Response of an alfalfa-timothy mixture grown in open-top chambers under ambient and elevated carbon dioxide

Jonathan Messerli

Department of Plant Science

McGill University

Montréal, Québec, Canada

April 2015

A thesis submitted to McGill University in partial fulfillment of the requirements for the degree of Master of Science

TABLE OF CONTENTS

LIST OF FIGURES **5**

LIST OF TABLES **6**

LIST OF ABBREVIATIONS **7**

ABSTRACT **8**

RÉSUMÉ **9**

ACKNOWLEDGMENTS **10**

CONTRIBUTION OF AUTHORS **11**

CHAPTER 1 ..... **12**

GENERAL INTRODUCTION **12**

1.1 OBJECTIVES 13
1.2 HYPOTHESES 13

CHAPTER 2 ..... **14**

LITERATURE REVIEW **14**

2.1 CLIMATE CHANGE 14
2.2 FIELD STUDIES 15
2.3 FORAGE MIXTURES 17
2.4 FORAGE GROWTH 18
2.4.1 Photosynthesis *18*
2.4.2 Forage Yield *21*
2.5 NUTRITIVE VALUE 22
2.5.1 Non-Structural Carbohydrates *23*
2.5.2 Nitrogen Concentration *24*
2.5.3 Fibre Concentration and Digestibility *26*
2.6 ORGANIC RESERVE ACCUMULATION AND WINTER SURVIVAL 28
2.6.1 Carbohydrates Reserves *28*
2.6.2 Nitrogen Reserves *30*
2.7 ROOT C:N RATIO AND DECOMPOSITION RATE 30

CONNECTING TEXT FOR CHAPTER 3 ..... **32**

CHAPTER 3 ..... **33**

PERFORMANCE OF LOW-COST OPEN-TOP CHAMBERS TO STUDY LONG-TERM EFFECTS OF CARBON DIOXIDE AND CLIMATE UNDER FIELD CONDITIONS **33**

3.1 ABSTRACT **33**

3.2 INTRODUCTION **34**

3.3 MATERIALS AND METHODS **36**

3.3.1 CHAMBER CONSTRUCTION 36
3.3.2 VENTILATION SYSTEM 37
3.3.3 CO_2 CONTROL SYSTEM 37

3.3.4 COST 38
3.3.5 ASSESSMENT OF ENVIRONMENTAL CONDITIONS WITHIN CHAMBERS 39
3.3.5.1 Plant material *39*
3.3.5.2 CO_2 concentration *39*
3.3.5 AIR TEMPERATURE 39
3.3.6 LIGHT TRANSMISSION 39
3.4 RESULTS AND DISCUSSION 40
3.4.1 COST 40
3.4.2 CO_2 CONSUMPTION 40
3.4.2 CO_2 CONCENTRATION 41
3.4.3 AIR TEMPERATURE 42
3.4.4 LIGHT TRANSMISSION 43
3.5 CONCLUSION 43
CONNECTING TEXT FOR CHAPTER 4 47
CHAPTER 4 48
ELEVATED CARBON DIOXIDE INCREASES THE YIELD BUT DECREASES THE NUTRITIVE VALUE OF AN ALFALFA-TIMOTHY MIXTURE 48
4.1 ABSTRACT 48
4.2 INTRODUCTION 49
4.3 MATERIALS AND METHODS 51
4.3.1 EXPERIMENTAL DESIGN 51
4.3.2 SAMPLE COLLECTION AND PREPARATION 53
4.3.3 LEAF PHOTOSYNTHESIS MEASUREMENT 54
4.3.4 TOTAL NON-STRUCTURAL CARBOHYDRATES ANALYSIS 54
4.3.4.1 Extraction *54*
4.3.4.2 Soluble Carbohydrates *55*
4.3.4.3 Fructans (timothy) *55*
4.3.4.4 Starch *55*
4.3.5 FREE AMINO ACIDS 56
4.3.6 MINERALS 56
4.3.7 FIBRE CONCENTRATION AND DIGESTIBILITY 57
4.3.8 ROOT COMPOSITION 57
4.3.9 ROOT DEGRADABILITY 58
4.3.10 STATISTICAL ANALYSES 58
4.4 RESULTS 58
4.4.1 FORAGE YIELD AND BOTANICAL COMPOSITION 58
4.4.2 LEAF PHOTOSYNTHESIS AND SUCROSE CONCENTRATION 59
4.4.3 NUTRITIVE VALUE 60
4.4.3.1 Non-Structural Carbohydrates *60*
4.4.3.2 Total Nitrogen *60*
4.4.3.3 Fibre and Digestibility *61*
4.4.4 FALL ORGANIC RESERVE ACCUMULATION 61

4.4.5 Root Composition and Degradability 62
4.5 DISCUSSION 62
4.5.1 Forage Yield and Botanical Composition 62
4.5.2 Photosynthesis and Sucrose Concentration 64
4.5.3 Nutritive Value 64
4.5.3.1 Non-Structural Carbohydrates 64
4.5.3.2 Total Nitrogen 65
4.5.3.3 Fibre and Digestibility 66
4.5.4 Fall Organic Reserve Accumulation 67
4.5.5 Root Composition and Degradability 68
4.6 CONCLUSION 69
CHAPTER 5 76
CONCLUSIONS AND SUMMARY 76
CHAPTER 6 78
RECOMMENDATION FOR FUTURE RESEARCH 78
LITERATURE CITED 79
APPENDICES 89

LIST OF FIGURES

FIGURE 3.1. (A) SCHEMATIC REPRESENTATION AND (B) PICTURES OF ONE OPEN-TOP CHAMBER, (C) THE MIXING BOX, (D) THE CO_2 CONTROL SYSTEM, AND (E) THE EXPERIMENTAL SITE IN QUÉBEC CITY, QC, CANADA. 45

FIGURE 3.2. LEFT PANEL: AVERAGE DAILY CO_2 CONCENTRATION (µMOL MOL^{-1}) WITHIN FOUR OPEN-TOP CHAMBERS UNDER THE ELEVATED-CO_2 TREATMENT (DOTTED LINE) AND WITHIN ONE CHAMBER UNDER THE AMBIENT-CO_2 TREATMENT (DASHED LINE); AND DAILY AVERAGE AIR TEMPERATURE DIFFERENCES (^{O}C) BETWEEN THE INSIDE OF OPEN-TOP CHAMBERS (MEAN OF EIGHT CHAMBERS) AND THE MIDDLE OF THE FOUR CONTROL PLOTS WITHOUT CHAMBERS (FULL LINE) RECORDED FROM MAY TO SEPTEMBER 2014. RIGHT PANEL: ZOOMING ON THE CO_2 CONCENTRATIONS (µMOL MOL^{-1}) AND AIR TEMPERATURE DIFFERENCES (^{O}C) DURING THREE DAYS, FROM AUGUST 21-23 2014. 46

FIGURE 4.1. TOTAL WEEKLY PRECIPITATIONS (BARS) AND AVERAGE WEEKLY AIR TEMPERATURE (LINES) MEASURED IN CONTROL PLOTS WITHOUT CHAMBERS FROM 14 JULY TO 26 OCTOBER 2013 AND FROM 5 MAY TO 11 OCTOBER 2014 IN QUÉBEC CITY, QC. ARROWS INDICATE TIME OF CUTTING OF THE ALFALFA-TIMOTHY FORAGE PLOTS. ..70

FIGURE 4.2. ABOVEGROUND YIELD AT EACH CUT (SIGNIFICANT CO_2 × CUT EFFECT, P<0.001) OF AN ALFALFA-TIMOTHY MIXTURE (BARS) GROWN IN OPEN-TOP CHAMBERS UNDER AMBIENT (NEAR 400 µMOL MOL^{-1}) AND ELEVATED (600 µMOL MOL^{-1}) CO_2 CONCENTRATION, AND WITHOUT AN OPEN-TOP CHAMBER (CONTROL). THE PROPORTION OF ALFALFA IN FORAGE DRY MATTER YIELD IS REPRESENTED BY THE SOLID LINE (SIGNIFICANT CUT MAIN EFFECT, P<0.001). DIFFERENT LETTERS INDICATE SIGNIFICANT (P≤0.05) DIFFERENCES WITHIN A CUT FOR DM YIELD AND ACROSS CUTS FOR THE CONTRIBUTION OF ALFALFA TO DM YIELD. STANDARD ERROR IS REPRESENTED BY ERROR BARS.....71

FIGURE 4.3. CONCENTRATIONS OF NON-STRUCTURAL CARBOHYDRATES (NSC) AND TOTAL FREE AMINO ACIDS (SIGNIFICANT YEAR EFFECT, P<0.001 AND P<0.002, RESPECTIVELY) IN THE CROWNS OF COLD-ACCLIMATED ALFALFA IN FALL 2013 COMPARED TO FALL 2014. PLANTS WERE GROWN IN OPEN-TOP CHAMBERS UNDER AMBIENT (NEAR 400 µMOL MOL^{-1}) AND ELEVATED (600 µMOL MOL^{-1}) [CO_2]. DIFFERENT LETTERS INDICATE SIGNIFICANT DIFFERENCES BETWEEN YEARS (P≤0.05). STANDARD ERROR IS REPRESENTED BY ERROR BARS. 72

FIGURE A.1. LOCATION OF OPEN-TOP CHAMBERS AND THEIR RESPECTIVE TREATMENTS AND OF CONTROL PLOTS ON THE EXPERIMENTAL SITE IN A COMPLETELY RANDOMIZED DESIGN. 89

LIST OF TABLES

TABLE 4.1. DRY MATTER FORAGE YIELD, CONCENTRATIONS OF NON-STRUCTURAL CARBOHYDRATES (NSC), TOTAL NITROGEN, ACID DETERGENT FIBRE (ADF), NEUTRAL DETERGENT FIBRE (NDF) AND ACID DETERGENT LIGNIN (ADL), AS WELL AS *IN VITRO* TRUE DIGESTIBILITY (IVTD) AND NDF DIGESTIBILITY (NDFD) IN THE BIOMASS OF AN ALFALFA-TIMOTHY MIXTURE GROWN IN OPEN-TOP CHAMBERS UNDER AMBIENT (NEAR 400 µMOL MOL^{-1}) AND ELEVATED (600 µMOL MOL^{-1}) CO_2 CONCENTRATIONS, AND WITHOUT AN OPEN-TOP CHAMBER (CONTROL). 73

TABLE 4.2. DRY MATTER FORAGE YIELD, LEAF PHOTOSYNTHESIS, AND CONCENTRATIONS OF SUCROSE AND TOTAL NITROGEN IN AN ALFALFA-TIMOTHY MIXTURE GROWN IN OPEN-TOP CHAMBERS UNDER AMBIENT (NEAR 400 µMOL MOL^{-1}) AND ELEVATED (600 µMOL MOL^{-1}) CO_2 CONCENTRATIONS, AND WITHOUT AN OPEN-TOP CHAMBER (CONTROL).....74

TABLE 4.3. CONCENTRATIONS OF CARBON, NITROGEN AND ACID DETERGENT LIGNIN (ADL), AS WELL AS DRY MATTER DEGRADABILITY MEASURED BY *IN VITRO* TRUE DIGESTIBILITY (IVTD) OF THE ROOTS OF ALFALFA AND TIMOTHY GROWN IN A MIXTURE IN OPEN-TOP CHAMBERS UNDER AMBIENT (NEAR 400 µMOL MOL^{-1}) AND ELEVATED (600 µMOL MOL^{-1}) CO_2 CONCENTRATIONS, AND WITHOUT AN OPEN-TOP CHAMBER (CONTROL).....75

TABLE A.1. COST OF MATERIALS AND EQUIPMENT USED FOR BUILDING EIGHT OPEN-TOP CHAMBERS (2013 PRICES, US $).....90

TABLE A.2. AVERAGE INCOMING PAR (µMOL M^{-2} SEC^{-1}) MEASURED IN THE MIDDLE OF EIGHT OPEN-TOP CHAMBERS (OTCS) AS COMPARED TO FOUR CONTROL PLOTS WITHOUT CHAMBER. PAR WAS MEASURED ABOVE PLANT CANOPY AT THREE DIFFERENT PERIODS OF THE DAY (AM, PM AND LATE PM), THREE TIMES BETWEEN 25 JUNE AND 11 AUGUST 2014, CORRESPONDING TO THREE DIFFERENT PLANT HEIGHTS (30 CM, 60 CM AND 90 CM FROM THE GROUND) N=5. LIGHT TRANSMISSION (%) WAS CALCULATED AS: (PAR OTCS / PAR CONTROL PLOTS) × 100 AND THE AVERAGE LIGHT TRANSMISSION WAS THE AVERAGE OF 15 OBSERVATIONS FOR EACH HEIGHT. 91

TABLE A.3. CONCENTRATIONS OF SUGARS (MG G^{-1} DM) IN THE BIOMASS OF ALFALFA GROWN IN OPEN-TOP CHAMBERS UNDER AMBIENT (NEAR 400 µMOL MOL^{-1}) AND ELEVATED (600 µMOL MOL^{-1}) CO_2 CONCENTRATIONS, AND WITHOUT AN OPEN-TOP CHAMBER (CONTROL).....92

TABLE A.4. CONCENTRATION OF SUGARS (MG G^{-1} DM) IN THE BIOMASS OF TIMOTHY GROWN IN OPEN-TOP CHAMBERS UNDER AMBIENT (NEAR 400 µMOL MOL^{-1}) AND ELEVATED (600 µMOL MOL^{-1}) CO_2 CONCENTRATIONS, AND WITHOUT AN OPEN-TOP CHAMBER (CONTROL).....93

TABLE A.5. CONCENTRATION OF SUGARS (MG G^{-1} DM) IN THE BIOMASS OF AN ALFALFA-TIMOTHY MIXTURE GROWN IN OPEN-TOP CHAMBERS UNDER AMBIENT (NEAR 400 µMOL MOL^{-1}) AND ELEVATED (600 µMOL MOL^{-1}) CO_2 CONCENTRATIONS, AND WITHOUT AN OPEN-TOP CHAMBER (CONTROL).....94

TABLE A.6. CONCENTRATION OF MINERALS (MG G^{-1} DM) IN THE BIOMASS OF AN ALFALFA-TIMOTHY MIXTURE GROWN IN OPEN-TOP CHAMBERS UNDER AMBIENT (NEAR 400 µMOL MOL^{-1}) AND ELEVATED (600 µMOL MOL^{-1}) CO_2 CONCENTRATIONS, AND WITHOUT AN OPEN-TOP CHAMBER (CONTROL).....95

TABLE A.7. CONCENTRATION OF SUGARS (MG G^{-1} DM) IN THE CROWNS OF ACCLIMATED ALFALFA AND TIMOTHY GROWN IN OPEN-TOP CHAMBERS UNDER AMBIENT (NEAR 400 µMOL MOL^{-1}) AND ELEVATED (600 µMOL MOL^{-1}) CO_2 CONCENTRATIONS, AND WITHOUT AN OPEN-TOP CHAMBER (CONTROL).....96

TABLE A.8. CONCENTRATION OF FREE AMINO ACIDS (µMOL G^{-1} DM) IN THE CROWNS OF ACCLIMATED ALFALFA AND TIMOTHY GROWN IN OPEN-TOP CHAMBERS UNDER AMBIENT (NEAR 400 µMOL MOL^{-1}) AND ELEVATED (600 µMOL MOL^{-1}) CO_2 CONCENTRATIONS, AND WITHOUT AN OPEN-TOP CHAMBER (CONTROL).....97

LIST OF ABBREVIATIONS

ADF - Acid detergent fibre
ADL - Acid detergent lignin
C - Carbon
Ca - Calcium
CP - Crude protein
[CO_2] - Carbon dioxide concentration
DM - Dry matter
HDP - High degree of polymerization
IVTD - *In vitro* true digestibility
K - Potassium
LDP - Low degree of polymerization
Mg - Magnesium
N - Nitrogen
NDF - Neutral detergent fibre
NDFd - Neutral detergent fibre digestibility
OTC - Open-top chamber
P - Phosphorus
PAR - Photosynthetically active radiation

ABSTRACT

The rise in atmospheric carbon dioxide concentration ([CO_2]) is expected to have significant effects on alfalfa and timothy, two forage crops widely grown as a mixture throughout Canada. In this study, we designed and built an open-top chamber system that was used to study the effects of elevated [CO_2] on the yield, nutritive value, fall organic reserve accumulation and root degradability of an alfalfa-timothy mixture. After two growing seasons of operation, the OTC system proved to be efficient at maintaining an elevated [CO_2] with minimal alteration of growing conditions. The yield of the alfalfa-timothy mixture increased in response to elevated [CO_2], particularly during the warmest months of the growing season. The nutritive value of the mixture was decreased through a slight reduction in total nitrogen concentration, a significant increase in fibre constituents and slightly reduced digestibility. Elevated [CO_2] did not affect fall organic reserve accumulation measured during cold acclimation. However, root degradability was significantly increased in alfalfa, indicating potentially reduced soil carbon storage under future conditions. This study validates the use of an OTC system as an effective and relatively inexpensive way to study the long-term effects of elevated [CO_2] on plants.

RÉSUMÉ

L'augmentation de la concentration en dioxide de carbone (CO_2) pourrait affecter la croissance de la luzerne et de la fléole, deux plantes fourragères généralement cultivées en mélange à travers le Canada. Dans cette étude, un système d' « open-top chambers » a été mis au point afin d'étudier les effets de l'augmentation de la concentration en CO_2 sur le rendement, la valeur nutritive, l'accumulation des réserves et la dégradabilité des racines d'un mélange pérenne composé de luzerne et de fléole des prés. Le système, après avoir été en fonction pendant deux saisons de croissance, s'est montré efficace à maintenir une concentration élevée en CO_2, tout en maintenant des conditions de croissance semblables à celles en champs. Le rendement du mélange a augmenté en réponse à l'augmentation du CO_2, plus particulièrement durant les mois les plus chauds de la saison de croissance. La valeur nutritive du fourrage a été diminuée en raison d'une légère baisse du contenu en azote totale, d'une augmentation significative du contenu en fibres et d'une légère baisse de la digestibilité. L'augmentation du CO_2 n'a toutefois pas affecté l'accumulation des réserves à l'automne chez les plantes. Cependant, la dégradabilité des racines de luzerne a été augmentée significativement, indiquant une possible diminution du carbone du sol sous des conditions climatiques futures. Cette étude valide l'utilisation d' « open-top chambers » comme un moyen efficace et relativement peu coûteux pour l'étude à long terme des effets du CO_2 sur les plantes.

ACKNOWLEDGMENTS

First of all, I would like to thank my M.Sc. supervisor Dr. Philippe Seguin for offering me the opportunity to work on this appealing project, as well as for his guidance and constructive comments. Also, I sincerely wish to thank my co-supervisor Dr. Annick Bertrand, for welcoming me into her research team and for all her guidance throughout all phases of project design, implementation and analysis. I am also grateful for the advice and recommendations of Dr. Gilles Bélanger, Dr. Yves Castonguay, Dr. Gaëtan F. Tremblay, Dr. Vern Baron and Dr. Alan Watson.

I thank Erik Hellou from Cryotech for his assistance in designing the CO_2 control system. I also wish to extend my warmest thanks to everyone from the Soils and Crops Research and Development Centre in Québec City who provided technical assistance in building the chambers, helped transplanting and harvesting the plants, and provided laboratory assistance. I would especially like to express my gratitude to Josée Bourassa, whose technical advice and assistance were invaluable to the project, as well as Mario Laterrière, Lucie Lévesque, Danielle Mongrain and Gabriel Lévesque. I also appreciate the contributions of Dr. Hélène Crépeau from Université Laval for her assistance in statistical analyses. Finally, I owe thanks to Dr. Vern Baron, Lacombe Research Center, AB, and all staff members and students who contributed their time and labor to the successful installation of the second experimental site.

CONTRIBUTION OF AUTHORS

The thesis was written in the form of manuscripts. The first manuscript was published in Agronomy Journal and was co-authored by the candidate; Dr. Annick Bertrand, Josée Bourassa, Dr. Gilles Bélanger, Dr. Yves Castonguay, Dr. Gaëtan F. Tremblay, Dr. Vern Baron and Dr. Philippe Seguin. The candidate was the primary author of the manuscript and built and operated the open-top chambers, performed all data collection, compiled and analyzed the results and wrote the manuscript. Dr. Annick Bertrand provided the funds, access to research facilities and equipment, supervision, as well as revised the manuscript. Josée Bourassa provided assistance in designing and building the open-top chambers. Additional input and direction was provided by Dr. Gilles Bélanger, Dr. Yves Castonguay, Dr. Gaëtan F. Tremblay, Dr. Vern Baron and Dr. Philippe Seguin who also reviewed and edited the manuscript.

The second manuscript was co-authored by the candidate; Dr. Annick Bertrand, Dr. Gilles Bélanger, Dr. Yves Castonguay, Dr. Gaëtan F. Tremblay and Dr. Philippe Seguin. The candidate was the primary author of the manuscript, performed all data collection, compiled and analyzed the results and wrote the manuscript. Dr. Annick Bertrand provided the funds, access to research facilities and equipment, supervision, as well as revised the manuscript. Dr. Gilles Bélanger and Dr. Yves Castonguay reviewed and edited the manuscript. Dr. Gaëtan F. Tremblay provided access to research facilities and equipment as well as reviewed and edited the manuscript. Dr. Philippe Seguin reviewed the manuscript and provided feedback.

CHAPTER 1

GENERAL INTRODUCTION

Tame hay ranks third in terms of acreage in Canada, with 19% of the agricultural area being devoted to that crop, behind wheat and canola (Statistics Canada 2014b). In Québec, the proportion is 38% (ISQ 2014), highlighting the economic importance of dairy and beef productions. Indeed, the main output of grasslands is forage fibre, which is a major constituent of dairy and beef cattle's diet. The latter typically ingest a greater proportion of their ration as forages, as opposed to lactating dairy cows which have higher energy requirements, generally filled from a combination of forages and supplements (Kawas et al. 1991). In Canada, the dairy and beef industries account for 5.9 and 6.8 billion dollars in farm cash receipts, respectively (Statistics Canada 2014a).

Climatological models predict that the atmospheric carbon dioxide concentration ($[CO_2]$) could reach up to 1000 μmol mol^{-1} by the end of the century, combined with an average annual temperature increase of 2.1 to 3.1^{o}C over eastern North America (IPCC 2013). Those altered environmental conditions will affect plant growth. In fact, Campbell and Stafford Smith (2000) report an average grassland yield increase of 17% in ecosystem-based experiments as a result of CO_2 doubling, although elevated temperature may reduce or cancel out this productivity improvement. Doubling the $[CO_2]$ may also alter the dynamics of species by favouring legumes, and decrease the nutritive value through a lower nitrogen concentration (Campbell and Stafford Smith 2000). Feeding lower nutritive value forage might reduce the economic viability of dairy and beef productions, by increasing supplementation costs in order to maintain milk or meat production per animal. Additionally, the winter survival of forage crops might be decreased by warmer climate conditions (Bélanger et al. 2002).

Little work has been done studying the effects of climate change on forages under field settings despite increasing concerns about climate change and their effects on plants and consequently ruminant animals. Several studies on the effects of climate change were done in controlled-environment growth chambers, which do not take into account the actual growing conditions encountered by field-grown perennial crops. Moreover, these experiments generally investigate individual plant species grown in pots and for a short period of time, which do not reflect natural growing conditions for perennial forage mixtures. On the other hand, field experiments are rare because of the technical difficulties and high costs associated with modifying air composition (Kimball et al. 1997).

1.1 Objectives

- To design and build an efficient and low-cost open-top chamber system.
- To assess the effects of elevated [CO_2] on yield, nutritive value, fall organic reserve accumulation and root degradability of an alfalfa-timothy mixture grown in open-top chambers during two growing seasons in Québec City, QC.

1.2 Hypotheses

Elevated atmospheric [CO_2]:

- Increases the yield of alfalfa-timothy mixtures while decreasing their nutritive value.
- Increases organic reserve accumulation during cold acclimation.
- Increases the potential for carbon sequestration of alfalfa-timothy mixtures.

