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INTERACTIVE EFFECTS OF WARMING AND NITROGEN DEPOSITION ON NET ECOSYSTEM CO2 FLUX IN A TEMPERATE OLD FIELD.

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INTERACTIVE EFFECTS OF WARMING AND NITROGEN DEPOSITION ON NET ECOSYSTEM CO_2 FLUX IN A TEMPERATE OLD FIELD.

(Spine-title: Warming and Nitrogen Effects on Net Ecosystem CO_2 Flux)

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by

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ABSTRACT

Global warming and increased atmospheric nitrogen deposition are expected to alter the carbon balances of ecosystems over the next century. My study measured net ecosystem CO_2 exchange (NEE) in response to warming and nitrogen addition in a temperate old field using open system canopy chambers. Additionally, I assessed the indirect effects of warming and nitrogen addition on NEE caused by changes in aboveground productivity. In both 2009 and 2010, the study system was a net source of carbon, with the highest CO_2 efflux during mid-summer. Warming and nitrogen addition did not affect NEE patterns in both years, notwithstanding a significant nitrogen effect on aboveground biomass in 2010. These findings suggest that the NEE of the study site was dominated by ecosystem respiration, which correlated strongly with soil temperature.

Keywords: atmospheric nitrogen deposition, climate change, CO_2 flux, plant productivity, warming.

CO-AUTHORSHIP STATEMENT

Hugh Henry will be a co-author on any manuscript(s) based on the contents of this thesis.

DEDICATION

I would like to dedicate this thesis to my mother, who always gave more than she had with hopes for a brighter future for my sister and me.

ACKNOWLEDGEMENTS

I would like to thank my supervisor Dr. Hugh Henry for all of his assistance during my studies here at The University of Western Ontario. These last two years would not have been the same without his academic guidance and I am grateful for the wonderful learning experience. More importantly, he was a great mentor who went above and beyond his call of duty to ensure that my experience was maximized both as an academic and an individual.

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List of Abbreviations

CIM - control interface module

GPP - gross primary production

NEE – net ecosystem exchange of CO_2

NEP – net ecosystem production

NPP – net primary productivity

PET – polyethylene terephthalate

R_A – autotrophic respiration

R_H – heterotrophic respiration

SOC – soil organic carbon

SOM – soil organic matter

VWC – volumetric water content

Chapter 1: Introduction

1.1 The global carbon cycle

The global carbon cycle is an important factor in the prediction of future climate conditions, yet further understanding of the natural fluxes between the major carbon reservoirs and anthropogenic perturbations to the cycle is needed. Through modeling and conducting field studies, information can be obtained to best mitigate human-caused CO_2 emissions through deliberate sequestration and abatement (Keller *et al.*, 2008). Global carbon pools can be divided into 3 separate but interacting components: the atmosphere, the oceans and terrestrial ecosystems, including stores in vegetation and soils (Houghton, 2007). The largest of the reservoirs is the oceans, which store 40,000 petagrams ($Pg = 1 \times 10^{15}$ grams) of carbon, followed by 2,100 Pg of labile carbon in the soils and vegetation (Zimov *et al.*, 2006). The atmosphere contains only 805 Pg of carbon, the concentration of which is approximately 380 ppmv (parts per million by volume) (Houghton, 2007).

Net flux between the atmosphere and the oceans is variable over time, though an estimated net oceanic uptake of 1.7 – 2.4 Pg of carbon in the recent decades has been suggested (Plattner *et al.*, 2002). The surface of the oceans exchanges carbon with the atmosphere through a diffusion process, in which gaseous CO_2 can be stored as carbonate and bicarbonate ions (Houghton, 2003). In addition, marine biota, such as phytoplankton and macrophytes, can take up atmospheric CO_2. Conversely, when the partial pressure of CO_2 is reduced at the surface of the ocean, net CO_2 release can occur.

Terrestrial ecosystems exchange carbon with the atmosphere through two major pathways: the biological pathway and the geological pathway (Keller *et al.*, 2008). Although geological formations serve as large reservoirs for carbon, flux occurs over a long time scale. Biological pathways occur over a short period of time and are calculated by determining the net balance of carbon exchange between the biosphere and the atmosphere (Heimann and Reichstein, 2008). The total amount of carbon captured by primary producers through photosynthesis is referred to as the gross primary production (GPP) (Fig. 1.1). Photorespiration and metabolic respiration by plants account for autotrophic respiration (R_A). Carbon is returned to the atmosphere through R_A, which when subtracted from GPP results in net primary production (NPP) (Equation 1). Heterotrophic respiration (R_H) encompasses metabolic respiration and the conversion of organic matter to CO_2. NPP minus R_H, minus carbon leaching from the system, is the net ecosystem productivity (NEP) (Ciais *et al.*, 2006) (Equation 2).

Equation 1. $NPP = GPP - R_A$

Equation 2. $NEP = NPP - R_H - \text{leachate}$

The vertical transfer of carbon between the atmosphere and the terrestrial ecosystem is defined as the net ecosystem exchange (NEE), which determines whether the system is a source or a sink of carbon (Kicklighter *et al.*, 1999). Terrestrial ecosystems can leach dissolved organic carbon, as well as dissolved inroganic carbon, through the soils (Qualls *et al.*, 1991). Dissolved CO_2 in soils can reach streams and lakes, where it is released back to the atmosphere. In addition, small amounts of carbon are laterally transferred between terrestrial ecosystems (Ciais *et al.*, 2006).

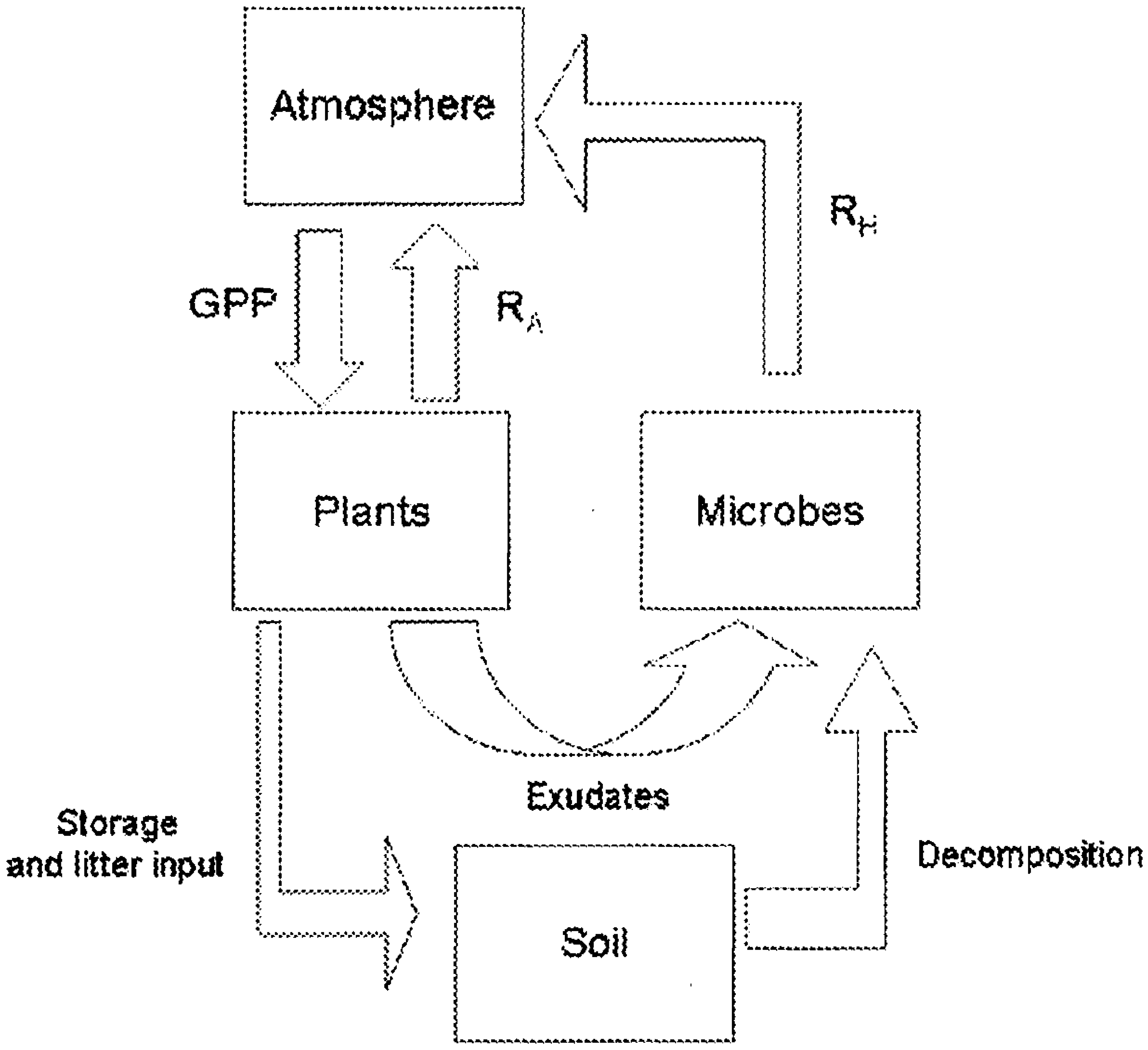

Figure 1.1 A simplified conceptual diagram of carbon flux through a terrestrial ecosystem. Not included in this figure are geological and lateral fluxes of carbon. (GPP = gross primary productivity, R_A = autotrophic respiration, R_H = heterotrophic respiration).

Many studies have examined the aforementioned components separately through bottom up approaches, as well as top-down approaches, to investigate the overall NEP of terrestrial ecosystems (Houghton, 2003) (Detailed in the next section). Models suggest that 16 - 34% of CO_2 emissions could potentially be offset by terrestrial systems in the future under climate change scenarios through feedbacks (enhancement of NEP) between warming, increased atmospheric CO_2 concentrations and primary productivity (Hungate *et al.*, 2003). Thus, the potential for terrestrial ecosystems to serve as large carbon sinks has driven much of the interest in the atmosphere-terrestrial coupling.

1.2 Global climate warming and terrestrial ecosystems

1.2.1 Temperature and carbon flux

Climate warming and the rate of anthropogenic emissions of greenhouse gases have accelerated in the last century (Solomon *et al.*, 2007). Major drivers include fossil fuel burning and industrial intensification, increasing CO_2 in the atmosphere (along with other trace gases: CH_4 and N_2O). As a result, mean global temperature is expected to increase by 1 – 6.4° C in the next 50 - 100 years (Van Vuuren *et al.*, 2008). It is recognized that this warming will differ in both severity and consequences across the globe, with high latitude regions experiencing the greatest warming (Houghton *et al.*, 2001). As well, warming is expected to be greater over land than the oceans and greater during winter than summer (Mellander *et al.*, 2007; Sabine *et al.*, 2004).

Temperature is a crucial component of ecosystem dynamics. Interconnectivity between temperature, primary production, decomposition, soil, water and nutrient dynamics has been extensively studied within the context of global climate change (Cox *et al.*, 2000; Bonan, 2008; Schuur *et al.*, 2008). However, the contribution of these interacting components to the overall response of ecosystems to warming has been difficult to study. In particular, the feedback between the warming climate and the ability of terrestrial ecosystems to mitigate this effect through carbon sequestration has been an elusive yet important question in climate change research (Schlesinger and Andrews, 2000).

