Foliar dark respiration: scaling gas exchange characteristics and isotopic signals from leaf to canopy and ecosystem level

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ABSTRACT

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The carbon balance of an individual plant or an ecosystem is determined by the small difference between two large fluxes, photosynthesis and respiration. Plant respiration consumes 30% to 70% of the photosynthetic products and is sensitive to temperature. Thus, climate change (*e.g.* global warming) can significantly influence plant respiration and the carbon balance status. Foliar respiration contributes up to two thirds of total plant carbon loss, so it is necessary to understand the response of leaf respiration to environmental factors at multiple temporal and spatial scales, given that we intend to predict the long-term effects of climate change. ~~On~~ At community level, species-specific changes in foliar carbon balance can affect the relative competitive ability and alter the community components. ~~On~~ At ecosystem level, a small change in foliar respiration efflux can lead to large effects on the net ecosystem CO_2 exchange (NEE) and may alter the carbon sink/ source status.

This thesis firstly addressed leaf respiration of *Quecus rubra* (red oak) and several understory shrubs, seasonally in a temperate deciduous forest, and then scales foliar respiration to the canopy level. The base leaf respiration rate (R_0, respiration at 10 ºC) of *Q. rubra* was significantly affected by season, site water availability, canopy height and their interactions, but the activation energy of respiration as a single reaction (E_0) was constant. Ignoring the season, site and canopy height effects on leaf respiration resulted

in upto a 130% error on the estimation of canopy foliar carbon loss (R_c), but canopy level model parameterizations could be simplified by assuming a constant E_0. In the understory, leaf respiration was compared and contrasted between invasive *Berberis thunbergii* (Japanese barberry, early leafing) and two native shrubs, *Kalmia latifolia* (mountain laurel, evergreen) and *Vaccinium corymobsum* (highbush blueberry, late leafing). A negative correlation between R_0 and E_0 was found in all three shrubs. The annual R_c per unit leaf area of *K. latifolia* was much higher than *B. thunbergii* and *V. corymobsum* respectively. Among the three shrubs, the effect of significant winter warming in southern New York state in the 20th century on R_c was the smallest in the evergreen *K. latifolia*, which is mainly attributed to the low E_0 in this species.

Longer-term (*e.g.* inter annual to decades), integrated environmental effects on ecosystem respiration can be reflected by stable carbon isotope signals. In ecosystem studies, it is widely assumed that $\delta^{13}C$ of plant respiratory CO_2 ($\delta^{13}C_R$) should reflect the $\delta^{13}C$ of plant organic carbon. Thus, I subsequently surveyed leaf $\delta^{13}C_R$ in five C_3 plants to test this assumption. In all cases leaf respiratory CO_2 was more ^{13}C enriched than leaf organic components, illustrating that $\delta^{13}C_R$ was 5.8 ‰ higher than leaf bulk organic matter ~~in~~ on average. However, due to the complex origin of ecosystem respiration, caution should be taken when predicting vegetation $\delta^{13}C$ on ecosystem level by scaling leaf level results.

In summary, this thesis demonstrated that leaf respiration can significantly affect canopy and ecosystem level processes (carbon efflux, invasion, etc.), so appropriate upscaling of leaf respiratory properties is critical to quantify these processes.

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Dedication

To my mother, who fought and survived lung cancer in the past four years,

my father, grandparents, and all relatives and friends,

who provided help when my family was in difficulty

INTRODUCTION

Rationale

Comparable to photosynthesis, respiration is a primary biological process regulating the carbon balance of individual plants and ecosystems. Globally, plant respiration, which comprises about half of the total respiration flux, releases approximately 50% of the carbon fixed through net photosynthesis (Amthor, 1989; Ryan, 1991). In plants, leaf respiration is the most commonly studied respiratory component. Leaf respiration accounts for 10-35% of daily photosynthesis (Ryan *et al.*, 1994; Van der Werf, Poorter & Lambers, 1994; Atkin & Lambers, 1998) and can affect leaf photosynthetic capacity (Turnbull, Murthy & Griffin, 2002; Turnbull *et al.*, 2005), and thus it plays a critical role in global carbon cycle.

Plant respiration is sensitive to temperature. Typically, respiration rates double for each successive 10°C increment in temperature ($Q_{10,}$ Ryan, 1991), but the value of Q_{10} can be highly variable, ranging between 1.1 and 4.2 (Azcon-bieto & Osmond, 1983; Azcon-bieto, 1992; Tjoelker, Oleksyn & Reich, 2001). Under field conditions, the temperature response of respiration can be affected by measurement temperature (Tjoelker *et al.*, 2001), species (Larigauderie & Korner, 1995), season (Stockfors & Linder, 1998; Atkin, Holly & Ball, 2000; Vose & Ryan, 2002; Damesin, 2003), growth temperature (Larigauderie & Korner, 1995; Atkin *et al.*, 2000), canopy position (Griffin *et al.*, 2001; Griffin, Turnbull & Murthy, 2002a; Turnbull *et al.*, 2003), soil characteristics (Turnbull *et al.*, 2005), water availability (Turnbull *et al.*, 2001), and leaf metabolic state (Azcon-

bieto & Osmond, 1983; Griffin *et al.*, 2002b). Plant respiration is also subject to thermal acclimation (*reviewed in* Atkin & Tjoelker, 2003), so it is a function of temperature and physiological history (Amthor, 1989; Atkin *et al.*, 2000). It is predicted that global temperatures will rise 1.4 to 5.8 °C by the end of this century (Hansen *et al.*, 1999; IPCC, 1999) and is likely to be more significant at night (Easterling *et al.*, 1997; Alward, Detling & Milchunas, 1999; IPCC, 1999), when respiration is the dominant physiological process in plants. Due to the thermal sensitivity, respiration is subjected to the global warming and may cause significant impact on large-scale ecological processes.

At the ecosystem level, a small change in foliar respiration efflux can lead to a large effect on net ecosystem CO_2 exchange (NEE) and may alter the carbon sink/ source status. In general, NEE is determined by the small difference between photosynthesis and respiration fluxes (Schimel, 1995; Hansen *et al.*, 1999; Field, 2001), and can be measured by eddy covariance or estimated by models. However, due to the insufficient understanding in the mechanistic processes that regulate the response of respiration to temperature and thermal acclimation, compared to photosynthesis, respiration is less represented in ecosystem models of NEE (Dewar, Medlyn & McMurtrie, 1999; Saxe *et al.*, 2001; Tjoelker *et al.*, 2001). Often, respiration is simplified as a "fixed" rate and exponential temperature response, which does not take into account the influence of growth environment and thermal acclimation (Ryan *et al.*, 1996; Tjoelker *et al.*, 2001), and thus it is possible for biased error to influence estimates of respiratory efflux. For these reasons, I argue that intensive spatial and temporal surveys of leaf respiratory

temperature responses are needed to parameterize canopy and ecosystem models, and to determine the potential errors introduced by specific simplifying assumptions.

At the community level, species-specific changes in foliar carbon balance can affect the relative competitive ability and alter the community composition. The rapid expansion and obvious competitive success of invasive species in the forest understory, indicates that these plants have a relative carbon balance advantage over the co-occurring natives. In deciduous forest, early leafing species can gain a significant spring carbon subsidy through photosynthetic carbon gain during periods of high irradiance, and this spring subsidy has been proposed to be an important mechanism of understory invasion (Harrington, Brown & Reich, 1989; Zotz, Franke & Woitke, 2000; Myers & Anderson, 2003). However, plant carbon balance is simultaneously determined as the net equilibrium between carbon gain and carbon loss, and thus respiration needs to be considered when explaining the advantage of understory invasive plants. Furthermore, in light-limited environments, the net carbon gain may be predominately determined by respiration (Walters & Reich, 1999), but comparative studies on respiratory properties between invasive species and the co-occurring natives are rare.

In ecosystem level studies, carbon stable isotope methods are widely used to integrate the long-term effect of environmental conditions on ecological processes. It is well known that carbon isotope discrimination takes place during plant photosynthetic CO_2 fixation, resulting in the organic carbon found in all higher plants being depleted in ^{13}C in relative to atmospheric CO_2, but studies on the carbon isotope ratio of respiratory CO_2 ($\delta^{13}C_R$) are

limited. In general, the magnitude of the potential isotope effect on dark respiration is unclear and the current data appear contradictory (O'leary, 1981; Lin & Ehleringer, 1997; Duranceau *et al.*, 1999; Duranceau, Ghashghaie & Brugnoli, 2001). However, in ecological and physiological studies, it is widely assumed that carbon fractionation in dark respiration is negligible (Flanagan & Ehleringer, 1998; Yakir & Sternberg, 2000; Ehleringer *et al.*, 2002). If, in fact, the $\delta^{13}C_R$ does not correctly reflect the $\delta^{13}C$ of the pool of respiratory substrates, the conclusions of these studies will need to be reconsidered and modified accordingly. Clearly, detailed information on the respiratory carbon isotope effect, and ultimately the mechanisms responsible for any observed effects are needed to gain insight into ecosystem level processes.

Although leaf respiration plays an important role in carbon cycle, our understanding in its response to varying environmental conditions is incomplete and insufficient. Given that we intend to predict the long-term effects of climate change, it is necessary to understand the response of leaf respiration to environmental factors at multiple temporal and spatial scales. This thesis addressed the seasonality of plant leaf respiration in a temperate deciduous forest in southern New York state and the carbon stable isotope of leaf respiration at the Biosphere 2 center. Then, these leaf level results were upscaled to interpret ecological processes happening in canopy and ecosystem scale.

Thesis Summary

The studies in the first three chapters of this thesis were completed in the Black Rock Forest, which is a 1500 ha preserve in Hudson Highlands of Southeastern New York

State, located at 41°24' N and 74°01' W with elevations ranging from 150 to 450m above sea level. The air temperature is strongly seasonal, with monthly average temperature ranges from -2.7°C in January to 23.4°C in July. The average annual precipitation is 1.2m. The forest is a *Quercus* dominated secondary growth forest that characterizes the northeastern United States. *Q. rubra* (red oak) is the most abundant species tree species. Common understory shrubs include *Gaylussacia baccata* L. (huckleberry), *Kalmia latifolia* L. (mountain laurel), *Rhododendron periclymenoides* L. (pink azalea), *Vaccinium spp.* (blueberries), and invasive *Berberis thunbergii* (Japanese barberry).

In chapter 1, I measured the leaf respiratory temperature response, leaf properties and analyzed the respiration – leaf property relationship of *Q. rubra* throughout the growing season of 2003. Measurements were made in both the upper and lower tree canopy and at two sites with different water availability. I found significant temporal and spatial heterogeneities in the leaf respiration rate and the temperature response of leaf respiration throughout the growing season. I also found that the leaf respiration rate was significantly correlated to leaf nitrogen and reducing monose. These results indicate that temporal and spatial heterogeneities of respiration need to be considered in ecosystem models.

In chapter 2, the leaf level respiratory parameters reported in chapter 1 were upscaled to estimate the canopy foliar carbon loss (R_{canopy}) of a virtual *Q. rubra* monoculture based on the stand leaf area index (LAI), and night temperature of the same two research sites. Since it is often not practical to obtain such detailed data to parameterize respiratory and/or ecosystem models, I further estimated the error caused by a series of simplified

parameterization scenarios, which respectively neglect the effect of specific environmental or biological factors. I found that, for *Q. rubra* stands, the variation in the base respiration rate (R_0) needs to be fully parameterized but E_0 can be assumed as a constant and night temperature fluctuation can be ignored without introducing unacceptable error (introduced error < 5%).

In chapter 3, the temperature response of leaf respiration and leaf properties were compared between invasive *Berberis thunbergii* (Japanese barberry), an early leafing understory shrub, and two native shrubs, *Kalmia latifolia* (mountain laurel), a broad leaf evergreen and *Vaccinium corymobsum* (highbush blueberry), a late leafing deciduous species. Then, the leaf level respiratory parameters were upscaled to address the annual canopy foliar carbon loss (R_c) of the three shrubs across 2004 and the effect of warming in the 20th century on the foliar carbon loss was estimated. Species-specific seasonal pattern of respiratory properties were observed and respiratory properties, rather than seasonal pattern of warming or phenology, is more important to determine the carbon loss of these shrubs. I also combine these results with information on photosynthetic properties (Appendix I) to assess the regulation of leaf carbon balance.

Studies in chapter 4 were completed at the Biosphere 2 center. I compared the stable carbon isotope ratios of leaf respiratory CO_2 ($\delta^{13}C_R$) and leaf organic components (soluble sugar, water soluble fraction, starch, protein and bulk organic matter) in 5 C_3 plants with leaf scale Keeling plots. The plants were grown in a greenhouse and inside Biosphere 2 (a 1.29 ha. glass enclosed mesocosum research facility in Oracle, Arizona,

with one ocean and five terrestrial biomes: tropical rainforest, mangrove, savanna, desert, and intensive temperate forest). A conceptual model was established to explain how $\delta^{13}C$ of ecosystem respiration is determined. In this study, leaf respiratory CO_2 was always more ^{13}C-enriched than leaf organic components, which indicates a widespread ^{13}C-enriched respiratory CO_2 in plants. However, due to the complex origin of ecosystem respiration, caution should be taken when attempting to predict the $\delta^{13}C$ of leaf respiratory CO_2 at the ecosystem scale by upscaling the relationship between leaf $\delta^{13}C_R$ and $\delta^{13}C$ of leaf organic components.

In addition to the main body of my thesis, which focuses on foliar respiration, in Appendix 1, I present a comparative study on the phenology and photosynthetic characteristics of *B. thunbergii*, *K. latifolia* and *V. corymbosum* done in conjuction with chapter 3. In this study, I found a clear temporal photosynthetic niche separation in these three shrubs. *B. thunbergii*'s apparent success over the co-occurring natives appears to be related to a significant spring carbon subsidy and the ability to acclimate to varying irradiance through active nitrogen allocation and leaf morphological modifications. In the northeastern United States, pronounced winter warming and nitrogen deposition may facilitate the carbon gain of *B. thunbergii* over the natives and may further contribute to its invasion in the forest understory.

In appendix 2, I used *Alternanthera philoxeriodes* as a model species to explore the growth response of plants to a simulated flue gas gradient in small, custom-made growth chambers. The goal of this study was to test the feasibility of using power plant flue gas

as a CO_2 source to enhance plant biomass yield in the greenhouses established on the power plant "buffer zone", which can potentially be a more cost-effective, commercially-viable carbon sequestration method than current biofuel profects. I found that plant biomass yield doubled in [CO_2] up to 1%, but acidic pollutants in flue gas will significantly offset the observed CO_2 growth enhancement. The demonstrated CO_2-enhanced biomass accumulation rate, if sustainably scaled up, would be comparable to the highest yields reported in other biofuel projects, and this may be a conservative estimate.