CHAPTER 2

LITERATURE REVIEW

2.1 Climate Change

Extensive burning of fossil fuels and deforestation since the beginning of the industrial era have released considerable amounts of greenhouse gases in the atmosphere, mainly CO_2, CH_4 and NO_2. Carbon dioxide is the most abundant atmospheric greenhouse gas. Its atmospheric concentration has increased from 280 μmol mol^{-1} at pre-industrial levels (Neftel et al. 1988; Raynaud and Barnola 1985) to 400 μmol mol^{-1} in 2013 (Monastersky 2013). Current projections for future atmospheric [CO_2] range between 400 to 1000 μmol mol^{-1} in eastern North America by the end of the century (IPCC 2013). Some plant species may respond to elevated [CO_2] by increasing photosynthetic rate (Xu et al., 2014) and decreasing stomatal conductance (Bunce 2004), leading to increased biomass production and water use efficiency.

Furthermore, greenhouse gases absorb and re-emit infrared radiations back to the Earth, causing a global warming of the planet (IPCC 2013). Consequently, the Intergovernmental Panel on Climate Change predicts that the average annual air temperature could increase by 2.1 to 3.1^{o}C by 2100 in eastern North America (IPCC 2013). Increased temperature is generally negatively correlated with yield and nutritive value of forages, particularly cool-season grasses such as timothy (Bertrand et al. 2008).

Precipitations could also increase slightly throughout the year in eastern North America, but in the western part of the continent the increase could be limited to the winter (IPCC 2013). A warmer winter climate coupled with more precipitations in the form of rain could increase alfalfa mortality through a lack of snow cover, soil heaving and ice encasement (Bélanger et al. 2002).

Another major effect of climate change is that climate extremes, such as heat waves and flash rains, are expected to become more frequent (IPCC 2013), which could periodically affect forage growth during the summer (Jing et al. 2013).

2.2 Field Studies

Many controlled-environment studies in growth chambers have investigated the effects of elevated [CO_2] on forage crops recently (Aranjuelo et al. 2009; Bertrand et al. 2007b; Piva et al. 2013; Xu et al. 2014; Yu et al. 2012). Lawlor and Mitchell (1991) reports that there is generally a larger number of controlled-environment studies compared to the number of field experiments and that they are usually concentrated on a few crops, especially soybean. This can be explained by the fact that controlled-environment studies provide precise, stable conditions and even allow combining temperature and CO_2 treatments. Clear differences between treatments are generally obtained, but those results cannot be directly extrapolated to an open field due to the natural fluctuating conditions encountered (temperature, light, moisture, nutrients, pests, etc.). Furthermore, controlled-environment studies generally focus on individual species grown in pots during a relatively short time span, while assessing the effects of climate change on plants requires no restriction on the rooting zone volume (Ainsworth et al. 2002) and, for perennials, multiple years.

Although field studies have looked at the effects of gas pollutants such as hydrogen fluoride (Mandl et al. 1973), sulfur dioxide (Weigel et al. 1990), nitrogen dioxide (Adaros et al. 1991), nitrous acid gas (Sakugawa and Cape 2007), ozone (Heagle et al. 1979) and carbon dioxide (Drake 2014) on plants, reliably and effectively modifying the air composition in open air still represents a technical challenge. Many systems have been designed to serve that purpose, but open-top chambers (OTCs) and free-air carbon dioxide enrichment (FACE) are the two

systems mostly used. Open-top chambers consist in small chambers with an inside volume restricted to a few cubic meters, generally equipped with a fan that blows air mixed with CO_2. On the other hand, FACE experiments generally involve a large unrestricted area (over 10 meters in diameter) where CO_2 is injected from the sides right above canopy level (Long et al. 2006).

The main advantage of FACE studies resides in the absence of a chamber effect. However, their wide unprotected area requires continuous injection of large quantities of CO_2, which greatly increases the cost of the project. On the other hand, OTCs are low-cost structures (Ashenden et al. 1992), portable (Drake et al. 1989), adaptable to most plants and even trees (Barton et al. 2010) and allow controlling air temperature inside the chamber much more easily than FACE experiments (Kimball et al. 1997). Nonetheless, OTCs do alter the growing environment in several ways, although these alterations vary depending on the conception of the chamber, as well as on the materials used for the frame and cover (D'Andrea and Rinaldi 2010). First, chamber intercept rainfall and light, and plant watering may be necessary (Cheesman and Winter 2013). The cover's attenuation of solar radiation typically ranges between -30 to -10% compared to the outside. Second, air temperature inside the OTC is usually 0.5 to 2.5^{o}C higher than outside (Kimball et al. 1997). Maintaining strong ventilation within the OTC may result in smaller temperature differences (Leadley and Drake 1993), although a continuous air flow produced by the fan renders air flow constant compared to what is found outside (Drake et al. 1989). In addition, walls provide a physical barrier against wind and thus modify the movement of air inside the chamber. They also produce an enclosed environment which limits the movement and favors the proliferation and of insect populations and diseases (Long et al. 2006).

Open-top chambers of various designs, shapes, sizes and materials have been built, but they are most commonly cylindrical or hexagonal, between 0.8 to 4.5 meters in diameter and

with varying heights depending on the plant species under study. The frame is generally built out of aluminum, steel or wood and covered with transparent PVC, plexiglas, polyester film or plastic (D'Andrea and Rinaldi 2010; Kimball et al. 1997; Leadley and Drake 1993). Wind incursions can cause a reduction and more variability in the [CO_2] within the chamber (Ashenden et al. 1992). Since all OTCs aim to maintain a fairly stable [CO_2] for the plants, it is very common to add a frustum, which consists in a piece of cover material angled at 45^o at the top of the chamber. Although it further alters the growing conditions by intercepting light and rainfall as well as increasing air temperature within the chamber (Kimball et al. 1997), it does help maintaining [CO_2] closer to target during windy days (Drake et al. 1989). The height of the chamber also helps maintaining adequate [CO_2] near canopy level (Ashenden et al. 1992).

2.3 Forage Mixtures

Alfalfa *(Medicago sativa* L.) and timothy (*Phleum pratense* L.) are two forage species widely grown throughout Canada. In Québec, they represent two out of three of the most sold forage species across the province (Allard et al. 1998). Also named "Queen of Forages", alfalfa is widely recognized for its ability to produce high yields and excellent forage quality. It also benefits from a symbiosis with nitrogen-fixing *Sinorhizobium* bacteria and from a deep taproot, conferring an advantage over other species to reach deeper soil moisture reserves (Michaud et al. 1988). On the other hand, timothy is a bunchgrass sensitive to drought and hot weather, but benefits from good winter hardiness, making it most productive in northern areas where cool and moist conditions prevail (Berg et al. 1996).

In order to benefit from the advantages of both species, alfalfa and timothy are often grown as mixtures rather than pure stands. Forage mixtures are very common throughout Canada. Bélanger et al. (2014) reported that they provide greater yields, optimized nutritive

value and reduced weed proportions in the forage mixture as compared to individual species. Grass-legume mixtures can yield more nitrogen than legume pure stands due to natural stimulation of nitrogen uptake from symbiotic and non-symbiotic sources (Nyfeler et al. 2011). Nitrogen fertilizer savings can reach up to 100 kg N ha^{-1} compared to a pure grass stand (Malhi et al. 2002).

2.4 Forage Growth

Cattle-raising operations require high forage yields in order to maximize milk or meat production per unit area. Since growing conditions will be altered by climate change, it is important to examine how it could affect the photosynthesis and yield of alfalfa-timothy mixtures and of their individual components.

2.4.1 Photosynthesis

Plants use atmospheric CO_2 for photosynthesis, a reaction catalyzed by the enzyme Rubisco. Under current atmospheric [CO_2], Rubisco is not saturated in C_3 plants, and the oxygenation reaction leading to photorespiration is favoured. Thus, the predicted rise in atmospheric [CO_2] will increase the [CO_2] within chloroplasts as well as the rate of carboxylation, which will stimulate photosynthesis (Williams et al. 1995). Nevertheless, temperatures above the optimum may inhibit carboxylation and promote the oxygenation reaction and photorespiration (Tingey et al. 2003). On the other hand, C_4 species have structures that increase the internal [CO_2] up to several times that of the ambient, which prevents a direct stimulation of photosynthesis and renders C_4 plants less responsive to atmospheric [CO_2] increase (Long et al. 2006).

2.4.1.1 Mixtures

There are very few experiments examining the net photosynthesis of forage mixtures. One of the difficulties comes from the fact that estimating the photosynthesis of a canopy has to account for lit and shaded leaves (Long 1991). In an OTC experiment examining the growth of natural plant communities, the net ecosystem CO_2 exchange per unit of green biomass was estimated to an average of +48% under elevated [CO_2] (Drake and Leadley 1991). Although it gives an idea of the performance of the mixture as a whole, individual responses of each species of the mixture remain unknown.

2.4.1.2 Alfalfa

As a C_3 legume, alfalfa has a greater photosynthetic response than C_3 grasses (Poorter 1993). In a controlled-environment experiment, photosynthesis was stimulated up to 50% as a result of [CO_2] doubling (Erice et al. 2006a). In that experiment, it was also observed that the greatest stimulation of photosynthesis happens after cutting, in response to a high root:shoot ratio and respiration, a situation that creates a strong sink for photosynthates. This effect was, however, not permanent. Alfalfa generally acclimates to elevated [CO_2] by the end of the vegetative growth period, as shown by a reduction of photosynthesis stimulation (Ainsworth et al. 2004). Elevated temperatures have been shown to further decrease alfalfa photosynthetic rate by reducing nitrogen fixation (Sanz-Sáez et al. 2013).

Photosynthesis of alfalfa generally increases with temperature under ambient [CO_2]. In an experiment, the highest rate of photosynthesis was attained at 30^{o}C, with an optimum between 25 and 30^{o}C (Brown and Radcliffe 1986), which corresponds to the optimum growing temperature of alfalfa reported in Greenfield and Smith (1973). However, under elevated [CO_2], photosynthesis of alfalfa was significantly increased at 15 and 20^{o}C, but not at higher

temperatures of 25 and 30°C, showing a strong interaction between these two factors (Ziska and Bunce 1994).

2.4.1.3 Timothy

In an eleven-year FACE experiment examining the response of 13 plants species to elevated [CO_2], five C_3 grasses maintained a 9% increase in leaf photosynthesis (Lee et al. 2011). A higher stimulation of leaf photosynthesis by elevated [CO_2] was observed under controlled conditions in timothy with 18% and 28% at first and second harvest, respectively (Piva et al. 2013). However, it was observed in a recent meta-analysis that the increase in biomass and grain yield of C_3 crops as a response to elevated [CO_2] is generally lower than the stimulation of photosynthesis (Bishop et al. 2014), which was also observed by Piva et al. (2013).

The photosynthetic response of crops exposed to elevated temperatures depends on the species. According to Murata and Iyama (1963), among twelve crops under study, crops belonging to the "northern type" generally had an optimum temperature for photosynthesis between 10 and 15°C and the photosynthetic rate decreased rapidly above that range. In fact, higher temperatures generally raise the optimum temperature for photosynthesis of C_3 plants, but also induce photorespiration, which results in a decline in CO_2 exchange rate (Salvucci and Crafts-Brandner 2004). However, this does not seem to always be the case since photosynthesis of timothy was found to be higher when grown under 25/15°C than 22/10°C day/night temperature (Piva et al. 2013). Furthermore, elevated [CO_2] combined with a temperature of 15, 20 and 25°C did not increase the photosynthetic rate of orchardgrass (*Dactylis glomerata* L.), but a lower photosynthetic rate was observed at 30°C compared to the other temperature treatments (Ziska and Bunce 1994). Similar results were also observed in a meta-analysis examining the

effects of elevated [CO_2] at normal, elevated and heat stress temperatures on other grass species (Wang et al. 2012).

2.4.2 Forage Yield

Forage yield is an important factor affecting net farm income, since it determines the number of animals that can be fed from a unit area. Since alfalfa-timothy mixtures represent a predominant source of forage and an essential part of cattle's diet, it is important to examine their response to climate change conditions.

2.4.2.1 Mixtures

Elevated [CO_2] concentrations are generally considered to stimulate aboveground growth of grasslands by 17% on average, as outlined in two reviews (Ainsworth and Long 2005; Campbell and Stafford Smith 2000). However, C_3 grasses and C_3 legumes do not respond the same way to elevated [CO_2]. In fact, C_3 nitrogen-fixing species generally respond more to elevated [CO_2] than other C_3 species because of their large sink strength (Ainsworth and Long 2005; Poorter 1993). This has been shown to alter mixture composition by significantly increasing the percentage of legumes (Hebeisen et al. 1997; Ross et al. 2004; Teyssonneyre et al. 2002). On the other hand, in response to elevated temperature, the biomass yield of perennial ryegrass (*lolium perenne* L.) was shown to increase in the spring and fall while it decreased during summer (Casella et al. 1996).

2.4.2.2 Alfalfa

Alfalfa usually shows a strong yield improvement when exposed to elevated [CO_2]. For instance, alfalfa yield increased by 50% at 600 μmol mol^{-1} compared to 350 μmol mol^{-1} in a FACE experiment in Switzerland (Lüscher et al. 2000). Similar yield increases were obtained under elevated [CO_2] and temperatures ranging from 15 to 30^{o}C in a controlled-environment

experiment (Ziska and Bunce 1994). However, Vough and Marten (1971) observed that alfalfa reached maturity earlier at 27/21^{o}C day/night temperatures compared to 16/10^{o}C, thereby reducing the cutting interval and decreasing yields. Also, the maximum growth rate of alfalfa is attained under day/night temperatures not exceeding 27/18°C (Greenfield and Smith 1973), above which a yield decrease generally results.

2.4.2.3 Timothy

C_3 grasses, like timothy, do not usually respond markedly to elevated [CO_2]. In fact, in a growth chamber experiment, timothy grown under an elevated [CO_2] of 600 µmol mol^{-1} had the same yield during two cuts than under an ambient [CO_2] of 400 µmol mol^{-1} (Piva et al. 2013). In another experiment, elevated [CO_2] reduced the growth of timothy in two out of three cuts (Sæbø and Mortensen 1995). The absence of biomass response from elevated [CO_2] in timothy is not an isolated case among C_3 grasses, since no yield stimulation was obtained from five out of seven grass species (Sæbø and Mortensen 1996). However, as a cool-season grass, timothy is very sensitive to day/night temperatures above 17/5^{o}C, which significantly decrease its yield (Bertrand et al. 2008). This could render timothy susceptible to predicted temperature increases. Combined increases in temperature and [CO_2] had no effect on timothy yield, showing that the adverse effect of temperature was compensated by elevated [CO_2] (Piva et al. (2013).

2.5 Nutritive Value

Forage nutritive value is the corner stone of dairy and beef productions, as it is essential to sustain high milk production and body weight gain. By increasing photosynthesis of C_3 forage crops, elevated [CO_2] will likely affect the concentrations of non-structural carbohydrates, nitrogen and fibre, as well as the digestibility of the forage.

2.5.1 Non-Structural Carbohydrates

Non-structural carbohydrates (NSC) are highly digestible and contribute to increase the nutritive value of the forage. The main NSC are starch, sucrose and fructans, which are all storage molecules (Moore and Hatfield 1994). Starch is the main storage molecule in a wide range of plants including legumes, whereas sucrose plays a leading role in carbohydrate transport and storage and is also involved in starch synthesis. Fructans are mainly found in temperate grasses and cereals as a storage molecule (Pollock 1986). In the rumen, NSC are quickly degraded to simple sugars and transformed into volatile fatty acids, which serve as an energy source for the cows (Moore and Hatfield 1994). A high NSC concentration in forages promotes feed intake, milk production and nitrogen-use efficiency in dairy cattle (Brito et al. 2008).

2.5.1.1 Mixtures

Very few experiments, if any, have measured the carbohydrates concentration of forage mixtures in response to elevated [CO_2] or temperature, but it could be expected that each species will contribute differently to the total NSC concentration of the mixture.

2.5.1.2 Alfalfa

Elevated [CO_2] typically induces an accumulation of starch (Erice et al. 2006b) and total soluble sugars and starch (Sanz-Sáez et al. 2010) in alfalfa. It has been suggested that increased plant carbohydrates concentration stimulates the symbiosis with nitrogen-fixing rhizobium, as commonly observed under elevated [CO_2], which creates a new carbon sink and prevents photosynthesis acclimation (Irigoyen et al. 2014). However, this response could depend on the rhizobial strain in association with alfalfa as has been shown in Bertrand et al. (2007a) and Sanz-Sáez et al. (2012b). Also, Smith (1969) observed that alfalfa grown under 18/10^{o}C

day/night temperature had significantly higher sugar, starch and NSC concentrations compared to 32/24^{o}C.

2.5.1.3 Timothy

Grasses exposed to elevated [CO_2] tend to accumulate NSC. As a matter of fact, fructans concentration in grasses grown under elevated [CO_2] was found to reach several times that of ambient [CO_2] (Casella and Soussana 1997; Piva et al. 2013; Read et al. 1997). The accumulation of fructans originates from the conversion of sucrose molecules into storage molecules under elevated [CO_2] (Read et al. 1997).

Piva et al. (2013) observed that soluble sugars, fructans and starch were decreased with elevated temperature, but only at the second cut. Similarly, Smith (1968) grew timothy under 29.5/21^{o}C and 18.5/10^{o}C day/night temperature and reported a higher concentration of NSC under cooler temperature, which was mainly the result of increased fructans concentration. An opposite trend was observed in Bertrand et al. (2008) as timothy grown at 28/15^{o}C day/night temperature had a higher sugar concentration compared to 22/10^{o}C and 17/5^{o}C, which was explained as a response to temperature stress inducing growth cessation.

2.5.2 Nitrogen Concentration

Protein is one of the most important nutrient for milk, muscle and wool production (Minson 1990). It is generally expressed in terms of crude protein (CP), which is the sum of true protein and non-protein nitrogen. Crude protein concentration is positively affected by nitrogen fertilization (Buxton 1996) and is closely linked with N concentration in forages. Legumes contain substantially more CP than grasses (Minson, 1990). However, CP concentration decreases with plant maturity irrespective of the species (Buxton 1996).

2.5.2.1 Mixtures

It is generally recognized that elevated [CO_2] decreases plant nitrogen concentration. In a meta-analysis examining 75 scientific articles, Cotrufo et al. (1998) observed that the reduction in nitrogen concentration is lower in legumes and C_4 plants (7%) compared to C_3 plants (16%). In fact, in an OTC experiment evaluating the growth of white clover and perennial ryegrass mixtures grown in pots, Schenk et al. (1997) reported a significant decrease in the nitrogen concentration of the grass component in response to elevated [CO_2], whereas the clover and the mixture did not experience any significant change.

2.5.2.2 Alfalfa

Elevated [CO_2] has been shown to reduce the plant nitrogen concentration in alfalfa (Bertrand et al. 2007b; Erice et al. 2006b; Sanz-Sáez et al. 2012b; Sanz-Sáez et al. 2010; Ziska and Bunce 1994). This response is attributed to the translocation of nitrogen from the stems to the increased rooting system rather than to a dilution effect (Cotrufo et al. 1998; Sanz-Sáez et al. 2012b). In fact, plants modify nitrogen allocation to optimize their energy costs, which increases their nitrogen use efficiency under elevated [CO_2] (Xu et al. 2013).

On the other hand, elevated temperatures generally increase CP concentration, which was found to be higher in alfalfa grown under 32/24^{o}C than 18/10^{o}C day/night temperature (Smith 1969). Smith (1970) observed that the day/night temperature at which protein concentration is maximized in alfalfa is 27/21^{o}C, and suggested that temperatures too far from this optimum do not favour protein accumulation. The combination of elevated [CO_2] and temperature had an opposite effect and significantly decreased total leaf soluble protein during vegetative growth of alfalfa (Erice et al. 2006b).

2.5.2.3 Timothy

In an OTC experiment in a shortgrass steppe in Colorado, two C_3 grasses showed a clear reduction in CP concentration, which was not compensated for by the increase in protein yield under elevated [CO_2] (Milchunas et al. 2005b). Also, as a cool-season grass, timothy exhibits a clear decrease in nitrogen concentration and, as such, in CP concentration, with temperature increase (Bertrand et al. 2008). However, it was observed in other grass species that a small temperature increase of +3^{o}C coupled with elevated [CO_2] could lead to a nitrogen concentration close to what is obtained under ambient [CO_2] and temperature (Seligman and Sinclair 1995; Soussana et al. 1996).

2.5.3 Fibre Concentration and Digestibility

The production of meat and milk from ruminant animals is heavily dependent on the amount of feed they consume, which is related to the concentration of cell wall material (Buxton 1996). Structural carbohydrates refer to plant cell wall constituents, such as cellulose, hemicellulose and lignin (Moore and Hatfield 1994), which are responsible for a plant's ability to stand in an upright position and withstand elements (Wilson 1994). Those three components are also known as neutral detergent fibre (NDF), while acid detergent fibre (ADF) is only composed of cellulose and lignin (Van Soest et al. 1991). Among structural carbohydrates, hemicellulose has the highest digestibility, followed by cellulose and lignin (Moore and Hatfield 1994), which is resistant to digestive enzymes (Van Soest et al. 1991). Also, as plants get mature, lignin, cellulose and hemicellulose increase while proteins and NSC concentrations decrease (Waite 1963), thus reducing the nutritive value and potential intake by animals.

2.5.3.1 Mixtures

Studies examining the effects of elevated [CO_2] on fibre concentration and digestibility of grass-legume mixtures are very few. In an experiment where perennial ryegrass and white clover were grown in a 75:25 mixture under doubled [CO_2], Schenk et al. (1997) observed a decreased crude fibre concentration throughout the season for both years of the study. More importantly, it fell below the minimal requirements for ruminant animals during some harvests, which could negatively affect animal health.

2.5.3.2 Alfalfa

A wide range of variation in the fibre constituents and digestibility of alfalfa grown under elevated [CO_2] was observed in controlled-environment experiments, which highlights the importance to conduct more studies in that area. Some of the resulting differences may come from the rhizobium strains in symbiosis with alfalfa, which could affect photosynthetic stimulation under elevated [CO_2] (Bertrand et al. 2007b; Sanz-Sáez et al. 2012b). However, no studies were undertaken to verify this effect under field conditions.

Smith (1969) observed that alfalfa grown under 18/10^{o}C day/night temperature had a higher digestibility but similar fibre concentration compared to 32/24^{o}C. Similarly, Vough and Marten (1971) reported higher digestibility and lower ADF and acid detergent lignin (ADL) in alfalfa grown at 16/10^{o}C compared to 27/21^{o}C day/night.

2.5.3.3 Timothy

Elevated [CO_2] seems to have a limited effect on fibre constituents in grasses. Barbehenn et al. (2004) reported an increase in NDF concentration in only two out of five grasses studied under elevated [CO_2]. In OTC experiments, Newman et al. (2003) and Milchunas et al. (2005b) reported a small decrease in the lignin concentration of tall fescue and three shortgrass steppe

species, respectively. On the other hand, the digestibility of grasses grown under elevated [CO_2] seems to be species specific. In an experiment where perennial ryegrass was grown at twice ambient [CO_2] in OTCs, no effect on digestibility was observed (Jones et al. 1996). In contrast, in another OTC experiment where shortgrass steppe was grown under doubled [CO_2], Milchunas et al. (2005b) observed a 14% and 10% reduction in digestibility during summer and fall, respectively.

As a cool-season grass, the digestibility of timothy is negatively affected by increasing temperature, although fibre constituents may or may not be affected (Bertrand et al. 2008; Thorvaldsson et al. 2007).

2.6 Organic Reserve Accumulation and Winter Survival

The predicted rise in temperatures and precipitations during winter under climate change could become problematic for forage survival. Decreased fall hardening, snow cover and cold hardiness could increase population losses due to winterkill (Bélanger et al. 2002). Those changes may have a negative effect on alfalfa persistence and may alter the composition of alfalfa-based mixtures over time under future climate change conditions. In fact, increased variability in weather events during the winter could favor species more resistant to ice encasement, such as timothy, over alfalfa (Gudleifsson 2010). Moreover, elevated atmospheric [CO_2] concentrations were found to decrease the freezing tolerance of native temperate grassland (Obrist et al. 2001), alfalfa (Bertrand et al. 2007a) and meadow fescue (Jurczyk et al. 2013), which could shorten the lifespan of forage mixtures.