The ability of terrestrial ecosystems to act as either sinks or sources of carbon across space and time further substantiates the importance of including climate feedbacks and their mechanisms when calculating the global carbon budget (Raich and Potter, 1995). Ecosystem characteristics that determine the direction of NEE are of great importance in climate change research.

1.2.2 Grassland systems

In North America, loss of carbon from soils due to landscape conversion for agriculture has prompted interest in restoration of natural habitats (e.g. tallgrass prairies) in an attempt to revert the process. Studies investigating the CO_2 flux of these systems are abundant, addressing both landscape level and fine scale questions (Suyker and Verma, 2001; Mielnick and Dugas, 2000; etc.). However, less attention has been given to old field systems (i.e. abandoned agricultural land), that can remain grass-dominated for decades before shrubs and trees take over. Old fields are prevalent in temperate regions and their carbon sequestration potential is poorly characterized in the literature. Studies in forests and tallgrass prairies suggest that biomass accumulation contributes to long term carbon storage, and presumably the

NEE of old field systems may respond similarly to enhanced biomass production in the future under warmer conditions.

1.3 Effects of warming on soil

Globally, about 1500 Pg of labile carbon is stored in the top 1 m of soil in soil organic matter (SOM) (Batjes, 1996). Recalcitrant carbon stored down to 2 m depth exceeds 2500 Gt (Jobbágy and Jackson, 2000). Carbon is returned to the atmosphere primarily through root respiration and microbial respiration (Lundegårdh, 1927), which are expected to change in a warming climate. In turn, understanding the mechanics of soil respiration under warming is quantitatively important in carbon budgeting. A steady-state view of soil carbon exchange suggests that with increasing temperature, stimulation of NPP would offset the increased carbon loss from soils caused by accelerated decomposition (Gifford, 1992). In addition, it has been suggested that increased soil carbon efflux is negligible compared to the large amount of carbon input from anthropogenic activities (Kirschbaum, 1995). On the other hand, NPP seems to be limited by more variables than soil respiration, such as light availability, because of its crucial role in photosynthesis (Kirschbaum, 1995). This indicates that warming may have greater impact on heterotrophic respiration than biological sequestration of carbon through NPP. Thus, experiments measuring effects of warming on soil respiration have asserted the importance of studying the temperature sensitivity of soils.

Temperature sensitivity of soil respiration has been well documented (Davidson and Janssens, 2006) and it has been attributed to the biological processes that contribute to soil respiration: microbial decomposition and root respiration. Many experiments combine the two factors due to difficulties in quantifying the contribution of each factor in isolation. The temperature sensitivity of soil respiration rate is highest at lower temperatures, and Q_{10} values (the temperature coefficient which measures the rate of change in a biological system in response to a 10 °C increase in temperature) of up to 12.5 have been reported near 4 °C, lessening to 2-2.5 near 30 °C (Schleser, 1982). Accordingly, warming is expected to have major influences on the carbon dynamics of high latitude systems where mean annual temperature fluctuates near 0 °C (Davidson and Janssens, 2006). This effect is exacerbated by the fact that these systems typically store large amounts of carbon due to permafrost and anaerobic conditions (Shaver *et al.*, 2000). Conversely, some studies have observed acclimatization of soil carbon efflux over time (Luo *et al.*, 2001). If true, this phenomenon suggests the biogeosphere's capacity to maintain a steady-state through various mechanisms; i.e. shifts in microbial communities, moisture and nutrient limitations and decreased root activity through reduction in NPP (Luo *et al.*, 2001).

Roots play a major role in the transfer of carbon to and from soils (Wan *et al.*, 2004). Both increases and decreases in root biomass allocation have been reported in warming studies (Pregitzer *et al.*, 2000; Kandeler *et al.*, 1998). The mechanisms are further complicated by the interaction between the rhizosphere, microbes and nutrient dynamics (Hu *et al.*, 1999). For instance, warming can stimulate increased nutrient turnover rates (as alluded to in the previous section) increasing R_H. Increased nutrient availability, however, can cause a reduction in belowground biomass allocation, decreasing belowground R_A (Cheng and Johnson, 1998).

Decrease in soil moisture has been linked to warming due to its link to increases in evapotranspiration. The effect of water limitation manifests differently to plants and microbial communities. Water limitation reduces R_H (Wan *et al.*, 2007); however, reduction in autotrophic carbon sequestration (GPP) as a result may counterbalance the reduction in R_H, making the overall carbon balance difficult to investigate (Flanagan and Johnson, 2005). At the global scale, climate warming is expected to increase precipitation (Fowler and Hennessy, 1995; Wentz *et al.*, 2007), which can potentially alleviate water limitations in some terrestrial ecosystems, such as temperate grasslands.

1.4 Effects of warming on plants

1.4.1 Phenology

Climate change can advance the onset of the growing season via increases in ambient air temperature (Menzel, 2003). Meta-analyses report earlier advancements of spring onset by 1 to 3 days per decade across many systems (Menzel, 2003). For example, spring advancement in deciduous forests of up to 3.5 days per °C has been observed (Menzel 2003). In Europe, advancements of up to 8 days per °C have been reported (Chmielewski and Rötzer, 2001). In northern temperate systems, plants are most sensitive to increase in mean February temperature (Fitter and Fitter, 2002). Reduced snowfall, shallower snow packs and earlier spring melt, all caused by increases in mean annual temperature cue emergence in alpine, subalpine and arctic systems (Inouye and McGuire 1991; Shaver and Kummerov 1992; Walker *et al.* 1995). Warmer summer and fall temperatures can delay senescence, while warm springs and mild late-season temperatures can have the opposite effect (Menzel, 2003). Overall, a steady increase in temperature can lengthen the growing season (Cleland *et al.*, 2006). Effects of warming on the availability of resources through space and time also alter the growth and reproductive responses of plants (Nord and Lynch, 2009). In addition to plasticity over the growing season, leaf-level changes (i.e. browning, self-pruning) can occur over much shorter periods of time. In addition to earlier emergence and flowering, plant development can accelerate resulting in alterations to population and community structure (Badeck *et al.*, 2004).

1.4.2 Productivity

Increase in growing season length tends to increase net primary productivity through early germination and prolonged growing seasons (Arft *et al.*, 1999). Landscape studies involving eddy covariance flux towers indicate a close correspondence of atypical seasonal changes in atmospheric CO_2 mixing ratio and the lengthening of the vegetation period (Chen *et al.*, 1999). Warming can eliminate snow cover allowing earlier germination (Hutchison and Henry, 2010), and increases in standing biomass of 125% - 200% have been reported in response to warming (Henry and Molau, 1997; Press *et al.*, 1998).

Although much of the increase in productivity can be attributed to changes in phenology, warming can stimulate growth through physiological changes (Johnson and Thornley, 1985). The flexible morphology and plasticity of herbaceous species allows them to increase nutrient uptake under warming (Shaver and Kummerov, 1992). Temperature sensitive downstream processes such as cell divisions can potentially be relieved of temperature limitations, allowing upstream processes including photosynthesis rates to be enhanced (Farrar, 1988). On the other hand, in C_3

plant dominated systems, increases in photorespiration due to warmer temperatures can offset increases in photoassimilation (Berry and Downton, 1982). Furthermore, warming can enhance microbial decomposition of litter (Section 1.3), allowing increased rates of nutrient mineralization and relieving nutrient stress (Rustad and Fernandez, 1998). With increased availability of nutrients, alterations to root morphology increase uptake, thereby enhancing primary productivity (BassiriRad, 2000).

Despite the potential to increase productivity, warming in both *in-situ* and field experiments has been negatively correlated with water availability. Warming is correlated with drying of soil and increased evapotranspiration causing water limitations (Korner and Larcher 1988). Hutchinson and Henry (2010) showed that warming did not have an effect on primary productivity when the plants were under water stress.

Decoupling of photoperiods and temperature can have damaging effects on the ontogeny of seedlings, because temperature increases will not affect the seasonal solar cycle (Ellis *et al.*, 1997). In addition, night temperature is expected to increase more than day time, which can have greater consequences on soil dynamics and dark respiration rather than enhancing photosynthetic capacity (Turnbull *et al.*, 2002). Despite these potential negative effects of warming on productivity, a meta-analysis by Rustad *et al.* (2001) showed a mean increase of 19% in productivity in 32 warming experiments. An important factor to consider, however, is the increased litter input following a productive growing season. The increased decomposition of increased litter mass may enhance CO_2 evolution, decreasing the carbon sequestration overall (Fontaine *et al.*, 2004).

1.5 Effects of nitrogen on ecosystems

The atmosphere is composed of 78% nitrogen (N_2), but most organisms cannot take up N_2 directly. NH_3 and NO_3^- are primarily processed by microorganisms through nitrogen fixation prior to uptake by plants (White, 1993). Nitrogen is an important nutrient for plants; it is a major component of proteins, amino acids, chlorophyll, as well as genetic and structural materials. High demand and low availability has rendered nitrogen as a limiting nutrient in most terrestrial ecosystems, determining their productivity, diversity and nutrient cycling dynamics (Vitousek *et al.*, 1997).

Human practices such as agriculture and industrial activity have led to abnormal increases in reactive nitrogen in the atmosphere near emission sources (Vitousek *et al.*, 1997). Nitrogen deposition is expected to reach 2 - 5 g N m^{-2} y^{-1} near industrial regions around the globe within the next 50 years. These figures approximate an increase in global N deposition from 156 Tg N y^{-1} in 1990 to 270 Tg N y^{-1} by 2050 (Galloway *et al.*, 2004). Two dominant forms of nitrogen in deposition are ammonium and nitrates, commonly deposited in areas near agricultural fields and industrial regions, respectively (Sickles and Shadwick, 2007).

Increasing nitrogen deposition and warming can have additive effects on primary production and ecosystem functioning (Hutchison and Henry, 2010). In addition, decomposition rates and soil dynamics can be altered as a result of chronic N deposition (Burton *et al.*, 2004). In studying the global carbon budget, we must consider the potential effects of both warming and nitrogen in conjunction to increase the precision of our flux estimates and, in particular, the ability of increased primary production to promote carbon sequestration may be limited by nitrogen (Hungate *et al.*, 1993).

1.5.1 Nitrogen and productivity

Although ecosystems show varying responses to added nitrogen, meta-analysis reveals a widespread enhancement of primary productivity (LeBauer and Treseder, 2008). When plants are under nitrogen limitation, nitrogen deposition alleviates this pressure and allows for enhanced growth until a different elemental limitation (often phosphorus) is imposed. However, despite the importance of this direct fertilization effect, it is the indirect and secondary effects of nitrogen fertilization that may determine the overall plant response and the ecosystem's carbon sequestration ability in the long term. For example, how additional N input alters microbial mineralization and immobilization or alters the C:N in litter fall can have strong effects on the overall primary productivity. Nitrogen deposition exceeding plants' uptake ability also leads to saturation and a decline in productivity caused by acidification and leaching (Aber *et al.*, 1989). Furthermore, the energetically-expensive reduction of NO_x to NH_3 within the plant is limited by photosynthesis, and this coupling can limit N assimilation by plants (Hewitt *et al.*, 1976).

