.41 ± 1.03
BI	Inter	30.45 ± 2.74
PS	None	115.02 ± 10.34
PS	Intra	52.78 ± 4.75
PS	Inter	30.60 ± 2.75

BI = *Bromus inermis*, PS = *Pascopyrum smithii*, None = Single/No-Competition, Intra = Intraspecific competition, Inter = Interspecific competition

Table 3.4 Effects of competition on the proportion of live propagules at each development stage per plant of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects)

Effect	Proportion of live propagules at each development stage per plant
Species	**$F_{1,522} = 4.6, P = 0.0325$**
Competition	$F_{2,522} = 0.85, P = 0.427$
Species*Competition	$F_{2,522} = 0.95, P = 0.3893$
Development Stage	**$F_{2,522} = 87.23, P < 0.0001$**
Species*Development Stage	$F_{2,522} = 2.14, P = 0.1183$
Competition*Development Stage	$F_{4,522} = 0.91, P = 0.4592$
Species*Competition*Development Stage	$F_{4,522} = 0.52, P = 0.7221$

[1]The values in bold represent significant effects with $P < 0.05$.

Table 3.5 Effects of competition on the proportion of live propagules at each development stage per plant of *B. inermis*, and *P. smithii* (Mean ± SE)

Species	Competition	Bud	Small Juvenile Tiller	Large Juvenile Tiller
BI	None	0.85 ± 0.29	0.08 ± 0.03	0.07 ± 0.02
BI	Intra	0.92 ± 0.31	0.04 ± 0.01	0.05 ± 0.02
BI	Inter	0.81 ± 0.27	0.12 ± 0.04	0.07 ± 0.02
PS	None	0.70 ± 0.24	0.14 ± 0.05	0.15 ± 0.05
PS	Intra	0.78 ± 0.26	0.11 ± 0.04	0.11 ± 0.04
PS	Inter	0.79 ± 0.27	0.13 ± 0.04	0.08 ± 0.03

BI = *Bromus inermis*, PS = *Pascopyrum smithii*, None = Single/No-Competition, Intra = Intraspecific competition, Inter = Interspecific competition

Table 3.6 Effects of competition on the number of live propagules per tiller by generation of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects)

Effect	Number of live propagules per tiller by generation
Species	**$F_{1,174} = 99.93$, $P < 0.0001$**
Competition	**$F_{2,174} = 3.14$, $P = 0.0459$**
Species*Competition	$F_{2,174} = 1.38$, $P = 0.2544$
Generation	$F_{1,165} = 2.79$, $P = 0.0966$
Species*Generation	$F_{1,165} = 3.78$, $P = 0.0535$
Competition*Generation	**$F_{2,165} = 3.44$, $P = 0.0342$**
Species*Competition*Generation	$F_{2,165} = 2.46$, $P = 0.0888$

The values in bold represent significant effects with $P < 0.05$.

Table 3.7 Effects of competition on the number of live propagules per tiller by generation of *B. inermis*, and *P. smithii* (Mean ± SE)

Species	Competition	Primary tiller	Secondary tiller
BI	None	1.20 ± 0.13	1.48 ± 0.14
BI	Intra	1.36 ± 0.14	0.98 ± 0.12
BI	Inter	1.32 ± 0.13	1.47 ± 0.14
PS	None	2.24 ± 0.18	3.06 ± 0.21
PS	Intra	1.9 ± 0.17	2.34 ± 0.20
PS	Inter	2.1 ± 0.17	2.24 ± 0.19

BI = *Bromus inermis*, PS = *Pascopyrum smithii*, None = Single/No-Competition, Intra = Intraspecific competition, Inter = Interspecific competition. Output for tertiary tiller generation not available because of no sufficient data

Table 3.8 Contrast of effects between intra-and interspecific competition, and species on the bud production, and propagule development of *B. inermis*, and *P. smithii*.