2.6.1 Carbohydrates Reserves

Poorter et al. (1997) found that the most important biochemical change in plants grown under elevated [CO_2] is the increase in NSC concentration, which could increase the freezing

tolerance of perennial plants. For instance, during the cold acclimation period in the fall, starch is converted to sucrose and other soluble sugars that accumulate in roots and crowns. This has crucial implications for perennial plants, since some soluble sugars, such as sucrose, stachyose and raffinose play a protective role against freezing at the cell level (Castonguay et al. 1995). Furthermore, their accumulation is linked to freezing tolerance in perennial forages (Castonguay et al. 1995).

2.6.1.1 Alfalfa

Bertrand et al. (2007a) did not observe a difference in sugar accumulation in crowns of alfalfa grown under elevated and ambient [CO_2]. It was concluded that alfalfa grown under elevated [CO_2] did not have a higher potential of winter survival through the accumulation of carbohydrate reserves. In fact, it was observed that elevated [CO_2] increased the metabolic rate of alfalfa acclimated at 2^{o}C, resulting in postponed cold acclimation and reduced cold tolerance. A similar observation was made in black spruce seedlings exposed to 1000 μmol mol^{-1} CO_2 (Margolis and Vézina 1990).

2.6.1.2 Timothy

Leaf freezing tolerance decreased in five native grasses exposed to six years of CO_2 enrichment at 600 μmol mol^{-1}, despite the fact that the concentration of soluble sugars, starch and NSC increased significantly (Obrist et al. 2001). Thus, the link between sugar accumulation and freezing tolerance may be more complex than initially thought, and elevated [CO_2] may in fact increase freezing damage to plants. However, elevated [CO_2] did not affect the freezing tolerance of winter wheat grown in OTCs, while elevated temperature (+2.5^{o}C) decreased the concentration of total sugars as well as freezing tolerance (Hanslin and Mortensen 2010). On the

other hand, the NSC concentration of grasses grown in OTCs in an alpine meadow of the Tibetan Plateau was not affected by warming (+0.83^{o}C) (Shi et al. 2015).

2.6.2 Nitrogen Reserves

Nitrogen reserves such as soluble proteins and total free amino acids, including proline, arginine and asparagine, accumulate in alfalfa taproots during the cold acclimation process (Dhont et al. 2003) and decline as they are used up for shoot growth in the spring (Dhont et al. 2006). Consequently, alfalfa plants with low concentrations of nitrogen reserves grow slower in the spring and generally do not withstand competition from other plants (Volenec et al. 1996). Few studies report the effect of atmospheric [CO_2] on the accumulation of amino acids in perennials. Bertrand et al. (2007a) reported a lower accumulation of total free amino acids and of proline, an amino acid linked with the acquisition of freezing tolerance, in alfalfa grown under elevated than ambient [CO_2]. It was concluded that this lower accumulation of nitrogen reserve could have a negative impact on freezing tolerance.

2.7 Root C:N ratio and Decomposition Rate

Perennial forages have the capacity to sequester carbon in their long-lived rooting systems. Alfalfa is widely known for its deep taproot, whereas timothy has a fibrous rooting system. Elevated [CO_2] has been shown to increase the C:N ratio in roots of perennials (Luo et al. 2006) and, as such, to decrease their decomposition rate (Silver and Miya, 2001). By this mechanism, elevated [CO_2] could affect the carbon sequestration potential of perennial forages.

2.6.1 Alfalfa

In an experiment using a root ingrowth bag technique, Jongen et al. (1995) observed that there was no difference in the C:N ratio of white clover root material grown under elevated than under ambient [CO_2]. However, the relative decomposition of that material was 1.8 to 3.6 times

lower under elevated [CO_2], depending on the nitrogen fertilization regime. Those results highlight legumes potential to store carbon in the soil through decreased root turnover, and may suggest that changes in lignin chemistry take place under elevated [CO_2] (Bertrand et al. 2006; Talbot et al. 2012).

2.7.2 Timothy

In an experiment examining the growth of timothy under elevated [CO_2] in controlled-environment chambers, Bertrand et al. (2014) observed a decrease in the nitrogen concentration of timothy roots as well as an increase in the C:N ratio, which translated into a lower root degradability assessed by IVTD. Similar results were observed in shortgrass steppe by Milchunas et al. (2005c) and in perennial ryegrass by Jongen et al. (1995), although root decomposition rate was slightly increased in the latter.

On the other hand, a day/night temperature of 25/15^{o}C compared to 22/10^{o}C increased the root nitrogen concentration and decreased the C:N ratio in the second harvest of timothy, with a negative effect on root degradability (Bertrand et al. 2014). Higher nitrogen concentration was also observed in an experiment with perennial ryegrass grown under elevated temperature (+3^{o}C) (Soussana et al. 1996).

CONNECTING TEXT FOR CHAPTER 3

Rising greenhouse gases concentrations will affect growing conditions for plants. Despite increasing preoccupations about predicted climate change and the key importance of forages in ruminant nutrition, few studies have examined the effects of elevated [CO_2] and climate on forage crops in field settings. The main difficulty comes from the inherent difficulty to modify air composition and temperature in open air. In order to expand research in this area, it is important that the experimental systems designed to study plant response in the field are relatively inexpensive to build and operate, while achieving a stable [CO_2]. The following chapter was published in Agronomy Journal (Messerli et al. 2015) and describes the construction and performance of the open-top chamber system that was used to study the growth of an alfalfa-timothy mixture under elevated [CO_2].

CHAPTER 3

PERFORMANCE OF LOW-COST OPEN-TOP CHAMBERS TO STUDY LONG-TERM EFFECTS OF CARBON DIOXIDE AND CLIMATE UNDER FIELD CONDITIONS

3.1 ABSTRACT

The increase in atmospheric carbon dioxide concentration ([CO_2]) and consequent increase in air temperature is expected to have significant effects on plant growth and nutritive value. Studies examining the effects of elevated [CO_2] on plants under field conditions have been limited by the inherent difficulty to modify air composition in open air. Here we describe an efficient and inexpensive open-top chamber (OTC) system designed to study the effects of elevated atmospheric [CO_2] and temperature on perennial alfalfa-timothy mixture. The design and construction of these OTCs are described in detail, along with cost estimation for each component. Eight OTCs, each with 1.2 m^2 of ground area (four with elevated [CO_2] and four with ambient [CO_2]) were fabricated and four control plots of the same dimension were established to assess the chamber effects on plant responses to CO_2. The [CO_2] in elevated-CO_2 chambers fell 93% of the time within ± 20% of the targeted 600 μmol mol^{-1} CO_2, based on 10 minute averages. The CO_2 consumption in elevated-CO_2 chambers averaged 3.0 kg CO_2 m^{-2} day^{-1}. To ensure that the environment within OTCs was similar to the surrounding field, growing conditions were determined in all chambers and control plots. Adequate light transmission was observed compared to control plots (93%) and the temperature increase was 0.7°C on average. After two growing seasons of continued use, this system has proven its effectiveness for studying the effects of CO_2 and climate change in the field at low cost.

3.2 INTRODUCTION

Human activities are increasing the [CO_2], which could reach between 400 to 1000 μmol mol^{-1} by the end of the century and lead to an annual temperature increase between 2.1 to 3.1^{o}C across Eastern North America (IPCC 2013). Some plant species may respond to elevated [CO_2] by increasing photosynthetic rate, leading to increased biomass production in the absence of other climatic constraints, such as drought or other extreme events (Xu et al. 2014). However, in the long term, plants may acclimate to elevated atmospheric [CO_2] and undergo photosynthesis down-regulation (Bloom et al. 2010). Moreover, in a meta-analysis examining the effects of elevated [CO_2] and temperature, Wang et al. (2012) observed that plant response varies according to functional type (legume vs non-legume) and growth form (herbaceous vs woody). Thus, changes in climatic conditions will likely affect species dynamics and ecosystems with repercussions on agricultural production, underscoring the need for studies examining plant response to elevated [CO_2] and climate effects.

Some experiments have looked at plant response to elevated [CO_2] and temperature in growth chambers (Piva et al. 2013). However, most growth chamber studies are designed to assess the impact of simulated conditions on individual plant species grown in pots and during a relatively short time span. Even though these experiments effectively assess plant responses, they need to be validated under field conditions without restricting the rooting zone volume (Ainsworth et al. 2002) and, for perennials, over multiple years. Growing plants under modified air composition by the addition of a gas, without further altering growing conditions, represents a challenge. Many systems have been designed to study the impact of gaseous pollutants and greenhouse gases, such as temperature-gradient tunnels and solar domes/glasshouses, but open-top chambers (OTCs) and free-air carbon dioxide enrichment (FACE) are the two systems that

provide a growing environment closest to field conditions. An OTC usually covers a small ground area (generally 1 – 15 m^2) and includes a fan that blows air mixed with CO_2, whereas FACE experiments involve a large open area (generally 100 - 3000 m^2) where CO_2 is continuously injected from the sides at canopy level. The main advantage of FACE over other systems is that they have a limited impact on growing conditions (Long et al. 2006) but the cost of CO_2 and of the control system make the FACE system prohibitive (Kimball et al. 1997). Mini-FACE facilities covering smaller area have also been used, but they have a similar constraint of high CO_2 consumption (Petersen et al. 2001). The OTCs, for their part, are low-cost structures (Ashenden et al. 1992) allowing the study of plant responses to elevated [CO_2] in the field without incurring the high cost of FACE experiments. Moreover, they can easily be adapted to any environment under study. Drake (2014) reported observations from a 28 year experiment using portable OTCs in a marsh subject to irregular flood tides. Barton et al. (2010) used OTCs large enough to accommodate whole trees. In some experiments, cooling/heating systems were added to control temperature within OTCs (Norby et al. 1997).

The last OTC experiment that studied the effects of atmospheric pollution on plants in Eastern Canada stopped functioning in the late 90s (Bertrand et al. 1999). To our knowledge, except for our study, there is currently no OTC experiment with addition of CO_2 in Canada, despite increasing concerns about greenhouses gases and their unpredictable repercussions on climate. This paper describes a low-cost OTC system that was designed to study the effects of elevated [CO_2] and temperature on an alfalfa-timothy mixture grown over a three-year period at an experimental site located in Québec City, QC. Although many OTC designs have been used for a long time, very few have been clearly described in detail with respect to construction, cost, performance and CO_2 consumption. Furthermore, our system takes advantage of recent

technological advances in CO_2 control systems and temperature sensors. The objective was to provide potential users with a simple, low-cost, and easily transposable turn-key OTC design that we adapted for the long-term study of elevated [CO_2] and temperature on perennials under realistic field conditions.

3.3 MATERIALS AND METHODS

3.3.1 Chamber construction

Eight hexagonal OTCs (four with elevated [CO_2] and four with ambient [CO_2]), each with 1.2 m^2 of ground area were built during spring 2013 and placed in a completely randomized design (Fig. A.1). Each side measured 0.65 m (inside) (Fig. 3.1A). The entire chamber height from ground to upper frame was 1 m. The frame was made out of 6 × 3.5 cm treated wood and covered with clear plastic around the outside circumference. The construction began by assembling the upper and lower hexagonal wood plates of the frame (Fig. 3.1B). Then, twelve uprights (0.93 m long) were cut lengthwise at a 30^o angle, assembled and placed vertically between upper and lower plates. A door-frame was placed within one side-panel of the hexagon and mounted on hinges for easier access to the plants. The chambers were anchored on each side by rebar, driven 0.5 m into the soil and attached to a wooden bracket fastened to the OTC frame. A 1 × 4.5 m piece of clear greenhouse plastic treated against adherence of dust particles on the outside and against condensation on the inside, was cut and stapled around the outside of the frame. Thin pieces of wood remaining from cutting the uprights were used as slats and screwed on each corner of the chambers to tighten the plastic. The plastic was removed in the fall to allow snow accumulation during winter, so that normal overwintering conditions occurred. New plastic

was used in the spring. Four control plots without chambers were established by anchoring wooden hexagons in the ground. Their dimensions and management were identical to OTCs.

3.3.2 Ventilation system

Each OTC had its own ventilation system which consisted of a fan (5.66 m^3 min^{-1} Powerventpro, Soler and Palau, Toronto, ON, Canada) placed in a ≈ 50 L plastic mixing box covered with a lid for weather protection. Within this mixing box, the fan mixed pure CO_2, fresh air coming from a grid-protected 10 cm hole drilled in the back of the plastic box, and air backflow from the chamber. These three air sources were located in the back of the fan (Fig. 3.1C). Then, the air was pushed into the OTC at a flow rate of 5.66 m^3 min^{-1}. The air backflow was supplied from a flexible PVC pipe (3.75 cm diameter × 2.15 m) left hanging in the middle of the chamber, 0.6 m from the ground and tightly connected to the back of the mixing box. The flexible PVC pipe also protected the CO_2 sensor, which was placed at the end of the pipe connected to the plastic box. This resulted in measurement of the [CO_2] in the air coming directly from the middle of the chamber. The recirculation of air from the chamber reduced the CO_2 consumption and maintained a stable [CO_2]. Air was ducted from the fan through galvanized pipes (30 cm long, 10 cm diameter), then split through a galvanized "T" connection, and distributed around the circumference of the chamber through 4.15 m long, 12 cm diameter "lay-flat" plastic tube, connected to the "T" using duct tape. The tube, placed at the bottom of each chamber was perforated every 5 cm with 0.5 cm-diameter holes made on two parallel rows along its length to provide an even air distribution around the chamber.

3.3.3 CO_2 control system

Each OTC with elevated [CO_2] had its own CO_2 control system comprised of a sensor-transmitter (0 - 2000 ppm), a power supply, a transformer and a solenoid valve, fixed inside a

PVC electrical box (Fig 3.1D). The target CO_2 concentration was of 600 μmol mol^{-1}. To reach this concentration, a non-dispersive infrared CO_2 sensor (GMP222, Vaisala Oyj, Helsinki, Finland) was connected to a transmitter (GMT220 Carbocap®, Vaisala Oyj, Fig. 3.1D) that switched open a solenoid valve when the [CO_2] was lower than 625 μmol mol^{-1} and closed it as the [CO_2] reached 630 μmol mol^{-1}. These trigger points were identified during preliminary tests made on the CO_2 control systems and were shown to maintain the most stable CO_2 concentration closest to 600 μmol mol^{-1}. Opening and closing the valves at 625 and 630 μmol mol^{-1} respectively account for the slight delays in CO_2 concentration adjustment due to the tubing length (9 m) between control valves and CO_2 probes. Each transmitter was connected to a data logger recording the [CO_2]. A transmitter was installed to measure the [CO_2] inside one ambient-CO_2 chamber. Each OTC with elevated [CO_2] was operated in parallel and each had its own CO_2 supply, in order to easily monitor the CO_2 consumption of each chamber. The CO_2 supply consisted of individual cylinders containing 22.68 kg of pure CO_2 gas (100095, Linde Canada, Mississauga, ON) (Fig 3.1E). Each cylinder had its own regulator (HRF 1425-580, Weldmark, Indianapolis, IN) allowing manual flow adjustment. Nylon tubing was used to carry CO_2 from the cylinders up to the transmitters, and then up to the back of the mixing boxes of the OTCs described earlier. Since the CO_2 sensor wire was not UV resistant, it was inserted, along with the CO_2 tubing, into pipe insulation to ensure its protection. Minimal electricity requirements were needed to run the above-mentioned equipment, as their total amperage was 15 A.

3.3.4 Cost

The total building cost for the eight OTCs on the experimental site was estimated around US $ 14,000 (See Table A.1 for detailed cost of material and equipment). This amount includes equipment for controlling the [CO_2] in four elevated-CO_2 OTCs and monitoring the [CO_2] in one

ambient-CO_2 OTC. However, the total excluded labour and electricity costs as well as the cost of a shed needed to house the CO_2-control equipment, a computer and CO_2 cylinders.

3.3.5 Assessment of environmental conditions within chambers

3.3.5.1 Plant material

The environmental conditions within OTCs were measured in the presence of perennial forages. A total of 330 alfalfa (*Medicago sativa* L.) and timothy (*Phleum pretense* L.) plants were transplanted in July 2013 in a uniformly distributed 50-50% mixture at a total density of 275 plants m^{-2}.

3.3.5.2 CO_2 concentration

The CO_2 concentration within one ambient and within all elevated-CO_2 OTCs was constantly monitored by a CO_2 sensor which analyzed air sampled directly from the middle of each chamber. Data points were recorded at 10 seconds intervals and averaged over 10 minutes.

3.3.5 Air temperature

Air temperature was continuously measured at canopy height in all OTCs and compared to ambient air temperature in control plots throughout the course of the growing season (from July to October 2013 and from May to September 2014) using data loggers (U23-004 HOBO ProV2, Onset, Bourne, MA) at 16 minutes reading interval. The height of the sensor was manually placed at the top of the canopy every week to avoid shading effects from the plants on surrounding air temperature. Canopy height varied from 7 cm after a cut to 90 cm when plants were fully grown.

3.3.6 Light transmission

To assess the difference in incoming light within OTCs and control plots, photosynthetically active radiation (PAR) was measured using a light meter (LI-250A LI-COR,

Lincoln, NE) at three different heights (30, 60 and 90 cm from the ground) on top of the canopy to avoid plant shading. The different heights of measurement correspond to the fact that plants were growing throughout the season and that canopy top reached 30 cm, then 60 cm and finally 90 cm height. Five measurements were made in the middle of each chamber and each control plot between 25 June and 11 August 2014 at three different time periods (AM, from 8:00 to 10:00 h, PM, from 12:00 to 14:00 h and late PM from 16:00 to 18:00h). The goal here was to assess the light interception from chamber framework and plastic only, so within-chamber measurements were compared to control plots.

3.4 RESULTS AND DISCUSSION

3.4.1 Cost

The total estimated cost of US $ 14,000 for 9.6 m^2 of open-top chambers including four with elevated [CO_2] and four with ambient [CO_2] (Table A.1) is similar to earlier reports of OTC cost and performance (Ashenden et al. 1992; Kimball 1992). In the 2000s, FACE experiments were undertaken to study the effects of greenhouse gases on plants. However, the complex infrastructures required in such experiments can be as expensive as US $ 100,000 (Pepin and Körner 2002). Open-top chamber experiments therefore represent a low cost alternative for the study of plant response to elevated [CO_2] in the field.

3.4.2 CO_2 consumption

The cost of the CO_2 supply was estimated at US $ 2,500 from May to September 2014 (153 days). We found that the use of 22.68 kg gas CO_2 cylinders was optimal for our needs. Depending on wind conditions, cylinders had to be replaced every 4-7 days. However, when experimental sites are difficult to reach, the possibility of using a single large liquid CO_2 tank

would be an option that reduces the transport to the site and the cost of CO_2. The average daily CO_2 consumption of 3.0 kg m^{-2} of elevated-CO_2 area reported here compares advantageously with the range of 4.0 – 6.5 kg m^{-2} reported in different FACE designs (Bunce 2011) and is comparable to OTCs of a similar size (Ashenden et al. 1992). The low CO_2 consumption obtained was likely the result of the recirculation of the air drawn from the center of the OTC. Indeed, our mixing box was specifically designed to mix fresh air and recirculated air in a 5:1 ratio, which reduced the amount of pure CO_2 to be added to reach the target concentration.

3.4.2 CO_2 concentration

The average [CO_2] and standard deviations measured were of 632 ± 59 (n = 4 OTCs) and of 423 ± 58 (n = 1 OTC) µmol mol^{-1} in elevated and in ambient-CO_2 chambers, respectively, over the 2013 and 2014 growing seasons (total of 254 days). The standard deviations were comparable or smaller than those of previously reported OTC experiments (Rogers et al. 1983; Whitehead et al. 1995). Moreover, standard deviations were similar between elevated and ambient-CO_2 OTCs, showing similar ranges of [CO_2] variation for both treatments.

In addition, the frequency of actual [CO_2] that fell within ± 20% of the 600 µmol mol^{-1} target (480-720 µmol mol^{-1}) was 93% of the time, while it was 66% for the within-10% target (450-660 µmol mol^{-1}). This is comparable to previously reported FACE experiments (Miglietta et al. 2001; Okada et al. 2001) showing clearly the efficiency of the design of the OTCs and of the CO_2 control system that we used. As shown in Fig. 3.2, a large difference in [CO_2] was maintained between elevated and ambient chambers. Nevertheless, the precision of the system could be improved by replacing the manual flow control with an automated one (Leadley et al. 1997). This would allow a quicker response to changes in weather conditions such as wind speed, and would spare users from doing daily checks and manual adjustments when needed.

3.4.3 Air temperature

For 2014, the overall average daily increase in air temperature in OTCs compared to control plots was of 0.7°C (Fig. 3.2). Although this temperature elevation is lower than what is predicted in 2050 for Eastern Canada by Jing et al. (2013), it is close to what is forecasted in other IPCC scenarios and as such, generates a realistic simulation of future conditions that could prevail during summer in Eastern Canada. Furthermore, it is within the range of temperatures (0.5 to 2.5°C) reported by Kimball et al. (1997) in a comparison of nine OTC studies with passive temperature increase. Open-top chamber experiments can therefore be considered as an appropriate approach to study climate change in the field and obtain information on plant response to increased temperature without a large investment in temperature control equipment.

The average midday (from 10:00 to 14:00 h) passive temperature increase in our experiment was of 1.05°C, whereas it was 0.43°C at night (from 22:00 to 2:00 h), during the whole 2014 growing season (data not shown). Similar results were obtained by Whitehead et al. (1995), who associated the higher temperature difference during midday with increased solar radiation, causing the warming of air in OTCs during mid-day. In our study, there was no temperature difference between elevated-CO_2 and ambient-CO_2 OTCs. This clearly shows that temperature elevation was only due to a chamber effect and not to the addition of CO_2.

Based on fan air flow (5.66 m^3 min^{-1}) and the air volume inside the chamber (1.2 m^3), the fan that we used made approximately five air exchanges per minute, which corresponds to a wind speed between 0.4 – 1.4 m s^{-1} within the OTC. This value is similar to Norris et al. (1996), who obtained within-chamber temperature increases of 2°C above ambient during warm clear summer days with high solar radiation. On the other hand, Whitehead et al. (1995) used a lower air exchange rate of two per minute, and obtained large air temperature increases (4.3°C above

ambient). Our results show that five air exchanges per minute was optimal under our experimental conditions to ensure an even distribution of CO_2 through a tall and dense forage mixture while limiting the temperature increase within a realistic range based on global change scenarios. Furthermore, this relatively strong ventilation contributed to decrease the difference between air and leaf temperature, which can sometimes be an issue in OTC experiments (De Boeck et al. 2012)

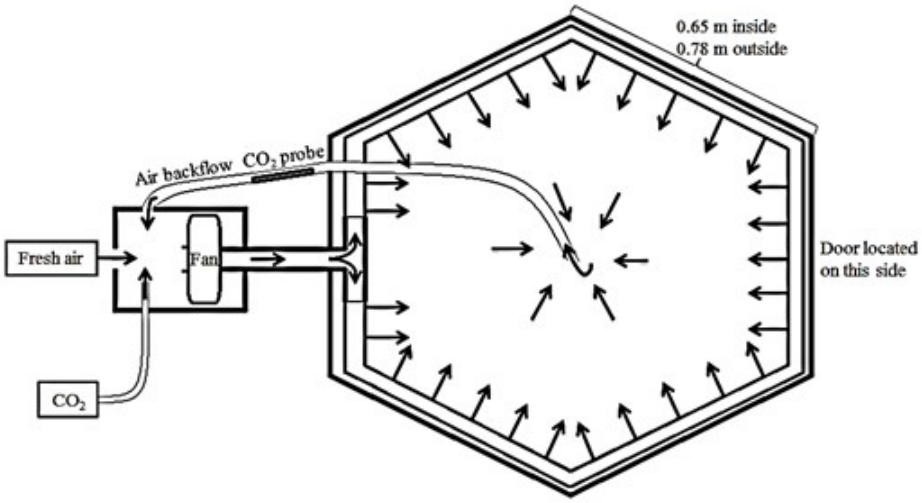
3.4.4 Light transmission

The overall average of 93% light transmission (Table A.2) was consistent with the range of values obtained in a review on chamber effects in OTC studies (Kimball et al. 1997). As observed here, Whitehead et al. (1995) demonstrated that sun position can affect light transmission. It is typically lower in the morning and late afternoon than during mid-day, as a result of lower zenith angle which increases shading by the frame and light intercepted by the plastic. Additionally, a lower light transmission was detected at a canopy height of 30 cm and 60 cm as compared to 90 cm due to shading by the framework. Whitehead et al. (1995) showed that one year old aged plastic contributes to decrease light transmission. In order to avoid this in our experiment, the plastic was removed in the fall and replaced in the spring. This also allowed snow, ice and rain to accumulate during winter, mimicking natural overwintering conditions for perennials.