Nitrogen fertilization can delay senescence in both leaf tissue and the whole plant (Bauer *et al.*, 2004). This can potentially extend the growing season, in conjunction with warming, to enhance productivity. In addition, nitrogen replete plants increase their shoot to root ratio, and allocate more resources to vegetative growth rather than sexual reproduction (Vogt *et al.*, 1990). Thus, greater foliage and higher foliar N concentration increases plant photosynthetic capacities, leading to additional carbon storage (Field and Mooney, 1986). However, fertilization studies in Harvard Forest showed a negative response to nitrogen, although higher foliar nitrogen concentrations were observed (Baeur *et al.*, 2004). Immediate immobilization of additional nitrogen entering the system partially explains the non-responsiveness of certain systems (Boyce *et al.*, 1996). Concordantly, decreased resource allocation to root biomass can reduce soil respiration by limiting plant-microbial interactions (Burton *et al.,* 2004). On the other hand, decreased root biomass may lead to less carbon being delivered to SOM.

Potential effects of nitrogen on phenology may be system dependent. For example, in a grassland system, nitrogen addition accelerated flowering in most forbs and delayed flowering in grasses (Dunne *et al.*, 2003). Phenology driven changes in community structure (e.g. reduction in diversity) can disrupt the productivity of some ecosystem (Huenneke *et al.*, 19900; Phoenix *et al.*, 2006).

1.5.2 Nitrogen and decomposition

Decomposition of SOM by microorganisms depends on the availability of C and N and their ratio (C:N) (Chapin *et al.*, 2002). Chronic N addition alters the C:N of SOM, which can retard decomposition rates by altering decomposer communities, acidifying soil and suppressing enzymatic activity (MacDonald *et al.*, 2002). This can ultimately reduce CO_2 efflux from the soil (Agren *et al.*, 2001, Reay *et al.*, 2008).

Reduction in C:N in leaves can lead to a positive feedback of N availability. For instance, low C:N litter provides excess N to microbes, which release it as organic N (Hodge *et al.*, 2000). Plants can directly benefit from additional N inputs, further reducing leaf C:N. However, it is not necessarily the case that plants increase foliar N concentrations in response to N addition.

1.6 Measuring the effects of warming and nitrogen on CO_2 exchange

It is clear that CO_2 exchange at the ecosystem level is highly dependent on interconnected components that respond both positively and negatively to warming and nitrogen (Shaver *et al.*, 2000). The combination of warming and nitrogen can potentially have additive, multiplicative or nullifying effect on NEE. Bottom-up approaches investigate specific components of NEE, such as photosynthesis, plant dark respiration, and soil respiration, separately (Knorr *et al.*, 2005; Pregitzer *et al.*, 2008; Luo *et al.*, 2004). As noted in the previous section, terrestrial systems vary greatly in the responses of these parameters over space and time. Thus, while these types of studies are useful to understanding the mechanisms of responses, amalgamation of such studies to predict ecosystem responses proves to be a challenge (Shaver *et al.*, 2000). Furthermore, mechanistic studies often isolate components of interest, e.g. soil respiration, from the remainder of the system due to logistics and technological limitations. In particular, plants in soil respiration collars are often clipped, or when possible, the soil collars are positioned in between plant shoots. Interconnectivity within ecosystems is sufficiently high such that isolation of components will provide confounded and sometimes inaccurate representation of reality (Luo *et al.*, 2001).

Integrative measures of plant and soil carbon flux can be performed using top-down approaches. The Eddy-covariance technique is used to determine the overall flux of gases over large landscapes (c.f. Suyker and Verma, 2001). While this technique provides useful data on the overall flux across an ecosystem, experimental treatments such as warming cannot be applied at the large scale over which eddy covariance towers perform their measurements. As a useful compromise between small scale measures (e.g. soil respiration collars) that can be used in warming experiments, but do not adequately integrate over plant and soil responses, and large scale measures (e.g. Eddy-covariance) that can provide useful integrative data, but over too large a spatial scale for warming experiments, open canopy chambers have recently been developed. Open-canopy chambers enclose sufficiently large areas to include vegetation and soils, yet they are small enough that they can be deployed in plots where experimental treatments such as nitrogen addition and warming can be applied.

1.7 Objectives and Hypotheses

My objectives were to examine the NEE of CO_2 in a temperate old field, and to explore the interactive effects of warming and N deposition on NEE. I also examined the correlation between NEE and changes in green plant biomass in response to the warming and nitrogen addition treatments.

I hypothesized that the old field system would be a net sink of carbon on an annual basis, and that warming and nitrogen would further increase seasonal carbon sequestration by enhancing plant growth. I predicted that the treatment effects on NEE and biomass accumulation would be additive, based on the assumption that nitrogen and warming enhance primary productivity through differing mechanisms.

Chapter 2: Materials and Methods

2.1 Site Description

I conducted my research as part of a long-term warming and nitrogen addition experiment located within the Agriculture and Agri-Food Canada's Southern Crop Protection and Food Research Centre in London, Ontario (43° 04' N, 81° 20'W, elevation 264 m) between March 2009 and October 2010. The field site was previously used as an agricultural field but had not been ploughed, fertilized or mowed in the past 25 years.

The soil is characterized as imperfectly drained silt loam glacial till with an average pH of 7.5 ± 0.1 (Bell et al., 2010). The mean annual temperature for the site is 7.5 ± 0.1 °C , with low of -6.3 ± 0.5 °C in January and a high of 20.5 ± 0.2 °C in July and mean annual precipitation is 595 mm (Environment Canada National Climate Data and Information Archive). Perennial C_3 grasses *Poa Pratensis* L. (Kentucky blue grass) and *Bromus inermis* Leyss. (smooth brome) dominate the site with *Cirsium arvense* L. (Canada thistle) and *Lotus corniculatus* L. (bird's-foot trefoil) present in patches within the plots. Other species found at the site are *Asclepias syriaca* L. (common milkweed), *Aster ericoides* L. var. *ericoides* (heath aster), and *Solidago canadensis* L. var. *scabra* (tall golden rod) (Hutchison and Henry, 2010) are also present but rare in the plots.

2.2 Design of the warming and nitrogen addition experiments

In 2006, prior to my arrival at Western, warming and nitrogen manipulations were set up at the field site as a randomized block split-plot design (n = 10; Fig. 2.1).

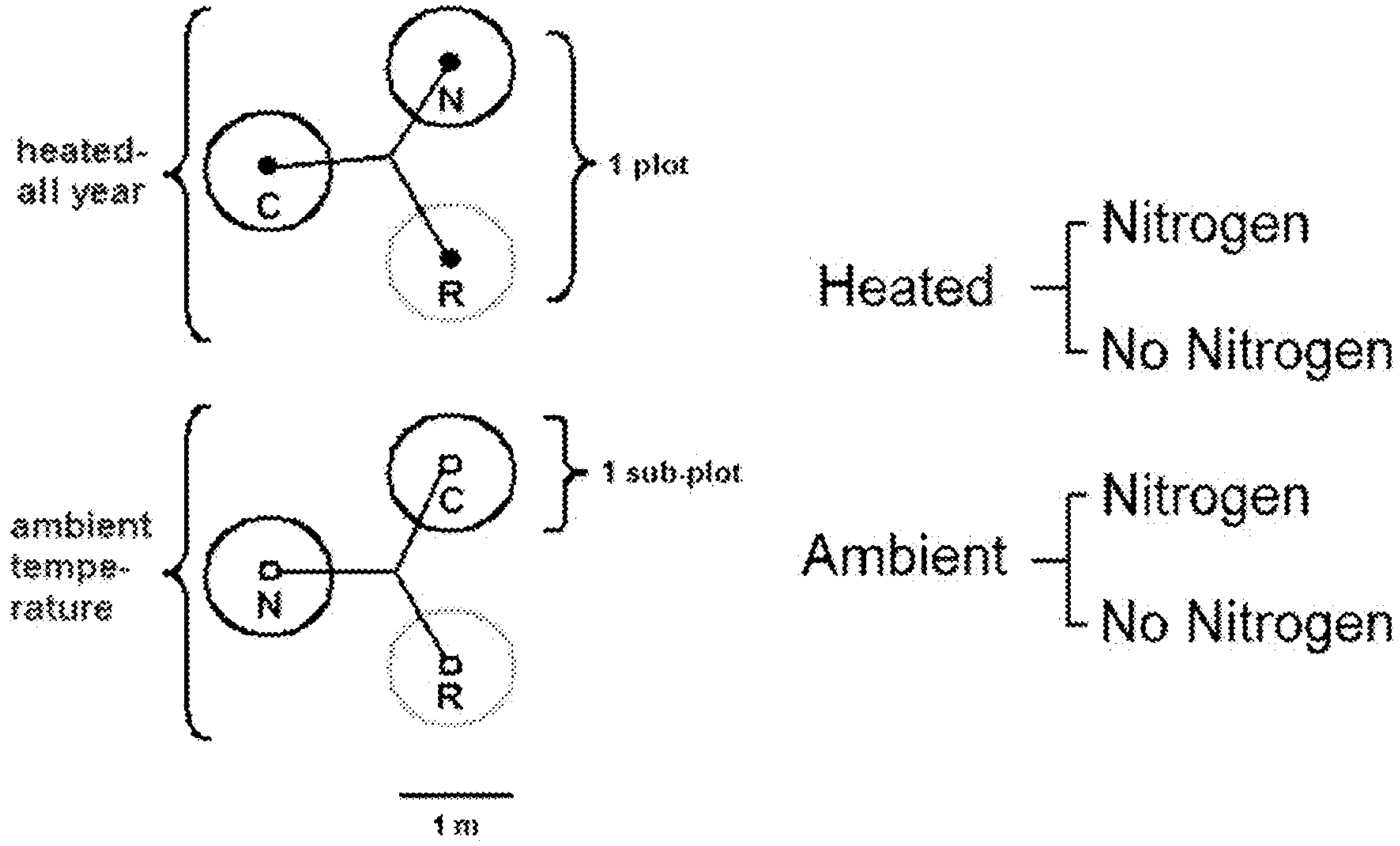

Figure 2.1 Experimental design showing 1 of 6 blocks. Each block contains 2 heating treatments: warmed all-year and control. Subplots consist of those receiving N additions (N), control (C), and reserve plots (R) which were not used.

Each block contained three plots (control, warmed and winter-warmed) with two nested nitrogen treatment sub-plots for a total of 6 sub-plots per block. Each circular subplot was 113 cm in diameter with a 10 cm buffer zone which also received experimental treatments. The treatments began in late November of 2006 and continue to present. Soil temperature was measured hourly, all-year, in each of the plots using 107-BAM-L temperature probes at both 1 and 5 cm depth. Soil moisture was measured using CS616-L time-domain reflectometry located at depths of 0-15 cm and 0-30 cm (both probes from Campbell Scientific Canada Corp., Edmonton, AB, Canada).

To provide warming, 150 W ceramic infrared heaters (Zoo-Med Laboratories, San Luis Obispo, CA, USA) were suspended 50 cm above the center of the sub-plots. As a control, non-warmed plots had the same ceramic heater brackets without active heaters. Ceramic infrared heaters were selected, because they do not emit photosynthetically active radiation.