Label	Number of live propagules per plant	Proportion of live propagules at each development stage per plant	Number of live propagules per tiller by generation
intra *vs.* inter competition across species	**$F_{1,174}$ = 5.89, P = 0.0163**	$F_{1,522}$ = 0.93, P = 0.3357	$F_{1,174}$ = 1.96, P = 0.1632
B. inermis vs. P. smithii excluding none competition	**$F_{1,174}$ = 72.95, P < 0.0001**	$F_{1,522}$ = 2.58, P = 0.1089	**$F_{1,174}$ = 54.09, P < 0.0001**

The values in bold represent significant effects with $P < 0.05$.

Table 3.9 Effects of competition on the number of new tillers established per tiller in each generation of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects)

Effect	Number of new tillers established per tiller
Species	**$F_{1,513} = 30.51, P < 0.0001$**
Competition	**$F_{2,513} = 16.4, P < 0.0001$**
Species*Competition	**$F_{2,513} = 12.82, P < 0.0001$**
Generation	**$F_{2,513} = 273.32, P < 0.0001$**
Species*Generation	**$F_{2,513} = 16.66, P < 0.0001$**
Competition*Generation	$F_{4,513} = 2.3, P = 0.0577$
Species*Competition*Generation	**$F_{4,513} = 2.47, P = 0.044$**

The values in bold represent significant effects with $P < 0.05$.

Table 3.10 Effects of competition on the number of new tillers established per tiller in each generation of B. inermis, and P. smithii (Mean ± SE)

Species	Competition	Primary tiller	Secondary tiller	Tertiary tiller
BI	None	4.80 ± 0.49	2.03 ± 0.17	0.15 ± 0.02
BI	Intra	3.50 ± 0.40	1.16 ± 0.13	0.01 ± 0.01
BI	Inter	4.37 ± 0.46	1.71 ± 0.16	0.19 ± 0.03
PS	None	6.37 ± 0.60	2.35 ± 0.19	0.57 ± 0.05
PS	Intra	3.97 ± 0.44	1.25 ± 0.13	0.51 ± 0.07
PS	Inter	4.07 ± 0.44	1.12 ± 0.12	0.34 ± 0.06

BI = *Bromus inermis*, PS = *Pascopyrum smithii*, None = Single/No-Competition, Intra = Intraspecific competition, Inter = Interspecific competition

Table 3.11 Contrast of effects between intra-and interspecific competition, and species the on number of new tillers established per tiller in each generation of *B. inermis*, and *P. smithii*.

Label	Number of new tillers established per tiller
intra *vs.* inter competition across species	**$F_{1,513} = 8.81$, $P = 0.0031$**
B. inermis vs. P. smithii excluding none competition	**$F_{1,513} = 16.24$, $P < 0.0001$**

The values in bold represent significant effects with $P < 0.05$.

Table 3.12 Effects of competition on the overall plant establishment traits of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects)

Effect	Number of total tillers per plant	Number of total rhizomes per plant	Total rhizome length(cm) per plant
Species	**$F_{1,174}$ = 28.18, P < 0.0001**	**$F_{1,174}$ = 29.81, P < 0.0001**	**$F_{1,174}$ = 116.61, P < 0.0001**
Competition	**$F_{2,174}$ = 122.23, P < 0.0001**	**$F_{2,174}$ = 13.97, P < 0.0001**	**$F_{2,174}$ = 3.72, P = 0.0261**
Species*Competition	**$F_{2,174}$ = 30.36, P < 0.0001**	$F_{2,174}$ = 2.22, P = 0.1122	$F_{2,174}$ = 1.44, P = 0.2397

The values in bold represent significant effects with $P < 0.05$.