3.5 CONCLUSION

The OTC design described above has proven its effectiveness as a way to study the effects of elevated CO_2 on plants over a prolonged period of time. It was built from widely available materials and assembling was simple, making these OTCs relatively inexpensive

compared to other systems. Minimal temperature elevation was observed, along with a light transmission of 93 %, which limited the alteration of growing conditions due to the chamber. The CO_2 control system used during the 2013 and 2014 growing seasons maintained the [CO_2] close to the target of 600 μmol mol^{-1} and no operational problems were encountered. We recently installed an identical experimental site in another location in Canada (Lacombe, AB), showing the facility to transpose this design to other location and allowing the comparison of CO_2 effects on plants under various climatic conditions.



A. Above view of open-top chamber and ventilation system

B. Open-top chamber

C. Mixing box

D. CO_2 control system

E. Experimental site and CO_2 storage

Figure 3.1. (A) Schematic representation and (B) pictures of one open-top chamber, (C) the mixing box, (D) the CO_2 control system, and (E) the experimental site in Québec City, QC, Canada.

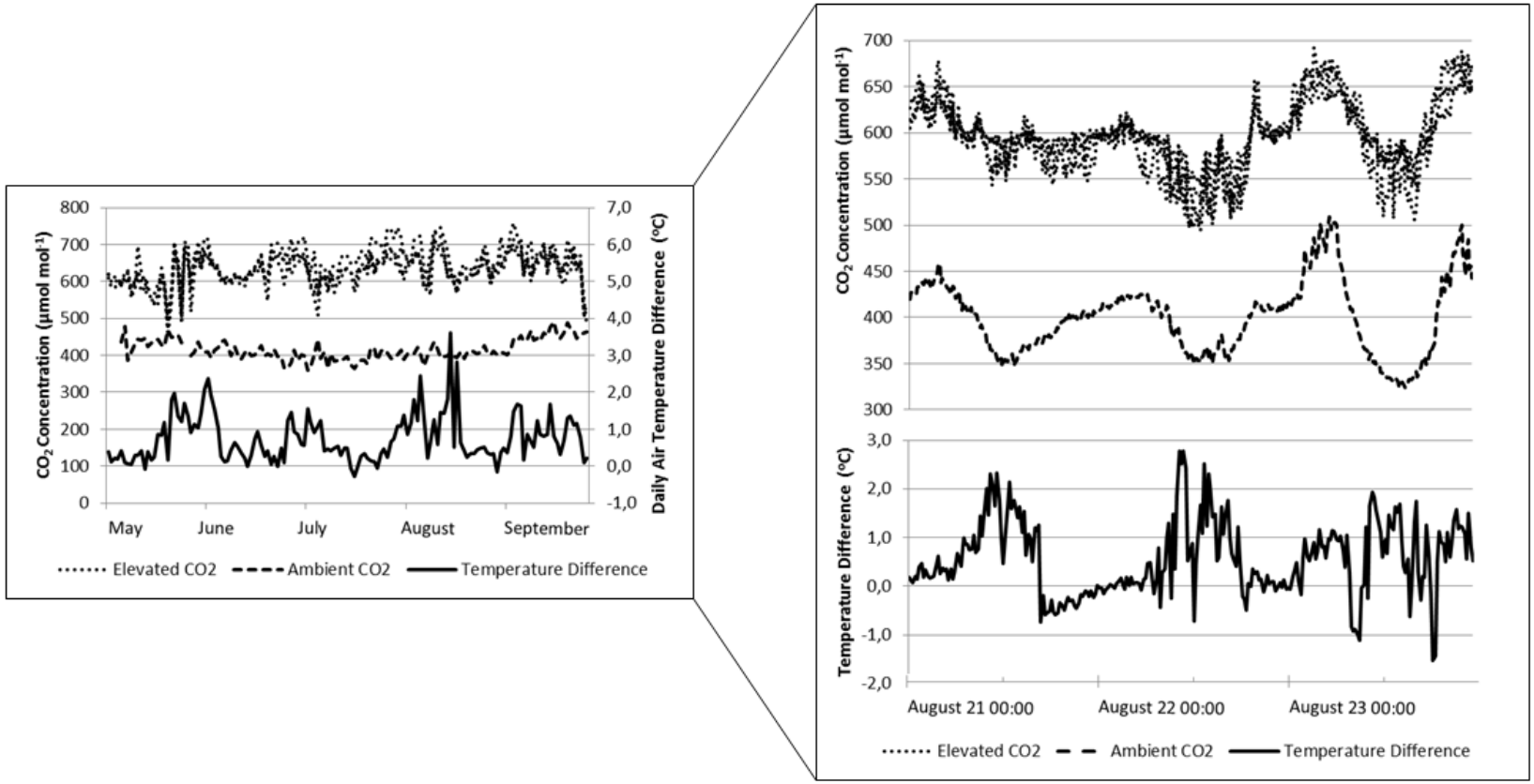


Figure 3.2. Left panel: Average daily CO_2 concentration (µmol mol^{-1}) within four open-top chambers under the elevated-CO_2 treatment (dotted line) and within one chamber under the ambient-CO_2 treatment (dashed line); and daily average air temperature differences (^oC) between the inside of open-top chambers (mean of eight chambers) and the middle of the four control plots without chambers (full line) recorded from May to september 2014. Right panel: Zooming on the CO_2 concentrations (µmol mol^{-1}) and air temperature differences (^oC) during three days, from August 21-23 2014.

CONNECTING TEXT FOR CHAPTER 4

Alfalfa-timothy mixtures are widely used to feed dairy and beef animals across Canada. Since forage yields and nutritive value can affect farm profitability, it is essential to examine the effects of elevated [CO_2] on these two variables, especially fibre constituents and soluble sugars, and their possible repercussions on animal performance. Furthermore, little is known about the effects of elevated [CO_2] on winter survival of legumes, and plant potential for carbon sequestration. The OTCs presented in Chapter 3 were thus used to grow an alfalfa-timothy mixture under ambient and elevated [CO_2] during two growing seasons and to assess the effects of [CO_2] on yield, nutritive value, fall organic reserve accumulation and root degradability.

CHAPTER 4

ELEVATED CARBON DIOXIDE INCREASES THE YIELD BUT DECREASES THE NUTRITIVE VALUE OF AN ALFALFA-TIMOTHY MIXTURE

4.1 ABSTRACT

The rise in atmospheric carbon dioxide concentration ($[CO_2]$) could significantly affect alfalfa (*Medicago sativa* L.) and timothy (*Phleum pratense* L.), two perennial forage crops commonly grown as a forage mixture throughout Canada. Very few long-term studies have looked at the effects of predicted future $[CO_2]$ on perennial forage crops under field conditions. In this experiment, we examined the effects of elevated $[CO_2]$ on forage DM yield and nutritive value, fall organic reserve accumulation and root degradability of an alfalfa-timothy mixture grown in open-top chambers (OTCs). Plants were transplanted in a uniformly distributed 50:50 mixture and were grown under ambient (near 400 μmol mol^{-1}) and elevated (600 μmol mol^{-1}) $[CO_2]$ during two growing seasons (2013 and 2014). An average yield increase of 18% under elevated $[CO_2]$ was accompanied by a significant increase in acid detergent fibre and neutral detergent fibre concentrations, and a slight decrease in *in vitro* true digestibility. Non-structural carbohydrates in the forage mixture were unaffected by elevated $[CO_2]$, and the total nitrogen concentration was slightly decreased, although the effect was only significant in alfalfa. Fall organic reserve accumulation was not affected by CO_2 treatments. Root degradability of alfalfa was increased under elevated $[CO_2]$ in spite of unaffected root carbon and nitrogen concentrations, indicating lower potential for carbon sequestration. Overall, the positive effect of increasing yields under elevated $[CO_2]$ were partly offset by a decreased forage digestibility. Elevated $[CO_2]$ increased root degradability, with nonetheless no effects on winter survival to this date.

4.2 INTRODUCTION

The global atmospheric carbon dioxide concentration ([CO_2]) has increased from 280 μmol mol^{-1} at pre-industrial levels (Neftel et al. 1988; Raynaud and Barnola 1985) to 400 μmol mol^{-1} in 2013 (Monastersky 2013). Depending on climate scenarios, current projections for future atmospheric [CO_2] range between 400 to 1000 μmol mol^{-1} by the end of the century (IPCC 2013), which could increase the annual air temperature by 2.1 to 3.1^{o}C by 2100 in eastern North America (IPCC 2013).

Tame hay is grown on 19% of the agricultural area throughout Canada (Statistics Canada 2014b) and 38% in the province of Québec (ISQ 2014). It represents a primary source of feed for ruminant animals. Alfalfa-based mixtures are predominant in hay fields across Canada, because of their ability to sustain higher yields of greater nutritive value and reduced weed competition compared to individual species (Bélanger et al. 2014). Despite increasing concerns about climate change and the importance of forage crops for dairy and beef farms, few experiments, if any, have studied the effects of elevated [CO_2] on the yield, nutritive value and fall organic reserve accumulation of perennial forage species, along with the potential for carbon (C) sequestration under North-American field conditions.

The effects of doubling the [CO_2] on forage yield of grasslands worldwide have been well reviewed (Ainsworth and Long 2005; Campbell and Stafford Smith 2000) and include an average 17% yield increase. However, a greater yield improvement is to be expected in alfalfa, compared to timothy. This comes from the fact that as a C_3 nitrogen-fixing species, alfalfa's yield response is not limited by nitrogen (Lüscher et al. 2014). This was illustrated by Erice et al. (2006a) who observed a 50% photosynthetic stimulation in alfalfa compared to 18 to 28% in timothy (Piva et al. 2013) under doubled [CO_2].

Forage nutritive value is a key element for ruminant animal nutrition. An increased concentration of non-structural carbohydrates (NSC) in forage is linked to improved silage conservation (Tremblay et al. 2014) and potentially increased milk production in dairy cows (Brito et al. 2008). Elevated [CO_2] increased the concentration of NSC in experiments with alfalfa (Erice et al. 2006b; Sanz-Sáez et al. 2010) and timothy (Casella and Soussana 1997; Piva et al. 2013; Read et al. 1997).

Nitrogen (N) is a very important nutrient to sustain high milk production. Its concentration has been shown to decrease both in alfalfa (Sanz-Sáez et al. 2012a) and grasses (Milchunas et al. 2005b) as a result of elevated [CO_2]. However, the decrease may be higher in grasses compared to legumes (Cotrufo et al. 1998). Similarly, Schenk et al. (1997) observed that the reduction in N concentration was only significant in the grass portion of a white clover-perennial ryegrass (*Trifolium repens* L.)-(*Lolium perenne* L.) mixture.

Increased forage fibre concentration and lower digestibility typically decrease forage intake by ruminants and thus negatively affect their milk and meat production (Buxton, 1996). Nevertheless, very few studies have examined the effects of elevated [CO_2] on fibre concentration and digestibility in a field setting. In an open-top chamber (OTC) experiment, Schenk et al. (1997) observed a decrease in the crude fibre concentration of a perennial ryegrass-white clover mixture for both years of the study. However, a wide range of responses was obtained in the fibre concentration and digestibility of alfalfa grown under controlled environment (Baslam et al. 2014; Bertrand et al. 2007b; Sanz-Sáez et al. 2012b). In contrast, in OTC experiments with grasses, a small decrease in the lignin concentration of tall fescue (Newman et al. 2003) and three shortgrass steppe species (Milchunas et al. 2005b) was observed.

Alfalfa is well-known to be more sensitive to harsh winter conditions than grasses. Fall organic reserves in the form of C (NSC) and N (free amino acids) typically accumulate in root material during acclimation and have been linked to winter survival and spring regrowth (Bélanger et al., 2006). Bertrand et al. (2007a) did not observe differences in root and leaf NSC between ambient and elevated treatments of alfalfa grown under simulated cold acclimation conditions. However, a decreased freezing tolerance and increased metabolic rate were reported, suggesting that elevated [CO_2] may delay cold acclimation and potentially increase winter mortality (Bertrand et al. 2007a)

Alfalfa has a deep taproot, whereas timothy has a fibrous rooting system. Since decreased N and increased lignin concentration has been reported in material grown under elevated [CO_2], the amount of C stored in soils could be affected by global change scenarios (Cotrufo et al. 1994). Also, some experiments examined the root decomposition of white clover and perennial ryegrass (Jongen et al. 1995) and timothy (Bertrand et al. 2014) under elevated [CO_2]. Increased C:N ratios were reported in these species, rendering roots generally less degradable.

Considering that climate change is altering the current growing conditions, an experiment was designed to study, over three years, the effects of elevated [CO_2] on an alfalfa-timothy mixture grown in OTCs. We hypothesized that elevated [CO_2] increases the yield of the forage mixture as well as fall organic reserve accumulation, while decreasing nutritive value and root degradability. After two years of growth under such conditions, the effects are reported here.

4.3 MATERIALS AND METHODS

4.3.1 Experimental Design

Alfalfa (*Medicago sativa* L. cv. Calypso) and timothy (*Phleum pratense* L. cv. AC Alliance) were seeded individually in 4.5 × 4.5 × 5 cm Jiffy pots and grown for 50 days in a

greenhouse. Then, on 18 July 2013, they were cut and transplanted in a uniformly distributed 50-50% mixture at a density of 275 plants m^{-2} into eight OTCs and four control plots without chambers in Quebec City, QC, Canada (46°78'N; 71°29'W). Construction of OTCs is described in detail by Messerli et al. (2015). Briefly, four OTCs were maintained at an elevated [CO_2] of 600 µmol mol^{-1} and four others had ambient [CO_2] (near 400 µmol mol^{-1}). Four control plots without chambers, at ambient [CO_2], were included in the experimental design to assess the chamber effect. The 12 experimental units each had a ground area of 1.2 m^2 and were placed randomly within the experimental site.

A single cut was taken during the establishment year on 28 August 2013 and four cuts were taken in 2014 on 11 June, 10 July, 19 August, and 9 October. The cut in 2013 and the first three cuts in 2014 were taken when alfalfa reached the 10% flowering stage of development, while the cut in October was taken approximately 500 growing degree-days (base 5°C) after the last summer cut, as recommended by Bélanger et al. (1999). Weekly temperatures and precipitations at the site during the 2014 growing season are presented in Fig. 4.1. Air temperature within chambers was on average 0.7°C higher than outside (Fig. 3.2). Plants were rainfed throughout the project, except for two weeks after transplantation and during a dry spell in June and July 2014, where all chambers and control plots were watered equally, based on soil moisture measured using a portable FieldScout TDR 100 moisture meter (Spectrum Technologies, Aurora, IL). No N fertilization was applied, following a standard management practice for field-grown alfalfa-timothy mixtures. Based on soil analysis, 2.5 Mg ha^{-1} of pure lime equivalent and 70 kg of K were incorporated in the soil before transplantation in 2013. In spring 2014, 1 kg ha^{-1} of B, 60 kg ha^{-1} of K and 1.5 Mg ha^{-1} of pure lime equivalent were

applied. Based on local recommendations, an additional application of 60 kg ha^{-1} of K was made after the third cut in 2014 (CRAAQ 2010).

4.3.2 Sample Collection and Preparation

At each cut, the developmental stage of timothy in each OTC was determined according to Simon and Park (1983), whereas that of alfalfa was determined based on Fick and Mueller (1989). Forage biomass within a permanent 40 × 40 cm quadrat located in the center of each OTC and control plot was cut at a height of 7 cm. The harvested forage samples were separated by species, dried at 55°C for 48 hours, weighed, and ground to pass a 1-mm sieve with a Wiley mill (model 3379-k35, Digital ED 5 Wiley Mill, Thomas Scientific Inc., Swedesboro, NJ). NSC and N were analyzed on both species separately to assess the contribution of each species to these variables. After those analyses were made, the two species were mixed back together and NSC, total N and P, minerals (K, Ca, Mg), fibre concentrations (acid detergent fibre, ADF; neutral detergent fibre, NDF; acid detergent lignin, ADL) and digestibility (*in vitro* true digestibility, IVTD; neutral detergent fibre digestibility, NDFd) were analyzed on the mixture. The weight of the samples was corrected for moisture by using a thermogravimetric analyzer (TGA701, LECO, St. Joseph, MI) to determine the dry matter concentration at 135°C.

During cold acclimation, five plants of each species located outside of the harvested quadrat were dug out in each OTC and control plot on 24 October in 2013 and on 9 October in 2014. They were washed, and roots and crowns were cut and separated. Roots were dried at 55°C and ground using a 1-mm sieve with the Wiley mill previously described, while crowns were freeze-dried (Freezone 12 model 7759040, Labconco, Kansas City, MO) and ground with a Mixer Mill (Retsch, Newton, PA). For both years of the project, sugars and free amino acids were analyzed in the crowns to determine fall organic reserve accumulation, while C and N were

analyzed in the roots. In 2014, the ADL concentration and IVTD of the roots were analyzed to measure the effects of elevated [CO_2] on the degradability of roots grown under long-term exposure to elevated [CO_2].

4.3.3 Leaf Photosynthesis Measurement

The day before each harvest, photosynthesis was measured on the youngest, fully expanded leaf (the middle leaflet of the trifoliate was used for alfalfa) of each species under their respective growth-[CO_2] of either elevated (600 μmol mol^{-1}) or ambient (400 μmol mol^{-1}). Three measurements on different plants of each species were taken in each OTC and control plots using a portable photosynthesis system (LI-6400XT, LI-COR, Lincoln, NE). Measurements were made between 10:00 h and 15:00 h, at ambient outside air temperature, under a PPFD of 1000 μmol m^{-2} s^{-1} provided by a Red/Blue Light Source (Model 6400-02B, LI-COR) and under either 600 or 400 μmol mol^{-1} depending on the CO_2 treatment.

4.3.4 Total Non-Structural Carbohydrates Analysis

4.3.4.1 Extraction

Soluble carbohydrates were extracted from the forage biomass and the crowns by adding 7 mL of deionized water to 200 mg of ground sample. Extracts were incubated at 100°C for 90 min, cooled in an ice-water bath, frozen at -80°C for 60 min and thawed in an ice-water bath. Extracts were centrifuged twice for 10 min at 3220 × *g* before 1 mL-subsamples of supernatant were taken. Soluble carbohydrates, fructans and starch were analyzed on the same extract using a Waters High-Performance Liquid Chromatography (HPLC) analytical system controlled by Empower II software (Waters, Milford, MA) and composed of a Model 1525 pump, a Model 2707 autosampler and a Model 2414 refractive index detector.

4.3.4.2 Soluble Carbohydrates

Subsamples were centrifuged at 13000 × *g* for 3 min, and then 200 µL were placed into vials kept at 4°C into the autosampler. Stachyose, raffinose, sucrose, glucose, fructose and pinitol (alfalfa) were separated on a Bio-Rad HPX-87P column (Bio-Rad, Richmond, CA) eluted isocratically at 80°C with deionized water at a flow rate of 0.5 mL min^{-1} and detected on a refractive index detector. Peak identity and sugar quantity were determined by comparison to standards.

4.3.4.3 Fructans (timothy)

Low degree of polymerization (LDP) fructans were separated on a Bio-Rad HPX-42A column (Bio-Rad, Richmond, CA), eluted isocratically at 25°C with deionized water at a flow rate of 0.5 mL min^{-1}. The degree of polymerization of LDP fructans was established by comparison with elution time of purified standards from Jerusalem artichoke (*Helianthus tuberosus* L.). High degree of polymerization (HDP) fructans were separated on a Shodex KS-804 column preceded by a Shodex KS-G precolumn (Shodex, Tokyo, Japan) eluted isocratically at 50°C with deionized water at a flow rate of 1.0 mL min^{-1}. The degree of polymerization of HDP fructans was estimated by reference to a standard curve established with seven polymaltotriose pullulan standards (Shodex Standard P-82) ranging from 0.58×10^4 to 85.3×10^4 of molecular weight. Both LDP and HDP fructans are expressed on an equivalent fructose basis.

4.3.4.4 Starch

Total starch was measured in the aqueous supernatant used for soluble carbohydrates analysis after an enzymatic digestion by amyloglucosidase (AGS) (Sigma A7255, Sigma-Aldrich, St. Louis, MO). For this purpose, 3 mL of AGS solution were added to the tubes that

were placed on a rotative incubator at 55°C and 80 RPM for 60 minutes, and then cooled down for 5 minutes in an ice-cold water bath. The concentration of soluble sugars was assessed by HPLC. The difference in glucose concentration after and before starch hydrolysis by AGS represents the amount of starch in the sample.

4.3.5 Free Amino Acids

Free amino acids were also analyzed from the 1 mL sub-sample used for soluble carbohydrates analysis. Twenty-one amino acids were separated and quantified using Waters ACQUITY ultra-performance liquid chromatography (UPLC) analytical system controlled by the Empower II software (WATERS, Milford, MA). Samples were prepared for ULPC analysis using the AccQ•Tag™ Ultra Derivatization Kit. The amino acids were derivatized using AccQ•Tag™ Ultra reagent (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate). The derivatives were separated on an AccQ Tag Ultra column (2.1 × 100 mm) and detected with Waters ACQUITY Tunable UV detector set at 260 nm under the chromatographic conditions described in Cohen (2000). Peak identity and amino acid quantity were determined by comparison to a standard mix containing the 21 amino acids.

4.3.6 Minerals

The concentration in N, P, K, Ca and Mg were extracted using a method adapted from Isaac and Johnson (1976). Ground samples (100 mg) were digested for 60 min at 380°C in a 1.5-mL solution of H_2SeO_3 and H_2SO_4 (1:42) plus 2 mL of 30% H_2O_2. After cooling, the solution was diluted to 75 mL with distilled water. Total N and P were then determined on an automated continuous-flow injection analyzer (Model QuickChem 8000 FIA, Zellweger Analytics Inc., Lachat Instruments, Milwaukee, WI) using the method 13-107-06-2-E. Concentrations of K, Ca,

and Mg were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES, Model 4300DV, Perkins Elmer, Shelton, CT).

4.3.7 Fibre Concentration and Digestibility

The concentrations of ADF, NDF and ADL in biomass samples were determined using ANKOM F57 filter bags (25-mm porosity) on an ANKOM 2000 Fibre Analyzer (ANKOM Technology, Macedon, NY). The concentration of NDF assayed with a heat-stable amylase was determined according to Mertens (2002), and heat-stable α-amylase and sodium sulphite were both added during extraction. The concentration of ADF was determined according to method 973.18 of the Association of Official Analytical Chemists (2005). The ADL concentration was determined according to Robertson and Van Soest (1981).

The IVTD was measured according to the method of Goering and Van Soest (1970), using 48-h incubation with buffered rumen fluid followed by an NDF wash of the post-digestion residues. The IVTD was performed with an ANKOM Daisy II incubator (ANKOM Technology, Macedon, NY) using rumen fluid from a lactating ruminally-fistulated dairy cow fed a total mixed ration. The IVTD was calculated as follows: IVTD (mg g^{-1} DM) = [1 − (post-digestion dry weight following NDF wash / pre-digestion dry weight)] × 1000. The NDF digestibility (NDFd) was calculated from IVTD and NDF values as follows: NDFd (mg g^{-1} NDF) = 1000 − [(1000 − IVTD) / (NDF / 1000)].

4.3.8 Root Composition

All root C and N analyses were performed by dry combustion (TruSpec CN, LECO, St. Joseph, MI) of 100 mg subsamples. The ADL concentration was determined according to same procedure than for aboveground material, as described above.

4.3.9 Root Degradability

Root dry matter (DM) degradability was assessed by IVTD following the procedure described above for forage IVTD. This method of assessing root degradability was validated on timothy material in a previous experiment (Bertrand et al. 2014).