In the nitrogen addition plots, aqueous ammonium nitrate was added in late March of 2009 and 2010 to simulate a rapid increase in nitrogen due to a pulse input during spring melt (2 g m^{-2}). An additional 4 g m^{-2} y^{-1} of nitrogen was added in late May using slow-release pellets (Florikan ESA, Sarasota, FL, USA) to simulate gradual atmospheric nitrogen deposition. Both warming and nitrogen deposition treatments were designed to reflect approximate conditions of this region by the year 2050 (Galloway *et al.*, 2004).

2.3 CO_2 flux chamber systems

Net ecosystem CO_2 flux was measured using four CPY-3 open canopy chambers manufactured by PP Systems (Figure 2.2; Amesbury, MA, USA). The system is composed of two main components: the polyethylene terephthalate (PET) chamber and the Control Interface Module (CIM). The PET chambers were cylindrical with aluminum base rings measuring 0.5 m in diameter and covering 1963 cm^2 of ground area. Height, although adjustable, was set at 1 meter in order to accommodate the growth of both common grass species *Bromus inermis* and *Poa pratensis*. The open system was maintained by an exhaust chimney at the top of the chamber and an intake valve at the base, allowing continuous flow of air, pumped and supplied by the connected CIM. In addition, two electric fans inside the chambers circulated the air within.

The control interface module was connected to the PET chamber through 3 distinct connections at the base ring: the air supply hose, the chamber electrical cable and the gas analysis tube. The CIM used an electric air pump inside the chimney unit to transfer ambient air through the air supply hose. A mass flow meter inside the chimney evaluated and controlled the volume of air. Flow rate was adjusted by a potentiometer allowing up to 20 litres of influx per minute. The electrical connection supplied power from the CIM to the chambers for the operation of the electric fans, the temperature sensor and the PAR sensor. In addition, the connection transfers data from the PAR, temperature and humidity sensors inside the chamber to the internal memory disk of CIM. Using the available AC power previously installed at our site, four 12-volt AC-DC power supplies were used to power the CIMs.

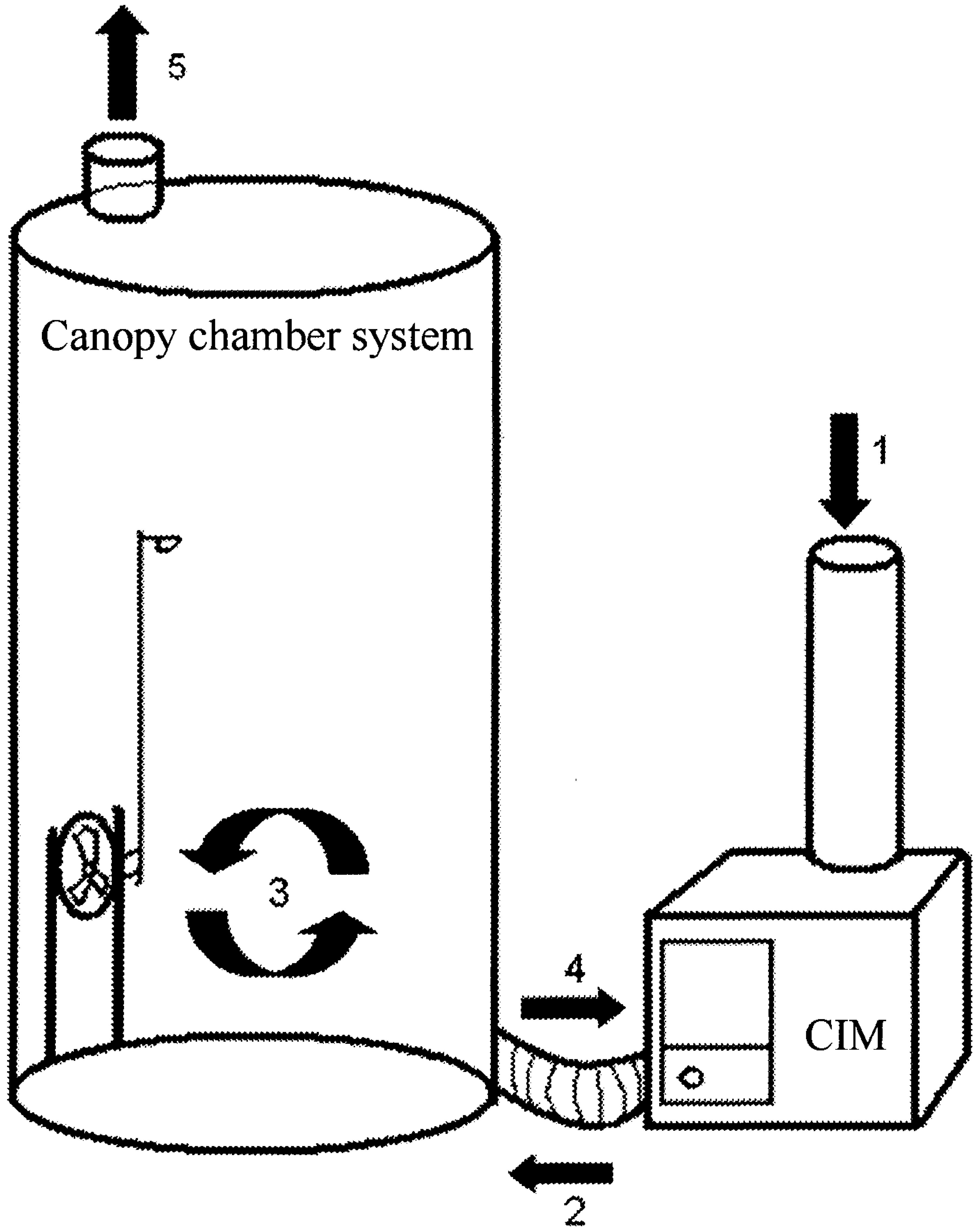

Figure 2.2 Schematic of the open canopy CO_2 flux chamber system. The chamber is attached to the control interface module (CIM).

1. Ambient air is regulated and pumped through the chimney of the CIM.
2. Initial CO_2 measurement is made using an infrared gas analyzer inside the CIM.
3. Ambient air circulates freely inside of the chamber.
4. A small volume of within chamber air is sampled back in the CIM.
5. Air is allowed to escape through the ventilation system.

The CIM used an infrared gas analyzer to measure the concentration of CO_2. Using a single gas analysis tube, the CIM used a solenoid valve to temporally separate the analysis of reference sample and analysis sample. The CO_2 concentration of the air sample entering the chamber through the supply hose was measured at a single point in time. After 30 seconds the solenoid valve switches and a second measurement is made by extracting the air sample through analysis tube, directly withdrawing the sample from within the chamber.

The 4 chambers correspond to the four treatment combinations present at each block during the measurement interval. In my experiment, CIMs were programmed to sample at 10 minute intervals. Diurnal measurements of CO_2 flux began at approximately 0930 hours. One block of 6 in the experimental design was measured each day, with the 4 chambers occupying the 4 treatments within a block. The chambers were then relocated to the next block at approximately 0830 hours the next day, and I and began recording CO_2 flux again by 0930 hours. I converted the standard flux output in μmols m^{-2} h^{-1} to g m^{-2} 600 s^{-1} and integrated the 10 minute interval data to calculate the daily flux (Figure 2.3). Each daily data set was plotted against time and anomalies were noted, with peaks arising from potential system errors were deleted. Peaks were determined to be erroneous if the two surrounding measurements were approximately less than 50% of the peak value. The 6 consecutive blocks were measured every other week throughout the growing season in 2009 and 2010 beginning at the end of April to the end of October. Fifteen weeks were sampled in 2009 and 10 weeks were sampled in 2010 (several weeks were lost in 2010 as a result of technical problems).

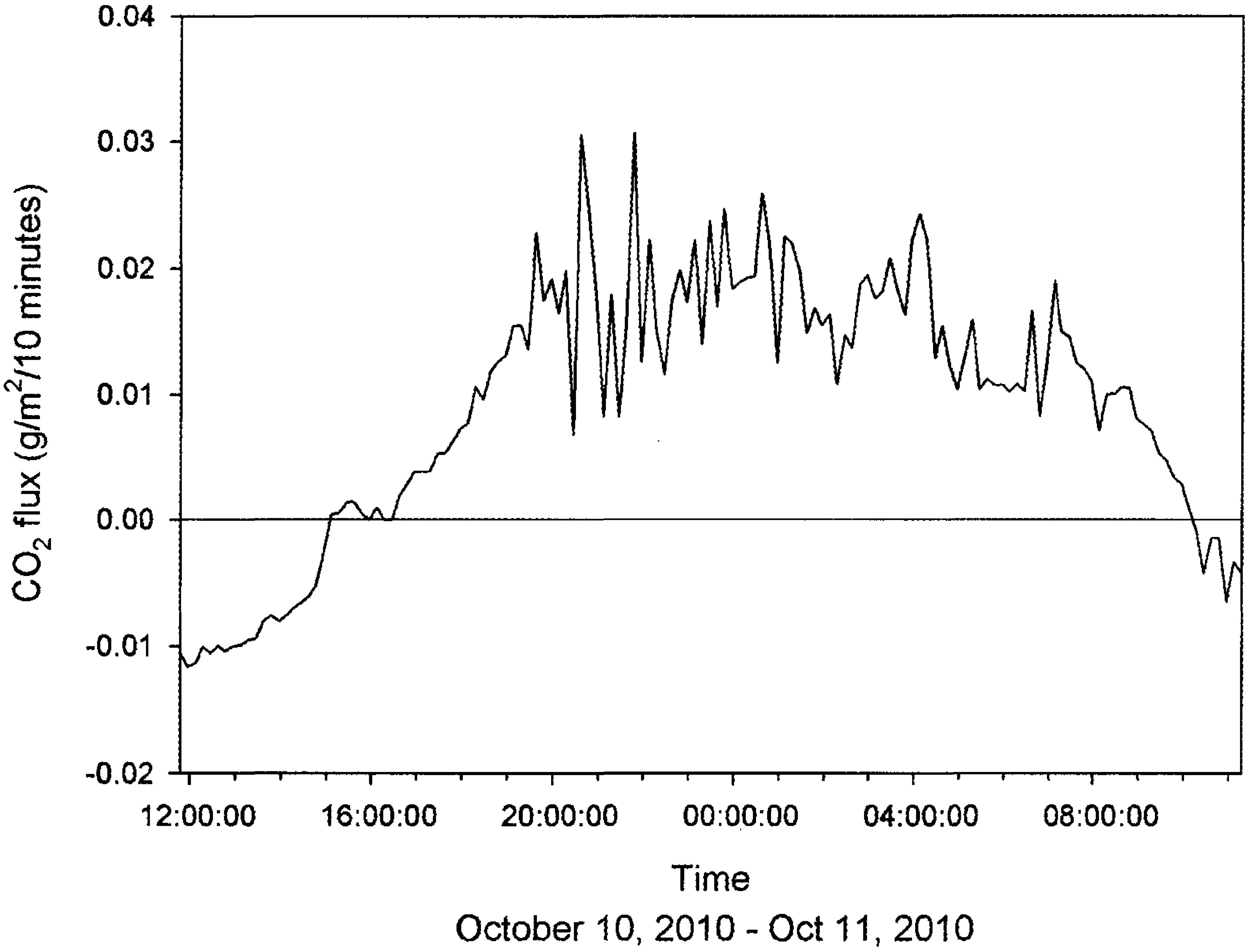

FIGURE 2.3 An example of a measurement of the CO_2 flux from a single plot over a 23-hour period. Positive values represent CO_2 efflux from the system. This measurement was taken between October 10th and October 11th of 2010 at a treatment plot (warming + nitrogen addition).