Table 3.13 Effects of competition on the overall plant establishment traits of *B. inermis*, and *P. smithii* (Mean ± SE)

Species	Competition	Number of total tillers per plant	Number of total rhizomes per plant	Total rhizome length (cm) per plant
BI	None	15.60 ± 0.83	7.23 ± 0.74	32.68 ± 5.70
BI	Intra	7.33 ± 0.53	4.33 ± 0.50	22.06 ± 3.85
BI	Inter	12.87 ± 0.74	5.37 ± 0.59	26.7 ± 4.66
PS	None	28.23 ± 1.23	12.37 ± 1.14	176.03 ± 30.72
PS	Intra	11.23 ± 0.68	8.23 ± 0.82	124.94 ± 21.80
PS	Inter	10.03 ± 0.64	6.63 ± 0.69	88.42 ± 15.43

BI = *Bromus inermis*, PS = *Pascopyrum smithii*, None = Single/No-Competition, Intra = Intraspecific competition, Inter = Interspecific competition

Table 3.14 Contrast of effects between intra-and interspecific competition, and species on the overall plant establishment traits of *B. inermis* and *P. smithii*

Label	Number of total tillers per plant	Number of total rhizomes per plant	Total rhizome length(cm) per plant
intra *vs.* inter competition across species	**$F_{1,174} = 12.36$, $P = 0.0006$**	$F_{1,174} = 0$, $P = 0.9918$	$F_{1,174} = 0.2$, $P = 0.6567$
B. inermis vs. P. smithii excluding none competition	$F1_{,174} = 1.93$, $P = 0.166$	**$F_{1,174} = 15.84$, $P = 0.0001$**	**$F_{2,174} = 70.52$, $P < 0.0001$**

The values in bold represent significant effects with $P < 0.05$.

Table 3.15 Effects of competition on the proportion of live propagules from tiller per plant of *B. inermis* and *P. smithii* (Type III Tests of Fixed Effects)

Effect	Proportion of live propagules from tiller per plant
Species	$F_{1,174}$ = 1.74, P = 0.1894
Competition	**$F_{2,174}$ =1.81, P = 0.01671**
Species*Competition	**$F_{2,174}$ = 21.27, P < 0.0001**

The values in bold represent significant effects with $P < 0.05$.

Table 3.16 Effects of competition on the proportion of live propagules from tiller per plant of *B. inermis* and *P. smithii* (Mean ± SE)

Species	Competition	Proportion of live propagules from tiller per plant
BI	None	0.80 ± 0.03
BI	Intra	0.81 ± 0.03
BI	Inter	0.51 ± 0.05
PS	None	0.68 ± 0.04
PS	Intra	0.53 ± 0.05
PS	Inter	0.80 ± 0.03

BI = *Bromus inermis*, PS = *Pascopyrum smithii*, None = Single/No-Competition, Intra = Intraspecific competition, Inter = Interspecific competition

Table 3.17 Effects of competition on the proportion of total tillers at each location per plant of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects)

Effect	Proportion of total tillers at each location per plant
Species	**$F_{1,522}$ = 20.74, P < 0.0001**
Competition	$F_{2,522}$ = 1.21, P = 0.2989
Species*Competition	$F_{2,522}$ = 2.76, P = 0.0641
Location	**$F_{2,522}$ = 235.91, P < 0.0001**
Species*Location	**$F_{2,522}$ = 80.69, P < 0.0001**
Competition*Location	$F_{4,522}$ = 1.05, P = 0.3783
Species*Competition*Location	$F_{4,522}$ = 2.35, P = 0.0533

The values in bold represent significant effects with $P < 0.05$.

Table 3.18 Effects of competition on the proportion of total tillers at each location per plant of *B. inermis*, and *P. smithii* (Mean ± SE)

Species	Competition	Crown	Rhizome	Rhizome tip
BI	None	0.60 ± 0.04	0.002 ± 0.002	0.4 ± 0.03
BI	Intra	0.52 ± 0.05	0.02 ± 0.01	0.46 ± 0.05
BI	Inter	0.62 ± 0.04	0.02 ± 0.01	0.36 ± 0.03
PS	None	0.66 ± 0.03	0.19 ± 0.02	0.15 ± 0.01
PS	Intra	0.74 ± 0.05	0.1 ± 0.02	0.16 ± 0.02
PS	Inter	0.73 ± 0.05	0.11 ± 0.02	0.16 ± 0.02