4.3.10 Statistical Analyses

A two-factor ANOVA with repeated measures was used to compare the effects of elevated [CO_2] on an alfalfa-timothy mixture cut five times. Repeated factor is the cut-year. The MIXED procedure in SAS, Version 9.2 (SAS Institute) was used with the REPEATED statement in order to model the correlation between measurements taken over time for the same experimental unit. The variance-covariance matrix, which minimizes Akaike's information criterion, was selected. The method of Kenward-Roger was used to calculate the degrees of freedom. A residual analysis was used to verify the assumptions of normality and homogeneity of the model. Multiple comparisons were made using Protected Fisher's LSD. In addition, a simple ANOVA with one factor was used to compare treatment effects for fall organic reserve accumulation and root composition and degradability. The GLM procedure of SAS was used and the assumptions of normality and homogeneity were validated using a residual analysis. Statistical significance was postulated at $P \leq 0.05$. Least square means are presented. Unless otherwise indicated, only significant effects are presented.

4.4 RESULTS

4.4.1 Forage Yield and Botanical Composition

The average DM yield of the forage mixture across the five cuts was increased by 18% under elevated compared to ambient [CO_2] (Table 4.1). However, an interaction between [CO_2]

and cut was observed. This reflected in a higher yield in the elevated-CO_2 treatment at all cuts but the fourth cut in 2014 (Fig. 4.2). The mixture grown under elevated [CO_2] produced 129% of the yield obtained under ambient [CO_2] in 2013, and then 142%, 137%, 109% and 73% for the four successive cuts taken in 2014. The DM yield obtained in the ambient-CO_2 treatment was not different at any cut than that obtained in the control plots without chambers. A cut main effect was observed for the proportion of alfalfa in forage DM yield (solid line in Figure 4.2). Even though the experiment was started with the same number of plants per species, the proportion of alfalfa started at 75% in 2013 and rapidly increased until it represented 100% of the stand at the second cut of 2014.

The DM yield was also calculated for each forage species present in the mixture (Table 4.2). The average yield of alfalfa across the five cuts was affected by the treatments but an interaction between [CO_2] and cut was observed. Alfalfa grown under elevated [CO_2] produced 127% of the yield obtained under ambient [CO_2] in 2013, and then 154%, 135%, 108% and 71% during the four cuts taken in 2014. Like for the mixture, the yield of alfalfa obtained in the ambient-CO_2 treatment was not different at any cut than that obtained in the control plots without chambers, except in 2013 where it was lower under ambient [CO_2]. In timothy, there were no differences between CO_2 treatments, but the yield obtained in the first cuts of 2013 and 2014 were significantly higher than the subsequent cuts, where there was virtually no timothy.

4.4.2 Leaf Photosynthesis and Sucrose Concentration

The leaf photosynthetic rate of alfalfa was greater (+22%) under elevated than under ambient [CO_2] and there was no difference between the ambient-CO_2 treatment and control plots without chambers (Table 4.2). For timothy, leaf photosynthesis was only measured during the first cuts of 2013 and 2014, because of the lack of plants afterwards. An interaction between

[CO_2] and cut was observed for photosynthesis of timothy. There were no differences between treatments in 2013, but in 2014 photosynthesis was 73% higher under elevated than under ambient [CO_2].

Sucrose concentration in alfalfa was 14% higher under elevated than ambient [CO_2], and 20% lower under ambient [CO_2] than in the control plots without chambers. In timothy, sucrose concentration was three times greater in 2014 than in 2013.

4.4.3 Nutritive Value

4.4.3.1 Non-Structural Carbohydrates

For NSC, there were no differences between CO_2 treatments, but a cut main effect was observed in the mixture, indicating that NSC concentration varied across cuts (Table 4.1). It was lowest in 2013 and in the third cut of 2014, highest in the first and second cuts of 2014, and intermediate in the fourth cut. Similar results were obtained in both species (Tables A.3 and A.4). Moreover, in alfalfa, the [CO_2] main effect was significant at P=0.055, showing that NSC concentration was higher in alfalfa grown in the control plots than within chambers (Table A.3).

4.4.3.2 Total Nitrogen

An interaction between [CO_2] and cut was observed for total N concentration (Table 4.1). Total N concentration of alfalfa-timothy forage was 12% lower under elevated than ambient [CO_2] in the first cut of 2013. Also, in the third cut, it was found to be 13% lower in both CO_2 treatments compared to the control plot. Total N concentration per species (Table 4.2) shows that the response is more pronounced in alfalfa than timothy. In the former, a [CO_2] main effect was observed: total N concentration was highest in the control plot, intermediate under ambient [CO_2] and lowest under elevated [CO_2]. On the other hand, a cut main effect was observed in timothy, as highlighted by the lower total N concentration in 2013 than 2014.

4.4.3.3 Fibre and Digestibility

A [CO_2] main effect was observed for ADF and NDF concentrations in alfalfa-timothy biomass (Table 4.1). For both variables, the ranking of concentration was as follows: elevated [CO_2] > ambient [CO_2] > control plot without chamber.

The ADL concentration increased from one cut to the other as plants grew older and became more lignified, except for the fourth cut where the ADL concentration actually decreased. Moreover, an interaction was observed between [CO_2] and cut, as only the ADL concentration in the third cut was significantly higher in the ambient-CO_2 treatment compared to control plots without chambers.

An interaction between [CO_2] and cut was observed for IVTD. In the first and second cuts of 2014, IVTD was lower under elevated than ambient [CO_2]. Also, it was lower under ambient [CO_2] compared to the control plots without chambers in the first and third cuts of 2014.

An interaction between [CO_2] and cut was observed for NDFd. Lower NDFd was observed under elevated than ambient [CO_2] in the first cut of 2014. In contrast, this effect was inverted in the fourth cut where a greater NDFd was detected under elevated than ambient [CO_2]. Also, in the third cut of 2014, a lower NDFd concentration was also observed under ambient [CO_2] compared to the control plots without chambers.

4.4.4 Fall Organic Reserve Accumulation

In alfalfa and timothy, there were no differences between CO_2 treatments for both NSC and total free amino acids measured in the crowns during cold acclimation in 2013 and 2014 (Tables A.7 and A.8). However, for alfalfa, their concentrations were lower in 2014 compared to 2013 (Fig. 4.3), which was associated with an overall 96% survival rate.

4.4.5 Root Composition and Degradability

In 2014, the C concentration in alfalfa was higher in the control plots without chambers compared to both ambient and elevated-CO_2 treatments (Table 4.3). Additionally, root DM degradability measured by IVTD was as follows: elevated [CO_2] > ambient [CO_2] > control plots without chambers.

4.5 DISCUSSION

4.5.1 Forage Yield and Botanical Composition

The average DM yield increase of the forage mixture observed under elevated [CO_2] in our study is within the range of 0 to 30% reported for permanent grasslands in a review of ecosystem-based experiments (Campbell and Stafford Smith 2000). The experimental design of our experiment, with successive forage cuts, allowed us to study the response of the forage mixture throughout a growing season. A gradual decrease in the response to elevated [CO_2] was observed at each cut, which can be associated with differences in growing conditions. For instance, the greatest yield stimulation by elevated [CO_2] was observed in the first and second cuts of 2014 (Fig 4.2), when growing conditions (air temperature and precipitations) were favorable for cool-season forage. However, the CO_2 effect faded in the third cut, and the effect on yield was negative in the fourth cut, which could be linked to shorter photoperiod and lower air temperatures, particularly at the last cut taken in October. This is consistent with findings from Newton et al. (1994) who collected ryegrass-white clover turves from a pasture and grew them under doubled [CO_2] while gradually increasing day/night air temperatures from 10/4^{o}C to 22/16^{o}C. They observed a significant yield increase due to elevated [CO_2] only under 22/16^{o}C. Consequently, a threshold temperature may be required for a positive effect of elevated [CO_2] on

yield, implying that the yield response to elevated [CO_2] could be mitigated by cooler temperatures that prevail at northern latitudes (Dodd 2013).

When looking at the response of each species to elevated [CO_2] (Table 4.2), a large stimulation of alfalfa DM yield was observed, similar to values obtained in a FACE experiment under 600 μmol mol^{-1} [CO_2] by Lüscher et al. (2000). They observed a yield enhancement up to 54% and this response was associated with the lack of growth limitation by N availability due to the plant's ability to fix nitrogen. The lack of significant DM yield response to elevated [CO_2] observed for alfalfa in the third cut, as well as the negative response in the fourth cut could be due to less favorable growing conditions, such as decreasing day length and lower temperatures. Alternatively, alfalfa could have acclimated to elevated CO_2 conditions, which makes plants less responsive to CO_2 enhancement in the long term (Sanz-Sáez et al. 2013).

The absence of yield stimulation observed in timothy under elevated [CO_2] conditions may be typical of that species, as a similar result was obtained in growth chambers under 400 and 600 μmol mol^{-1} [CO_2] (Piva et al. 2013). On the other hand, a gradual decrease in the proportion of timothy was observed in the forage mixture for all treatments (Fig. 4.2). This could be explained by the fact that time of cutting was determined based on the growth stage of alfalfa, which resulted in timothy being cut at the elongation stage, both in 2013 and 2014. This stage is typically associated with low reserves in grasses as well as the absence of basal tillers to initiate regrowth (Smith et al. 1973). Therefore, timothy was not able to compete against fast-growing alfalfa. A dry period during regrowth in 2014 (June and July) could also have contributed to timothy mortality.

4.5.2 Photosynthesis and Sucrose Concentration

Elevated [CO_2] increased the leaf photosynthesis of alfalfa by an average of 22% (Table 4.2), which is consistent with the range of values reported in previously published experiments (Bertrand et al. 2007b; Erice et al. 2006a; Sanz-Sáez et al. 2012b). As a consequence of the higher photosynthetic rate under elevated [CO_2], an increase in C temporarily stored as sucrose was observed in alfalfa. Interestingly, the highest sucrose concentration was detected in control plots without chambers, which could be linked to lower temperatures (Fig. 3.2) or higher irradiance (Table A.2) measured in these control plots.

Leaf photosynthesis of timothy grown under elevated [CO_2] was increased by 73% as compared to the ambient-CO_2 treatment in the first cut of 2014. This also explains the three-fold increase in sucrose concentration compared to 2013, where photosynthesis was not stimulated. This higher than expected response in 2014 can be associated with the fact that instead of being measured the day before cutting, photosynthesis was measured after two weeks of regrowth because of thermic stress at cutting. In an experiment where 13 grassland species were grown under elevated [CO_2] for 11 years, a higher photosynthetic response resulting from the absence of photosynthetic acclimation was observed in plants after cutting (Lee et al. 2011). Similar observations were reported in alfalfa regrowth after cutting in another experiment under elevated [CO_2] (Erice et al. 2006a). However, we did not observe such effect in alfalfa in our study.

4.5.3 Nutritive Value

4.5.3.1 Non-Structural Carbohydrates

Elevated [CO_2] typically induces an accumulation of NSC, particularly starch in alfalfa (Erice et al. 2006b; Sanz-Sáez et al. 2010) and fructans in grasses (Casella and Soussana 1997; Piva et al. 2013; Read et al. 1997). However, in this experiment, no significant differences in

NSC, starch and fructans concentrations of the mixture and of both species were observed between elevated [CO_2] and the ambient-CO_2 treatment (Tables 4.1, A.3, A.4 and A.5). Our results are similar to those obtained by Bertrand et al. (2007a) and Sanz-Sáez et al. (2012b) in experiments under controlled conditions where the NSC concentration of alfalfa forage was unaffected by elevated [CO_2]. The higher NSC concentration in alfalfa grown in the control plots compared to alfalfa grown within open-top chambers can be explained by the slightly higher air temperature in the latter, which has been shown to reduce the NSC concentration in alfalfa (Smith 1969).

4.5.3.2 Total Nitrogen

The negative effect of elevated [CO_2] on total N concentration was much more pronounced in alfalfa than in the mixture (Tables 4.1 and 4.2), which concurs with multiple experiments where a decreased N concentration was observed in alfalfa under elevated [CO_2] (Bertrand et al. 2007b; Erice et al. 2006b; Sanz-Sáez et al. 2012b; Sanz-Sáez et al. 2010). This decrease is generally attributed to the translocation of N to a stronger sink effect of the rooting system (Cotrufo et al. 1998; Sanz-Sáez et al. 2012b). However, there was no [CO_2] effect in timothy, which contrasts with an OTC experiment in which the growth of white clover and perennial ryegrass mixtures grown in pots was evaluated (Schenk et al. 1997). In this study, a significant decrease in the N concentration of the grass component was reported, whereas the clover and the mixture did not experience any significant change. However, our results are similar to that obtained in a temperate grass sward, where a small temperature increase of 3^{o}C coupled with elevated [CO_2] led to a N concentration close to what was obtained under ambient [CO_2] and temperature (Soussana et al. 1996).

No effects of elevated [CO_2] on the NSC concentration of the mixture coupled with a slight decrease in total N concentration could increase the NSC:N ratio of the forage. Similar results were reported by Bertrand et al. (2007b) in alfalfa grown under elevated [CO_2]. An increased NSC:N ratio has proven to be beneficial for dairy cows through improved balance and synchrony of C and N (Miller et al. 2001), and has been associated with increased milk production (Brito et al. 2008).

4.5.3.3 Fibre and Digestibility

Significantly higher ADF and NDF concentrations observed under elevated [CO_2] as compared to the ambient-CO_2 treatment caused a decrease in IVTD (Table 4.1). This is very similar to results reported in an OTC experiment in shortgrass steppe (Morgan et al. 2004) and with one of two rhizobium strains used in a controlled-conditions experiment with alfalfa (Bertrand et al. 2007b). However, in our experiment, an interaction between [CO_2] and cut was observed for both IVTD and NDFd, indicating that the effect of elevated [CO_2] varied across cuts. Elevated [CO_2] decreased NDFd in the first cut of 2014, but increased it in the fourth cut. This may be attributed to cooler weather in the fall which may have altered fibre composition, mainly through an 8 mg kg^{-1} reduction in ADL concentration between the third and fourth cut.

However, the greater reduction in IVTD compared to NDFd indicates that total dry matter, rather than NDF, was less digestible. This could be the result of a change in the leaf-stem ratio, supported by a positive correlation between fibre concentration and DM yield and a negative correlation between IVTD and dry DM yield, as highlighted by Bélanger et al. (2001). Very few experiments have examined the effects of elevated [CO_2] on fibre concentration and digestibility of field-grown plants. Since milk and meat productions are strongly dependent on

the amount of feed consumed by cattle, slight changes in forage fibre concentration could have important repercussions on animal performance.

Photosynthetically active radiation (PAR) was decreased by 7% within chambers (Table A.2), but, most importantly, air temperature was increased by 0.7^{o}C (Fig. 3.2). The negative effect of a higher temperature on forage nutritive value has been reported previously in alfalfa (Smith 1969; Vough and Marten 1971) and timothy (Bertrand et al. 2008; Thorvaldsson et al. 2007). In these studies, a higher air temperature, although greater than observed in this experiment, was associated with increased fibre concentration and decreased digestibility. Also, constant wind flow within chambers could have induced a higher fibre accumulation in those plants as a result of the abrasive action of wind (Grace 1988).

4.5.4 Fall Organic Reserve Accumulation

In this study, fall accumulation of NSC and free amino acids concentrations in overwintering crowns of both species did not differ between CO_2 treatments (Tables A.7 and A8). A similar observation was made for NSC and free amino acids in the roots of alfalfa grown under elevated [CO_2] in controlled-environment chambers (Bertrand et al. 2007a). Since fall organic reserve accumulation has a strong seasonal effect (Castonguay et al. 2011), it could have overcome the CO_2 effect. Also, organic reserve accumulation is strongly affected by cutting management (Dhont et al. 2002; Dhont et al. 2003). In this experiment, a lower concentration in fall 2014 than in fall 2013 was observed (Fig. 4.3), which could be due to three cuts taken in 2014 as compared to one cut in 2013. Therefore, the fourth cut taken afterwards in October, combined with lower reserves, might play a role in alfalfa survival, as well as regrowth potential the next spring. However, total NSC and N amounts in plant roots, rather than concentrations,

have been shown to be better determinant factors of alfalfa spring regrowth (Dhont et al. 2002; Dhont et al. 2003).

4.5.5 Root Composition and Degradability

The root C and N concentrations of alfalfa were not affected by elevated CO_2 treatments, but root degradability measured by IVTD in 2014 increased in response to elevated [CO_2] (Table 4.3). Jongen et al. (1995) also observed no difference in the C:N ratio of white clover root material grown under elevated [CO_2] but, contrary to our results, they observed a 1.8 to 3.6 times lower relative decomposition, depending on N fertilization. It also differs from experiments on timothy (Bertrand et al. 2014) and perennial ryegrass (Jongen et al. 1995) where N concentration was decreased and linked to increased C:N ratio and decreased IVTD, indicating that material grown under elevated [CO_2] was more recalcitrant to breakdown. Therefore, the difference in root dry matter degradability in response to elevated [CO_2] seems to be species specific, as previously reported by Cotrufo et al. (1994). However, in order to obtain a better estimate of the carbon sequestration potential, total C stored in roots should be measured. In fact, elevated [CO_2] has been shown to increase the root:shoot ratio of alfalfa up to 57% (MacDowall 1982), which could offset the increase in degradability observed here.

In timothy, no differences in root C and N concentrations between CO_2 treatments were observed in 2013. It contrasts with Bertrand et al. (2014) and Milchunas et al. (2005a) who observed a decrease in N concentration in timothy and shortgrass steppe, respectively. However, the lack of CO_2 effect in our experiment might be due to the fact that plants were transplanted in mid-summer and were only briefly exposed to elevated [CO_2] before roots were sampled.

4.6 CONCLUSION

Elevated [CO_2] increased the yield of the mixture, particularly during the warmest month of the season. However, timothy did not persist in the mixture because growth conditions were favourable to alfalfa which out-competed timothy. There was a significant increase in ADF and NDF concentrations of the forage mixture grown under elevated [CO_2], which was associated with a slight reduction of the IVTD. However, no significant effect of elevated [CO_2] on the NSC concentration of the mixture was observed and there was only a slight decrease in total N concentration. Fall organic reserve accumulation in the crowns was not affected by elevated [CO_2], but was lower in 2014 compared to 2013, likely as the effect of three cuts taken in 2014. Root composition of alfalfa and timothy was unaffected by elevated [CO_2], but root degradability of alfalfa was increased.

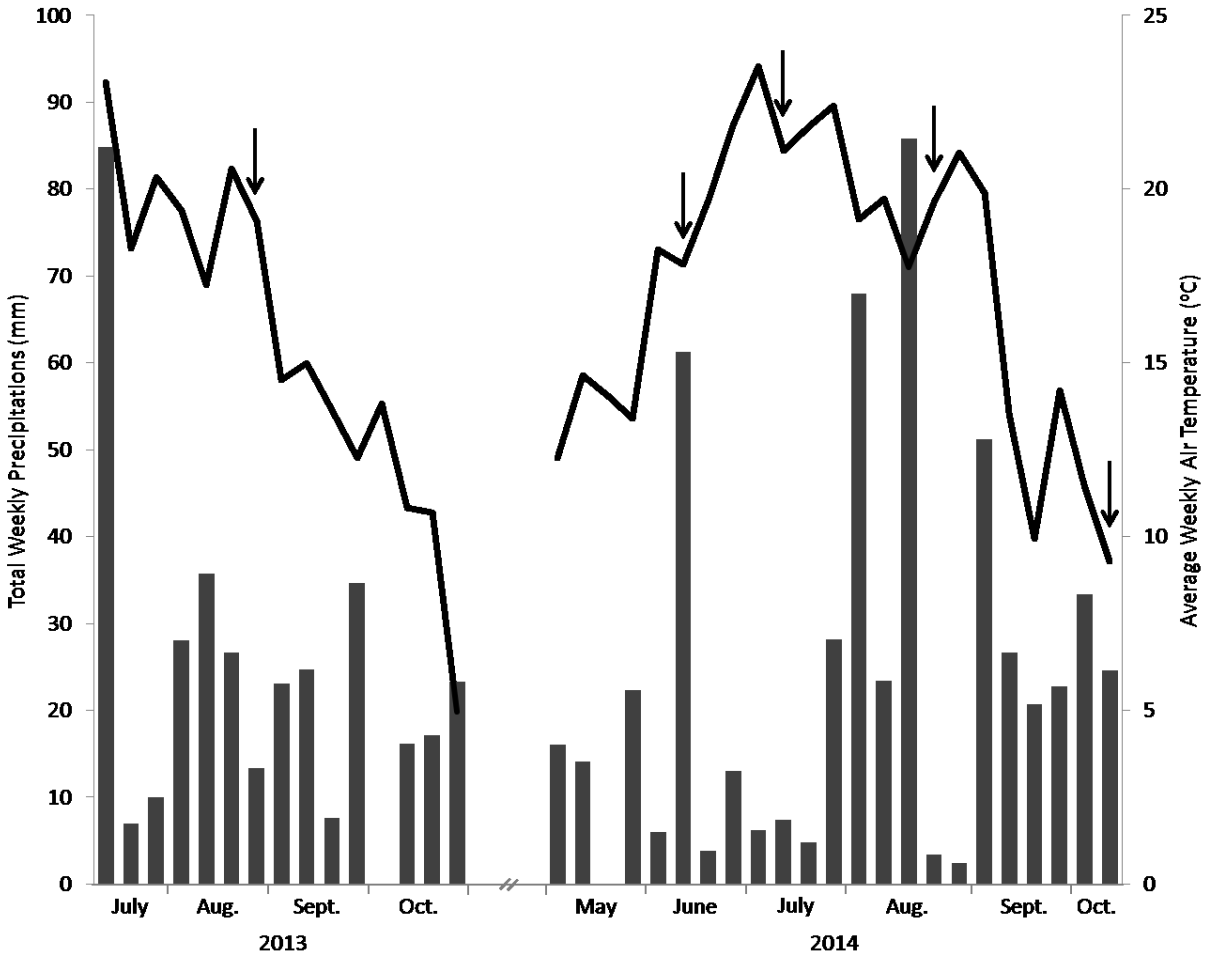


Figure 4.1. Total weekly precipitations (bars) and average weekly air temperature (lines) measured in control plots without chambers from 14 July to 26 October 2013 and from 5 May to 11 October 2014 in Québec City, QC. Arrows indicate time of cutting of the alfalfa-timothy forage plots.

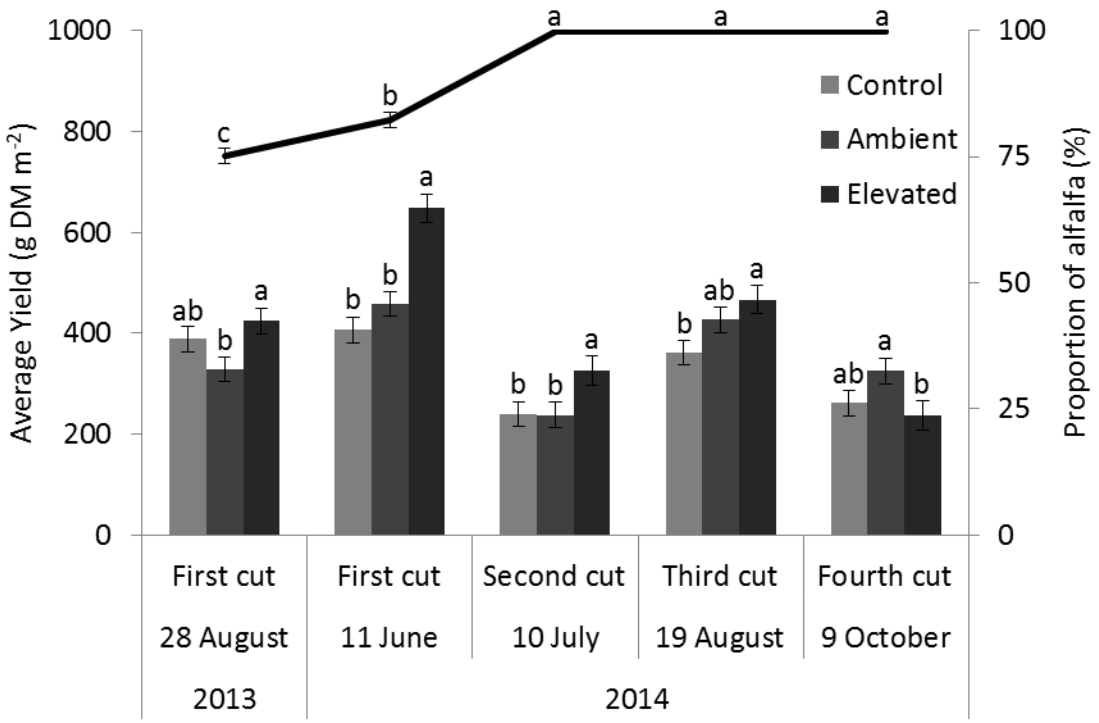


Figure 4.2. Aboveground yield at each cut (significant CO_2 × cut effect, $P<0.001$) of an alfalfa-timothy mixture (bars) grown in open-top chambers under ambient (near 400 μmol mol^{-1}) and elevated (600 μmol mol^{-1}) CO_2 concentration, and without an open-top chamber (control). The proportion of alfalfa in forage dry matter yield is represented by the solid line (significant cut main effect, $P<0.001$). Different letters indicate significant ($P\leq0.05$) differences within a cut for DM yield and across cuts for the contribution of alfalfa to DM yield. Standard error is represented by error bars.