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Chapter 1:

Seasonal variation in the temperature response of leaf respiration in *Quercus rubra* I: foliage respiration and leaf properties

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Abstract

Leaf respiratory temperature responses and general leaf properties of *Quercus rubra* were measured throughout the 2003 growing season in a deciduous forest in northeastern USA. Measurements were made in the upper and lower portions of the canopy at two sites with different soil water availability. Correlations among respiration and various leaf properties were examined. At a set temperature (10 ºC and 20 ºC), leaf respiration rates were higher in both early and late growing season than in mid growing season. Upper canopy leaves generally had higher respiration rates then lower canopy leaves. At the drier site, a more significant seasonal pattern in respiration was observed, while at the more mesic site, a stronger canopy position effect was detected. E_0, a model variable related to the over-all energy of activation of respiration, only varied slightly (52 ± 5 kJ mol^{-1} K^{-1}), and was not influenced by season, site, or canopy position. Leaf properties (specific leaf area, nitrogen, soluble sugars) also varied across season, site and canopy position. Leaf nitrogen and reducing monose were significantly correlated to the leaf respiration rate. After isolating single factors (season, site, canopy position), reducing monose could partially explain the seasonality in respiration, and leaf nitrogen (N_{area}) was well correlated to canopy position effect. Our results suggest that the temporal and spatial heterogeneities of respiration need to be considered in ecosystem models, but significant simplifications may be made in *Q. rubra* by assuming a constant temperature coefficient (E_0) or predicting the base respiration rate (R_0) from well-understood leaf properties.

Introduction

Warming will raise the global temperatures 1.4 to 5.8 °C by the end of this century (Hansen *et al.*, 1999; IPCC, 1999). Furthermore, warming is likely to be more significant at night (Easterling *et al.*, 1997; Alward, Detling & Milchunas, 1999; IPCC, 1999), when respiration is the dominant physiological process in plants. Comparable to photosynthesis, respiration is a primary biological process regulating the exchange of carbon between the atmosphere and the terrestrial biosphere and it is the small difference between these two large fluxes (net photosynthesis 122 GT C $year^{-1}$, and autotrophic respiration 64 GT C $year^{-1}$ + heterotrophic respiration 58 GT C $year^{-1}$, Schimel, 1995; Hansen *et al.*, 1999; Field, 2001) that determines the carbon balance of an ecosystem. Globally, plant respiration, which comprises about half of the total respiration flux, releases approximately 50% of the carbon fixed through net photosynthesis (Amthor, 1989; Ryan, 1991) and of this, 80% of plant respiratory CO_2 is attributable to forest trees (Hall & Scurlock, 1993; Houghton, 1993). Because plant respiration is highly sensitive to temperature, global warming could dramatically influence the size of the respiratory flux and potentially carbon storage in forest ecosystems. Therefore, understanding the temperature response of tree respiration is critical if we are to estimate the potential future forest carbon sink.

Foliar respiration makes up to two thirds of total tree respiration (Hagihara & Hozumi, 1991; Ryan, Lavigne & Gower, 1997) and is the most commonly studied respiratory component of a tree's carbon budget. Typically, leaf respiration rates double for each successive 10 °C increment in temperature ($Q_{10,}$ Ryan, 1991), but the value of Q_{10} can be

highly variable, ranging between 1.1 and 4.2 (Azcon-bieto & Osmond, 1983; Azcon-bieto, 1992; Tjoelker, Oleksyn & Reich, 2001). Although widely used for its simplicity, Q_{10} does not have a strong theoretical justification and may be temperature-dependent itself (Johnson & Thornley, 1985; Tjoelker *et al.*, 2001). Recently, models based on an Arrhenius function have been introduced to better describe the response of respiration to temperature in a more mechanistic basis (Lloyd & Taylor, 1994; Turnbull *et al.*, 2001). Under natural field conditions, leaves are exposed to a range of environmental conditions which may influence their respiration rates and temperature responses (*e.g.* growth temperature, canopy position, soil moisture, Turnbull *et al.*, 2001, 2003). In response, plants can regulate leaf respiration and the temperature response by altering chemical components and physiological activities, resulting in respiratory acclimation and/ or adaptation. Although not completely elucidated, close relationships between respiration and leaf characteristics have been found. For example, a leaf nitrogen-respiration relationship exists across terrestrial ecosystems, functional groups and canopy levels (Ryan, 1991, 1995; Reich, Oleksyn & Tjoelker, 1996; Ryan *et al.*, 1996a; Reich *et al.*, 1998a; Reich *et al.*, 1998b; Griffin *et al.*, 2001; Griffin, Turnbull & Murthy, 2002; Turnbull *et al.*, 2003). Similarly, soluble carbohydrate concentrations in leaves may regulate the temperature response by limiting the formation of respiratory substrates (Atkin, Holly & Ball, 2000; Griffin *et al.*, 2002).

In order to scale up leaf level results to the tree, canopy or ecosystem, a better understanding of the pattern and regulation of spatial and temporal variation in the respiratory temperature response is needed. On the individual tree level, previous studies

found that within-tree distribution of respiration was highly dependent on the canopy depth (Griffin *et al.*, 2001; Griffin *et al.*, 2002; Tissue *et al.*, 2002; Turnbull *et al.*, 2003; Whitehead *et al.*, 2005). At the ecosystem level, spatial heterogeneity (*e.g.* water availability) in environmental resources have been shown to influence both leaf respiration and leaf properties (Turnbull *et al.*, 2001, 2003). Long term studies also show significant seasonal and annual variation of respiration in conifer forests and seedlings (Stockfors & Linder, 1998; Atkin *et al.*, 2000; Vose & Ryan, 2002). However, to the best of our knowledge, there are no studies that integrate temporal, spatial, canopy and their interactive effects on tree respiratory temperature response. Furthermore, it is unclear whether the respiration – leaf property correlations, which were originally described across biomes and functional groups (Reich *et al.*, 1998b), will be useful to explain spatial and temporal variations of respiratory temperature response in individual plant species from specific landscapes.

The Northeastern deciduous forest of the US is regenerating rapidly and believed to be an important carbon sink in the northern hemisphere (Myneni *et al.*, 2001; Hooker & Compton, 2003). In this study, we measured the leaf respiratory temperature response, leaf properties and the respiration – leaf property relationship of *Quercus rubra* in a Northeastern deciduous forest in New York State throughout the growing season of 2003. Measurements were made in both the upper and lower tree canopy and at two sites with different water availability. We expected that leaf respiration rates and general leaf properties (specific leaf area, leaf nitrogen, leaf carbohydrates etc.) would all vary with season, site, canopy level or their interactions. For a better mechanistic understanding, we

further examined (1) whether the model parameters of leaf respiratory temperature response would vary with season, site or canopy position and (2) whether the temporal and spatial effects could be explained by different leaf properties.

Material and Methods

Study site and field plots

Black Rock Forest is a 1500 ha preserve in Southeastern New York State, located at 41°24' N and 74°01' W with elevations ranging from 150 to 450 m above sea level. The air temperature is strongly seasonal, with monthly average temperature ranges from -2.7 °C in January to 23.4 °C in July. The average annual precipitation is 1.2 m (Black Rock Forest climate database). Black Rock Forest is a *Quercus* dominated secondary growth forest that characterizes the Northeastern United States. Dominant tree species include red oak (*Quercus rubra*, 42.3% basal area), chestnut oak (*Q. prinus*, 23.8% basal area) and red maple (*Acer rubrum*, 7.6%, basal area, Turnbull *et al.*, 2001). The soils are typically brown forest soils, acidic and low in nutrients (Lorimer, 1981), with granite gneiss bedrock or glacial till parent material at 0.25-1m depth (Olsson, 1981).

The Cascade Brook watershed is a 135-hectare plot in the southeastern portion of Black Rock Forest, with elevation from 210 to 430 m. Two 0.1 ha permanent research sites were established in 1999 at a 270m lowland and at a 410m upland site. The two sites differed significantly in respect of water availability, and the distribution of these species along this elevation gradient follows their drought tolerance (Engel *et al.*, 2002). In this study, the stable carbon isotope of leaf tissue was measured as an indicator of soil water availability (see "leaf analysis" below). Tree density at the upper elevation site is 760 trees ha^{-1} and the basal area is 23.7 m^2 ha^{-1}. Chestnut oak, white oak (*Quercus alba* L.) and red oak together comprised 78% of the basal area. Although chestnut oak is the most numerous species in the overstory, red oak dominates the canopy comprising at least 54%

of the leaf area index (data not shown). The lower site has 650 trees per hectare and a basal area of 24.9 m^2 ha^{-1}. Red oak is the most abundant *Quercus* species, which made the greatest contribution to basal area and dominates the overstory together with red maple. For detailed descriptions of the sites, refer to Turnbull *et al.* (2001) and Engel *et al.* (2002). Meteorological conditions of the forest are continuously measured and recorded by two standard meteorological stations run by the Black Rock Forest staff.

Respiration measurements

Physiological measurements were made four times during the 2003 growing season, June 11th to 16th, July 30th to August 1st, September 17th to 18th, and October 20th to 23rd. At each site, leaf dark respiration was measured on 6 fully expanded leaves from 3 trees, respectively from the sunlit upper canopy and the shaded lower canopy. Sampled trees were generally representative in height and crown size.

Dark respiration was measured with infrared gas analysis systems (LI-6400, Li-Cor, Inc., Lincoln NE) equipped with CO_2 and temperature control modules. Large branches from trees were excised under water in the field in late afternoon and dark acclimated for at least 1.5 hour before measurements. All measurements were made during 5 PM to 2 AM in a growth chamber with temperature control (Conviron E15, Winnipeg, Canada). Respiration rates were measured at 5 to 7 temperature set points between 5 and 35 °C (typically 10, 15, 20, 25 and 30 °C), which covered the typical night temperature range of the growing season. Temperature within the cuvette was controlled to match the ambient temperature in the growth chamber. CO_2 partial pressure in the cuvette was maintained at

400 ppm. At each temperature set point, the leaves were left for 15-20 minutes to stabilize the respiration rate before being recorded. The measurements were made when respiratory gas exchange had equilibrated (taken to be when the rate of CO_2 efflux was visually stable and the coefficient of variation for CO_2 partial pressure differential between the sample and reference was < 0.3%). Previous studies had shown no differences in respiration between *in situ* leaves and leaves from detached branches in *Q. rubra* (Mitchell, Bolstad & Vose, 1999) and such test was also repeated in *Q. rubra* at our research site (Turnbull & Griffin unpublished data). The respiration rate was reported in area-, mass- and nitrogen- based units.

The temperature response curves were analyzed using a modified Arrhenius equation described by Lloyd & Taylor (1994), which had been applied to *Q. rubra* by Turnbull *et al.* (2001):

$$R = R_0 e^{\frac{E_0}{R_g}\left(\frac{1}{T_0} - \frac{1}{T_a}\right)} \quad (1)$$

where R_0 is the respiration rate at base temperature T_0 (10 °C 283 K in our study), T_a is the measurement temperature (K) of R, R_g is the ideal gas constant (8.314 J mol^{-1} K^{-1}). Originally, this type of model was used to describe the temperature response of a simple chemical reaction and E_0 is the energy of activation (kJ mol^{-1}). When applying the model to respiration, we thus simplify and treat the overall chemical processes of respiration as a single reaction. By doing so, E_0 is equivalent to the overall energy of activation, similar but not identical to the energy of activation for a single enzyme reaction. The value of E_0 may not be a constant and may be influenced directly by the leaf's biochemical and

physiological status (*e.g.* substrate level, reaction pathways) or indirectly by environmental conditions (*e.g.* water availability, light level). Nevertheless, previous studies indicated that E_0 appears constant over the physiological temperature range of temperate species (Lyons & Raison, 1970). When using this model, the temperature response curve can be described by the intercept (base respiration rate), which is represented by the parameter R_0, while the curvature (sensitivity of respiratory temperature response) is represented by both R_0 and E_0. The model was fitted with SigmaPlot 2001 (SPSS Inc., Chicago, IL, USA). Besides R_0 (Respiration at 10 °C), respiration rate at 20 °C (R_{20}), and ± 7-day night average temperature during the measurement period (R_{ave}), were also calculated.

The commonly used Q_{10}, which is a simple parameter to measure respiratory temperature response, can be linked to this model by:

$$Q_{10} = e^{\frac{E_0}{R_g}\left(\frac{1}{T_2}-\frac{1}{T_1}\right)} \qquad (2)$$

and

$$T_1 - T_2 = 10\ (^{\circ}C) \qquad (3)$$

Clearly, as defined by this model, Q_{10} is temperature dependent (Atkin & Tjoelker, 2003) and is determined by E_0 at a set temperature. In this study, a Q_{10} of 15 – 25ºC was calculated to facilitate comparison with other studies reporting only Q_{10} values.

Leaf analysis

All analyses were performed on the same leaf material used for respiration measurements. Following the dark respiration measurements, several leaf disks were

immediately frozen in liquid nitrogen for carbohydrate analysis. The area of the remaining leaf material (excluding the mid rib and the petiole) was determined using a leaf area meter (Li-3000, Li-Cor Inc. Lincoln NE, USA) and then dried in a 60 °C oven for a minimum of 48 hrs. The dried leaf material was weighed and ground to fine powder for nitrogen and carbon stable isotope ratio ($\delta^{13}C$) analysis with an Europa 20/20 continuous flow isotope ratio mass spectrometer (CF-IRMS) coupled with an ANCA NT combustion system (Europa, Cheshire, UK) at Lamont-Doherty Earth Observatory. Specific leaf area (SLA) was calculated from the leaf area and dry weight. Leaf soluble carbohydrates (sucrose and reducing monose, (the latter included glucose and fructose) in the harvested leaf disks were determined colorimetrically using the ethanol extraction technique of Hendrix (1993) as described by Griffin, Sims & Seemann (1999), with required modifications. Since Sigma-Aldrich (St. Louis, USA) glucose kit #115A in the original protocol is no longer commercially available, glucose kit GATK-20 was substituted and the carbohydrate contents were determined by measuring the absorption at 340 nm. All samples were analyzed in triplicate and reported as the mean value. Leaf nitrogen results were reported on an area (N_{area}) and mass basis (N_{mass}). Leaf soluble carbohydrates were reported on an area ($monose_{area}$, suc_{area}, $sugar_{area}$), mass ($monose_{mass}$, suc_{mass}, $sugar_{mass}$) and nitrogen ($monose_N$, suc_N, $sugar_N$) basis.

Statistical analysis

A three-way ANOVA was used to test for the main effects and interactions of season, site and canopy position on all respiration parameters and leaf properties (Datadesk 6.0, Data Description Inc. Ithaca, NY, USA). The respiratory parameters (E_0, R_0) were compared

among different season / site / canopy position combinations by simple t-test (Excel, Microsoft, Seattle, WA, USA). Differences were considered significant if the probabilities were less than 0.05. Multi-variant regression was used to analyze the relationship between respiration at 20 ºC (R_{20}) and leaf properties (Datadesk 6.0, Data Description Inc. Ithaca, NY, USA). For certain leaf properties, the correlation to respiration rate was considered significant if the probabilities of the partial coefficient was less than 0.05. For the above analysis, all data were log transformed to best fulfill the assumption of normality and homoscedasticity.

Results

Environmental conditions of the research site in 2003

The night length increased throughout our four measurement periods from 9 hours to 13 hours (Figure 1a). The 14-day (± 7 days bracketing the measurement days) average night temperature across the measurement period peaked in late July (21°C) and then dropped to 5°C in late October, but there was no difference between the two research sites (Figure 1b).

The carbon stable isotope ratios ($\delta^{13}C$) of upper canopy leaves were constantly heavier in the upper site across the entire growing season, indicating higher water use efficiency and lower water availability. In contrast, $\delta^{13}C$ of the lower canopy leaves did not show a site effect since water availability is less likely to affect stomatal openness in the shady, cool lower canopy. Across the growing season, leaf $\delta^{13}C$ dropped about 1‰ in all site/ canopy position combinations (Figure 1c).