BI = *Bromus inermis*, PS = *Pascopyrum smithii*, None = Single/No-Competition, Intra = Intraspecific competition, Inter = Interspecific competition

Table 3.19 Contrast of effects between intra-and interspecific competition, and species on the investment in phalanx and guerilla growth of *B. inermis*, and *P. smithii*

Contrast	Proportion of live propagules on tiller per plant	Proportion of total tillers at each location per plant
intra *vs.* inter competition across species	$F_{1,174} = 0.16$, $P = 0.6919$	$F_{1,522} = 0$, $P = 0.9982$
B. inermis vs. P. smithii excluding none competition	$F_{1,174} = 0.01$, $P = 0.9248$	**$F_{1,522} = 8.25$, $P = 0.0042$**

The values in bold represent significant effects with $P < 0.05$.

Table 3.20 Effects of competition on the aboveground, rhizome, and total biomass plant of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects)

Effect	Aboveground biomass (g) per plant	Rhizome biomass (g) per plant	Total biomass (g) per plant
Species	$F_{1,54}$ = 0.01, P = 0.9227	**$F_{1,54}$ = 15.55, P = 0.0002**	**$F_{1,54}$ = 4.1, P = 0.0478**
Competition	**$F_{2,54}$ = 90.9, P < 0.0001**	**$F_{2,54}$ = 3.22, P = 0.0476**	**$F_{2,54}$ = 90.11, P < 0.0001**
Species*Competition	**$F_{2,54}$ = 9.05, P = 0.0004**	$F_{2,54}$ = 0.07, P = 0.9311	**$F_{2,54}$ = 9.99, P = 0.0002**

The values in bold represent significant effects with $P < 0.05$.

Table 3.21 Effects of competition on the aboveground, rhizome, and total biomass plant of *B. inermis*, and *P. smithii* (Mean ± SE)

Species	Competition	Aboveground biomass (g) per plant	Rhizome biomass (g) per plant	Total biomass (g) per plant
BI	None	6.27 ± 0.32	0.37 ± 0.11	6.64 ± 0.34
BI	Intra	2.21 ± 0.32	0.19 ± 0.06	2.26 ± 0.34
BI	Inter	4.12 ± 0.32	0.21 ± 0.06	4.33 ± 0.34
PS	None	6.96 ± 0.32	0.99 ± 0.29	7.96 ± 0.34
PS	Intra	3.02 ± 0.32	0.44 ± 0.13	3.82 ± 0.34
PS	Inter	2.54 ± 0.32	0.60 ± 0.18	3.14 ± 0.34

BI = *Bromus inermis*, PS = *Pascopyrum smithii*, None = Single/No-Competition, Intra = Intraspecific competition, Inter = Interspecific competition

Table 3.22 Contrast of effects between intra-and interspecific competition, and species on the aboveground, rhizome, and total biomass plant of *B. inermis*, and *P. smithii*

Label	Aboveground biomass (g) per plant	Rhizome biomass (g) per plant	Total biomass (g) per plant
intra *vs.* inter competition across species	**$F_{1,54} = 5.15$, $P = 0.0273$**	$F_{1,54} = 0.42$, $P = 0.5202$	**$F_{1,54} = 4.19$, $P = 0.0456$**
B. inermis vs. P. smithii excluding none competition	$F_{1,54} = 1.47$, $P = 0.2299$	**$F_{1,54} = 10.08$, $P = 0.0025$**	$F_{1,54} = 0.3$, $P = 0.5866$

The values in bold represent significant effects with $P < 0.05$.