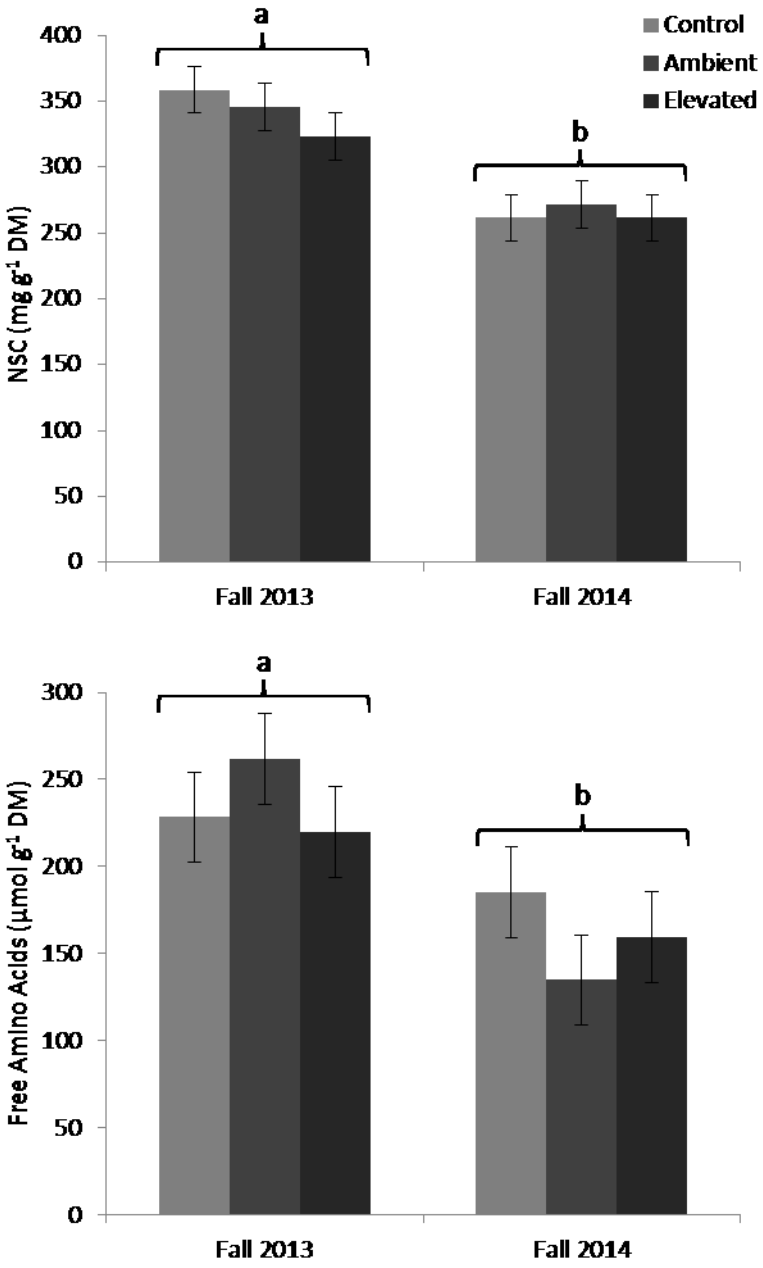


Figure 4.3. Concentrations of non-structural carbohydrates (NSC) and total free amino acids (significant year effect, $P<0.001$ and $P<0.002$, respectively) in the crowns of cold-acclimated alfalfa in fall 2013 compared to fall 2014. Plants were grown in open-top chambers under ambient (near 400 µmol mol^{-1}) and elevated (600 µmol mol^{-1}) [CO_2]. Different letters indicate significant differences between years ($P \leq 0.05$). Standard error is represented by error bars.

Table 4.1. Dry matter forage yield, concentrations of non-structural carbohydrates (NSC), total nitrogen, acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL), as well as *in vitro* true digestibility (IVTD) and NDF digestibility (NDFd) in the biomass of an alfalfa-timothy mixture grown in open-top chambers under ambient (near 400 μmol mol^{-1}) and elevated (600 μmol mol^{-1}) CO_2 concentrations, and without an open-top chamber (control).

Treatment effect	Year	Cut	Treatment	Yield (g DM m^{-2})	NSC	Nitrogen	ADF	NDF	ADL	IVTD	NDFd (mg g^{-1} NDF)
						(mg g^{-1} DM)					
$[CO_2]$											
			Control	331.3 b[1]	66.1	32.9 a	287 c	356 c	67 c	812 a	468 a
			Ambient	354.9 b	61.1	32.3 ab	300 b	369 b	70 b	800 b	456 b
			Elevated	420.0 a	60.4	31.1 b	318 a	388 a	74 a	786 c	448 b
Cut											
	2013	First		379.9 c	49.0 c	30.1 b	308 b	407 a	57 d	828 a	577 a
	2014	First		503.6 a	76.6 a	28.7 c	302 b	388 b	69 c	779 c	430 c
		Second		267.9 d	74.5 a	32.8 a	286 c	338 c	73 bc	796 b	398 d
		Third		418.1 b	50.3 c	34.0 a	330 a	387 b	80 a	770 c	407 d
		Fourth		274.2 d	62.5 b	34.9 a	284 c	334 c	73 b	824 a	473 b
$[CO_2]$ × cut											
	2013	First	Control	387.9 ab[2]		31.8 a			56 a	837 a	588 a
			Ambient	327.9 b		31.2 a			58 a	830 ab	580 a
			Elevated	423.9 a		27.4 b			58 a	817 b	564 a
	2014	First	Control	406.3 b		29.6 a			64 b	803 a	463 a
			Ambient	457.3 b		28.4 ab			67 ab	780 b	440 a
			Elevated	647.3 a		28.1 b			75 a	753 c	388 b
		Second	Control	239.6 b		33.5 a			69 a	810 a	398 a
			Ambient	237.9 b		33.5 a			75 a	802 a	403 a
			Elevated	326.1 a		31.5 a			75 a	777 b	392 a
		Third	Control	361.4 b		37.5 a			72 b	794 a	431 a
			Ambient	426.7 ab		32.7 b			82 a	766 b	392 b
			Elevated	466.0 a		31.7 b			85 a	752 b	399 b
		Fourth	Control	261.4 ab		32.0 a			73 ab	818 a	460 b
			Ambient	324.5 a		35.8 a			70 b	823 a	464 b
			Elevated	236.8 b		37.0 a			77 b	830 a	495 a
P value	$[CO_2]$			0.02	NS[3]	0.032	<0.001	<0.001	<0.001	<0.001	0.007
	Cut			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	$[CO_2]$ x Cut			<0.001	NS	0.016	NS	NS	0.018	0.001	<0.001

[1] Different letters within a column and for a given treatment effect indicate significant differences ($P \leq 0.05$).
[2] Values are presented only when the interaction is significant.
[3] Non-significant with $P>0.05$.

Table 4.2. Dry matter forage yield, leaf photosynthesis, and concentrations of sucrose and total nitrogen in an alfalfa-timothy mixture grown in open-top chambers under ambient (near 400 µmol mol^{-1}) and elevated (600 µmol mol^{-1}) CO_2 concentrations, and without an open-top chamber (control).

Treatment effect	Year	Cut	Treatment	Alfalfa				Timothy			
				Yield (g DM m^{-2})	Photosynthesis (µmol CO_2 m^{-2} sec^{-1})	Sucrose (mg g^{-1} DM)	Nitrogen (mg g^{-1} DM)	Yield (g DM m^{-2})	Photosynthesis (µmol CO_2 m^{-2} sec^{-1})	Sucrose (mg g^{-1} DM)	Nitrogen (mg g^{-1} DM)
$[CO_2]$											
			Control	295.6 b[1]	21.4 b	23.1 a	32.7 a	35.8	12.9 ab	25.3	21.9
			Ambient	319.7 b	19.5 b	18.4 c	31.4 b	35.2	10.8 b	26.9	22.1
			Elevated	377.9 a	23.7 a	21.0 b	30.3 c	37.9	14.9 a	27.6	21.8
Cut											
	2013	First		284.9 b	26.7 a	14.7 d	31.2 b	95.0 a	12.0	12.4 b	20.9 b
	2014	First		418.3 a	23.7 b	24.0 b	27.5 c	83.6 a	13.7	40.8 a	23.0 a
		Second		265.3 b	21.2 c	20.4 c	31.2 b	1.2 b	n.d.[4]	n.d.	n.d.
		Third		415.1 a	20.8 bc	17.8 cd	33.5 a	0.8 b	n.d.	n.d.	n.d.
		Fourth		271.7 b	15.3 d	27.3 a	34.0 a	0.8 b	n.d.	n.d.	n.d.
$[CO_2]$ × cut											
	2013	First	Control	299.9 a[2]			32.7 a		13.2 a		
			Ambient	244.2 b			31.8 a		11.1 a		
			Elevated	310.5 a			29.2 b		11.7 a		
	2014	First	Control	317.2 b			28.9 a		12.5 b		
			Ambient	369.4 b			27.9 a		10.5 b		
			Elevated	568.2 a			25.7 b		18.2 a		
		Second	Control	239.0 b			32.3 a		n.d.		
			Ambient	236.8 b			31.5 ab		n.d.		
			Elevated	320.2 a			29.8 b		n.d.		
		Third	Control	360.2 b			35.8 a		n.d.		
			Ambient	425.1 ab			32.0 b		n.d.		
			Elevated	460.0 a			32.6 b		n.d.		
		Fourth	Control	261.4 ab			33.8 a		n.d.		
			Ambient	323.1 a			33.9 a		n.d.		
			Elevated	230.5 b			34.3 a		n.d.		
P value	$[CO_2]$			0.021	0.001	<0.001	<0.001	NS	0.012	NS	NS
	Cut			<0.001	<0.001	<0.001	<0.001	<0.001	NS	<0.001	0.034
	$[CO_2]$ x Cut			<0.001	NS[3]	NS	NS	NS	0.014	NS	NS

[1] Different letters within a column and for a given treatment effect indicate significant differences ($P \leq 0.05$).
[2] Values are presented only when the interaction is significant.
[3] Non-significant with $P > 0.05$.
[4] Not determined.

Table 4.3. Concentrations of carbon, nitrogen and acid detergent lignin (ADL), as well as dry matter degradability measured by *in vitro* true digestibility (IVTD) of the roots of alfalfa and timothy grown in a mixture in open-top chambers under ambient (near 400 μmol mol^{-1}) and elevated (600 μmol mol^{-1}) CO_2 concentrations, and without an open-top chamber (control).

Year	Species	Treatment	Carbon	Nitrogen	ADL	IVTD
			---------- (mg g^{-1} DM) ----------			
2013	Alfalfa	Control	457	22.1	n.d.[2]	n.d.
		Ambient	453	20.4	n.d.	n.d.
		Elevated	456	21.5	n.d.	n.d.
	Timothy	Control	465	58.7	n.d.	n.d.
		Ambient	462	64.2	n.d.	n.d.
		Elevated	464	65.4	n.d.	n.d.
2014	Alfalfa	Control	466 a[1]	21.1	40	858 c
		Ambient	460 b	20.2	36	872 b
		Elevated	459 b	20.2	38	898 a

[1] Different letters within a column and for a given treatment effect indicate significant differences ($P \leq 0.05$).
[2] Not determined.

CHAPTER 5

CONCLUSIONS AND SUMMARY

Alfalfa-timothy mixtures are widely grown throughout Canada, but information on their response to elevated [CO_2] is limited due to technical and cost limitations. Therefore, our study aimed to fabricate a low-cost yet efficient system, allowing us to assess the effects of elevated [CO_2] on a perennial alfalfa-timothy mixture.

The first objective was to build an efficient and low-cost OTC system on an experimental site located in Québec City, QC. Building OTCs from widely available materials made them relatively inexpensive compared to other systems. The chamber had minimal effects on air temperature and light transmission, which limited the alteration of growing conditions within OTCs. The system demonstrated its effectiveness by maintaining a stable [CO_2] and minimizing CO_2 consumption. An identical system was installed in Lacombe, AB during spring 2014, showing the facility to transpose this design to another location.

The second objective was to assess the effects of elevated [CO_2] on yield, nutritive value, organic reserve accumulation and potential for carbon sequestration of an alfalfa-timothy mixture grown in OTCs during two growing seasons in Québec City, QC. Elevated [CO_2] increased the yield of the mixture, particularly during the warmest months of the growing season. The increase in yield was greater in alfalfa than timothy, which proportion decreased gradually in the mixture. The NSC concentration of the mixture and both species was not affected by elevated [CO_2]. The total N concentration was slightly decreased in the mixture, and that effect was significant in alfalfa but not in timothy. Significantly higher ADF and NDF concentrations were found in the mixture, which slightly reduced the digestibility of the forage. Fall organic reserve accumulation measured in the crowns of both species during a period of cold acclimation

was not affected by elevated [CO_2]. In spite of unaffected root C and N concentrations in both species, root degradability of alfalfa was increased under elevated [CO_2].

CHAPTER 6

RECOMMENDATION FOR FUTURE RESEARCH

Our study highlighted the importance to further investigate the effects of elevated [CO_2] in forages grown in field and point out major knowledge gaps that should be fulfilled to mitigate the impacts of climate change at farm level. First of all, long-term field studies with a duration similar to the life span of perennial forages are necessary to understand the actual plant response to elevated [CO_2]. Those studies could, among other things, assess if forages acclimate to elevated [CO_2], translating in a transient positive effect of elevated [CO_2] on photosynthesis and growth. Also, the effects of cutting regime on fall organic reserve accumulation and winter survival should be pursued on three to four years to understand the effects of elevated [CO_2] on forage persistence. Future experiments should also evaluate the effects of elevated [CO_2] on different forage species in a field setting. The yield, nutritive value, persistence and root degradability of each species could be compared and serve as a basis to determine the best options for farmers under predicted climate conditions. Moreover, different forage mixtures could also be evaluated.

Most importantly, our study, by describing in detail the design and construction of efficient open-top chambers, provide an easily transposable turn-key system which could be installed under various climatic regions. These low-cost open-top chambers provide a basis for long-term multi-site comparison of plant response to elevated [CO_2] and climate.

LITERATURE CITED

Adaros, G., Weigel, H. and Jäger, H. 1991. Concurrent exposure to SO_2 and/or NO_2 alters growth and yield responses of wheat and barley to low concentrations of O_3. New phytologist 118(4):581-591.

Ainsworth, E. A., Davey, P. A., Bernacchi, C. J., Dermody, O. C., Heaton, E. A., Moore, D. J., Morgan, P. B., Naidu, S. L., Yoo Ra, H. S. and Zhu, X. G. 2002. A meta-analysis of elevated [CO_2] effects on soybean (Glycine max) physiology, growth and yield. Global Change Biology 8(8):695-709.

Ainsworth, E. A. and Long, S. P. 2005. What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. New Phytolologist 165(2):351-71.

Ainsworth, E. A., Rogers, A., Nelson, R. and Long, S. P. 2004. Testing the "source–sink" hypothesis of down-regulation of photosynthesis in elevated [CO_2] in the field with single gene substitutions in Glycine max. Agricultural and Forest Meteorology 122(1-2):85-94.

Allard, G., Pellerin, D., Michaud, R. and Perron, M. 1998. Les fourrages: quelque chose à ne pas manquer. Proceedings of the Symposium québécois sur les bovins laitiers.

Aranjuelo, I., Irigoyen, J. J., Nogués, S. and Sánchez-Díaz, M. 2009. Elevated CO_2 and water-availability effect on gas exchange and nodule development in N_2-fixing alfalfa plants. Environmental and Experimental Botany 65(1):18-26.

Ashenden, T., Baxter, R. and Rafarel, C. 1992. An inexpensive system for exposing plants in the field to elevated concentrations of CO_2. Plant, Cell & Environment 15(3):365-372.

Association of Official Analytical Chemists. 2005. Official methods of analysis. 17th ed. Association of Official Analytical Chemists, Washington, DC.

Barbehenn, R. V., Chen, Z., Karowe, D. N. and Spickard, A. 2004. C_3 grasses have higher nutritional quality than C_4 grasses under ambient and elevated atmospheric CO_2. Global Change Biology 10(9):1565-1575.

Barton, C. V., Ellsworth, D. S., Medlyn, B. E., Duursma, R. A., Tissue, D. T., Adams, M. A., Eamus, D., Conroy, J. P., McMurtrie, R. E. and Parsby, J. 2010. Whole-tree chambers for elevated atmospheric CO_2 experimentation and tree scale flux measurements in south-eastern Australia: The Hawkesbury Forest Experiment. Agricultural and Forest Meteorology 150(7):941-951.

Baslam, M., Antolín, M. C., Gogorcena, Y., Muñoz, F. and Goicoechea, N. 2014. Changes in alfalfa forage quality and stem carbohydrates induced by arbuscular mycorrhizal fungi and elevated atmospheric CO_2. Annals of Applied Biology 164(2):190-199.

Bélanger, G., Castonguay, Y., Bertrand, A., Dhont, C., Rochette, P., Couture, L., Drapeau, R., Mongrain, D., Chalifour, F.-P. and Michaud, R. 2006. Winter damage to perennial forage crops in eastern Canada: Causes, mitigation, and prediction. Canadian journal of plant science 86(1): 33-47.

Bélanger, G., Castonguay, Y. and Lajeunesse, J. 2014. Benefits of mixing timothy with alfalfa for forage yield, nutritive value, and weed suppression in northern environments. Canadian Journal of Plant Science 94(1):51-60.

Bélanger, G., Kunelius, T., McKenzie, D., Papadopoulos, Y., Thomas, B., McRae, K., Fillmore, S. and Christie, B. 1999. Fall cutting management affects yield and persistence of alfalfa in Atlantic Canada. Canadian Journal of Plant Science 79(1):57-63.

Bélanger, G., Michaud, R., Jefferson, P., Tremblay, G. and Brégard, A. 2001. Improving the nutritive value of timothy through management and breeding. Canadian Journal of Plant Science 81(4):577-585.

Bélanger, G., Rochette, P., Castonguay, Y., Bootsma, A., Mongrain, D. and Ryan, D. A. 2002. Climate change and winter survival of perennial forage crops in eastern Canada. Agronomy Journal 94(5):1120-1130.

Berg, C. C., McElroy, A. R. and Kunelius, H. T. 1996. Timothy. *In:* L. E. Moser, D. R. Buxton, M. D. Casler, eds. Cool-Season Forage Grasses. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, WI. pages 643-664.

Bertrand, A., Piva, A., Tremblay, G. F., Bélanger, G., Castonguay, Y. and Seguin, P. 2014. Elevated CO_2 and Temperature Affect Composition and in vitro Degradability of Timothy Roots. Crop Science 54(6):2893-2902.

Bertrand, A., Prevost, D., Bigras, F. J. and Castonguay, Y. 2007a. Elevated atmospheric CO_2 and strain of rhizobium alter freezing tolerance and cold-induced molecular changes in alfalfa (Medicago sativa). Annals of Botany 99(2):275-84.

Bertrand, A., Prévost, D., Bigras, F. J., Lalande, R., Tremblay, G. F., Castonguay, Y. and Bélanger, G. 2007b. Alfalfa response to elevated atmospheric CO_2 varies with the symbiotic rhizobial strain. Plant and Soil 301(1-2):173-187.

Bertrand, A., Robitaille, G., Nadeau, P. and Castonguay, Y. 1999. Influence of ozone on cold acclimation in sugar maple seedlings. Tree physiology 19(8):527-534.

Bertrand, A., Tremblay, G. F., Pelletier, S., Castonguay, Y. and Bélanger, G. 2008. Yield and nutritive value of timothy as affected by temperature, photoperiod and time of harvest. Grass and Forage Science 63(4):421-432.

Bertrand, I., Chabbert, B., Kurek, B. and Recous, S. 2006. Can the biochemical features and histology of wheat residues explain their decomposition in soil? Plant and Soil 281(1-2):291-307.

Bishop, K. A., Leakey, A. D. B. and Ainsworth, E. A. 2014. How seasonal temperature or water inputs affect the relative response of C_3 crops to elevated [CO_2]: a global analysis of open top chamber and free air CO_2 enrichment studies. Food and Energy Security 31(1):33-45.

Bloom, A. J., Burger, M., Asensio, J. S. R. and Cousins, A. B. 2010. Carbon dioxide enrichment inhibits nitrate assimilation in wheat and Arabidopsis. Science 328(5980):899-903.

Brito, A., Tremblay, G., Bertrand, A., Castonguay, Y., Bélanger, G., Michaud, R., Lapierre, H., Benchaar, C., Petit, H. and Ouellet, D. 2008. Alfalfa cut at sundown and harvested as baleage improves milk yield of late-lactation dairy cows. Journal of dairy science 91(10):3968-3982.

Brown, R. and Radcliffe, D. 1986. A comparison of apparent photosynthesis in sericea lespedeza and alfalfa. Crop science 26(6):1208-1211.

Bunce, J. A. 2004. Carbon dioxide effects on stomatal responses to the environment and water use by crops under field conditions. Oecologia 140(1):1-10.

Bunce, J. A. 2011. Performance characteristics of an area distributed free air carbon dioxide enrichment (FACE) system. Agricultural and Forest Meteorology 151(8):1152-1157.

Buxton, D. R. 1996. Quality-related characteristics of forages as influenced by plant environment and agronomic factors. Animal Feed Science and Technology 59(1):37-49.

Campbell, B. and Stafford Smith, D. 2000. A synthesis of recent global change research on pasture and rangeland production: reduced uncertainties and their management implications. Agriculture, ecosystems & environment 82(1):39-55.

Casella, E. and Soussana, J. 1997. Long-term effects of CO_2 enrichment and temperature increase on the carbon balance of a temperate grass sward. Journal of Experimental Botany 48(6):1309-1321.

Casella, E., Soussana, J. and Loiseau, P. 1996. Long-term effects of CO_2 enrichment and temperature increase on a temperate grass sward. Plant and Soil 182(1):83-99.

Castonguay, Y., Bertrand, A., Michaud, R. and Laberge, S. 2011. Cold-induced biochemical and molecular changes in alfalfa populations selectively improved for freezing tolerance. Crop Science 51(5):2132-2144.

Castonguay, Y., Nadeau, P., Lechasseur, P. and Chouinard, L. 1995. Differential accumulation of carbohydrates in alfalfa cultivars of contrasting winterhardiness. Crop Science 35(2):509-516.

Cheesman, A. W. and Winter, K. 2013. Elevated night-time temperatures increase growth in seedlings of two tropical pioneer tree species. New Phytologist 197(4):1185-1192.

Cohen, S.A. 2000. Amino acid analysis using precolumn derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate. *In*: C. Cooper, et al., eds. Amino Acid Analysis Protocols. Humana Press, Totowa, NJ. p. 39-47.

Cotrufo, M.F., Ineson, P. and Rowland, A.P. 1994. Decomposition of tree leaf litters grown under elevated CO_2: Effect of litter quality. Plant and Soil 163(1):121-130.

Cotrufo, M. F., Ineson, P. and Scott, A. 1998. Elevated CO_2 reduces the nitrogen concentration of plant tissues. Global Change Biology 4(1):43-54.