E_0

In fourteen out of the sixteen season/site/canopy position combinations, the observed differences in E_0 were not statistically significant (Table 1, $P>0.05$, t-test). E_0 was unaffected by season, site, canopy position, and by all two-way interactions (Table 2). Only the upper canopy leaves from the lower site showed a distinctively high (mid June) or low (mid September) E_0, which led to the significant effects of season × site × canopy position interaction (Table 2). Averaged across all seasons, sites and canopy positions, E_0 was 52.5 kJ mol^{-1}. The deviation of the mean of E_0 for any season/site/canopy

combination was within a range of ± 10% (52 ± 5 kJ mol^{-1} K^{-2}). Due to the functional relationship between Q_{10} and E_0 (equation 2), the pattern of Q_{10} was the same as E_0. Q_{10} (15 – 25 ºC) of all season / site /canopy position combinations ranged from 1.93 – 2.24, and the average was 2.09.

Respiration

In all site/canopy position combinations, area-based leaf respiration (R_{area}, at 10 °C and 20 °C) displayed a strong and consistent seasonal pattern (Table 1, 2; Figure 2a). R_{area} in late-October and mid June was significantly higher than that in late July and mid September. The canopy effect on R_{area} was also obvious, displaying higher rates in upper canopy leaves in both sites (Table 2). However, the site effect on R_{area} displayed a more complex pattern. Although site alone did not have a significant effect on R_{area}, site × canopy position and season × site interaction were all significant (Table 2). In general, leaves from the upper site showed more seasonal variation, while leaves from the lower site showed more canopy position variation (Table 2, Figure 2a).

The seasonal trends in R_{mass} and R_N are similar to, but stronger than, the trends in R_{area} (Table 1, 2; Figure 2a-c). Canopy position effects were much smaller in both R_{mass} and R_N, although still highly significant (Table 1 & 2, Figure 2). R_N was significantly affected by site (Table 2) as leaves from the lower site displayed slightly higher N-based respiration rates (25% on average) than leaves from the upper site.

Respiration rates estimated at the average field night temperatures corresponding to the measurement periods (± 7 days) shed light on the actual *in situ* respiration rates. In general, respiration rates gradually declined through the growing season, reflecting the combined effects of respiratory acclimation and temperature change (Figure 1). Furthermore, the seasonal variation of R_{ave} was smaller than respiration rates at a set temperature (*e.g.* at 20 °C, Figure 2).

Leaf nitrogen

Area-based leaf nitrogen (N_{area}) was significantly affected by season, site, and canopy position (Table 2). The seasonal pattern in N_{area} was uniform across all site and canopy position combinations, but the inverse of the pattern in respiration rates (Figure 3). The site and canopy effects were also clear. Upper site leaves and upper canopy leaves had higher N_{area} (37% and 72% higher than lower site and lower canopy leaves respectively). The seasonal variation was more significant in upper canopy leaves (season × canopy position effect, Table 2, Figure 3), which may experience larger seasonal variation in water availability or light. On the other hand, in the lower site, leaves displayed stronger canopy position effects (site × canopy position interaction), which is expected due to a denser canopy at the lower site (Table 2, Figure 3).

Different seasonal patterns were observed when nitrogen was expressed on a mass basis. From June to September, N_{mass} varied only slightly, but then declined significantly in late October. Significant site and canopy position effects on N_{mass} were observed, but the

magnitude of these differences was smaller than that in N_{area} (23% and 7% for site and canopy position respectively).

Leaf sugars

Leaves of *Q. rubra* contained similar amounts of sucrose and reducing monose (e.g. range in 0.5 – 4 g m^{-1} throughout the growing season), but the seasonal patterns were clearly different. Sucrose continually accumulated in leaves through the growing season till late October, when the concentration dropped; while reducing monose showed the inverse pattern (Figure 4). In combination, the low sucrose and high reducing monose levels in the leaves during mid June are consistent with active leaf growth during this period; while the decline of sucrose and increase in reducing monose in late October can be attributed to translocation prior to leaf loss. The canopy and site effects were more complex. On an area or a mass basis, sucrose concentration was similar in all canopy/site combinations at the beginning of the growing season, but accumulated much faster in upper canopy leaves through the growing season, especially at the lower site. On a nitrogen basis (suc_N), the site and canopy effects on sucrose were all absent (Table 2, Figure 4b, c). Canopy position had little effect on $monose_{area}$, but significantly changed $monose_{mass}$ and $monose_N$ (Table 2, Figure 4e, f).

Leaf ontogeny

There was a significant effect of canopy position on leaf thickness (Figure 1d, Table 2) and, as expected, lower canopy leaves had a much higher specific leaf area, SLA, (40% higher than upper canopy leaves in the dry site and 80% in wetter site). Although the

leaves were visually mature in mid June, higher SLA indicated that the leaves were still actively growing, which is also reflected in the change of leaf nitrogen and soluble sugars from mid June to late July. During the remainder of the growing season, SLA increased only slightly from late July to October. Additionally, in the lower canopy, leaves from the upper site were significantly thicker.

Correlations between respiration rate and leaf properties

A multi-variant regression was first applied to all data throughout the season, site and canopy positions to examine the general relationship between leaf respiration (at 20 ºC, area, mass, and nitrogen based, $R_{20(area)}$, $R_{20(mass)}$, $R_{20(N)}$) and leaf properties (nitrogen, reducing monose and sucrose). Then, regressions were performed on particular subsets of the data to isolate the three individual factors. For example, in order to isolate the seasonal effect, regressions were run on leaf data sets of four site / canopy position combinations respectively (UU, UL, LU, LL, Table 3). The multiple correlation coefficients of regressions and P values of partial correlation coefficients of each leaf property are presented in Table 3.

Multi-variant regression on all data illustrated that $R_{20(area)}$ was significantly correlated to leaf nitrogen and reducing monose, but $R_{20(mass)}$ was correlated only with reducing monose. Sucrose, the storage and transport sugar, was not significantly correlated with leaf respiration. However, only a small part of the overall variation in leaf respiration was explained by the leaf properties examined (25 – 36 %). Once specific factors were isolated, regression results demonstrated that correlations among respiration and leaf

properties were specific to the measurement time (season), site or canopy position. Seasonal variation in the respiration rates were partially related to variations in reducing monose, especially for $R_{20(N)}$. On the other hand, canopy position effects on respiration were well explained by N_{area} (7 significant effects out of 8 cases, $R^2 > 90\%$), but N_{mass} could not explain the canopy effects on R_{mass}. The ANOVA analysis indicated that the site effect on R_{20} was significant only on a nitrogen basis (Table 2), so the area- and mass-based regressions were not run. However, site variation in $R_{20(N)}$ could not be well explained by nitrogen or soluble sugars, with the exception of the late October measurements, when reducing monose explained majority of the variation in $R_{20(N)}$. In most cases, sucrose was not significantly correlated to respiration.

Model fitting of respiratory temperature response

On an area basis, respiration in upper canopy leaves was more responsive to temperature than in lower canopy leaves (steeper fitting curve, Figure 5a, b) at both sites. The change of respiration rate between 10 – 30ºC was similar at two sites (0.5 – 2.5 μmol CO_2 m^{-2} s^{-1} for upper canopy leaves and 0.2 – 1.6 μmol CO_2 m^{-1} s^{-1} for lower canopy leaves), but upper site leaves showed more significant seasonal variation. During the early and late growing season (mid June and late October), the respiration rates were more sensitive to temperature than during the mid growing season (late July and mid September). On a mass basis, the canopy effect on respiratory temperature response curves was less significant, especially at the upper site, but the seasonal effect was enhanced at the lower site (Figure 5e-h).

Discussion

Model parameters of respiratory response to temperature

In the model we used to analyze our data, the response of respiration to leaf temperature is partially represented by the parameter E_0, which linearly determines ln R. Variations in E_0 are related to the cumulative change in the energy of activation for respiration as an overall reaction and shed light on possible biochemical / physiological adjustments in respiration (*e.g.* temperature acclimation). In our study, however, E_0 was not influenced by season, site, canopy position, or two-way interactions (Table 2) and the deviation from the mean value was small (10%, Table 1). The effect of the three-way interaction was caused by two distinct values (Table 2). The average E_0 is very similar to previously reported values for *Q. rubra* (Turnbull *et al.*, 2003) measured early in the growing season (June). This constant E_0 suggests that the energy of activation of dark respiration as an overall reaction is stable and only slightly influenced by environmental conditions in *Q. rubra*, indicating that the substrate composition and reaction pathways of dark respiration were uniform throughout the growing season. On the other hand, R_0 determines not only the base respiration rate (intercept of modeled temperature response), but also affects the respiratory temperature response. The variation in the respiratory temperature response observed in this experiment appears to be mainly related to a significant variation in R_{10} (Table 1, 2). The constant E_0 and variable R_0 is consistent with some previous observations (Bolstad, Mitchell & Vose, 1999, but see Griffin *et al.*, 2001), and further studies are needed to examine whether the pattern is generalizable in diverse plant species and growth conditions. If expressed as a Q_{10} (15 *vs* 25 ºC), the average respiratory

temperature response is 2.09, also comparable to recent studies on red oak and other related species (Bolstad *et al.*, 1999; Amthor, 2000; Turnbull *et al.*, 2001, 2003).

Respiratory Acclimation to Seasonal Temperature Change

The leaf respiratory response to temperature is known to be a function of both temperature and physiological history (Amthor, 1989; Atkin *et al.*, 2000). Seasonal variation and thermal acclimation of respiration have mostly been reported in conifers or in tree seedlings (Stockfors & Linder, 1998; Atkin *et al.*, 2000; Oleksyn *et al.*, 2000; Vose & Ryan, 2002). Here we observed similar patterns in ~100 year old *Q. rubra*, characterized by reduced leaf respiration (at a set temperature, *e.g.* 10 or 20 ºC) and the sensitivity of temperature response in warm mid growing season, while a significantly higher respiration rate and more sensitive temperature response in the cooler early and late growing season (Figure 1, 3). The obvious thermal acclimation partly offset the effect of seasonal temperature variation on *in situ* leaf respiration rates (indicated by the ± 7-day average night temperature), which shows a relatively stable pattern throughout the growing season. In general, the respiration rates at the ± 7-day average night temperature gradually decrease in the 5-month period (Figure 3), indicating declined leaf level carbon loss and physiological activities throughout the growing season.

Temperature acclimation of respiration has been suggested to be of two types (Atkin & Tjoelker, 2003). Type I acclimation is predominantly characterized by a change in Q_{10} (which can be calculated from E_0 in the model used in this study – equation 2 and 3), with little or no change in the respiration rate at a base temperature (R_0), and is probably

affected by substrate availability, adenylate restriction or both. By contrast, Type II acclimation is associated with a change in both R_0 and the respiration rate at moderately higher temperatures (e.g. 20 °C) and has been attributed to temperate mediated changes in respiratory capacity. In our study, a constant E_0 and variable R_0 across the growing season suggests that the respiration rate in *Q. rubra* leaves has typical Type II acclimation to the seasonal variation in temperature. Therefore, we speculate that mechanisms directly influencing respiratory capacity, such as enzyme activity and capacity, or overall demand for respiratory products, are likely to be primarily responsible for the seasonal variation of respiratory response to temperature in *Q. rubra*. For example, active growth in mid June and material translocation in late fall would require more energy and carbon skeletons, which are mainly products of respiratory processes.

Respiration – Leaf Property Relationships

Correlations among leaf respiration, nitrogen and soluble sugars have been reported in many studies (Ryan, 1991, 1995; Reich *et al.*, 1996; Ryan *et al.*, 1996a; Noguchi & Terashima, 1997; Reich *et al.*, 1998a; Reich *et al.*, 1998b; Atkin *et al.*, 2000; Griffin *et al.*, 2001; Griffin *et al.*, 2002; Tissue *et al.*, 2002; Vose & Ryan, 2002; Turnbull *et al.*, 2003). Although it has been suggested that respiration is determined by multiple factors (Tissue *et al.*, 2002), most previous work investigated the relationships between respiration and a individual leaf properties (but see Tjoelker, Reich & Oleksyn, 1999). Since the effects of multiple factors can be interactive, a simple correlation analysis may be biased. Here, we used multi-variant regression analysis to investigate the relationships

among respiration and the various leaf properties measured. In general, our findings are consistent with the previously reported positive correlation between respiration, nitrogen and soluble sugars and further confirmed the relationships while using a more mathematically strict means of analysis. Furthermore, by isolating the effects of the various environmental factors, we found that the season and canopy effects are associated with different leaf properties. In this case, N_{area} was well correlated to the canopy position effect on $R_{20(area)}$, while reducing monose, a direct substrate of respiration, was well correlated to R_{20}, especially on a nitrogen basis, over the course of the growing season (Table 3).

A general relationship between leaf nitrogen and respiration observed in our study (Table 3, first row) is consistent with previously reported results (Ryan, 1991, 1995; Reich *et al.*, 1996; Ryan *et al.*, 1996a; Reich *et al.*, 1998a; Reich *et al.*, 1998b; Griffin *et al.*, 2001; Griffin *et al.*, 2002; Tissue *et al.*, 2002; Turnbull *et al.*, 2003). Changes in N_{area} were also well correlated to the respiratory variation caused by canopy effects, but seasonal variations in respiration, in contrast, were poorly correlated with leaf nitrogen (Table 3). Similar results have also been reported in photosynthesis. Despite the widely reported relationship between maximum photosynthesis and leaf nitrogen (Field & Mooney, 1986; Dejong, Day & Johnson, 1989; Reich, Walters & Tabone, 1989; Abrams & Mostoller, 1995), in some cases temporal variation in photosynthetic capacity can not be explained by leaf nitrogen (Wilson, Baldocchi & Hanson, 2000; Dungan, Whitehead & Duncan, 2003). These previous studies attribute the lack of correlation to a seasonally dependent fractional allocation of leaf nitrogen to Rubisco (Wilson *et al.*, 2000). This mechanism,

however, is not likely to apply to respiration, since the concentration of respiratory enzymes is generally in excess for the observed respiration rates, and the proportion of respiratory enzymes in total protein is so low that total nitrogen availability is not likely to affect it significantly (Amthor, 1991).

It has been proposed that the relationship between respiration rate and nitrogen is derived from the more general relationship between nitrogen and protein concentration, which is linked to maintenance respiration (Ryan, 1991; Vose & Ryan, 2002). In light of this model, we speculate that, in our study, the N_{area} did not explain the seasonal variation in $R_{20(area)}$ due to the involvement of non-maintenance respiration components. Seasonally, many other physiological processes (*e.g.* growth, translocation, nitrogen metabolism, herbivore defense, etc.), can override the nitrogen – maintenance respiration relationship since they also depend on products of respiration (*e.g.* energy and secondary metabolites). In contrast to our result, a strong relationship between nitrogen and respiration was observed in an evergreen white pine forest (*Pinus strobes*) across extended periods of time (Vose & Ryan, 2002). However, the effect of non-maintenance respiration should be more significant for deciduous species like *Q. rubra* since leaves must complete their life cycle within one growing season. Interestingly we found that N_{area} but not $N_{mass,}$ is well correlated to the variation in R_{area} with canopy position (Table 3), which matches the observation of Tissue *et al.* (2002) in *Liquidambar styraciflua*. This pattern indicates that the $R_{20(area)} - N_{area}$ relationship may be mainly derived from the variation in leaf thickness or cellular density in the different canopy heights. This pattern is also consistent with the general nitrogen – maintenance respiration model since thicker

leaves would contain more nitrogen per unit area and have a higher demand for maintenance respiration on an area basis.