2.4 Measurements of green aboveground biomass

I estimated the aboveground green biomass monthly from May to September at the end of each month for the two grass species: *B. inermis* and *P. pratensis*, which accounted for more than 95% of the aboveground biomass. Each sub-plot contained a permanent 113 cm^2 sampling ring made of plastic tubing. I measured the heights of nine random *P. pratensis* leaves and measured all *B. inermis* tiller heights (measured at the highest point of the tallest leaf) present in each sampling ring. Heights of both species' inflorescences were measured. I also counted the number of *P. pratensis* leaves present. I estimated leaf mass from logarithmic height-mass allometric equations. To develop these equations, I harvested approximately 30 tillers and 30 inflorescences of both species from an area outside of the experimental plots. The samples were returned to the lab and dried at 70 °C for at least 48 hours and weighed. I plotted the logarithmic height against weight to obtain a linear equation of height-weight allometry. Using the equation "log(mass) = slope × log(height) – intercept", I estimated average leaf/tiller biomass for each plot, and multiplied this value by leaf/tiller abundance to estimate total biomass. A new allometric equation was calculated for each sampling period.

2.5 Statistical analyses

I performed statistical analyses in JMP 4.0 (SAS Institute, Inc., Cary, NC, USA). Daily net CO_2 flux estimates from the four treatment plots were used to evaluate treatment effects. Means of the six daily sums in each treatment combinations were used as individual replicates and were square-root transformed to achieve a Gaussian distribution and homogeneity of variance. I used a repeated measures analysis (Randomized complete block split-plot ANOVA) using "Date" as a fixed factor, warming as a fixed between-plot factor, nitrogen addition as a fixed within-plot factor, the interaction term for their treatment combination and block number as a random factor. Error terms for significance tests were as specified in Kirk (1995) and I ran the analyses using the REML method of the Fit Model platform. In 2009, 15 sampling points were included in the analyses. In 2010, 10 sampling points were included.

For both years, I estimated the total annual flux by determining the mean daily flux rate and multiplying it by 199 days of the growing season (April 15th – October 31st). Using the sunrise and sunset data from Environment Canada's database (Environment Canada National Climate Data and Information Archive), dark respiration was filtered from the measurements taken during daylight hours. A linear regression (ordinary least squares) was performed to determine the effects of nightly mean temperature on total night time respiration for 2009 and 2010 separately.

Repeated measures analysis was performed to assess treatment effects on green aboveground biomass (the same model used to analyze CO_2 flux). Three analyses were conducted: total green aboveground biomass, *P. pratensis* and *B. inermis* biomass. These data were square-root transformed to satisfy the assumptions of normal distribution and homogeneity of variances.

Chapter 3: Results

In 2009, the mean growing season temperature and precipitation were close to the climate normals, whereas winter preceding the growing season featured higher than normal precipitation (Table 3.1). In 2010, the mean growing season temperature was approximately 2 °C warmer than the climate normals with precipitation approximately 75 mm higher than normal, and the winter preceding the 2010 growing season was mild (mean air temperature of 0.6 °C) and featured low precipitation (195.2 mm) relative to normal (Table 3.1).

In the experimental plots, the warming treatment increased the plot soil temperature by 1-4 °C (at 1 cm depth), with the highest temperature differences occurring between April and May (Figure 3.1). The warming effect was reversed during winters, decreasing the soil temperature of the treatment plots by approximately 1 °C relative to control plots. With respect to volumetric water content, warming reduced soil moisture slightly, but this effect was small relative to the seasonal changes in soil moisture experiences across all plots.

3.1 CO_2 Flux

3.1.1 Total annual flux estimates

In both years, the study system was a source of carbon, with a total estimated annual efflux of 493 $gCO_2\ m^{-2}$ in 2009 and 480 $gCO_2\ m^{-2}$ in 2010. Daily net CO_2 efflux started near zero in early spring, but rose exponentially with plant biomass until late June, at which point it remained relatively stable over the rest of the

TABLE 3.1 Mean temperature and total precipitation over the 2008-2009 and 2009-2010 winters (Nov 1st to Apr 1st) and the 2009 and 2010 growing seasons (Apr 1st to Oct 31st) for the experimental site relative to 1971-2000 climate normals (Environment Canada, National Climate Data and Information Archive).

	1971 – 2000	2009	2010
Mean winter temperature (°C)	-2.4 ± 1.72	-2.8	-0.6
Total winter precipitation (mm)	392 ± 5.57	483	195
Mean growing season temperature (°C)	14.5 ± 0.7	14.5	16.3
Total growing season precipitation (mm)	595 ± 2.38	619	669

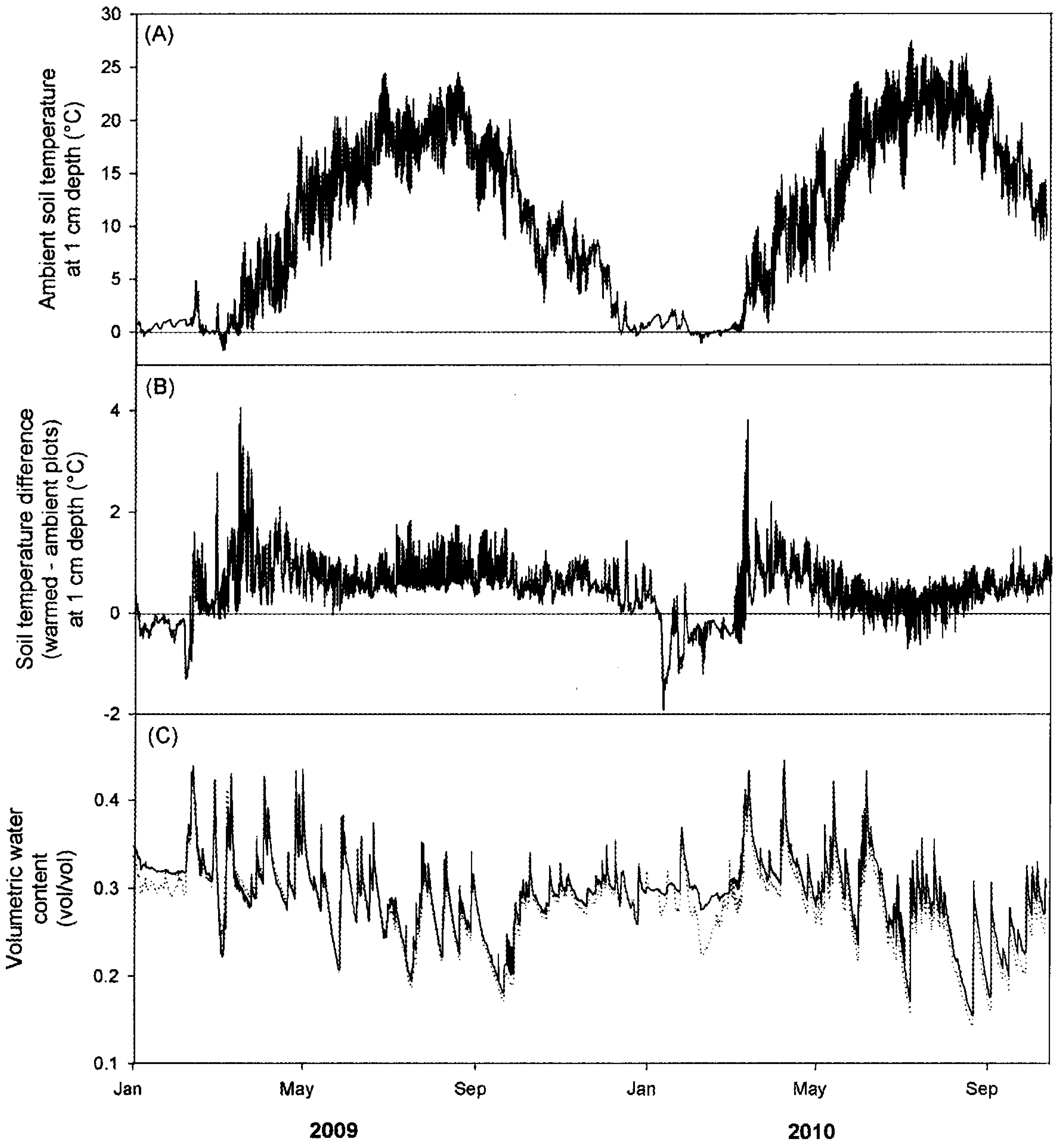

FIGURE 3.1 A Ambient soil temperature at 1 cm depth and **B** soil temperature difference at 1 cm depth between year-round warmed and ambient temperature plots. **C** The volumetric water content from 0 to 15 cm depth in ambient plots (*solid line*) and warmed plots (*dotted line*). The x-axis ticks denote the first day of each month.