CRAAQ. 2010. Reference guide for crop fertilization. (In French.) 2nd ed. Centre de Référence en Agriculture et Agroalimentaire du Québec, Québec, QC, Canada.

D'Andrea, L. and Rinaldi, M. 2010. Systems to evaluate the effects of atmospheric CO_2 concentration on field crops: a review of open top chambers. Italian Journal of Agrometeorology 15(1):23-34.

De Boeck, H. J., De Groote, T. and Nijs, I. 2012. Leaf temperatures in glasshouses and open-top chambers. New Phytologist 194(4):1155-1164.

Dhont, C., Castonguay, Y., Nadeau, P., Bélanger, G. and Chalifour, F.-P. 2002. Alfalfa root carbohydrates and regrowth potential in response to fall harvests. Crop Science 42(3):754-765.

Dhont, C., Castonguay, Y., Nadeau, P., Bélanger, G. and Chalifour, F.-P. 2003. Alfalfa root nitrogen reserves and regrowth potential in response to fall harvests. Crop Science 43(1):181-194.

Dhont, C., Castonguay, Y., Nadeau, P., Belanger, G., Drapeau, R., Laberge, S., Avice, J.-C. and Chalifour, F.-P. 2006. Nitrogen reserves, spring regrowth and winter survival of field-grown alfalfa (*Medicago sativa*) defoliated in the autumn. Annals of Botany 97(1):109-120.

Dodd, M. B. 2013. Elevated CO_2 impacts on grazed pasture: long-term lessons from the New Zealand FACE. Proceedings of the 22nd International Grassland Congress, Sydney, Australia. pages 1279-1284.

Drake, B. and Leadley, P. 1991. Canopy photosynthesis of crops and native plant communities exposed to long-term elevated CO_2. Plant, Cell & Environment 14(8):853-860.

Drake, B., Leadley, P., Arp, W., Nassiry, D. and Curtis, P. 1989. An open top chamber for field studies of elevated atmospheric CO_2 concentration on saltmarsh vegetation. Functional Ecology 3(3):363-371.

Drake, B. G. 2014. Rising sea level, temperature, and precipitation impact plant and ecosystem responses to elevated CO_2 on a Chesapeake Bay wetland: Review of a 28 year study. Global Change Biology.

Erice, G., Irigoyen, J. J., Pérez, P., Martínez-Carrasco, R. and Sánchez-Díaz, M. 2006a. Effect of elevated CO_2, temperature and drought on dry matter partitioning and photosynthesis before and after cutting of nodulated alfalfa. Plant Science 170(6):1059-1067.

Erice, G., Irigoyen, J. J., Pérez, P., Martínez-Carrasco, R. and Sánchez-Díaz, M. 2006b. Effect of elevated CO_2, temperature and drought on photosynthesis of nodulated alfalfa during a cutting regrowth cycle. Physiologia Plantarum 126(3):458-468.

Fick, G. W. and Mueller, S. C. 1989. Alfalfa: Quality, maturity, and mean stage of development. Department of Agronomy, College of Agriculture and Life Sciences, Cornell University.

Goering, H. K. and Van Soest, P. J. 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications). USDA Agronomy Handbook.

Grace, J. 1988. Plant response to wind. Agriculture, Ecosystems & Environment 22/23:71-88.

Greenfield, P. and Smith, D. 1973. Influence of temperature change at bud on composition of alfalfa at first flower. Agronomy Journal 65(6):871-874.

Gudleifsson, B. E. 2010. Ice tolerance and metabolite accumulation of herbage crops in Iceland and impact of climate change. Iceland Agriculture Science 23:111-122.

Hanslin, H. M. and Mortensen, L. M. 2010. Autumn growth and cold hardening of winter wheat under simulated climate change. Acta Agriculturae Scandinavica Section B - Soil and Plant Science 60(5):437-449.

Heagle, A., Philbeck, R., Rogers, H. and Letchworth, M. 1979. Dispensing and monitoring ozone in open-top field chambers for plant-effects studies. Phytopathology 69(1):15-20.

Hebeisen, T., Lüscher, A., Zanetti, S., Fischer, B., Hartwig, U., Frehner, M., Hendrey, G. and Blum, H. 1997. Growth response of Trifolium repens L. and Lolium perenne L. as monocultures and bi-species mixture to free air CO_2 enrichment and management. Global Change Biology 3(2):149-160.

IPCC. 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Irigoyen, J. J., Goicoechea, N., Antolin, M. C., Pascual, I., Sanchez-Diaz, M., Aguirreolea, J. and Morales, F. 2014. Growth, photosynthetic acclimation and yield quality in legumes under climate change simulations: An updated survey. Plant Science 226:22-29.

Isaac, R.A., Johnson, W.C., 1976. Determination of total nitrogen in plant tissue, using a block digestor. Journal Association of Official Analytical Chemists. 59:98-100.

ISQ. 2014. Superficie des grandes cultures, rendement à l'hectare et production, par région administrative, Québec, 2014 [Online]. Available at http://www.stat.gouv.qc.ca/statistiques/agriculture/grandes-cultures/gc_2014.htm (verified 03 March 2015).

Jing, Q., Bélanger, G., Qian, B. and Baron, V. 2013. Timothy yield and nutritive value under climate change in Canada. Agronomy Journal 105(6):1683-1694.

Jones, M. B., Jongen, M. and Doyle, T. 1996. Effects of elevated carbon dioxide concentrations on agricultural grassland production. Agricultural and Forest Meteorology 79(4):243-252.

Jongen, M., Jones, M. B., Hebeisen, T., Blum, H. and Hendrey, G. 1995. The effects of elevated CO_2 concentrations on the root growth of *Lolium perenne* and *Trifolium repens* grown in a FACE system. Global Change Biology 1(5):361-371.

Jurczyk, B., Rapacz, M. and Krępski, T. 2013. Short-term growth of meadow fescue with atmospheric CO_2 enrichment decreases freezing tolerance, modifies photosynthetic apparatus performance and changes the expression of some genes during cold acclimation. Acta Physiologiae Plantarum 35(5):1543-1554.

Kawas, J., Jorgensen, N. and Danelon, J. 1991. Fiber requirements of dairy cows: optimum fiber level in lucerne-based diets for high producing cows. Livestock Production Science 28(2):107-119.

Kimball, B., Pinter, P., Wall, G. W., Garcia, R., LaMorte, R., Jak, P., Frumau, K. and Vugts, H. F. 1997. Comparisons of responses of vegetation to elevated carbon dioxide in free-air and open-top chamber facilities. Advances in Carbon Dioxide Effects Research (ASA Special Publication) 61(5):113-130.

Kimball, B. A. 1992. Cost comparisons among free-air CO_2 enrichment, open-top chamber, and sunlit controlled-environment chamber methods of CO_2 exposure. Critical Reviews in Plant Sciences 11(2-3):265-270.

Lawlor, D. and Mitchell, R. 1991. The effects of increasing CO_2 on crop photosynthesis and productivity: a review of field studies. Plant, Cell & Environment 14(8):807-818.

Leadley, P., Niklaus, P., Stocker, R. and Körner, C. 1997. Screen-aided CO_2 control (SACC): a middle ground between FACE and open-top chambers. Acta Oecologica 18(3):207-219.

Leadley, P. W. and Drake, B. G. 1993. Open top chambers for exposing plant canopies to elevated CO_2 concentration and for measuring net gas exchange. Vegetatio 104/105:3-15.

Lee, T. D., Barrott, S. H. and Reich, P. B. 2011. Photosynthetic responses of 13 grassland species across 11 years of free-air CO_2 enrichment is modest, consistent and independent of N supply. Global Change Biology 17(9):2893-2904.

Long, S. 1991. Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO_2 concentrations: has its importance been underestimated? Plant, Cell & Environment 14(8):729-739.

Long, S. P., Ainsworth, E. A., Leakey, A. D., Nösberger, J. and Ort, D. R. 2006. Food for thought: lower-than-expected crop yield stimulation with rising CO_2 concentrations. Science 312(5782):1918-1921.

Luo, Y., Hui, D. and Zhang, D. 2006. Elevated CO_2 stimulates net accumulations of carbon and nitrogen in land ecosystems: a meta-analysis. Ecology 87(1):53-63.

Lüscher, A., Hartwig, U. A., Suter, D. and Nösberger, J. 2000. Direct evidence that symbiotic N_2 fixation in fertile grassland is an important trait for a strong response of plants to elevated atmospheric CO_2. Global Change Biology 6(6):655-662.

Lüscher, A., Mueller-Harvey, I., Soussana, J., Rees, R. and Peyraud, J. 2014. Potential of legume-based grassland-livestock systems in Europe: a review. Grass and Forage Science 69(2):206-228.

MacDowall, F. 1982. Effects of light intensity and CO_2 concentration on the kinetics of 1st month growth and nitrogen fixation of alfalfa. Canadian Journal of Botany 61(3):731-740.

Malhi, S., Zentner, R. and Heier, K. 2002. Effectiveness of alfalfa in reducing fertilizer N input for optimum forage yield, protein concentration, returns and energy performance of bromegrass-alfalfa mixtures. Nutrient Cycling in Agroecosystems 62(3):219-227.

Mandl, R., Weinstein, L., McCune, D. and Keveny, M. 1973. A cylindrical, open-top chamber for the exposure of plants to air pollutants in the field. Journal of Environmental Quality 2(3):371-376.

Margolis, H. A. and Vézina, L.-P. 1990. Atmospheric CO_2 enrichment and the development of frost hardiness in containerized black spruce seedlings. Canadian Journal of Forest Research 20(9):1392-1398.

Mertens, D. R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. Journal of Association of Official Analytical Chemists International 85(6):1217-1240.

Messerli, J., Bertrand, A., Bourassa, J., Bélanger, G., Castonguay, Y., Tremblay, G., Baron, V. and Seguin, P. 2015. Performance of low-cost open-top chambers to study long-term effects of carbon dioxide and climate under field conditions. Agronomy Journal. Published March 6th 2015.

Michaud, R., Lehman, W. F. and Rumbaugh, M. D. 1988. World Distribution and Historical Development. *In:* A. A. Hanson, D. K. Barnes, R. R. Hill, eds. Alfalfa and Alfalfa Improvement. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, WI. pages 25-91.

Miglietta, F., Peressotti, A., Vaccari, F. P., Zaldei, A., DeAngelis, P. and Scarascia-Mugnozza, G. 2001. Free-air CO_2 enrichment (FACE) of a poplar plantation: the POPFACE fumigation system. New Phytologist 150(2):465-476.

Milchunas, D., Morgan, J., Mosier, A. and LeCain, D. 2005a. Root dynamics and demography in shortgrass steppe under elevated CO_2, and comments on minirhizotron methodology. Global Change Biology 11(10):1837-1855.

Milchunas, D., Mosier, A., Morgan, J., LeCain, D., King, J. and Nelson, J. 2005b. Elevated CO_2 and defoliation effects on a shortgrass steppe: Forage quality versus quantity for ruminants. Agriculture, Ecosystems & Environment 111(1):166-184.

Milchunas, D., Mosier, A., Morgan, J., LeCain, D., King, J. and Nelson, J. 2005c. Root production and tissue quality in a shortgrass steppe exposed to elevated CO_2: using a new ingrowth method. Plant and Soil 268(1):111-122.

Miller, L., Moorby, J. M., Davies, D. R., Humphreys, M. O., Scollan, N. D., MacRae, J. C. and Theodorou, M. K. 2001. Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.): milk production from late-lactation dairy cows. Grass and Forage Science 56(4):383-394.

Minson, D. J. 1990. Forage in ruminant nutrition. Academic Press. New-York.

Monastersky, R. 2013. Global carbon dioxide levels near worrisome milestone. Nature 497(7447):13.

Moore, K. J. and Hatfield, R. D. 1994. Carbohydrates and Forage Quality. *In* G. C. Fahey, ed. Forage Quality, Evaluation, and Utilization. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America. pages 229-280.

Morgan, J. A., Mosier, A. R., Milchunas, D. G., LeCain, D. R., Nelson, J. A. and Parton, W. J. 2004. CO_2 enhances productivity, alters species composition, and reduces digestibility of shortgrass steppe vegetation. Ecological Applications 14(1):208-219.

Murata, Y. and Iyama, J. 1963. Studies on the Photosynthesis of Forage Crops: II. Influence of air-temperature upon the photosynthesis of some forage and grain crops. Japanese Journal of Crop Science 31(4):315-322.

Neftel, A., Oeschger, H., Staffelbach, T. and Stauffer, B. 1988. CO_2 record in the Byrd ice core 50, 000-5, 000 years bp. Nature 331(6157):609-611.

Newman, J., Abner, M., Dado, R., Gibson, D., Brookings, A. and Parsons, A. 2003. Effects of elevated CO_2, nitrogen and fungal endophyte-infection on tall fescue: growth, photosynthesis, chemical composition and digestibility. Global Change Biology 9(3):425-437.

Newton, P., Clark, H., Bell, C., Glasgow, E. and Campbell, B. 1994. Effects of elevated CO_2 and simulated seasonal changes in temperature on the species composition and growth rates of pasture turves. Annals of Botany 73(1):53-59.

Norby, R., Edwards, N., Riggs, J., Abner, C., Wullschleger, S. and Gunderson, C. 1997. Temperature-controlled open-top chambers for global change research. Global Change Biology 3(3):259-267.

Norris, T., Wilkinson, D., Lockwood, A., Belay, A., Colls, J. and Bailey, B. 1996. Performance of a controlled-ventilation open-top chamber for climate change research. Agricultural and Forest Meteorology 78(3):239-257.

Nyfeler, D., Huguenin-Elie, O., Suter, M., Frossard, E. and Lüscher, A. 2011. Grass-legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources. Agriculture, Ecosystems & Environment 140(1):155-163.

Obrist, D., Arnone, J. and Körner, C. 2001. *In situ* Effects of Elevated Atmospheric CO_2 on Leaf Freezing Resistance and Carbohydrates in a Native Temperate Grassland. Annals of Botany 87(6):839-844.

Okada, M., Lieffering, M., Nakamura, H., Yoshimoto, M., Kim, H. and Kobayashi, K. 2001. Free-air CO_2 enrichment (FACE) using pure CO_2 injection: system description. New Phytologist 150(2):251-260.

Pepin, S. and Körner, C. 2002. Web-FACE: a new canopy free-air CO_2 enrichment system for tall trees in mature forests. Oecologia 133(1):1-9.

Peterbauer, T. and Richter, A. 2001. Biochemistry and physiology of raffinose family oligosaccharides and galactosyl cyclitols in seeds. Seed Science Research 11(03):185-197.

Petersen, B. M., Jensen, B. and Christensen, B. T. 2001. A movable miniature free air CO_2 enrichment (Mini-FACE) facility for field-grown crops. Acta Agriculturae Scandinavica, Section B - Plant Soil Science 51(3):167-172.

Piva, A., Bertrand, A., Bélanger, G., Castonguay, Y. and Seguin, P. 2013. Growth and physiological response of timothy to elevated carbon dioxide and temperature under contrasted nitrogen fertilization. Crop Science 53(2):704-715.

Pollock, C. 1986. Tansley review no. 5. Fructans and the metabolism of sucrose in vascular plants. New Phytologist 104(1):1-24.

Poorter, H. 1993. Interspecific variation in the growth response of plants to an elevated ambient CO_2 concentration. Vegetatio 104(1):77-97.

Poorter, H., Berkel, Y. v., Baxter, R., Hertog, J. d., Dijkstra, P., Gifford, R., Griffin, K., Roumet, C., Roy, J. and Wong, S. 1997. The effect of elevated CO_2 on the chemical

composition and construction costs of leaves of 27 C_3 species. Plant, Cell & Environment 20(4):472-482.

Raynaud, D. and Barnola, J. 1985. An Antarctic ice core reveals atmospheric CO_2 variations over the past few centuries. Nature 315(6017):309-311.

Read, J., Morgan, J., Chatterton, N. and Harrison, P. 1997. Gas exchange and carbohydrate and nitrogen concentrations in leaves of *Pascopyrum smithii* (C_3) and *Bouteloua gracilis* (C_4) at different carbon dioxide concentrations and temperatures. Annals of Botany 79(2):197-206.

Robertson, J.B., and P.J. Van Soest. 1981. The detergent system of analysis and its applications to human foods. *In*: W.P.T. James and O. Theander, eds. The Analysis of Dietary Fiber. Marcel Dekker, New York, NY. pages 123-158.

Rogers, H., Bingham, G., Cure, J., Smith, J. and Surano, K. 1983. Responses of selected plant species to elevated carbon dioxide in the field. Journal of Environmental Quality 12(4):569-574.

Ross, D., Newton, P. and Tate, K. 2004. Elevated [CO_2] effects on herbage production and soil carbon and nitrogen pools and mineralization in a species-rich, grazed pasture on a seasonally dry sand. Plant and Soil 260(1-2):183-196.

Sæbø, A. and Mortensen, L. M. 1996. The influence of elevated CO_2 concentration on growth of seven grasses and one clover species in a cool maritime climate. Acta Agriculturae Scandinavica B - Plant Soil Sciences 46(1):49-54.

Sæbø, A. and Mortensen, L. M. 1995. Growth and regrowth of *Phleum pratense, Lolium perenne, Trifolium repens* and *Trifolium pratense* at normal and elevated CO_2 concentration. Agriculture, Ecosystems & Environment 55(1):29-35.

Sakugawa, H. and Cape, J. N. 2007. Harmful effects of atmospheric nitrous acid on the physiological status of Scots pine trees. Environmental Pollution 147(3):532-534.

Salvucci, M. E. and Crafts-Brandner, S. J. 2004. Inhibition of photosynthesis by heat stress: the activation state of Rubisco as a limiting factor in photosynthesis. Physiologia Plantarum 120(2):179-186.

Sanz-Sáez, Á., Erice, G., Aguirreolea, J., Irigoyen, J. J. and Sánchez-Díaz, M. 2012a. Alfalfa yield under elevated CO_2 and temperature depends on the *Sinorhizobium* strain and growth season. Environmental and Experimental Botany 77:267-273.

Sanz-Sáez, Á., Erice, G., Aguirreolea, J., Munoz, F., Sánchez-Díaz, M. and Irigoyen, J. J. 2012b. Alfalfa forage digestibility, quality and yield under future climate change scenarios vary with *Sinorhizobium meliloti* strain. Journal of Plant Physiology 169(8):782-788.

Sanz-Sáez, Á., Erice, G., Aranjuelo, I., Aroca, R., RuÍz-Lozano, J. M., Aguirreolea, J., Irigoyen, J. J. and Sanchez-Diaz, M. 2013. Photosynthetic and molecular markers of CO_2-mediated photosynthetic downregulation in nodulated alfalfa. Journal of Integrative Plant Biology 55(8):721-734.

Sanz-Sáez, Á., Erice, G., Aranjuelo, I., Nogués, S., Irigoyen, J. J. and Sánchez-Díaz, M. 2010. Photosynthetic down-regulation under elevated CO_2 exposure can be prevented by nitrogen supply in nodulated alfalfa. Journal of Plant Physiology 167(18):1558-1565.

Schenk, U., Jäger, H. J. and Weigel, H. J. 1997. The response of perennial ryegrass/white clover mini-swards to elevated atmospheric CO_2 concentrations: effects on yield and fodder quality. Grass and Forage Science 52(3):232-241.

Seligman, N. a. G. and Sinclair, T. R. 1995. Global environment change and simulated forage quality of wheat II. Water and nitrogen stress. Field Crops Research 40(1):29-37.

Shi, C., Silva, L. C., Zhang, H., Zheng, Q., Xiao, B., Wu, N. and Sun, G. 2015. Climate warming alters nitrogen dynamics and total non-structural carbohydrate accumulations of perennial herbs of distinctive functional groups during the plant senescence in autumn in an alpine meadow of the Tibetan Plateau, China. Agricultural and Forest Meteorology 200:21-29.

Silver, W. L., and Miya, R. K. 2001. Global patterns in root decomposition: comparisons of climate and litter quality effects. Oecologia 129(3):407-419.

Simon, U. and Park, B. 1983. A descriptive scheme for stages of development in perennial forage grasses. *In*: J.A. Smith and V.W. Hays, eds. Proceedings of the 14th International Grassland Congress, Lexington, KY. 15-24 Jun. 1981. Westview Press, Boulder, CO. pages 416-418.

Smith, D. 1968. Carbohydrates in grasses. IV. Influence of temperature on the sugar and fructosan composition of timothy plant parts at anthesis. Crop Science 8(3):331-334.

Smith, D. 1969. Influence of temperature on the yield and chemical composition of 'Vernal'alfalfa at first flower. Agronomy Journal 61(3):470-472.

Smith, D. 1970. Influence of temperature on the yield and chemical composition of five forage legume species. Agronomy Journal 62(4):520-523.

Smith, D., Jacques, A. and Balasko, J. 1973. Persistence of several temperate grasses grown with alfalfa and harvested two, three, or four times annually at two stubble heights. Crop Science 13(5):553-556.

Soussana, J., Casella, E. and Loiseau, P. 1996. Long-term effects of CO_2 enrichment and temperature increase on a temperate grass sward. Plant and soil 182(1):101-114.

Statistics Canada. 2014a. Field and special crops (seeded area) [Online]. Available at http://www.statcan.gc.ca/tables-tableaux/sum-som/l01/cst01/prim11a-eng.htm (verified 03 March 2015).

Statistics Canada. 2014b. Farm cash receipts [Online]. Available at http://www.statcan.gc.ca/tables-tableaux/sum-som/l01/cst01/agri03a-eng.htm (verified 03 March 2015).

Talbot, J. M., Yelle, D. J., Nowick, J. and Treseder, K. K. 2012. Litter decay rates are determined by lignin chemistry. Biogeochemistry 108(1-3):279-295.

Teyssonneyre, F., Picon-cochard, C., Falcimagne, R. and Soussana, J. F. 2002. Effects of elevated CO_2 and cutting frequency on plant community structure in a temperate grassland. Global Change Biology 8(10):1034-1046.

Thorvaldsson, G., Tremblay, G. F. and Tapani Kunelius, H. 2007. The effects of growth temperature on digestibility and fibre concentration of seven temperate grass species. Acta Agriculturae Scandinavica Section B - Soil and Plant Science 57(4):322-328.

Tingey, D. T., Mckane, R. B., Olszyk, D. M., Johnson, M. G., Rygiewicz, P. T. and Henry Lee, E. 2003. Elevated CO_2 and temperature alter nitrogen allocation in Douglas-fir. Global Change Biology 9(7):1038-1050.

Tremblay, G. F., Morin, C., Bélanger, G., Bertrand, A., Castonguay, Y., Berthiaume, R. and Allard, G. 2014. Silage Fermentation of PM- and AM-cut alfalfa wilted in wide and narrow swaths. Crop Science 54(1):439-452.

Van Soest, P., Robertson, J. and Lewis, B. 1991. Symposium: carbohydrate methodology, metabolism, and nutritional implications in dairy cattle. Journal of Dairy Science 74(10):3583-3597.

Volenec, J., Ourry, A. and Joern, B. 1996. A role for nitrogen reserves in forage regrowth and stress tolerance. Physiologia Plantarum 97(1):185-193.

Vough, L. and Marten, G. 1971. Influence of soil moisture and ambient temperature on yield and quality of alfalfa forage. Agronomy Journal 63(1):40-42.

Waite, R. 1963. Botanical and chemical changes in maturing grass and their effect on its digestibility. Agricultural Progress 38:50-56.

Wang, D., Heckathorn, S. A., Wang, X. and Philpott, S. M. 2012. A meta-analysis of plant physiological and growth responses to temperature and elevated CO_2. Oecologia 169(1):1-13.

Weigel, H., Adaros, G. and Jäger, H. 1990. Yield responses of different crop species to long-term fumigation with sulphur dioxide in open-top chambers. Environmental Pollution 67(1):15-28.

Whitehead, D., Hogan, K., Rogers, G., Byers, J., Hunt, J., McSeveny, T., Hollinger, D., Dungan, R., Earl, W. and Bourke, M. 1995. Performance of large open-top chambers for long-term field investigations of tree response to elevated carbon dioxide concentration. Journal of Biogeography 22(2-3):307-313.