It has been suggested that at moderately high temperatures, respiration rates can be limited by the availability of substrates (Atkin & Tjoelker, 2003), and thus we examined variation in leaf non-structural carbohydrates as a factor possibly regulating respiration and thermal acclimation. Previous studies had found a positive correlation between leaf soluble sugar or total non-structural carbohydrates (TNC) and the rate of respiration (Noguchi & Terashima, 1997; Atkin *et al.*, 2000; Griffin *et al.*, 2001; Turnbull *et al.*, 2003). In our study, overall, reducing monose was significantly correlated to respiration, but sucrose was not, indicating that the pool of reducing monose is more directly influencing respiration rates (Table 3). Furthermore, reducing monose levels explained the seasonal variation of respiration better than it explained the site or canopy position effects (Table 3), so it appears to be more closely related to seasonal thermal acclimation or phenology than to general physiological function. This observation is consistent with the model of Dewar, Medlyn & McMurtrie (1999), who found that adjustments in leaf sugars are responsible for the thermal acclimation and constant respiration to photosynthesis ratio (R/P). However, only very few previous studies have examined the relationship between respiration and particular pools of soluble sugars (e.g. Azcon-bieto & Osmond, 1983). We suggest that further studies in diverse species are warranted to establish the generality of this relationship. In order to predict leaf-level respiration rates from substrate concentrations, careful attention needs to be given as to which sugar pools to use.

Site and Canopy Position Effects on Respiration Rate

Overall respiration rates in upper and lower canopy leaves in our study are comparable (but slightly lower) to those previously reported at this site (Turnbull *et al.*, 2001, 2003), and the canopy position effect was consistent with what observed in Turnbull *et al.* (2001, 2003). Site differences in respiration rates also have been previously reported at these sites (Turnbull *et al.*, 2001), and was attributed to differences in water availability or demand for energy associated with leaf maintenance. By extending the measurements to the entire growing season, we found that the site and canopy depth could influence the leaf respiration in a more complex way. In general, leaf respiration was more strongly affected by canopy position at the lower, more mesic site, but more significant seasonal variations were found at the upper site (Figure 3). The effects can primarily be attributed to the light environment at these two sites, which indirectly affect the respiration rates by influencing the spatial distribution of photosynthetic machinery and the demand for maintenance metabolism. At the lower site, where the tree canopy is much deeper (~ 30 m), the lower canopy leaves are in a relatively constant low light environment. Thus, less photosynthetic machinery would be invested into the lower canopy leaves, so the demand for respiratory products to support growth and the associated maintenance costs would also be lower. At the upper site, the lower canopy experiences a more dramatic seasonal variation in light, since the canopy is much shallower (~ 10m) and more light can penetrate the semi-open canopy in early summer and late fall. During this period, upper site trees tend to allocate more photosynthetic machinery to lower canopy leaves and this would result in higher demand for growth / maintenance respiration. Finally, the canopy

depth and tree height in these two sites are determined by the long-term difference of soil water availability, which is derived from local topography (Engel *et al.*, 2002; Shaman *et al.*, 2002). Following this logic, our results shed light on how the local topographic heterogeneity can shape tree respiratory fluxes. Other observations, like the consistently higher leaf nitrogen concentrations at the upper site (Figure 4) and thinner lower canopy leaves at the lower site (Figure 1d), further support this deduction.

Indications to ecosystem modeling

Ecosystem modelers are aware of the temperature response of respiration and draw from gas exchange measurements to parameterize their models (Foley, 1994; Dewar *et al.*, 1999; Melillo, 1999). However, studies on temporal and spatial heterogeneity of the temperature response of respiration in forests are limited. Typically in these models, one fixed respiration rate is used and then adjusted by a Q_{10} (usually assumed as 2, see review Ryan *et al.*, 1996b). Our results found significant effects of season, site, canopy position and their interactions on respiration temperature responses and suggest that more elaborate gas exchange measurements are required to parameterize the complicated temporal and spatial variation of leaf respiration to correctly estimate plant respiratory CO_2 efflux.

In the Arrhenius equation we used to describe the leaf respiratory response temperature, two parameters, R_0 and E_0, would affect the thermal sensitivity. In this study, E_0 was nearly constant, and most of the temporal and spatial variations in leaf respiratory temperature response were determined by changes in R_0. If such a pattern is proved to be

widespread, it could simplify the treatment of respiration in ecosystem models by assuming a constant E_0 (52.5 kJ mol^{-1} K^{-1} for *Q. rubra* in our study). In contrast, detailed measurements should be made on R_0, which is relatively easy to assess, to parameterize the models. Based on the respiration – leaf properties correlations, it may also be possible to predict R_0 from some leaf properties (*e.g.* nitrogen or reducing monose). Such simplifications may apply in typical Northeastern deciduous forest dominated by *Q. rubra* and other *Quercus* species with similar physiological characteristics (Mitchell *et al.*, 1999; Turnbull *et al.*, 2001, 2003). In chapter 2, we used the parameters generated from this study to model the canopy respiration in a virtual monoculture forest of *Q. rubra* throughout the 2003 growing season and tested the applicability of several simplifications (*e.g.* assuming constant E_0, fixed parameter throughout season / site / canopy).

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Table 1. Model parameters of respiratory temperature response in all season/site/canopy positions combinations. E_0 is a parameter equivalent to the energy of activation for respiration as an overall reaction, and is similar but not identical to the energy of activation for a single enzyme reaction. R_0 (on an area basis and a mass basis) is the base respiration rate at 10ºC. Values shown are means (±SEM), where n=6. The values followed by the same letter are not significantly different at P=0.05 level (t test).

Model Parameter	Sampling Period	Upper Site		Lower Site	
		Upper Canopy	Lower Canopy	Upper Canopy	Lower Canopy
E_0 (kJ mol^{-1})	06/11 -- 06/16	51.0 (3.0)^{abc}	54.8 (1.9)^{ab}	57.6 (1.1)^{a}	51.2 (1.3)^{bc}
	07/30 -- 08/01	52.5 (1.9)^{bc}	52.1 (1.3)^{bc}	49.5 (2.4)^{bc}	56.7 (3.1)^{ab}
	09/17 -- 09/18	55.4 (1.5)^{ab}	55.1 (2.7)^{abc}	47.0 (2.4)^{c}	55.6 (1.9)^{ab}
	10/20 -- 10/23	52.0 (1.2)^{bc}	49.7 (2.7)^{bc}	51.5 (2.8)^{abc}	48.2 (3.2)^{bc}
R_0 (area, 10°C) (μmol m^{-2} s^{-1})	06/11 -- 06/16	0.64 (0.06)^{a}	0.43 (0.06)^{bcd}	0.51 (0.03)^{ab}	0.28 (0.03)^{e}
	07/30 -- 08/01	0.39 (0.01)^{d}	0.20 (0.02)^{f}	0.45 (0.05)^{bcd}	0.21 (0.03)^{ef}
	09/17 -- 09/18	0.39 (0.04)^{d}	0.21 (0.02)^{ef}	0.60 (0.05)^{a}	0.17 (0.01)^{f}
	10/20 -- 10/23	0.59 (0.04)^{a}	0.47 (0.06)^{abcd}	0.64 (0.06)^{a}	0.40 (0.03)^{cd}
R_0 (mass, 10°C) (μmol kg^{-1} s^{-1})	06/11 -- 06/16	8.5 (0.42)^{a}	8.0 (0.57)^{ab}	7.9 (0.42)^{ab}	6.1 (0.44)^{cd}
	07/30 -- 08/01	3.9 (0.19)^{ef}	2.9 (0.18)^{h}	4.4 (0.29)^{e}	3.6 (0.43)^{efgh}
	09/17 -- 09/18	3.6 (0.25)^{efg}	3.2 (0.29)^{fgh}	5.6 (0.32)^{d}	3.0 (0.20)^{gh}
	10/20 -- 10/23	6.3 (0.39)^{cd}	7.4 (0.54)^{abc}	7.1 (0.47)^{bc}	7.6 (0.53)^{abc}

Table 2. ANOVA statistics of the effects of season, site and canopy position on the respiration parameters and characteristics of leaves of *Quercus rubra*. Original data were log transformed. ("*" = significant at P<0.05, "**" = highly significant at P<0.01 and "***" = extremely significant at P<0.001.)

Source	Season	Site	Canopy Position	Season×Site	Season× Canopy Position	Site× Canopy Position	Season× Site× Canopy Position
E_0	0.19ns	0.48ns	0.46ns	0.33ns	0.09ns	0.55ns	**0.015***
$R_{10\ (area)}$	**<0.0001*****	0.29ns	**<0.0001*****	**0.02***	**0.0003*****	**0.003****	0.16ns
$R_{10\ (mass)}$	**<0.0001*****	0.13ns	**<0.0001*****	**0.0014****	**<0.0001*****	**0.012***	**0.02***
$R_{10\ (N)}$	**<0.0001*****	**<0.0001*****	**0.0008*****	**<0.0001*****	**0.0014****	0.08ns	0.07ns
$R_{20\ (area)}$	**<0.0001*****	0.15ns	**<0.0001*****	**0.02***	**0.0008*****	**0.0016****	0.15ns
$R_{20\ (mass)}$	**<0.0001*****	0.10ns	**<0.0001*****	**0.0001*****	**<0.0001*****	**0.003****	**0.0007*****
$R_{20\ (N)}$	**<0.0001*****	**<0.0001*****	**0.0003*****	**<0.0001*****	**0.005****	0.08ns	**0.009****
SLA	**<0.0001*****	**0.0001*****	**<0.0001*****	0.95ns	**0.02***	**0.02***	0.66ns
N_{area}	**<0.0001*****	**<0.0001*****	**<0.0001*****	0.06ns	**0.012***	**0.009****	0.78ns
N_{mass}	**<0.0001*****	**<0.0001*****	**0.0002*****	**<0.0001*****	**0.003****	0.13ns	0.81ns
Suc_{area}	**<0.0001*****	**0.0011****	**<0.0001*****	0.10ns	0.09ns	**0.0005*****	0.90ns
Suc_{mass}	**<0.0001*****	**0.013***	**0.011***	0.08ns	0.39ns	**0.003****	0.92ns
Suc_N	**0.00023****	0.75ns	0.053ns	0.24ns	0.45ns	**0.007****	0.89ns
$Monose_{area}$	**<0.0001*****	**0.006****	0.37ns	**0.003****	0.28ns	0.62ns	0.86ns
$Monose_{mass}$	**<0.0001*****	**0.0001*****	**<0.0001*****	**0.002****	0.22ns	0.89ns	0.79ns
$Monose_N$	**<0.0001*****	**<0.0001*****	**<0.0001*****	**0.0001*****	0.11ns	0.65ns	0.85ns

Table 3. Summary of multi-variant correlation analysis between R at 20 °C (R_{20}) and leaf properties (leaf nitrogen, reducing monose and sucrose) in *Quercus rubra*. Original data were log transformed. Multiple correlation coefficient (R^2), P values for the partial correlation coefficients of each leaf property and significance levels are shown ("*" = significant at P<0.05, "**" = significant at P<0.01 and "***" = significant at P<0.001). The abbreviations are: UU, upper site, upper canopy; UL, upper site, lower canopy; LU, lower site, upper canopy; LL, lower site, lower canopy; US, upper site; LS, lower site; UC, upper canopy; LC, lower canopy. Bold fonts highlight significant R^2 or P values. For P values of the partial correlations, only those with positive coefficients were bolded, and the negative partial correlations, which does not match the current model predications (Ryan, 1991; Atkin *et al.*, 2000; Vose & Ryan, 2002; Turnbull *et al.*, 2003), were underlined.

Effects Isolated	Data Group	$R_{20(area)}$ (μmol CO_2 m^{-2} s^{-1})				$R_{20(mass)}$ (μmol CO_2 kg^{-1} s^{-1})				$R_{20(N)}$ (μmol CO_2 gN^{-1} s^{-1})		
		R^2	P value			R^2	P value			R^2	P value	
			N_{area} (mmolN m^{-2})	$Monose_{area}$ (g m^{-2})	Suc_{area} (g m^{-2})		N_{mass} (mmolN g^{-1})	$Monose_{mass}$ (g g^{-1})	Suc_{mass} (g g^{-1})		$Monose_N$ (g gN^{-1})	Suc_N (g gN^{-1})
--	All	**0.36***	**0.0004***	**<0.0001***	0.94ns	**0.22***	0.77ns	**0.002****	0.26ns	**0.42***	**<0.0001***	0.73ns
Season	UU	**0.40***	0.39ns	**0.009****	0.76ns	**0.66***	0.13ns	**0.02***	0.007**	**0.48****	**0.004****	0.26ns
	UL	0.29ns	0.44ns	**0.04***	0.86ns	**0.44****	0.21ns	0.08ns	0.38ns	**0.41***	**0.005****	0.85ns
	LU	0.23ns	0.23ns	0.07ns	0.22ns	**0.34***	0.66ns	0.16ns	0.10ns	**0.57***	**0.0001***	0.27ns
	LL	**0.64***	0.40ns	**<0.0001***	0.53ns	**0.66***	0.24ns	**0.0001***	0.62ns	**0.80***	**<0.0001***	0.59ns
Canopy position	13-June US	**0.73***	0.053ns	0.39ns	**0.04***	0.19ns	0.92ns	0.86ns	0.24ns	0.41ns	0.37ns	0.09ns
	13-June LS	**0.91***	**<0.0001***	0.82ns	0.02*	0.54ns	0.49ns	0.03*	0.06ns	0.44ns	0.07ns	0.03*
	31-July US	**0.91***	**0.0012****	0.09ns	0.96ns	*0.73**	0.15ns	0.006**	0.77ns	**0.50***	0.02ns	0.93ns
	31-July LS	**0.92***	**0.002****	0.74ns	0.53ns	0.32ns	0.17ns	0.72ns	0.67ns	0.12ns	0.90ns	0.38ns
	21-Sept US	**0.91***	**0.03***	0.42ns	0.20ns	0.43ns	0.12ns	0.59ns	0.26ns	0.13ns	0.58ns	0.27ns
	21-Sept LS	**0.99***	**<0.0001***	0.05ns	0.87ns	**0.87****	**0.004****	0.02*	0.91ns	*0.56**	0.05*	0.20ns
	17-Oct US	**0.92***	**0.0002***	0.34ns	0.06ns	*0.73**	0.09ns	0.25ns	0.12ns	0.54ns	0.27ns	0.14ns
	17-Oct LS	**0.92***	**0.0004***	0.07ns	0.46ns	0.21ns	0.34ns	0.72ns	0.74ns	0.47ns	**0.03***	0.35ns
Site	13-June UC	Site effect on $R_{20(area)}$ is not significant (Table 2)				Site effect on $R_{20(mass)}$ is not significant (Table 2)				0.43ns	**0.05***	0.41ns
	13-June LC									0.02ns	0.91ns	0.72ns
	31-July UC									0.13ns	0.61ns	0.29ns
	31-July LC									0.33ns	0.12ns	0.75ns
	21-Sept UC									0.17ns	0.44ns	0.26ns
	21-Sept LC									0.32ns	0.07ns	0.20ns
	17-Oct UC									**0.82***	**0.0002***	0.82ns
	17-Oct LC									**0.77****	**0.002****	0.72ns

Figure Legends

Figure 1. Seasonal variation of environmental conditions and specific leaf area. a) 14-day average night length during the period of measurement; b) 14-day average night temperature during the period of measurement (●, upper site; ○, lower site) ; c) $\delta^{13}C$ of leaf bulk organic material, as an indicator of tree water use efficiency, and soil water availability (●, upper site, upper canopy; ○, lower site, lower canopy; ▼, lower site, upper canopy; ▿, lower site, lower canopy); d) specific leaf area. Values shown are means (± standard error of the mean, SEM), where n = 14 for a and b, and n = 6 for c and d.