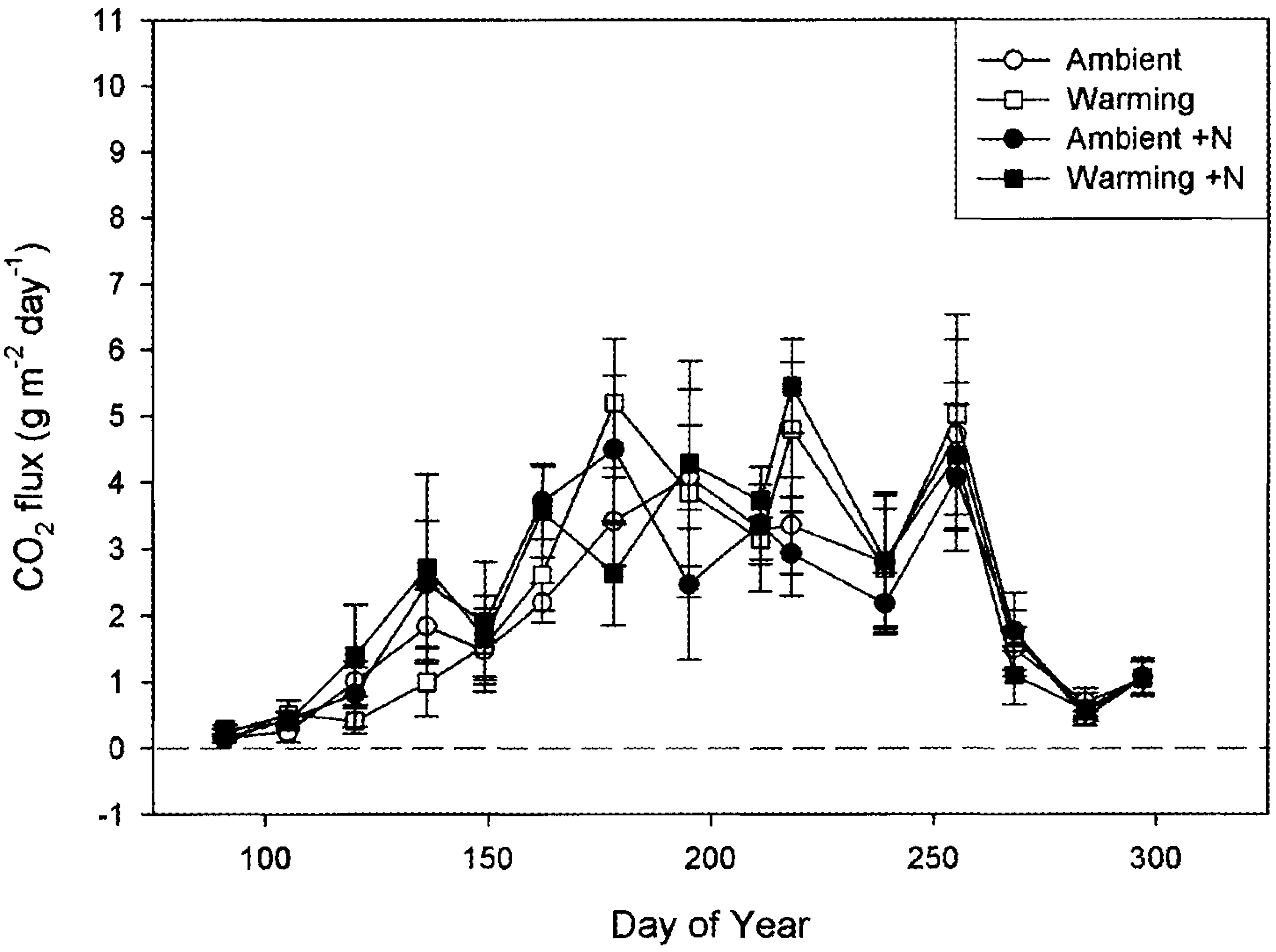

FIGURE 3.2 Net CO_2 flux over the 2009 growing season. Positive values indicate CO_2 efflux from the system. Data points denote the beginning of each 6-day sampling period. Data are presented for ambient temperature plots (*circles*) and for warmed plots (*squares*) both with added N (closed symbols) and without added N (open symbols) (n = 6). Raw means of the six replicate blocks are displayed and error bars denote standard error and are symmetrical. Associated significance tests are displayed in Table 4.2.

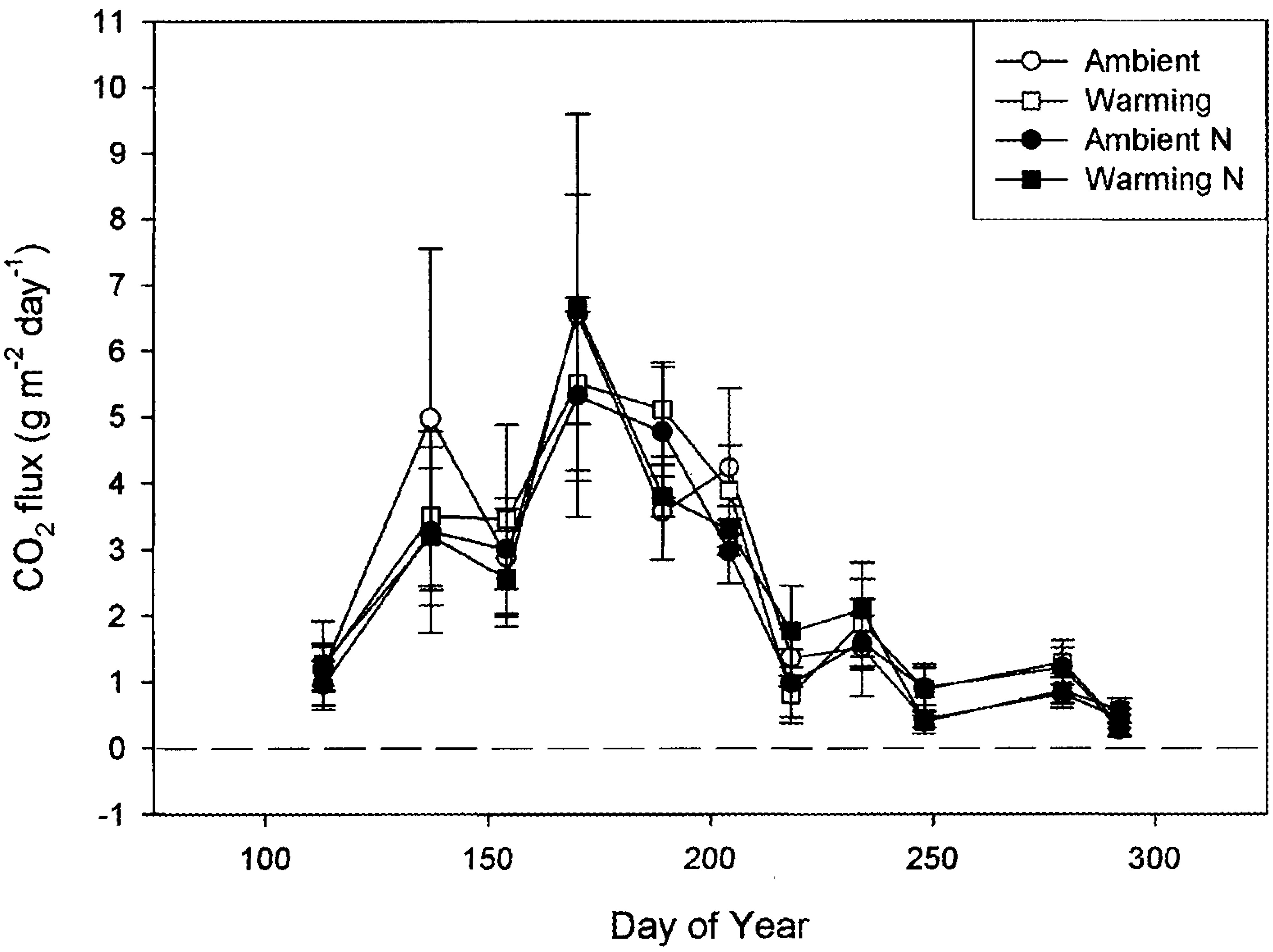

FIGURE 3.3 Net CO_2 flux over the 2010 growing season. Positive values indicate CO_2 efflux from the system. Data points denote the beginning of each 6-day sampling period. Data are presented for ambient temperature plots (*circles*) and for warmed plots (*squares*) both with added N (closed symbols) and without added N (open symbols) (n = 6). Raw means of the six replicate blocks are displayed and error bars denote standard error and are symmetrical. Associated significance tests are displayed in Table 4.2.

summer, then fell sharply in mid-September (Figure 3.2; Figure 3.3). In late-June, at the peak of green plant biomass, instantaneous measures of net CO_2 flux only became negative (indicating net ecosystem C influx) for several hours at midday. Contrary to my prediction, the majority of CO_2 sequestration occurred during during daylight hours in the late spring and early fall. Overall, the contribution of dark respiration to net daily CO_2 flux was high, and temperature was strongly correlated to mean dark respiration in 2009 ($r^2= 0.590$; Fig. 3.4A). This relationship was not as apparent in 2010 ($r^2 = 0.272$; Fig. 3.4B). Changes in net CO_2 flux during daylight hours was difficult to evaluate due to confounds resulting from a combination of photosynthetic C fixation and increased soil respiration (the latter driven by increased daytime soil temperature). The patterns of NEE were different between the two years observed. In 2009, efflux of CO_2 rapidly declined in mid-September. However, in 2010, the rapid decline in the CO_2 efflux occurred in mid-August and remained relatively low until the end of the growing season.

3.1.2 Effects of nitrogen and warming on CO_2 flux

In 2009, there were no significant effects of N addition or warming on net ecosystem CO_2 flux (Table 3.2; $P_W = 0.413$, $P_N = 0.769$, $P_{W\times N} = 0.958$). As indicated above, there was a strong effect of date on net CO_2 flux, but date did not interact significantly with the treatments ($P_D < 0.0001$, $P_{W\times D} = 0.815$, $P_{N\times D} = 0.499$). Similarly, in 2010, there were no significant treatment effects on net ecosystem CO_2 flux ($P_W = 0.259$, $P_N = 0.897$, $P_{W\times N} = 0.946$), and the effect ofdate was significant, but did not interact significantly with the treatments ($P_D < 0.0001$, $P_{W\times D} = 0.989$, $P_{N\times D} = 0.950$).

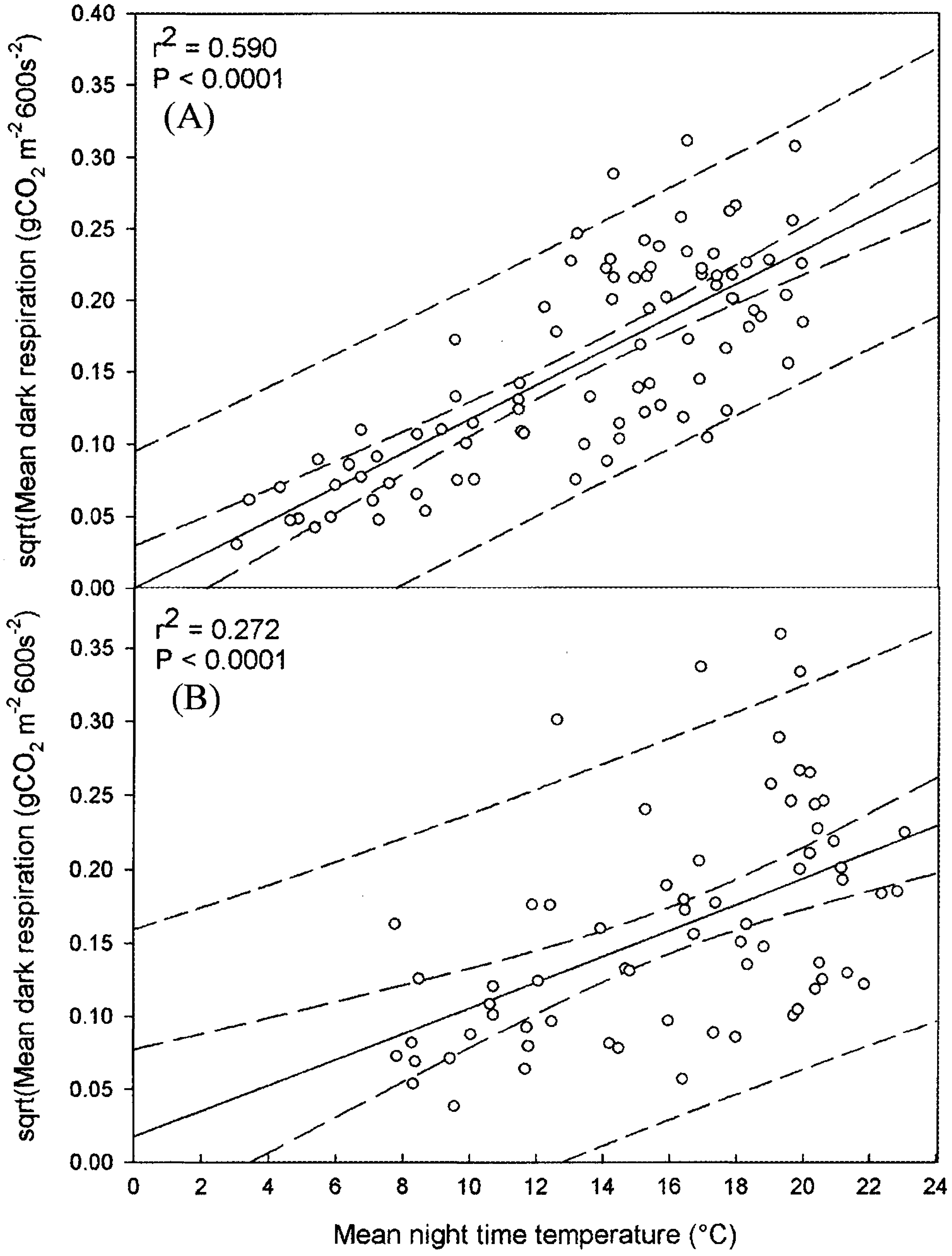

FIGURE 3.4 A Regression of mean night time temperature and mean dark respiration in 2009. Each dot represents a mean of four net CO_2 measurements (each chamber) between sunset and sunrise. **B** Regression of mean night time temperature and mean dark respiration in 2010. Each dot represents a mean of four average CO_2 measurements (each chamber) per night. The long dashes are the 95% confidence interval and the long dashes are the prediction interval.