Table 3.23 Effects of competition on the RII values (with respect to number of total tillers, number of total rhizomes, rhizome length, and number of live propagules per plant) of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects)

Effect	RII (number of total tillers per plant)	RII (number of total rhizomes per plant)	RII (total rhizome length(cm) per plant)	RII (number of live propagules per plant)
Species	**$F_{1,116} = 54.48$, $P < 0.0001$**	$F_{1,116} = 1.08$, $P = 0.3011$	$F_{1,116} = 1.18$, $P = 0.2799$	**$F_{1,116} = 42.76$, $P < 0.0001$**
Competition	**$F_{1,116} = 12.32$, $P = 0.0006$**	$F_{1,116} = 0.13$, $P = 0.7241$	$F_{1,116} = 0.27$, $P = 0.6045$	**$F_{1,116} = 4$, $P = 0.048$**
Species*Competition	**$F_{1,116} = 17.24$, $P < 0.0001$**	$F_{1,116} = 0.73$, $P = 0.3952$	$F_{1,116} = 0.62$, $P = 0.4337$	**$F_{1,116} = 30.31$, $P < 0.0001$**

The values in bold represent significant effects with $P < 0.05$.

Table 3.24 Effects of competition on the RII values (with respect to number of total tillers, number of total rhizomes, rhizome length, and number of live propagules per plant) of *B. inermis*, and *P. smithii* (Mean ± SE)

Species	Competition	RII (number of total tillers per plant)	RII (number of total rhizomes per plant)	RII (total rhizome length(cm) per plant)	RII (total live propagules per plant)
BI	Intra	-0.08 ± 0.03	-0.13 ± 0.08	-0.08 ± 0.10	-0.32 ± 0.06
BI	Inter	-0.35 ± 0.03	-0.09 ± 0.08	-0.06 ± 0.10	0.11 ± 0.06
PS	Intra	-0.48 ± 0.03	-0.15 ± 0.08	-0.11 ± 0.10	-0.37 ± 0.06
PS	Inter	-0.46 ± 0.03	-0.25 ± 0.08	-0.23 ± 0.10	-0.57 ± 0.06

BI = *Bromus inermis*, PS = *Pascopyrum smithii*, None = Single/No-Competition, Intra = Intraspecific competition, Inter = Interspecific competition

Table 3.25 Effects of competition on the RII values (with respect to aboveground, rhizome, and total biomass per plant) of *B. inermis*, and *P. smithii* (Type III Tests of Fixed Effects)

Effect	RII (aboveground biomass(g) per plant)	RII (rhizome biomass(g) per plant)	RII (total biomass(g) per plant)
Species	**$F_{1,36} = 4.25$, $P = 0.0466$**	$F_{1,36} = 1.2$, $P = 0.2799$	$F_{1,36} = 1.38$, $P = 0.2475$
Competition	**$F_{1,36} = 6.63$, $P = 0.0143$**	$F_{1,36} = 0.02$, $P = 0.8957$	**$F_{1,36} = 6.11$, $P = 0.0183$**
Species*Competition	**$F_{1,36} = 12.62$, $P = 0.0011$**	$F_{1,36} = 0.35$, $P = 0.5562$	**$F_{1,36} = 13.49$, $P = 0.0008$**

The values in bold represent significant effects with $P < 0.05$.

Table 3.26 Effects of competition on the RII values (with respect to aboveground, rhizome, and total biomass per plant) of *B. inermis*, and *P. smithii* (Mean ± SE)

Species	Competition	RII (aboveground biomass (g) per plant)	RII (rhizome biomass (g) per plant)	RII (total biomass (g) per plant)
BI	Intra	-0.48 ± 0.05	-0.05 ± 0.17	-0.49 ± 0.05
BI	Inter	-0.20 ± 0.05	-0.13 ± 0.17	-0.21 ± 0.05
PS	Intra	-0.41 ± 0.05	-0.34 ± 0.17	-0.37 ± 0.05
PS	Inter	-0.46 ± 0.05	-0.22 ± 0.17	-0.43 ± 0.05

BI = *Bromus inermis*, PS = *Pascopyrum smithii*, None = Single/No-Competition, Intra = Intraspecific competition, Inter = Interspecific competition