Figure 2. Seasonal variation of dark respiration rates estimated from fitted temperature response curves (see Figure 2) for *Quecus rubra* leaves in four site/canopy position combinations. Parameters R_{area} (upper panel), R_{mass} (middle panel) and R_N (lower panel) are area, biomass and nitrogen based dark respiration rates calculated from the fitted responses. Respiration rates at 20°C (a – c) or at the 14-day average night temperature during the measurement period (d – f) are plotted. Values shown are means (± SEM), where n = 6. The legends are the same as in figure 1c-d.

Figure 3. Seasonal variation in leaf nitrogen in the four site/canopy position combinations. a,b), leaf nitrogen expressed on an area and mass basis, respectively. Values shown are means (± SEM) where n = 6. The legends are the same as in figure 1c-d.

Figure 4. Seasonal variation in leaf sucrose and reducing monose (including glucose and fructose) in the four site/canopy position combinations. Concentrations are presented on an area basis (upper panel), a mass basis (middle panel) and nitrogen basis (lower panel). Values shown are means (± SEM) where n = 6. The legends are the same as in figure 1c-d.

Figure 5. Seasonal variation in the dark respiration-temperature response curves of *Quecus rubra* leaves. Data shown are modeled responses based on the mean parameters from 6 replicate response curves at each site/canopy position combination (individual curves are fitted by Equation 1). a – d, area based estimates, e – h, mass-based estimates. For parameters, see Table 1.

Figure 1.

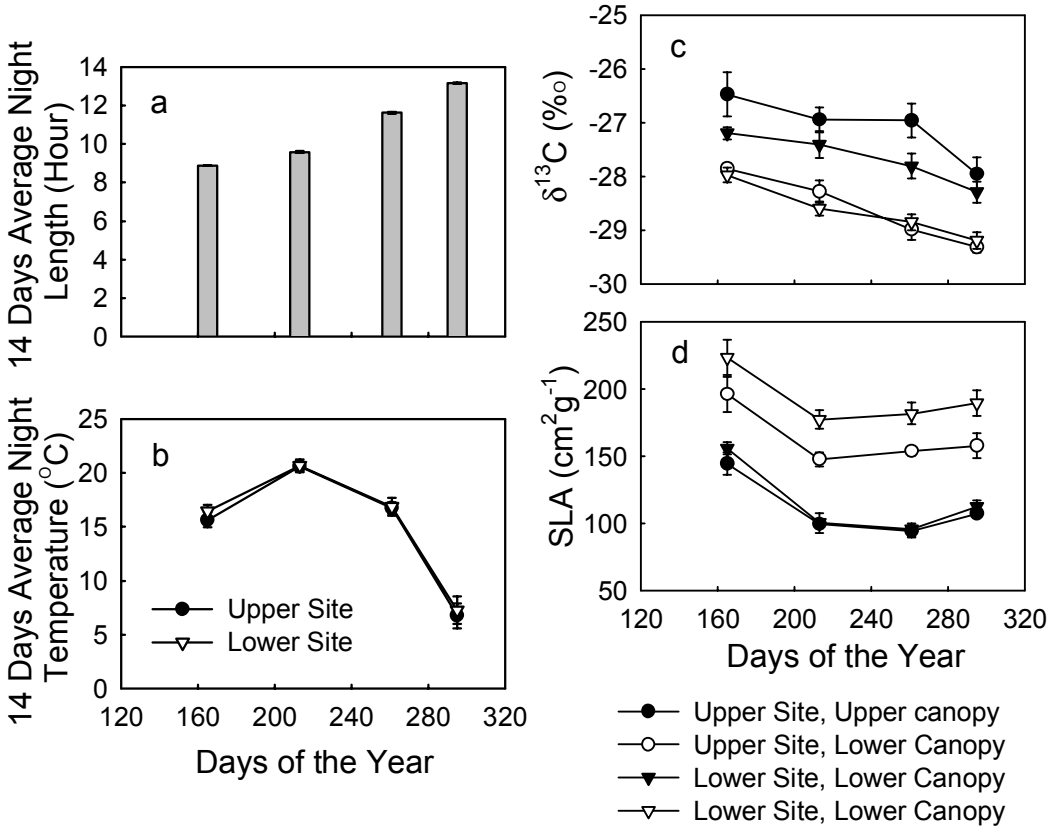

a
14 Days Average Night Length (Hour)
b
14 Days Average Night Temperature (°C)
Upper Site
Lower Site
Days of the Year
c
δ13C (‰)
d
SLA (cm2g-1)
Days of the Year
Upper Site, Upper canopy
Upper Site, Lower Canopy
Lower Site, Lower Canopy
Lower Site, Lower Canopy

Figure 2.

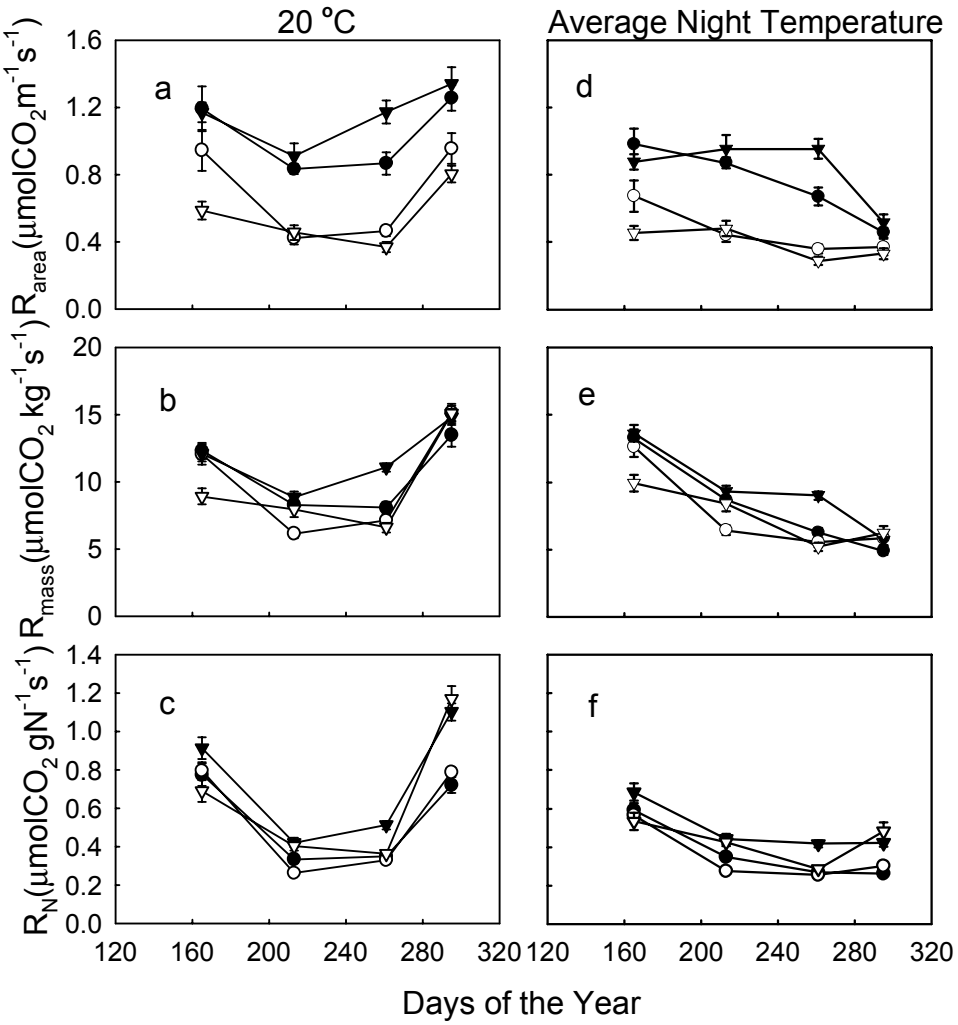

20 °C
Average Night Temperature
a
b
c
d
e
f
R_{area}(μmolCO_2 m^{-1} s^{-1})
R_{mass}(μmolCO_2 kg^{-1} s^{-1})
R_N(μmolCO_2 gN^{-1} s^{-1})
Days of the Year

Figure 3.

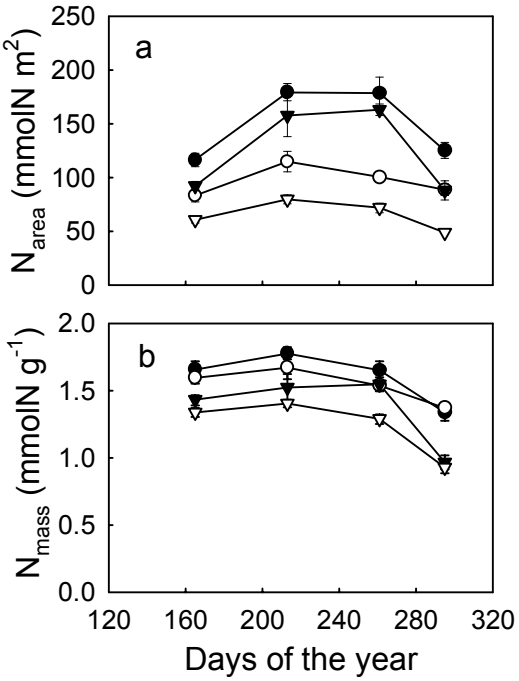

a
b
N_{area} (mmolN m^2)
N_{mass} (mmolN g^{-1})
250
200
150
100
50
0
2.0
1.5
1.0
0.5
0.0
120
160
200
240
280
320
Days of the year

Figure 4.

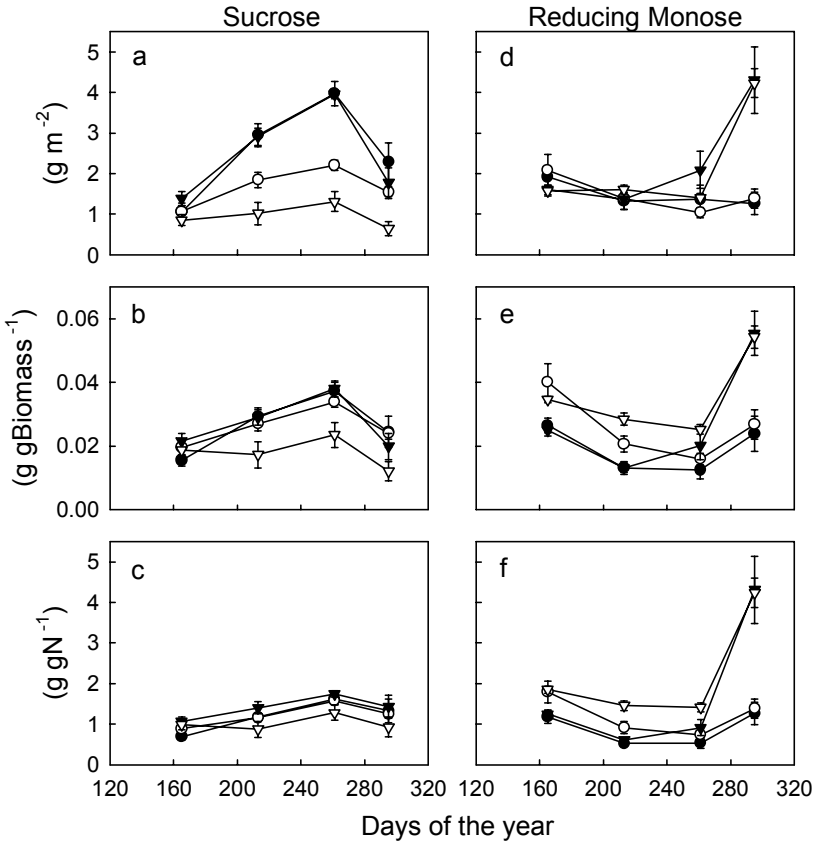
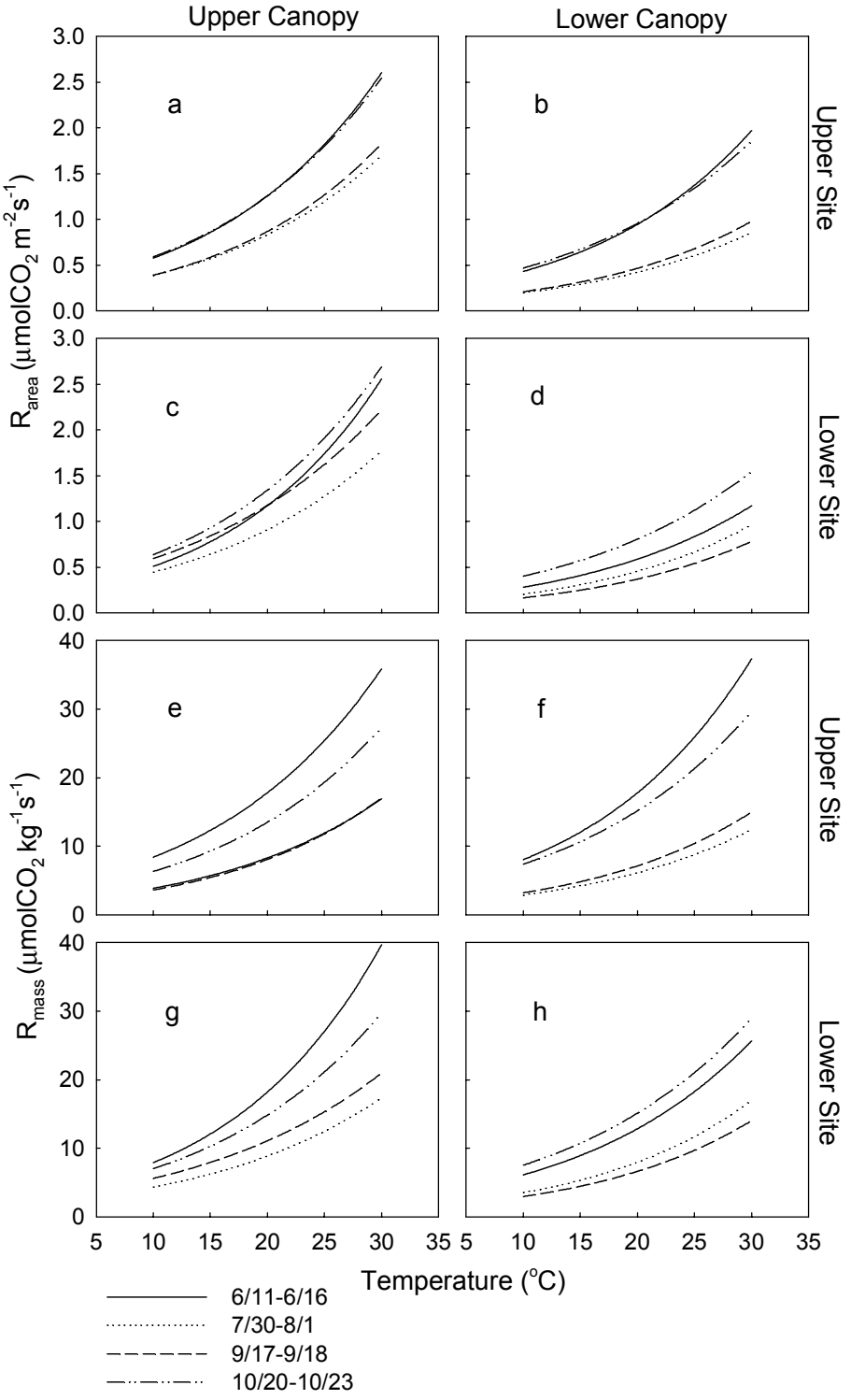

Sucrose
Reducing Monose
a
b
c
d
e
f
(g m-2)
(g gBiomass-1)
(g gN-1)
Days of the year

Figure 5.