TABLE 3.2 Summary of ANOVA *P*-values for effects of treatment and date on daily CO_2 flux.

Effect	2009	2010
	Flux	Flux
Between-subjects		
$W_{(1, 20)}$	0.413	0.259
$N_{(1, 20)}$	0.769	0.897
$W \times N_{(1, 20)}$	0.958	0.946
Within-subjects		
$D_{(14, 319)(9, 172)}{}^{\dagger}$	0.0002***	0.0001***
$W \times D_{(14, 319)(9, 172)}{}^{\dagger}$	0.814	0.989
$N \times D_{(14, 319)(9, 172)}{}^{\dagger}$	0.499	0.950
$W \times N \times D_{(14, 319)(9, 172)}{}^{\dagger}$	0.957	0.567
Repeated Measures	15	10

W warming; N nitrogen; D date.

*Asterisks denote significance (*0.05-0.01; **0.01-0.001, ***<0.001).*

Degrees of freedom are displayed in parentheses after the effects.

† *2010 analysis*

3.2 Aboveground green biomass

In 2009, there were no significant effects of N addition and warming on green biomass (Table 3.3; $P_N = 0.379$, $P_W = 0.294$, $P_{W \times N} = 0.379$). Averaged across all plots, total aboveground biomass was 619 ± 29 g m^{-2} (*P. pratensis* – 303 ± 13 g m^{-2}, *B. inermis* – 316 ± 34 g m^{-2}) during late June (the peak green biomass period). A second peak was observed in late August in 2009 (attributed to a second growth phase of *P. pratensis*), whereas a rapid decline in green biomass was evident in 2010 during the same time period (Figure 3.5, Figure 3.6).

In 2010, there was a significant effect of N addition on total aboveground biomass ($P_N = 0.022$). Nitrogen added plots on average had almost 160% more aboveground green biomass than the control plots (Nitrogen plots = 682 ± 30 g m^{-2}, Control plots = 432 ± 39 g m^{-2}) in late June. This effect was caused primarily by increases in *P. pratensis* biomass, whereas *B. inermis* biomass did not significantly increase with nitrogen addition (*P. pratensis* – $P_N = 0.002$, *B. inermis* – $P_N = 0.274$). There was a significant interaction between nitrogen and date on *P. pratensis* biomass ($P_{N \times D} = 0.002$). Warming did not have a significant effect on total aboveground biomass ($P_W = 0.652$) (Table 3.3, Figure 3.6). During peak growth in 2010 (late June), the average standing aboveground biomass was 541 ± 67 g m^{-2} across all plots (*P. pratensis:* 327 ± 42 g m^{-2}, *B. inermis:* 214 ± 41 g m^{-2}).

TABLE 3.3 Summary of ANOVA *P*-values for effects of treatment and date on green biomass estimates.

Effect	2009			2010		
	Total	*P. pratensis*	*B. inermis*	Total	*P. pratensis*	*B. inermis*
Between-subjects						
$W_{(1, 22)}$	0.294	0.325	0.557	0.652	0.567	0.742
$N_{(1, 22)}$	0.379	0.814	0.447	0.022*	0.002**	0.274
$W \times N_{(1, 22)}$	0.379	0.919	0.187	0.930	0.860	0.887
Within-subjects						
$D_{(4, 292)}$	<0.0001***	<0.0001***	<0.0001****	<0.0001***	<0.0001***	<0.0001***
$W \times D_{(4, 73)}$	0.800	0.889	0.517	0.215	0.825	0.543
$N \times D_{(4, 73)}$	0.289	0.380	0.277	0.215	0.002**	0.443
$W \times N \times D_{(4, 73)}$	0.793	0.507	0.818	0.240	0.999	0.890
Repeated Measures	5	5	5	5	5	5

W warming; N nitrogen; D date.

*Asterisks denote significance (*0.05-0.01; **0.01-0.001, ***<0.001).*

Degrees of freedom are displayed in parentheses after the effects.

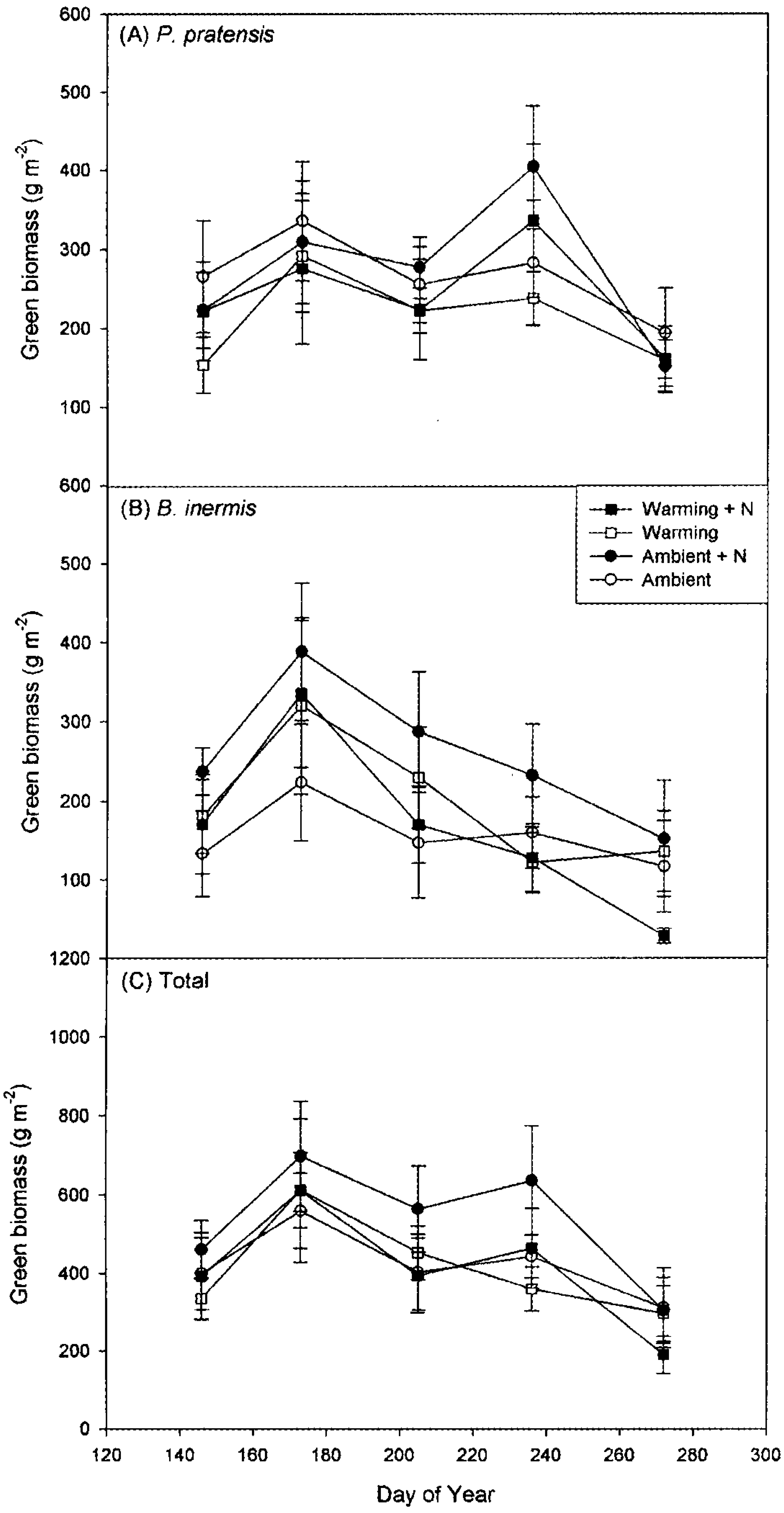

FIGURE 3.5 Aboveground green biomass of **A** *Poa pratensis* and **B** *Bromus inermis* up until each sampling date for the 2009 growing season. Data are presented for ambient temperature plots (*circles*) and for warmed plots (*squares*) both with added N (closed symbols) and without added N (open symbols) (N = 6). Raw means are displayed and error bars denote standard error and are symmetrical. Associated significance tests are displayed in Table 4.3.