Williams, M., Shewry, P., Lawlor, D. and Harwood, J. 1995. The effects of elevated temperature and atmospheric carbon dioxide concentration on the quality of grain lipids in wheat (*Triticum aestivum* L.) grown at two levels of nitrogen application. Plant, Cell & Environment 18(9):999-1009.

Wilson, J. 1994. Cell wall characteristics in relation to forage digestion by ruminants. The Journal of Agricultural Science 122(02):173-182.

Xu, Z., Shimizu, H., Ito, S., Yagasaki, Y., Zou, C., Zhou, G. and Zheng, Y. 2014. Effects of elevated CO_2, warming and precipitation change on plant growth, photosynthesis and peroxidation in dominant species from North China grassland. Planta 239(2):421-435.

Xu, Z., Shimizu, H., Yagasaki, Y., Ito, S., Zheng, Y. and Zhou, G. 2013. Interactive effects of elevated CO_2, drought, and warming on plants. Journal of Plant Growth Regulation 32(4):692-707.

Yu, J., Du, H., Xu, M. and Huang, B. 2012. Metabolic responses to heat stress under elevated atmospheric CO_2 concentration in a cool-season grass species. Journal of the American Society for Horticultural Science 137(4):221-228.

Ziska, L. H. and Bunce, J. A. 1994. Increasing growth temperature reduces the stimulatory effect of elevated CO_2 on photosynthesis or biomass in two perennial species. Physiologia Plantarum 91(2):183-190.

APPENDICES

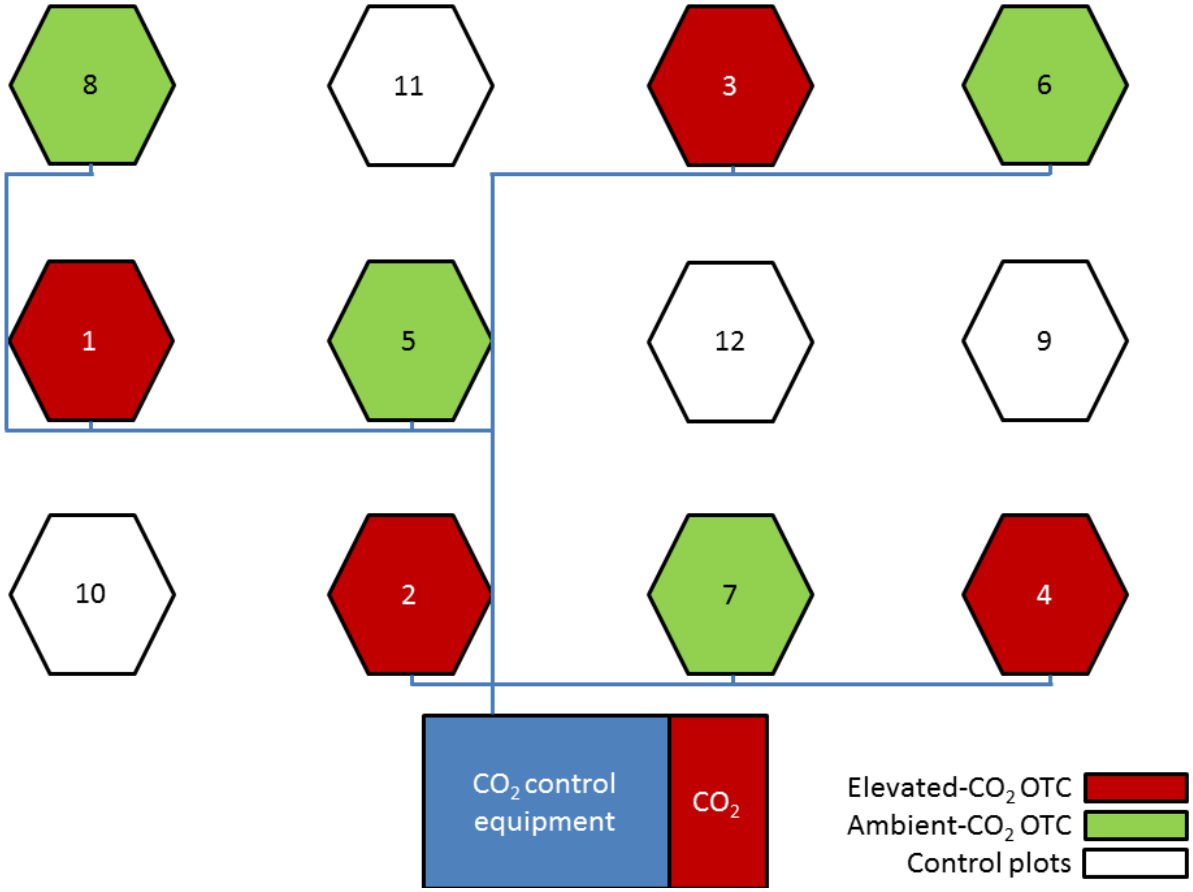


Figure A.1. Location of open-top chambers and their respective treatments and of control plots on the experimental site in a completely randomized design.

Table A.1. Cost of materials and equipment used for building eight open-top chambers (2013 prices, US $).

Material/Equipment	Make/Model	Quantity	Unit Cost	Total
Chamber construction				
Treated wood	6 cm × 3.5 cm	88	2.96	260.48
Greenhouse plastic	Clear, 6 mil (5.5 × 13.7 m)	1	63.02	63.02
Others (screws, hinges, rebar, etc.)	-	-	-	89.77
			Sub-total	413.27
Ventilation system				
Plastic box	33 cm × 50 cm × 33 cm high	8	4.48	35.84
Fan	Powerventpro (5.66 m^3 min^{-1})	8	111.29	890.32
Flexible PVC pipe	3.75cm inside diameter (2.15 m per OTC)	17 m	6.07	103.19
Galvanized pipe	10 cm diameter × 15 cm	16	1.34	21.44
Galvanized "T"	10 cm × 10 cm × 10 cm	8	1.09	8.72
Plastic "lay-flat" tube	12 cm diameter, clear (4.15 m per OTC)	33.2 m	0.53	17.60
			Sub-total	1077.11
CO_2 control system				
PVC box	Arlington EB1212	5	26.92	134.60
Power supply	Lovato Electric PSL1 010 24	5	73.52	367.60
Control transformer	HPS SP50PR	4	23.87	95.48
CO_2 solenoid	ASCO Red-Hat 8003HE	4	155.05	620.20
CO_2 sensor	Vaisala GMP222	5	1366.28	6831.40
CO_2 transmitter	Vaisala Carbocap GMT220			
Communication wire	Vaisala 19040GM Serial COM Adapter	1	107.72	107.72
CO_2 flow regulator	Weldmark HRF 1425-580	4	258.03	1032.12
CO_2 data logger	Kimo KT210 (has four entries)	2	369.86	739.72
Wires CO_2 data logger	Kimo KT210	5	68.99	344.95
CO_2 tubing	Nylon tubing 32.320.01 (100 m)	1	114.32	114.32
Pipe insulation	1.27 cm × 1.83 m	25	1.43	35.75
Others (wires, brackets, etc.)	-	-	-	89.77
			Sub-total	10513.63
Temperature Monitoring				
Temperature data loggers	Onset U23-004 HOBO PRO V2	12	116.25	1395.00
Data Carrier Onset	Onset U-DTW-1	1	212.57	212.57
			Sub-total	1607.57
Softwares				
Software Kimo	Kimo KT210	1	214.91	214.91
Software Onset	Onset BHW-PRO-CD	1	88.85	88.85
			Sub-total	303.76
			TOTAL	13915.34

Table A.2. Average incoming PAR (µmol m^{-2} sec^{-1}) measured in the middle of eight open-top chambers (OTCs) as compared to four control plots without chamber. PAR was measured above plant canopy at three different periods of the day (AM, PM and late PM), three times between 25 June and 11 August 2014, corresponding to three different plant heights (30 cm, 60 cm and 90 cm from the ground) n=5. Light transmission (%) was calculated as: (PAR OTCs / PAR control plots) × 100 and the average light transmission was the average of 15 observations for each height.

Height		**AM (8:00 - 10:00 h)**					**PM (12:00 - 14:00 h)**					**Late PM (16:00 - 18:00 h)**					**Average light transmission**
30 cm	Average PAR in OTCs	858	953	848	1607	975	1354	1070	1824	1837	1692	356	850	1044	647	968	
	Average PAR control plots	963	1077	954	1636	1067	1459	1192	1834	1842	1795	462	940	1293	724	1060	
	Light transmission	89%	88%	89%	98%	91%	93%	90%	99%	100%	94%	77%	90%	81%	89%	91%	91%
60 cm	Average PAR in OTCs	828	1106	499	1107	242	1960	1952	1144	1637	987	350	594	624	596	207	
	Average PAR control plots	1047	1051	616	1099	273	1986	2026	1334	1800	1056	391	654	761	706	229	
	Light transmission	79%	105%	81%	101%	89%	99%	96%	86%	91%	93%	90%	91%	82%	84%	90%	91%
90 cm	Average PAR in OTCs	1274	1091	595	1080	292	623	2130	1756	1031	1813	658	720	622	222	1002	
	Average PAR control plots	1231	1149	647	1030	309	634	2151	1807	1087	1823	746	758	694	225	1049	
	Light transmission	103%	95%	92%	105%	94%	98%	99%	97%	95%	99%	88%	95%	90%	99%	96%	96%
	Average light transmission			93%					95%					89%			93%

Table A.3. Concentrations of sugars (mg g^{-1} DM) in the biomass of alfalfa grown in open-top chambers under ambient (near 400 µmol mol^{-1}) and elevated (600 µmol mol^{-1}) CO_2 concentrations, and without an open-top chamber (control).

Treatment effect	Year	Cut	Treatment	Raffinose	Glucose	Pinitol	Fructose	SC[4]	Starch	NSC[5]
$[CO_2]$										
			Control	1.8	4.1 a	28.4 b	3.6 a	61.0	7.5 a	68.5
			Ambient	1.8	3.5 b	32.3 a	2.7 b	58.6	4.3 b	62.9
			Elevated	1.9	3.6 b	28.3 b	3.1 ab	57.9	5.3 b	63.1
Cut										
	2013	First		3.3 a[1]	2.6 b	35.2 a	1.1 d	57.1 c	5.9 a	63.0 b
	2014	First		1.1 d	4.6 a	36.3 a	5.0 a	71.0 a	5.1 a	76.1 a
		Second		1.7 b	4.5 a	35.5 a	3.6 b	65.8 b	7.1 abc	72.9 a
		Third		1.7 b	2.6 b	18.8 c	2.1 c	43.0 d	6.6 b	49.6 c
		Fourth		1.3 c	4.2 a	22.5 b	3.7 b	59.1 c	3.5 c	62.5 b
$[CO_2]$ × cut										
	2013	First	Control	3.0 a[2]		27.3 b			16.2 a	
			Ambient	3.3 a		41.0 a			0.2 b	
			Elevated	3.6 a		37.5 ab			1.5 b	
	2014	First	Control	0.8 b		39.3 a			5.3 ab	
			Ambient	1.0 ab		37.0 a			4.4 b	
			Elevated	1.4 a		32.6 b			5.7 b	
		Second	Control	1.7 a		38.5 a			7.4 a	
			Ambient	1.5 a		37.0 a			7.1 a	
			Elevated	2.0 a		31.0 b			6.7 a	
		Third	Control	1.9 a		16.6 b			5.0 b	
			Ambient	1.8 a		22.1 a			6.4 ab	
			Elevated	1.5 a		17.7 b			8.3 a	
		Fourth	Control	1.4 a		20.3 b			3.7 a	
			Ambient	1.3 ab		24.5 a			3.2 a	
			Elevated	1.2 b		22.7 ab			3.5 a	
P value	$[CO_2]$			NS[3]	0.015	0.048	0.003	NS	0.003	NS
	Cut			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	$[CO_2]$ x Cut			0.039	NS	<0.001	NS	NS	0.001	NS

[1] Different letters within a column and for a given treatment effect indicate significant differences ($P \leq 0.05$).
[2] Values are presented only when the interaction is significant.
[3] Non-significant with $P > 0.05$.
[4] Soluble carbohydrates = raffinose + sucrose + glucose + pinitol + fructose.
[5] Non-structural carbohydrates = SC + starch.

Table A.4. Concentration of sugars (mg g^{-1} DM) in the biomass of timothy grown in open-top chambers under ambient (near 400 µmol mol^{-1}) and elevated (600 µmol mol^{-1}) CO_2 concentrations, and without an open-top chamber (control).

Treatment effect	Year	Cut	Treatment	Raffinose	Glucose	Fructose	SC[4]	Starch	NSC[5]
$[CO_2]$									
			Control	2.4	7.2	4.7	39.6	7.2	50.0
			Ambient	2.7	7.1	4.8	41.5	7.4	49.1
			Elevated	2.9	6.9	5.3	42.6	6.9	49.8
Cut									
	2013	First		4.7 a[1]	5.3 b	4.0 b	26.3 b	7.6	34.3 b
	2014	First		0.7 b	8.8 a	5.8 a	56.2 a	6.7	62.9 a
$[CO_2]$ × cut									
	2013	First	Control					6.5 a[2]	
			Ambient					8.1 a	
			Elevated					8.1 a	
	2014	First	Control					7.8 a	
			Ambient					6.6 ab	
			Elevated					5.6 b	
P value	$[CO_2]$			NS[3]	NS	NS	NS	NS	NS
	Cut			<0.001	<0.001	<0.001	<0.001	NS	<0.001
	$[CO_2]$ x Cut			NS	NS	NS	NS	0.021	NS

[1] Different letters within a column and for a given treatment effect indicate significant differences ($P \leq 0.05$).
[2] Values are presented only when the interaction is significant.
[3] Non-significant with $P > 0.05$.
[4] Soluble carbohydrates = raffinose + sucrose + glucose + fructose.
[5] Non-structural carbohydrates = SC + starch.

Table A.5. Concentration of sugars (mg g^{-1} DM) in the biomass of an alfalfa-timothy mixture grown in open-top chambers under ambient (near 400 μmol mol^{-1}) and elevated (600 μmol mol^{-1}) CO_2 concentrations, and without an open-top chamber (control).

Treatment effect	Year	Cut	Treatment	Raffinose	Sucrose	Glucose	Pinitol	Fructose	SC[4]	Starch
[CO_2]										
			Control	1.3	23.6 a	4.2 a	26.9	3.4 a	59.4	6.7 a
			Ambient	1.3	20.7 b	3.6 b	29.6	2.7 b	56.5	4.7 b
			Elevated	1.2	19.2 b	3.7 b	26.3	2.9 b	55.0	5.5 ab
Cut										
	2013	First		0.9 b[1]	10.9 c	1.9 e	27.0 c	1.7 c	42.3 c	6.6 a
	2014	First		0.9 b	27.9 a	5.5 a	31.8 b	4.8 a	71.1 a	5.5 a
		Second		1.5 a	21.0 b	4.6 b	37.1 a	3.4 b	67.7 a	6.8 a
		Third		1.6 a	18.2 b	3.3 d	19.5 e	1.9 c	44.6 c	5.7 a
		Fourth		1.5 a	27.8 a	3.9 c	22.7 d	3.2 b	59.1 b	3.4 b
[CO_2] × cut										
	2013	First	Control			1.8 b[2]	22.8 a	1.2 b	39.5 a	13.4 a
			Ambient			1.6 b	29.8 a	1.5 ab	41.8 a	2.6 b
			Elevated			2.3 a	28.5 a	2.4 a	45.8 a	3.8 b
	2014	First	Control			6.5 a	34.0 a	5.7 a	76.9 a	5.8 a
			Ambient			5.1 b	31.5 a	4.3 b	67.2 b	4.9 a
			Elevated			5.0 b	29.9 a	4.6 b	69.1 ab	5.8 a
		Second	Control			5.5 a	40.0 a	4.5 a	76.1 a	6.8 a
			Ambient			4.1 b	39.1 a	2.7 b	65.4 b	7.0 a
			Elevated			4.3 b	32.2 b	2.9 b	61.8 b	6.6 a
		Third	Control			3.2 a	17.5 b	1.9 a	42.9 a	4.1 b
			Ambient			3.7 a	22.9 a	2.7 a	48.1 a	5.8 ab
			Elevated			2.9 a	18.1 b	2.9 a	42.7 a	7.3 a
		Fourth	Control			4.1 a	20.2 b	1.9 a	61.9 a	3.5 a
			Ambient			3.7 a	24.7 a	2.2 a	59.9 a	3.0 a
			Elevated			3.8 a	23.1ab	1.7 a	55.6 a	3.8 a
P value	[CO_2]			NS[3]	<0.001	0.005	NS	0.008	NS	0.009
	Cut			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	[CO_2] x Cut			NS	NS	0.018	0.011	0.005	0.029	0.005

[1] Different letters within a column and for a given treatment effect indicate significant differences ($P \leq 0.05$).
[2] Values are presented only when the interaction is significant.
[3] Non-significant with $P > 0.05$.
[4] Soluble carbohydrates = raffinose + sucrose + glucose + pinitol + fructose

Table A.6. Concentration of minerals (mg g^{-1} DM) in the biomass of an alfalfa-timothy mixture grown in open-top chambers under ambient (near 400 μmol mol^{-1}) and elevated (600 μmol mol^{-1}) CO_2 concentrations, and without an open-top chamber (control).

Treatment effect	Year	Cut	Treatment	Phosphorus	Potassium	Calcium	Magnesium
$[CO_2]$							
			Control	3.1 a[1]	31.9	13.6	2.3
			Ambient	2.7 b	32.3	14.2	2.3
			Elevated	2.8 b	32.7	13.3	2.2
Cut							
	2013	First		3.0 b	28.8 c	16.7 a	2.5 a
	2014	First		2.7 c	33.5 b	15.1 b	2.3 b
		Second		2.5 d	30.1 c	12.7 c	2.5 a
		Third		2.8 bc	32.5 b	10.8 d	2.1 c
		Fourth		3.3 a	36.9 a	13.2 c	2.0 c
$[CO_2]$ × cut							
	2013	First	Control	3.3 a	28.2 a	14.2 b	2.5 ab
			Ambient	3.0 ab	29.7 a	17.7 a	2.7 a
			Elevated	2.7 a	28.4 a	18.1 a	2.4 b
	2014	First	Control	2.8 a	33.6 a	15.6 a	2.3 a
			Ambient	2.8 a	35.0 a	15.1 a	2.2 a
			Elevated	2.5 a	31.8 a	14.5 a	2.2 a
		Second	Control	2.8 a	29.7 a	12.7 a	2.5 a
			Ambient	2.4 b	30.6 a	13.5 a	2.5 a
			Elevated	2.3 b	29.9 a	12.0 a	2.4 a
		Third	Control	3.3 b	34.1 a	12.1 a	2.1 a
			Ambient	2.4 c	29.9 a	10.7 a	2.0 a
			Elevated	2.8 b	33.4 a	9.5 a	2.0 a
		Fourth	Control	3.3 ab	34.0 b	13.3 a	2.0 a
			Ambient	3.1 b	36.5 ab	14.1 a	2.1 a
			Elevated	3.6 a	40.2 a	12.2 a	2.0 a
P value	$[CO_2]$			0.019	NS[2]	NS	NS
	Cut			<0.001	<0.001	<0.001	<0.001
	$[CO_2]$ x Cut			<0.001	0.001	0.024	0.025

[1] Different letters within a column and for a given treatment effect indicate significant differences ($P \leq 0.05$).
[2] Non-significant with $P > 0.05$.

Table A.7. Concentration of sugars (mg g^{-1} DM) in the crowns of acclimated alfalfa and timothy grown in open-top chambers under ambient (near 400 µmol mol^{-1}) and elevated (600 µmol mol^{-1}) CO_2 concentrations, and without an open-top chamber (control).

Year	Species	Treatment	Stachyose	Raffinose	Sucrose	Glucose	Pinitol	Fructose	Maltose	SC[2]	Starch	HDP[3] Fructans	NSC[4]
2013	Alfalfa	Control	4.3	2.3	102.1	2.7	4.	0.3	99.6	216.2	142.3	0.0	358.6
		Ambient	5.7	2.4	120.2	2.8	5.7	0.3	97.3	234.4	111.4	0.0	345.8
		Elevated	5.7	2.3	108.3	2.5	6.0	0.2	88.3	213.3	109.9	0.0	323.2
	Timothy	Control	0.0	0.1	22.6	1.2	0.0	2.0	0.0	25.8	4.6 ab	238.4	268.8
		Ambient	0.0	0.2	21.6	1.4	0.0	2.4	0.0	25.7	5.2 a	223.3	254.2
		Elevated	0.0	0.3	23.2	1.4	0.0	2.4	0.0	27.2	3.9 b	233.2	264.3
2014	Alfalfa	Control	1.7	1.0	71.0	3.1 a[1]	4.3	0.6	80.8	162.6	98.8	0.0	261.4
		Ambient	2.0	1.1	45.3	2.8 b	4.7	0.6	83.8	170.4	101.0	0.0	271.3
		Elevated	1.6	0.9	76.8	2.8 b	4.3	0.6	65.5	152.3	109.2	0.0	261.5

[1] Within a given column and for a given species, means with different letters differ at $P \leq 0.05$.
[2] Soluble carbohydrates = stachyose + raffinose + sucrose + glucose + pinitol + fructose + maltose.
[3] High degree of polymerization.
[4] Non-structural carbohydrates = SC + starch + HDP fructans.

Table A.8. Concentration of free amino acids (µmol g^{-1} DM) in the crowns of acclimated alfalfa and timothy grown in open-top chambers under ambient (near 400 µmol mol^{-1}) and elevated (600 µmol mol^{-1}) CO_2 concentrations, and without an open-top chamber (control).

Year	Species	Treatment	His	Asn	Ser	Gln	Arg	Gly	Asp	Glu	Thr	Ala	GABA
2013	Alfalfa	Control	8.3	130.8	4.0	0.0	57.4	0.5	9.5	7.6	1.1	4.3	10.6
		Ambient	7.0	111.5	3.6	0.0	55.6	0.5	6.0	7.5	0.9	4.8	8.5
		Elevated	9.1	108.0	3.0	0.0	53.1	0.5	6.0	5.4	0.9	3.2	7.0
	Timothy	Control	0.4	42.6	2.4	5.7	1.7	0.2	3.6	4.5	1.1	3.3	2.2
		Ambient	0.3	32.5	2.4	4.5	2.8	0.2	3.0	4.0	0.8	3.0	2.1
		Elevated	0.3	32.1	2.2	4.8	2.5	0.2	2.3	3.0	0.8	2.5	1.6
2014	Alfalfa	Control	4.2	71.1	2.9	0.4	21.7	0.2	4.5	4.8	1.1	3.1	4.2
		Ambient	5.8	107.5	3.5	0.4	26.8	0.2	5.2	4.9	1.4	3.1	4.9
		Elevated	4.3	88.6	3.1	0.5	23.3	0.3	4.0	5.3	1.2	4.3	6.7

Year	Species	Treatment	Pro	AABA	Orn	Lys	Tyr	Met	Val	Ile	Leu	Phe	Total
2013	Alfalfa	Control	24.3	0.0	0.5	0.6	0.2	0.1	0.8	0.6	0.5	0.5	262.0
		Ambient	19.5	0.0	0.7	0.5	0.2	0.0	0.5	0.4	0.3	0.3	228.3
		Elevated	20.2	0.0	0.3	0.4	0.2	0.0	0.7	0.5	0.4	0.4	219.6
	Timothy	Control	2.0	0.0	0.08 b[1]	1.2	0.0	0.0	1.0	0.3	0.3	0.3	74.4
		Ambient	1.6	0.0	0.14 a	0.9	0.0	0.0	1.0	0.3	0.3	0.3	59.2
		Elevated	0.9	0.0	0.16 a	0.9	0.0	0.0	0.8	0.2	0.2	0.3	56.1
2014	Alfalfa	Control	13.1	0.2	0.5	0.3	0.1	0.1	0.8	0.6	0.6	0.4	134.7
		Ambient	15.7	0.2	0.7	0.4	0.1	0.2	1.3	1.2	0.8	0.6	185.0
		Elevated	13.1	0.2	0.7	0.4	0.1	0.2	1.0	0.8	0.7	0.4	159.2

[1] Within a given column and for a given species, means with different letters differ at $P \leq 0.05$.