Upper Canopy
Lower Canopy
Upper Site
Lower Site
a
b
c
d
e
f
g
h
R_{area} ($\mu molCO_2\ m^{-2}s^{-1}$)
R_{mass} ($\mu molCO_2\ kg^{-1}s^{-1}$)
Temperature (°C)
6/11-6/16
7/30-8/1
9/17-9/18
10/20-10/23

Chapter 2:

Seasonal variation in the temperature response of leaf respiration in *Quercus rubra* II: scaling foliar respiration to the stand level throughout the 2003 growing season

CHENGYUAN XU AND KEVIN L. GRIFFIN

Abstract

Stand-level canopy foliar carbon loss (R_{canopy}) was modeled for a virtual *Quercus rubra* monoculture in two sites with different soil water availability of a northeastern deciduous forest in USA throughout the 2003 growing season. Previously reported foliar respiratory temperature responses of *Q. rubr*a were used to parameterize a "full distributed physiology model", which estimates R_{canopy} by integrating the effects of season, site, and canopy position. The model sensitivity to five simplified parameterization scenarios was tested and a reasonable procedure of simplification was established. Neglecting the season, site or canopy position effects on respiration results in considerable error in the estimation of R_{canopy}, but assuming a constant E_0, a model variable related to the over-all energy of activation of respiration, or constant night temperature (average nighttime temperature) results in only a small error. From June 8th to October 28th of 2003, the modeled R_{canopy} of the virtual *Q. rubra* monoculture was 5.4 mol m^{-2}, (averaging 37.5 mmol C m^{-2} $night^{-1}$ or 302 mmol C kg^{-1} $night^{-1}$) and 12.6 mol m^{-2} (averaging 88.0 mmol C m^{-2} $night^{-1}$ or 338 mmol C kg^{-1} $night^{-1}$), at the drier and mesic sites respectively. To model R_{canopy} of *Q. rubra* (or other *Quercus* species with similar respiratory properties), the variation in the base respiration rate (R_0) needs to be fully parameterized but E_0 can be assumed as a constant. Modeling R_{canopy} at the average nighttime temperature would not cause significant error either.

Introduction

The carbon balance or net CO_2 exchange of an ecosystem (NEE), is determined by the small difference between photosynthesis and respiration fluxes, and can be measured by eddy covariance or estimated by models. Plant respiration not only consumes 30% - 70% of the photosynthetic products (Amthor, 1989; Ryan, 1991; Ryan *et al.*, 1996a), but is thought to be more strongly influenced by global warming (Amthor, 1997), which has been shown to be more significant at night (Easterling *et al.*, 1997; Alward, Detling & Milchunas, 1999; IPCC, 1999). However, there is some concern about the reliability of experimental measurements of ecosystem respiration (Law *et al.*, 2001). Nocturnal eddy covariance, a direct measurement of ecosystem respiration, is problematic under the low turbulence conditions, which are common after sundown, and thus this technique often underestimates ecosystem respiration. In addition, partitioning respiration between soil and plant components is difficult. Therefore, it is critical to scale up the respiratory chamber measurements (leaf, stem, soil etc.) to build predictive models, and to make comparisons with eddy covariance results (Ryan *et al.*, 1996b).

The mechanistic processes that regulate the response of respiration to temperature and thermal acclimation is unclear, and as a result, compared to photosynthesis, respiration is less represented in ecosystem models of net CO_2 exchange (Cannell & Thornley, 2000; Dewar, 2000; Tjoelker, Oleksyn & Reich, 2001). Often, respiration is functionally partitioned into "growth" and "maintenance" components with growth respiration assumed to be a constant proportion of biomass accumulation, and maintenance respiration is set at a fixed rate and related to temperature (*reviewed in* Ryan *et al.*,

1996b). However, these simplifications, a "fixed" respiration rate and exponential temperature response, do not take into account the growth environment or allow for physiological and biochemical regulation, both of which can strongly affect the *insitu* rate of respiration. It is well known that the respiration rate and respiratory temperature response can be affected by measurement temperature (Tjoelker *et al.*, 2001), species (Larigauderie & Korner, 1995), season (Stockfors & Linder, 1998; Atkin, Holly & Ball, 2000; Vose & Ryan, 2002; Damesin, 2003), growth temperature (Larigauderie & Korner, 1995; Atkin *et al.*, 2000), canopy position (Griffin *et al.*, 2001; Griffin, Turnbull & Murthy, 2002; Turnbull *et al.*, 2003), soil characteristics (Turnbull *et al.*, 2005), water availability (Turnbull *et al.*, 2001), and leaf metabolic state (Berry & Raison, 1981; Azcon-bieto & Osmond, 1983). Respiration is also subject to thermal acclimation (*reviewed in* Atkin & Tjoelker, 2003), which is not explicitly described in most models (Ryan *et al.*, 1996b). Neglecting these environmental and physiological factors can greatly affect our estimation of respiratory efflux and possibly lead to biased error.

Respiration is most commonly studied in leaves. In natural and controlled systems, leaf respiration could account for 10-35% of daily photosynthesis (Ryan *et al.*, 1994; Van der Werf, Poorter & Lambers, 1994; Atkin & Lambers, 1998), comprising an important part of plant / ecosystem carbon balance. There is considerable interest in scaling foliage respiration properties spatially and temporally to predict the impacts of climate change (*e.g.* warming) on the global carbon balance (Lloyd *et al.*, 1995; Sellers *et al.*, 1997). In such models, the parameters may be improved by intensive spatial and temporal surveys of leaf respiratory temperature responses. Such empirical efforts provide the least biased

pathway of model parameterization given the current limited understanding of the mechanism of respiratory regulation and thermal acclimation. With abundant data on leaf respiratory properties, it is also possible to determine the potential error introduced by specific simplifying assumptions such as neglecting the known variation in respiration associated with the growth season, canopy height, or leaf physiological status. Further analysis of these errors can subsequently be used to establish the empirical basis of a robust simplified model.

In chapter 1 (Xu & Griffin unpublished data), we reported leaf respiratory temperature responses of *Quecus rubra* in a northeastern deciduous forest in New York State, which were repeatedly measured from upper and lower canopy leaves at two sites with different water availability throughout the growing season of 2003. Here, we scaled up these foliar respiration measurements to estimate the canopy foliar carbon loss (R_{canopy}) according to stand leaf area index (LAI), and night temperature of these two research sites. The leaf level results were used to parameterize an Arrhenius function model (Lloyd & Taylor, 1994) that integrated season, site, and canopy position effects on leaf respiratory temperature responses (modified from Griffin *et al.*, 2002; Turnbull *et al.*, 2003) to estimate R_{canopy} for a virtual monoculture forest of *Q. rubra* in the growing season of 2003. Since it is often not practical to obtain such detailed data to parameterize respiratory and/or ecosystem models, we further (1) estimated the error caused by a series of simplified parameterization scenarios, which respectively neglect the effect of specific environmental or biological factors and (2) established a reasonable simplifying procedure for the model. Firstly, we modeled R_{canopy} during four 14-day periods

bracketing the days when leaf respiratory temperature responses were measured. Secondly, the sensitivity of the model was tested to five simplified parameterization scenarios, in which respiratory parameters and specific leaf area were assumed to be constant throughout (1) the growing season, (2) canopy depth, (3) the whole virtual *Q. rubra* stand (neglecting site heterogeneity) respectively; moreover (4) E_0 was assumed to be constant across all season/site/canopy position combinations; and (5) the temperature fluctuations during each night were ignored. The magnitudes of the resulting errors were determined for the four 14-day periods mentioned above. Finally, a reasonable simplification was made to model R_{canopy} for the 2003 growing season (June 8th to Oct 28th, day 159-301). We expect that the result given by this well parameterized empirical model can potentially be used to evaluate yet to-be-developed mechanistic models in the future.

Methods

Respiratory temperature response and research site environmental measurements

Black Rock Forest (BRF) is a 1500 ha preserve of oak dominated deciduous forest in Southeastern New York State. Two permanent research sites were established in the Cascade Brook watershed in 1999 at a 270m lowland site and at a 410m upland site. Detailed descriptions of the sites were presented by Turnbull *et al.* (2001) and Engel *et al.* (2002). Respiratory temperature responses of *Q. rubra* leaves (from upper and lower canopy respectively) were measured in these two sites across the growing season of 2003 (June 11th to 16th, July 30th to August 1st, September 17th to 18th, and October 20th to 23rd) and fitted to an Arrhenius respiration-temperature response model (Turnbull *et al.*, 2001):

$$R = R_0 e^{\frac{E_0}{R_g}\left(\frac{1}{T_0} - \frac{1}{T_a}\right)} \quad (1)$$

Details regarding the parameters (R_0 at 10 ºC, and E_0) of the model were reported in chapter 1 (Xu & Griffin, unpublished data) and the mean parameter values were used to parameterize the stand level model. To assess the use of the average E_0 value within the exponential term of the Arrhenius function, the stand level model was further parameterized with an E_0 estimate derived from a single fit to the entire data set of leaf replicates. These two methods resulted in only a negligible difference (~1%, data not shown) in the estimated model result. Thus, the averaged parameters were used in this study (Table 1) to maintain constancy within chapter 1 and 2.

The leaf area index (LAI) of the research sites was determined by hemispheric photography (Gap Light Analysis, Simon Frazer Univ. BC, Canada & Institute of ecosystem studies, NY, USA) and a canopy analyzer (LAI-2000, Licor Inc. Lincoln NE, USA). These two methods showed about 15 % constant difference in LAI estimation throughout the growing season, so the average LAI estimates from these two methods were used to derive a stand canopy foliar carbon loss model parameter (Table 1). At the upland site, LAI was measured after each set of leaf respiratory temperature responses. At the lowland site, LAI was directly measured only in mid June and it was assumed that the seasonal variation in the LAI at lower site follows the same time course as that observed at the upper site. Meteorological conditions of the forest are continuously measured and recorded as hourly averages at two standard meteorological stations run by the Black Rock Forest staff (Figure 1). Although we assume that the stand is a virtual monoculture of *Q. rubra*, the model results could be further parameterized and then easily extended to multi-species forest by weighing the monoculture R_{canopy} of each species by its LAI proportion in the canopy.

Modeling stand foliar carbon loss

The *in situ* leaf respiration rates were predicted from a respiratory temperature response model (Lloyd & Taylor, 1994) and was scaled to stand level (R_{canopy}) with the modified version developed by Griffin *et al.* (2002) and Turnbull *et al.* (2003).

$$R_{canopy} = \sum_i R_{0i} e^{\frac{E_{0i}}{R_g}\left(\frac{1}{T_0}-\frac{1}{T_a}\right)} \times LAI_i \qquad (2)$$

The modified model integrated the seasonal, local spatial (site), and canopy position effects on leaf respiratory temperature responses of *Q. rubra* (full distributed physiology model). Stand foliar carbon loss (R_{canopy}) was modeled at the two sites during four 14-day periods bracketing the gas exchange measurements by considering the vertical distribution of leaf respiratory properties during these time periods. To do this, the canopy was separated into two layers (upper canopy 71% and lower canopy 29%) and the leaf area distribution was assumed to be the same as that previously used in canopy physiology models at this site (Whitehead *et al.*, 2004). To model R_{canopy}, we assumed that the leaf temperature at night was the same as the ambient temperature and thus ambient temperatures were used to drive equation 2 with the base temperature set at 10 ºC. R_{canopy} was calculated hourly by multiplying the instantaneous respiration rates by the LAI and then summing these values for each night, which was designated as the period between sunset and sunrise (Data services of Astronomical application department, U.S. Naval observatory). The modeled R_{canopy} was expressed on a ground area basis (R_{can-a}, mmol C m^{-2} $night^{-1}$) and an estimate of respiration per unit leaf mass (R_{can-m}, mmol C kg^{-1} $night^{-1}$) was further calculated by correcting R_{can-a} for the LAI and SLA .

Test the model error to simplification scenarios

After modeling R_{canopy}, we were able to quantify the model sensitivity to five simplified parameterization scenarios:

Scenario 1: Respiratory physiological parameters (R_0 and E_0) and specific leaf area (SLA) were assumed to be constant throughout the growing season (*seasonally constant*

physiology model - with the parameters being determined only once during the growing season: mid June, late July – early August, mid September or late October respectively)

Scenario 2: The vertical distribution of respiratory parameters in the canopy were assumed to be constant (*canopy constant physiology model* - with the model parameters of all leaves in the canopy assumed to be those of the leaves in upper or lower canopy respectively).

Scenario 3: Respiratory parameters were assumed to be constant throughout the entire virtual *Q. rubra* stand (*site constant physiology model* – with the model parameters from either the upper or lower site applied to all trees).

Scenario 4: The model parameter E_0 was assumed to be constant and the season/site/canopy position effects on the respiratory temperature responses were only reflected by variations in R_0 (*constant E_0 model* - $E_0 = 52 \pm 5$ kJ mol^{-1}, Xu & Griffin, unpublished data).

Scenario 5: The temperature fluctuations during each night were ignored (*constant night T model*). In this model, the instantaneous stand level foliar respiration was calculated at the average night temperature of each day and then multiplied night length to obtain an estimate of nightly R_{canopy}. This scenario accounts for the fact that while average temperature data are available in most metrological databases, but the instantaneous temperature record often are not.

Stand foliar carbon loss was modeled at two sites during four 14-day periods based on these five simplifying model scenarios (above). Then the results were compared with

R_{caonpy} generated by *full distributed physiology model* and the errors caused by simplifications were calculated as:

$$\text{Error} = \left[\frac{R_{canopy}\ (\text{simplified models})}{R_{canopy}\ (\text{full distributed physiology model})} - 1\right] \times 100\% \qquad (3)$$

A positive value indicates that the simplification overestimates stand foliar carbon loss and conversely a negative value indicates that the simplification results in an underestimation of stand foliar carbon loss.

Modeling R_{canopy} for 2003 growing season with the simplified model

According to the results of the simplification scenarios, we derived a "simplified distributed physiology model" and used it to estimate the R_{canopy} during the 2003 growing season. The simplified model fully considered the variation of R_0, R_{canopy} was separately modeled in upper and lower sites throughout the growing season and the canopy was split into two layers (upper and lower canopy). On the other hand, a constant E_0 (52.5 kJ mol^{-1}) and nightly average temperatures were used as appropriate simplifications. Since we found the error caused by "constant night T model" was correlated to the night temperature range (see result below), the modeled R_{canopy} was corrected accordingly. The results were expressed on both a ground area and a leaf mass basis.

The seasonal variation in R_0, SLA and LAI were fitted by a second-order polynomial equation throughout the growing season (log transformed to fit normality and homoscedasticity Table 3). Night lengths were calculated for each day as mentioned above. A small portion (<10 days) of meteorological data in upper site was lost due to equipment failure and the average night temperatures of those days was estimated by the

correlation between upper and lower site night average temperatures. Since it may be unreliable to extrapolate R_0 and LAI to a very early or late growing season empirically, the modeled period was limited to June 8th through October 28th (day 159-301, Figure 1), which covered at least 2/3 of the normal growing season. The 14-day average R_{canopy} was also calculated throughout the period and plotted for reference. Furthermore, R_{canopy} per unit LAI was calculated for comparison between the two sites.