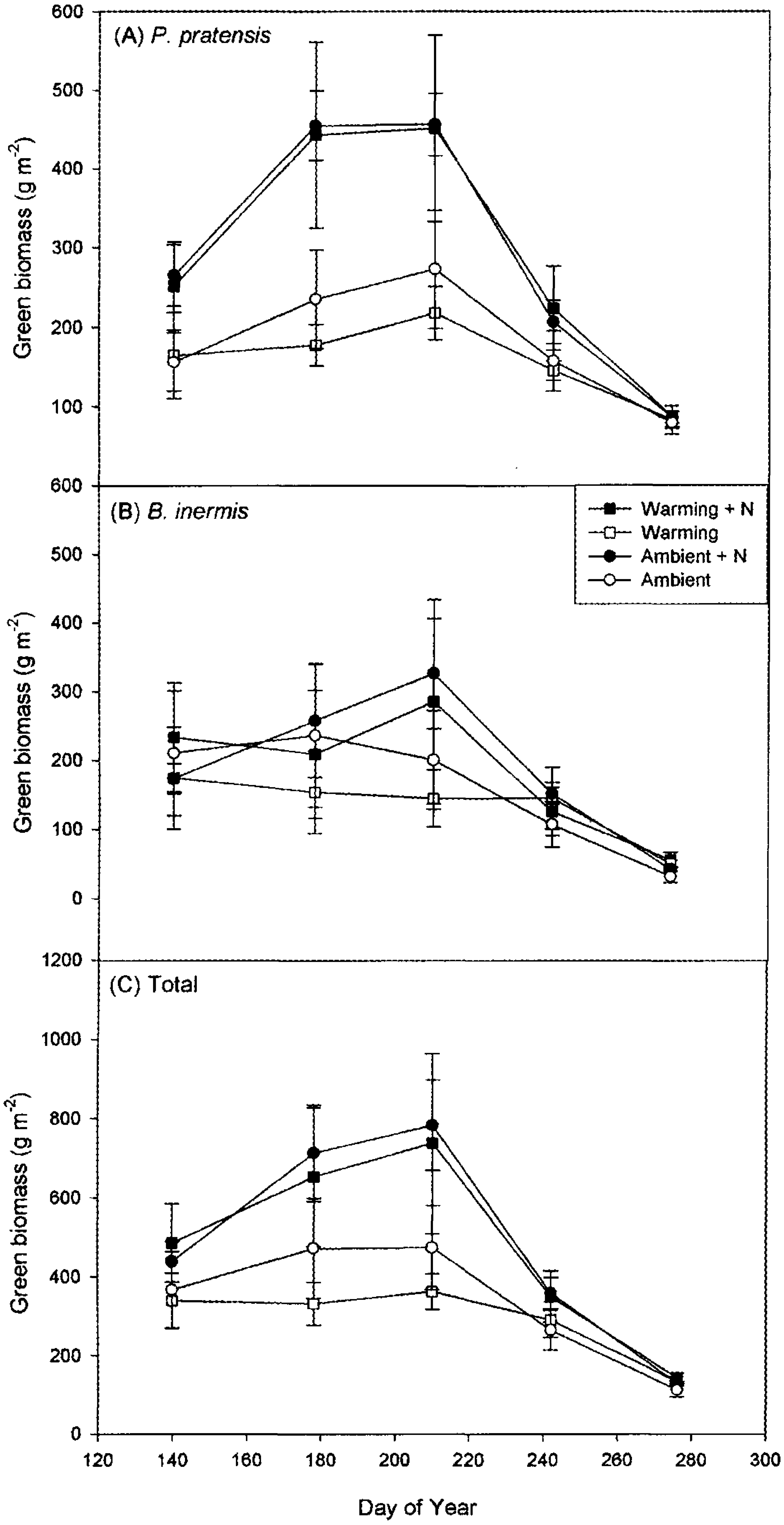

FIGURE 3.6 Aboveground green biomass of **A** *Poa pratensis* and **B** *Bromus inermis* up until each sampling date for the 2010 growing season. Data are presented for ambient temperature plots (*circles*) and for warmed plots (*squares*) both with added N (closed symbols) and without added N (open symbols) (N = 6). Raw means are displayed and error bars denote standard error and are symmetrical. Associated significance tests are displayed in Table 4.3.

Chapter 4: Discussion

4.1 Net Ecosystem Exchange

Although contrary to my hypothesis that the study system would be a net carbon sink, my estimates of annual net ecosystem exchange of 493 gCO_2 m^{-2} and 480 gCO_2 m^{-2} in 2009 and 2010, respectively, were close to the range of -1700 gC m^{-2} y^{-1} to 442 g C m^{-2} y^{-1} reported for other grassland systems (Raich and Potter, 1995; Norman *et al.*, 1992; Mielnick and Dugas, 2000). For the latter studies, high interannual variation was often observed in response to weather variability, in some cases with sites switching from being net sources to net sinks in alternate years, yet my study system remained a strong carbon source in both years. In addition, despite the advantage of using an open flux chamber to include the plant photoassimilation component of NEE, the contribution of dark respiration dominated the total flux values. Even at the point of peak green plant biomass in mid-summer, the contribution of photosynthesis to NEE was overwhelmed by increased respiration (the combination of R_H and R_A) under warmer temperatures, and the plots only served as marginal net carbon sinks during the peak daylight hours, if at all.

There are two possible explanations for why the study site was consistently a source of CO_2. First, vegetation-type has a large impact on the carbon flux of ecosystems (Raich and Potter, 1995), and the largest net carbon influxes are typically reported for forested systems, not grasslands (Wofsy *et al.*, 1993). This high capacity for carbon storage in forests is attributed to long term carbon accumulation in woody tissue through NPP (Goulden *et al.*, 2004). Among grass-dominated systems, large amounts of carbon sequestration have been reported for tall grass prairies, where plant root to shoot ratios are relatively high (Suyker and Verma, 2001; Suyker *et al.*, 2003). Other types of temperate grasslands show some propensity to sequester carbon, but large variability exists from site to site with respect to NEE (Novick *et al.*, 2004). Vertical distribution of SOC is largely dependent on soil composition and the depth of rooting system due to rhizosphere interactions that drive belowground C cycles (Jobbágy and Jackson, 2000). The dominant species at my study site, the grasses *Poa pratensis* and *B. inermis*, both have relatively shallow maximum rooting depths and low belowground biomass allocation compared to species generally found in tall grass prairies (e.g. *Andropogon gerardii* Vitrman., *Sorghastrum nutans* Nash.; Bookman and Mack, 1982; Canadell *et al.*, 1996). Biomass allocation to shallow soil depths effectively increases the organic carbon pool that remains labile near the surface of the soil (Raich and Potter, 1995). Rapid decomposition of this short-term carbon store adds to the large relative R_H and could diminish net carbon sequestration (Gu *et al.*, 2004).

While rapid decomposition of plant biomass could explain the potential carbon neutrality of the system over the long term, explanations regarding site history must be invoked in order to explain why the system persists as a carbon source. Given that the study site was a former agricultural field, and only the aboveground biomass of the crops (rotations of corn, soy and wheat) were harvested, belowground biomass could have contributed large carbon inputs to the soil prior to abandonment. In high shoot:root systems such as temperate grasslands, belowground processes may be limited in providing ideal conditions for the incorporation soil organic carbon into permanent aggregates for long term storage (Jobbágy and Jackson, 2000). Although lateral transport of soluble or dissolved carbon in leachate or runoff can also provide carbon inputs to some systems (Ciais *et al.*, 2006) my study site was relatively flat. Thus, the legacy effect of leftover soil carbon from agriculture may best explain why the system remains a net source of carbon despite the presence of the grasses.

4.2 Nitrogen and Warming Effects on CO_2 flux

As I predicted, nitrogen addition increased aboveground biomass in 2010. However, there was no correlation between increased biomass production and CO_2 flux. It should be noted that the green biomass measurements did not correspond directly with NPP, because of leaf senescence and losses to herbivory. This was in part due to my assumption that actively photosynthesizing aboveground biomass would be more closely correlated with NEE than NPP would be with NEE. As with the seasonal trend, the low contribution of plant biomass to NEE could be explained by the large role of R_H in determining total flux. While previous studies have shown decreases in microbial respiration with N limitation (e.g. Hu *et al.*, 2000; Carreiro *et al.*, 2000; Chung *et al.*, 2007; etc.), direct effects of N addition on NEE were not detected in my study. The lack of a significant difference in NEE between N addition plots and control plots could indicate that the study site soil may not be N limited with respect to soil microbes. Indeed, previous observations from the study site indicated no significant shifts in microbial extracellular enzymatic activity or microbial biomass carbon in response to nitrogen addition (Bell *et al.*, 2010). Alternatively, potential increases in microbial decomposition could have been counterbalanced by increased photo-assimilation. That is, while microbial activity may have increased in N addition plots, both directly or through litter chemistry changes, it was not reflected in the NEE due to enhanced aboveground productivity in these plots. The latter explanation is unlikely, because there were no significant treatment effects on aboveground biomass in 2009. In addition, an increased assimilation effect would have corresponded with increased plant dark respiration, which did not occur in nitrogen addition plots.

There was also no evidence of warming influencing aboveground production or CO_2 flux in either 2009 or 2010. This result was inconsistent with observations of increased plant biomass in response to warming in the same experiment in 2008 (Hutchison and Henry, 2010). However, the warming treatment only increased the biomass of *B. inermis* in 2008, and the dominance of *P. pratensis* in our plots in 2009-2010 could potentially explain this inconsistency. It was surprising that warming did not significantly influence CO_2 exchange in the plots, because heterotrophic respiration is typically highly correlated with temperature (Lloyd and Taylor, 1994). A lack of a warming effect could be explained by the heaters being turned off during the CO_2 flux measurements (because the heating infrastructure did not fit in the chambers), which caused the temperatures in the heated plots to quickly equilibrate with those of the ambient temperature plots over the course of the chamber measurements. In addition, the heaters warmed the plots by only 1-2 °C throughout the growing season, and the degree of warming (half of that predicted by most climate projections for our region for the end of the next century) may not have been sufficiently large for significant differences to be detected over the natural variation among plots. For the latter, within a given week of sampling, block to block variation was quite large. NEE appeared to respond most to day to day temperature fluctuations rather than to specific plot attributes such as standing biomass.

Warming and nitrogen addition treatments did not appear to interact directly to affect the NEE. However I observed a significant interaction between date and N in 2010. This was caused by a more rapid biomass accumulation in N plots relative to control plots between May and June. In addition, this growth phase was followed by a slow but steady increase into July, while control plots continued to accumulate aboveground green biomass at faster rate. This effect was most likely caused by intraspecific competition, as greater biomass in N plots experienced stunted biomass accumulation into July as a result of higher density (Chu *et al.*, 2008). With respect to the NEE, it would have been possible for warming and nitrogen to alter the NEE in opposite directions and effectively cancel each other out. However, this would have been detected in the measurement of my single treatment plots (nitrogen only and warming only treatments) relative to the control plots. While it is possible that the potential treatments effects were lost due to more complex interactions between warming and nitrogen, my system was more responsive to seasonal variations driven by stronger environmental predictors of NEE.

4.3 Drivers of CO_2 flux

Overall, although there was no treatment effect of warming, the NEE of the study system was sensitive to variation in temperature at multiple time scales: seasonal and daily. Because temperature effects on NEE during the day were confounded by changes in photosynthetically active radiation, dark respiration was the best parameter for assessing the temperature sensitivity of NEE, and it was strongly correlated with temperature. The relationship between dark respiration and temperature remained strong even when observations were pooled across the entire growing season. The nonlinear relationship between temperature and NEE (square-root transformation in Figure 3.4) is consistent with the results of respiration responses in temperature manipulation experiments (Schlesser, 1982).

Similar precipitation patterns (higher than climatic normals) over the two summers gave little insight into how warming or nitrogen addition might interact with variability in precipitation. Over each summer, volumetric water content and NEE were negatively correlated, which contradicts the general relationship between water availability and soil respiration. However, soil water availability was confounded with increases in soil temperature throughout the summer, such that dry soil during hot summer days corresponded with high CO_2 efflux. Nevertheless, NEE response to water may be limited to near drought conditions or flooding, neither of which was observed in 2009 or 2010.

4.4 Conclusions

My study system served as a carbon source despite moderately high primary production, which may be explained in part by the dominance of shallow-rooting grasses and the legacy effects of agriculture. These two factors combined released abundant and labile soil organic carbon from the system with no indication of long term carbon storage. Furthermore, nitrogen fertilization enhanced aboveground productivity without affecting CO_2 flux, which indicated a relatively low contribution of photo assimilation to NEE. Nevertheless, variation in ambient temperature remained an important factor in explaining CO_2 flux in my system. Further investigation into NEE responses to a gradient of temperature treatments is clearly needed, and likewise, the use of open canopy flux chambers across a wider variety of grass-dominated systems may reveal a more important contribution of plant cover and photosynthesis to NEE responses than indicated in my study.

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