Results

Stand canopy foliar carbon loss during four 14-day periods

Comparing R_{canopy} between the four 14-day periods bracketing measurement nights highlighted significant seasonal variation of R_{canopy} in both sites. At the upper site, R_{can-a} and R_{can-m} displayed the same seasonal trend: the highest rates occuring in mid June (159– 172 day), with a 14-day average of 47mmol C m^{-2} per night or 444 mmol C kg^{-1} per night, and decreased gradually throughout the growing season (Table 2, Figure 2). In late October, close to the end of the growing season, R_{canopy} was the lowest (24 mmol C m^{-2} $night^{-1}$ or 253 mmol C kg^{-1} $night^{-1}$).

At the lower site, R_{can-a} and R_{can-m} displayed a different seasonal pattern (Table 2, Figure 2). The 14-day average R_{can-a} was moderate (81.9 mmol C m^{-2} $night^{-1}$) in the early growing season, increased through September (254 – 267 day, 107 mmol C m^{-2} $night^{-1}$), and then dropped to 51mmol C m^{-2} $night^{-1}$ in late October. By comparison, the seasonal pattern of R_{can-m} was similar to that at the upper site: R_{can-m} displayed highest value (426 mmol C kg^{-1} $night^{-1}$) in mid June (159 – 172 day) and then decreased throughout the growing season. In general, R_{can-a} displayed much larger seasonal variation than area based R_{can-m} at the lower site. The ratio of the 14-day average maximum to minimum R_{can-a} was 2.1, but only 1.4 for R_{can-m}.

Summing the canopy foliar carbon loss for the four 14-day periods modeled, R_{can-a} at the lower site was approximately 2.2 times larger than that at the upper site, mainly attributable to the higher LAI (approximately 2.06 times of that in upper site), but the

biomass based R_{canopy} was similar in both sites. The day-to-day variation in R_{canopy} was especially large in late October, with the coefficient of variance (CV, (standard deviation / mean) × 100%) of 35% regardless of the basis of expression (Figure 2), which is mainly caused by a large day-to-day temperature fluctuation during this period.

Sensitivity of the model to five simplified parameterization scenarios

R_{canopy} modeled by the seasonally constant physiology model deviated significantly from the results of the full distributed physiology model (the average absolute value of error > 20% for both sites, Table 2). In general, extrapolating respiratory parameters (R_0 and E_0) derived from either the early or late growing season throughout the year caused a large positive error during mid growing season, while extrapolating respiratory parameters derived from mid growing season caused negative error during mid June and late October. The error introduced by the "seasonally constant physiology model" was more significant at the upper site, especially for $R_{can\text{-}m}$ (the average absolute value of error ~ 60%, Table 2), which could be attributed to stronger seasonal variation of respiratory temperature response in upper site (Xu & Griffin unpublished data).

The lower-canopy constant physiology model consistently underestimated, while the upper-canopy constant physiology model consistently overestimated R_{canopy} compared to the full distributed physiology model. For *Q. rubra*, more leaves are distributed in the upper canopy than in the lower canopy, so the modeled R_{canopy} of the upper-canopy constant physiology model was closer to the result of the full distributed physiology

model (approximately ±10%). Furthermore, when using the canopy constant physiology model, R_{can-m} had a smaller error than R_{can-a} (Table 2).

The site constant physiology model generated a moderate level of error. The upper-site constant physiology model overestimated lower site R_{canopy} in mid June (+23% for R_{can-a} and +10% for R_{can-m}), but underestimated it from late July through early August to late October. The inverse was true of the lower-site constant physiology model which underestimated upper site R_{canopy} in June (-19% for R_{can-a} and -11% for R_{can-m}) but overestimated R_{canopy} from late July through early August to mid September (Table 2).

R_{canopy} estimated from the constant E_0 model was very similar to the full distributed physiology model, with a small error term (± 5%), throughout the growing season, regardless the site or unit of expression. By comparison, the constant night T model systematically underestimate R_{canopy}, but again the error was generally very small (<3% during the four modeled 14-day periods). Furthermore, once log-transformed, the error of the constant night T model was linearly correlated to the log-transformed night ambient temperature fluctuation (Figure 3).

R_{canopy} during the 2003 growing season, modeled by the simplified distributed physiology model

The total R_{canopy} during June 8th to Oct 28th was 5.4 mol m^{-2} at the upper site and 12.6 mol m^{-2} at the lower site. The differences in R_{can-a} between the two sites were mainly attributed to the difference of canopy LAI and leaf SLA at the two sites. After correcting

for LAI and SLA, the two sites displayed similar stand canopy foliar respiration, with the $R_{can\text{-}m}$ of the lower site being approximately 12% higher than that of upper site, indicating that the red oak stand was physiologically more active at the relatively mesic lower site. On average, nightly R_{canopy} was 37.5 mmol C m^{-2} $night^{-1}$ (or 302 mmol C kg^{-1} $night^{-1}$) at the upper site and 88.0 mmol C m^{-2} $night^{-1}$ (or 338 mmol C kg^{-1} $night^{-1}$) at the lower site (Table 4). In general, R_{canopy} declined throughout the growing season, with the lowest rate occurring in October (Figure 4).

Discussion

R_{canopy} modeling

The value and annual trend of modeled R_{canopy} in this study closely matches previous observations. For example, once corrected for differences in LAI, the values of ground based 14-day R_{canopy} in mid June (30 mmol C m^{-2} $night^{-1}$ per unit LAI for the upper site and 26 mmol C m^{-2} $night^{-1}$ per unit LAI for the lower site) are comparable to that reported by Turnbull *et al.* (2003) in BRF during similar period of the year 2000 (28 mmol C m^{-2} $night^{-1}$ per unit LAI). The annual trend of R_{canopy} is also similar to that previously reported in temperate deciduous (Whitehead *et al.*, 2005) and coniferous (Stockfors & Linder, 1998; Damesin, 2003) forests. In general, R_{canopy} is high in early summer, coinciding with active growth and relatively high temperature. On the contrary, the lowest R_{canopy} occurred in the late growing season, when both temperature and LAI are low. Therefore, empirically parameterized model can give stable results of R_{canopy} and may potentially be used as an evaluation standard for a mechanistic model in future.

Thermal acclimation of plant respiration is commonly observed in nature and reduces the temperature sensitivity of plant respiration over the growing season (*reviewed in* Atkin & Tjoelker, 2003). However, the effect of thermal acclimation has not been mechanistically included in models of ecosystem carbon efflux (Ryan *et al.*, 1996b). By modeling R_{canopy} with foliar respiratory parameters measured throughout the growing season, our study empirically integrates long term seasonal respiratory temperature acclimation to derive the stand level estimates (*see also* Stockfors & Linder, 1998; Damesin, 2003). A recent study in BRF (Whitehead *et al.* 2005) modeled the stand foliar carbon loss using two

different methods, a "fixed respiration model" and a "variable respiration model" which corrected night respiration by the total photosynthesis during that day. The latter, in fact, might include some effects of respiratory thermal acclimation. According to a substrate-based model proposed by Dewar, Medlyn & McMurtrie (1999), respiratory thermal acclimation can be led by the dynamic of the supply of carbohydrates, which is fixed by photosynthesis and thus is subjected to the influence of season temperature variation. The result of Whitehead *et al.* (2005) found that the estimate from the "variable respiration model" was about 23% lower than that of the "fixed respiration model". The large difference suggests that neglecting thermal acclimation may lead to a large error in modeled respiratory efflux. Future studies should compare empirical models and the "variable respiration model" to estimate how well the latter integrates the seasonal respiratory acclimation to temperature.

Applicability of simplified scenarios

Many previous studies have modeled long term R_{canopy} by using constant respiratory parameters (R_0 and E_0 or Q_{10}) or assuming a conservative respiration to photosynthesis ratio (R/A, e.g. Dewar *et al.*, 1999; Gifford, 2003; Dungan *et al.*, 2004; Whitehead *et al.*, 2005). Such treatments, although reasonable when available data is limited, did not take into account the seasonal variation in the respiratory temperature response. Among the five simplified parameterization scenarios tested in this study, the "seasonally constant physiology model" caused the largest error in estimated R_{canopy} of *Q. rubra*. These results thus indicate that caution is needed when using the respiration rate measured at a single time of the year to estimate respiration over the entire growing season. The seasonal

variation in leaf respiratory parameters can be attributed to the respiratory acclimation to variation in ambient temperature throughout the season and other seasonally-related physiological activities that require respiratory products (e.g. growth or translocation, Ryan *et al.*, 1996b). For temperate deciduous forests, both effects can be significant since the annual temperature amplitude is generally large and the leaf span is relatively short (e.g. approximately 6 months for *Q. rubra*). Thus, it may be particularly important to incorporate the seasonal variation of respiratory parameters into carbon efflux models. However, it is possible that the seasonally constant physiology model may better approximate respiratory CO_2 efflux for ecosystems dominated by evergreen species whose leaf physiology status is more stable throughout the growing season (e.g. Northwestern conifer forest) or ecosystems with limited seasonal temperature variation (e.g. tropical rain forest).

The "canopy constant physiology model" introduced considerable error in our study (Table 2) and the pattern is similar to that observed in several previous studies (Griffin *et al.*, 2002; Turnbull *et al.*, 2003). In general, the upper-canopy constant physiology model overestimated, while the lower-canopy constant physiology model underestimated R_{canopy}. Meanwhile, the error of $R_{can\text{-}m}$ is much smaller than that of $R_{can\text{-}a}$, since a significant part of the respiratory gradient along canopy height is caused by leaf thickness or cell density (Xu & Griffin unpublished result), which can be further attributed to the leaf maintenance demand determined by the light-gradient-driven photosynthetic machinery allocation (Bolstad, Mitchell & Vose, 1999; Griffin *et al.*, 2001; Tissue *et al.*, 2002; Turnbull *et al.*, 2003). In NEE models, the canopy gradient of photosynthetic

properties has been well addressed and most canopy models are parameterized for gradients in light availability and/ or leaf N as related to light (Leuning *et al.*, 1995; dePury & Farquhar, 1997; Medlyn *et al.*, 2003). Here, our results suggest that the respiratory gradient along the canopy height should also be fully considered to model R_{canopy}. We conclude that whenever possible, multi-layer sampling in the canopy should be done to avoid a biased estimation of R_{canopy}. In cases where gas exchange measurements are limited, a mass based calculation, corrected by the relatively easily obtained SLA gradient, will provide a more robust estimate than ground area based estimations based on sampling a single canopy layer. For *Q. rubra*, the upper canopy constant physiology model caused a relatively small error since more than 70% of the leaves are distributed in the upper 1/2 part of the canopy. Nevertheless, this pattern can be species and/or forest specific, depending on the particular canopy architecture and site conditions.

The site effect observed in our study is complex, (especially when site×canopy and site×season are significant, Xu & Griffin unpublished data). Here, neglecting the site effect also produced considerable error in the estimation of R_{canopy}. Site difference in respiratory temperature responses have been reported previously (Stockfors & Linder, 1998; Turnbull *et al.*, 2001; Turnbull *et al.*, 2005) and it has been proposed that they are based on the indirect effects of soil nutrients and water availability on growth rate of the stands, and the demand for respiratory products associated with foliage maintenance (Turnbull *et al.*, 2005). Obviously, the heterogeneity of the soil conditions is specific to the particular research site. Therefore, wide surveys or meta-analytical approaches may

be required in species diverse or spatially heterogeneous environments, to determine in which ecosystems these simplifications are most applicable.

In this study, only a small variation in E_0 was observed in *Q. rubua* leaves, and thus, the error of "constant E_0 model" was less than 5%. A constant E_0 was also observed in broad leaf trees from the Southern Appalachians (Bolstad *et al.*, 1999), but many other studies indicated that E_0 could be subject to environment and variable (or Q_{10} at set temperature) (Stockfors & Linder, 1998; Tjoelker *et al.*, 2001; Griffin *et al.*, 2002; Damesin, 2003; Turnbull *et al.*, 2003; Turnbull *et al.*, 2005). Therefore, it appears that the regulation of E_0 may be species specific and perhaps depends on the growth condition.

The simplification of the "constant night T model" causes systematic negative error, which is derived from the concavity of the leaf respiratory temperature response curve. However, in a small night temperature fluctuation range (e.g. <10 ºC), the leaf temperature response curve would be close to a straight line and thus the "constant night T model" only leads to a small error (<3%). Furthermore, the magnitude of the error was predictable from the strong correlation between the error and night temperature range. It is possible that in situations where the amplitude in the night temperature fluctuation is not large, the simplification can be extended to an even longer time scale with acceptable error (e.g. constant monthly/yearly night T model), which is appropriate for longer time series models. Moreover, the simplification should also apply to other formats of respiratory temperature response models (Ryan, 1991; Lloyd & Taylor, 1994; Tjoelker *et al.*, 2001) since those models all predict an exponential concavity for a leaf respiratory

temperature response under a physiologically relevant temperature range (10 – 30ºC). Such simplifications will make it easier to model foliar respiratory efflux when climate data are limited. The temperature norm (average, maximum and minimum of a particular period) is usually available, but it can be much harder to obtain more frequent temperature data. On the other hand, in ecosystems with small temperature variation (e.g. tropical rain forest), it may be possible to use monthly, even yearly average night temperature to estimate annual R_{canopy} of the forest stand without introducing unacceptable levels of error. On the contrary, the constant night T model may not be useful in ecosystems with significantly large nocturnal temperature fluctuations on a daily basis, which are in fact not common in nature.

Since NEE is determined by the small difference between two large fluxes of photosynthesis and respiration, errors in the estimated respiratory flux can become magnified in the final output of NEE models. Our results demonstrate that extreme caution should be taken when assuming a "fixed" base respiration as a model parameter since it may cause considerable error. In summary, the results of the test of the five simplified parameterization scenarios can be classified into three categories. First, the canopy gradient of respiratory properties should not be neglected in the models since the canopy gradient of leaf respiratory temperature response is mainly caused by the indirect influence of light gradient in canopy, a factor almost impossible to avoid. Second, the applicability of the "seasonally / site constant physiology models" and the "constant E_0 model" is ecosystem / species specific, so it needs to be appropriately applied depending on the particular system studied. Third, the "constant night T model" can be generalized

to most models and even be extended to longer time scales. Together, these three responses suggest that with care a simplified respiratory model can be constructed to give accurate predictions of carbon fluxes and to assist in the assessment of forest carbon budgets and canopy physiology.

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Table 1. Parameters used for stand canopy foliar carbon loss modeling (distributed physiology model, Xu & Griffin unpublished data).

Parameter	R_0 (10°C) (μmol CO_2 m^{-2} s^{-1})				R_0 (10°C) (μmol CO_2 kg^{-1} s^{-1})				E_0 (kJ mol^{-1})				LAI	
Site	Upper		Lower		Upper		Lower		Upper		Lower		Upper Site	Lower Site
Canopy Position	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower		
13-June	0.604	0.432	0.509	0.281	8.69	8.07	7.91	6.13	50.92	54.80	57.60	51.21	1.58	3.20
31-July	0.389	0.199	0.448	0.205	3.88	2.90	4.35	3.56	52.47	52.08	49.54	56.74	1.64	3.32
17-Sept	0.391	0.211	0.596	0.166	3.63	3.24	5.63	2.97	55.43	55.08	47.02	55.58	1.52	3.08
21-Oct	0.591	0.471	0.640	0.404	6.34	7.42	7.07	7.57	52.03	49.65	51.51	48.19	1.08	2.11

LAI distribution: Upper Canopy, 71%; Lower Canopy 29%

Average E_0: (52.5 kJ mol^{-1})

Table 2. Test of model sensitivity to simplified parameterization scenarios (E_0 and R_0). The 14-day average nightly R_{canopy} modeled by full distributed physiology model (see text), which integrates seasonal and canopy position effects at both sites in the first row. The remainder of the table presents the errors caused by particular simplifying scenarios (% of distributed physiology model results). The first column on the left lists the simplifying scenarios. For example, 13-June means that E_0 and R_0 measured during mid June were assumed to be representative throughout the whole growing season; Upper Canopy means that E_0 and R_0 of upper canopy leaves were assumed to be representative for the whole canopy. Upper Site means that E_0 and R_0 of upper site leaves were assumed to be representative in both sites. Constant E_0 model means that E_0 was assumed to be constant (the mean value of all season/site/canopy positions combinations). The upper part of the table lists the result of area based respiration and the lower part lists mass based respiration.

Sites and Days		Upper Site					Lower Site				
		159-172	206-219	254-267	288-301	All 4 periods	159-172	206-219	254-267	288-301	All 4 periods
14-Day Average R_{can-a} (mmol C m^{-2} $night^{-1}$)		47.0	44.7	39.4	23.8	38.7	81.9	100.8	107.6	51.3	85.4
Seasonally Constant Physiology Model	13-June	--	+68.4%	+62.2%	+5.4%	+36.4%	--	+28.8%	+1.0%	-22.4%	+5.4%
	31-July	-41.0%	--	-4.2%	-38.4%	-14.9%	-19.3%	--	-19.1%	-32.7%	-15.5%
	17-Sept	-38.6%	+6.0%	--	-38.2%	-20.5%	+0.03%	+22.0%	--	-13.9%	+5.8%
	21-Oct	-5.0%	+60.0%	+54.0%	--	+37.6%	+20.8%	+50.3%	+21.2%	--	+27.2%
Canopy Constant Physiology Model	Upper Canopy	+6.7%	+11.5%	+10.8%	+4.3%	+7.2%	+11.3%	+11.4%	+17.1%	+8.3%	+10.7%
	Lower Canopy	-25.4%	-43.3%	-40.5%	-16.3%	-32.3%	-38.4%	-42.8%	-64.1%	-31.3%	-44.2%
Site Constant Physiology Model	Upper Site	--	--	--	--	--	+22.6%	-9.1%	-23.9%	-4.3%	-5.7%
	Lower Site	-18.8%	+10.1%	+32.1%	+4.5%	+6.5%	--	--	--	--	--
Constant E_0 Model		-3.9%	+0.1%	-3.0%	4.1%	-2.1%	-4.2%	+3.5%	+5.5%	-0.2%	+3.5%

Table 2. (Continuous)

Sites and Days		Upper Site					Lower Site				
		159-172	206-219	254-267	288-301	All 4 periods	159-172	206-219	254-267	288-301	All 4 periods
14-Day Average R_{can-m} (mmol C kg^{-1} $night^{-1}$)		444.0	290.6	266.1	253.4	313.5	426.5	335.7	336.4	297.5	349.0
Seasonally Constant Physiology Model	13-June	--	+136%	+136%	+36.7%	+40.4%	--	+94.1%	+74.7%	+5.2%	+29.4%
	31-July	-57.9%	--	-0.6%	-43.0%	-43.6%	-46.5%	--	-7.2%	-39.4%	-33.6%
	17-Sept	-57.8%	+2.2%	--	-44.9%	-42.9%	-42.2%	+6.3%	--	-32.5%	-23.4%
	21-Oct	-26.8%	+73.0%	+72.7%	--	+20.0%	-10.9%	+67.1%	+54.6%	--	+22.1%
Canopy Constant Physiology Model	Upper Canopy	+0.4%	+4.2%	+1.8%	-2.7%	+1.0%	+4.2%	+1.2%	+4.0%	-1.0%	+2.5%
	Lower Canopy	-2.0%	-23.0%	-10.8%	+14.8%	-6.3%	-22.6%	-8.2%	-32.3%	+6.1%	-19.6%
Site Constant Physiology Model	Upper Site	--	--	--	--	--	10.0%	-12.4%	-18.8%	-10.2%	-7.4%
	Lower Site	-9.5%	+14.2%	+23.8%	+11.5%	+7.1%	--	--	--	--	--
Constant E_0		-1.6%	+0.1%	-3.0%	+4.1%	-0.4%	-4.2%	+3.5%	+5.5%	-0.2%	+0.9%

Table 3. Parameters fitted by second-order polynomial curves throughout the growth season (June 4th- October 30th , 155 – 303 day of the year). In the equations, y_1, y_2 and y_3 and x respectively symbolize area based R_0, mass based R_0, LAI and days of 2003. Since LAI in lower site was measured only in June, we assume that the seasonal variation of LAI in lower site was proportional to that in upper site. Before running the regressions, the data were log transformed to best fit the assumptions of normality and homoscedasticity.

Parameters	Site	Canopy	Equation	R^2	P
R_0 (10°C, area based, μmol m^{-2} s^{-1})	Upper	Upper	$\ln y_1 = 5.8938\ln^2 x - 63.8104\ln x + 171.6985$	0.582	<0.001
	Upper	Lower	$\ln y_1 = 10.343\ln^2 x - 111.6247\ln x + 299.4338$	0.671	<0.001
	Lower	Upper	$\ln y_1 = 2.3699\ln^2 x - 25.11\ln x + 65.7265$	0.297	0.025
	Lower	Lower	$\ln y_1 = 7.8251\ln^2 x - 84.1318\ln x + 224.3143$	0.480	0.001
R_0 (10°C, mass based, μmol kg^{-1} s^{-1})	Upper	Upper	$\ln y_2 = 8.6853\ln^2 x - 94.4059\ln x + 257.7727$	0.847	<0.001
	Upper	Lower	$\ln y_2 = 12.5105\ln^2 x - 135.3041\ln x + 366.7903$	0.874	<0.001
	Lower	Upper	$\ln y_2 = 6.1371\ln^2 x - 66.3579\ln x + 180.8705$	0.711	<0.001
	Lower	Lower	$\ln y_2 = 9.5784\ln^2 x - 103.3136\ln x + 279.6418$	0.652	<0.001
SLA (cm^2 g^{-1})	Upper	Upper	$\ln y_3 = 2.7916\ln^2 x - 30.5955\ln x + 88.3767$	0.673	<0.0001
	Upper	Lower	$\ln y_3 = 1.9488\ln^2 x - 21.3613\ln x + 63.5239$	0.669	0.002
	Lower	Upper	$\ln y_3 = 3.7672\ln^2 x - 41.2479\ln x + 117.4466$	0.732	<0.0001
	Lower	Lower	$\ln y_3 = 1.7533\ln^2 x - 19.1819\ln x + 57.6302$	0.388	0.006
LAI	Upper	--	$\ln y_4 = -2.611\ln^2 x + 27.5739\ln x - 72.3288$	0.664	<0.0001
	Lower	--	$\ln y_4 = (-2.611\ln^2 x + 27.5739\ln x - 72.3288) \times 2.06$	--	--

Table 4. Summary of modeled stand canopy foliar carbon loss (R_{canopy}) using the simplified distributed model at the two sites from June 8th – Oct 28th (159– 301 days of the year).

Site and Unit Basis	Upper Site		Lower Site	
	Ground Area Based (mmol C m^{-2} $night^{-1}$)	Biomass Based (mmol C kg^{-1} $night^{-1}$)	Ground Area Based (mmol C m^{-2} $night^{-1}$)	Biomass Based (mmol C kg^{-1} $night^{-1}$)
Average R_{canopy}	37.5	301.8	88.0	337.6
Maximum R_{canopy}	77.6	716.5	152.7	624.9
Minimum R_{canopy}	11.1	116.2	28.2	155.4
CV (%)	32.8%	35.2%	29.7%	26.0%

Figure Legends:

Figure 1. Daily temperature variation during 2003 at the research sites in the Black Rock forest, showing growing season, and the periods when canopy respiration was modeled by the distributed physiology model with the full or simplified parameterization.

Figure 2. Distributed physiology model results of nightly canopy respiration in four ±7-day periods from when leaf respiratory parameters and LAI were measured (solid circles, upper site; empty circles, lower site).

Figure 3. The sensitivity of the model to simplified parameterization of night temperature (constant night T model). R_{canopy} was modeled for the four 14-day periods presented in table 2 and figure 2 (solid circles, upper site; empty circles, lower site) respectively by hourly temperature record ($R_{nightly\ T}$, full parameterization) or nightly average temperatures ($R_{hourly\ T}$, simplified parameterization). The simplification causes systematically negative error, which is the same to both $R_{can\text{-}a}$ and $R_{can\text{-}m}$ (expressed by 1-$R_{nightly\ T}$ / $R_{hourly\ T}$ to obtain a positive error value so that the axis could be log transformed to fit the assumptions of normality and homoscedasticity) and the error was correlated to the night temperature range. The difference of the error – night temperature range regression between upper and lower sites was not significant, so data from the two sites were merged to run one single linear regression. The equations of fitted line was $\log_{10}y=1.9666\log_{10}x-1.6397$.

Figure 4. Average night temperature and nightly canopy respiration rates modeled by simplified model from the 159th to the 301st day of 2004 (solid circles, upper site; empty circles, lower site). The solid lines show 14-day average stand respiration. Temperature in upper (smooth) and lower (dotted) sites were shown in grey lines for reference.

Figure 1.

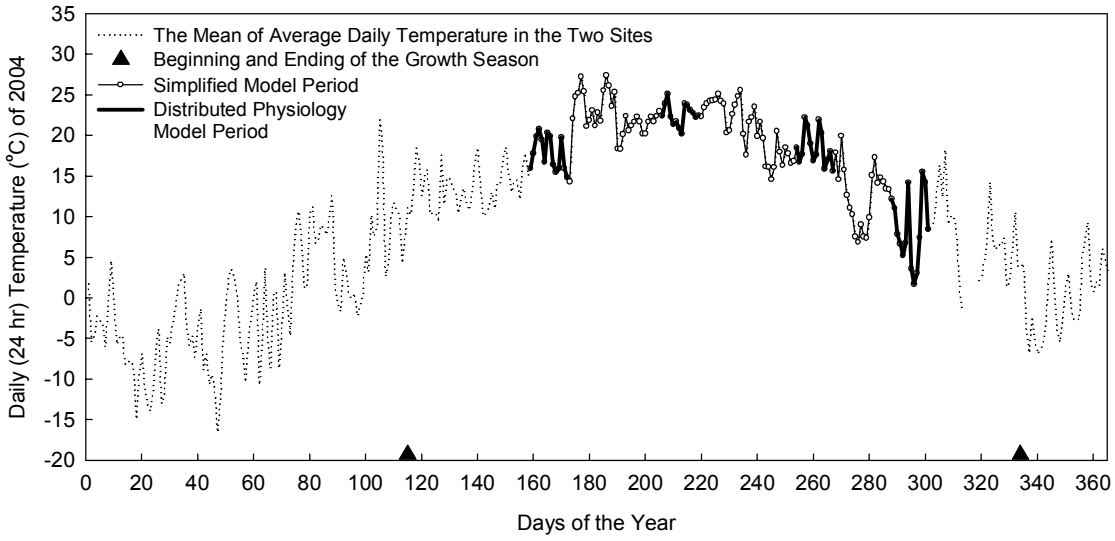

The Mean of Average Daily Temperature in the Two Sites
Beginning and Ending of the Growth Season
Simplified Model Period
Distributed Physiology Model Period
Daily (24 hr) Temperature (°C) of 2004
Days of the Year
35
30
25
20
15
10
5
0
-5
-10
-15
-20
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360

Figure 2.

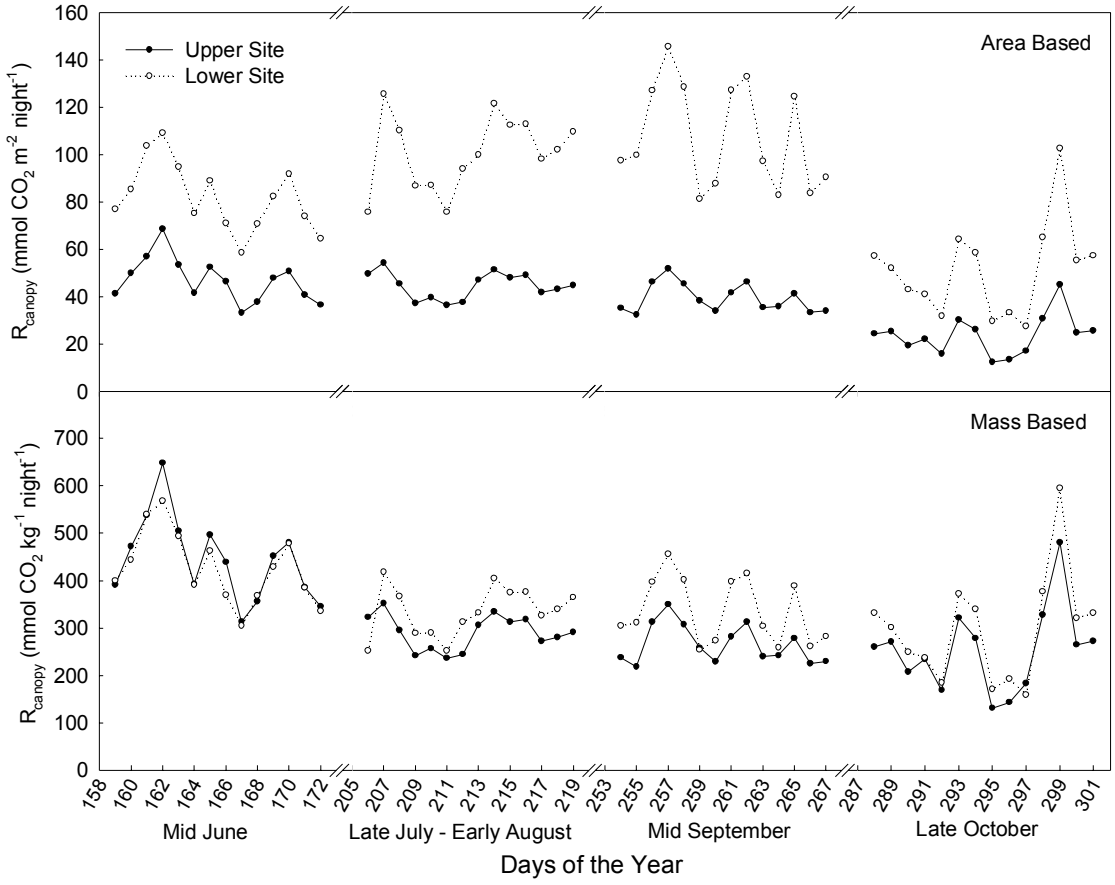

Upper Site
Lower Site
Area Based
Mass Based
R_{canopy} (mmol CO_2 m^{-2} $night^{-1}$)
R_{canopy} (mmol CO_2 kg^{-1} $night^{-1}$)
Mid June
Late July - Early August
Mid September
Late October
Days of the Year

Figure 3.

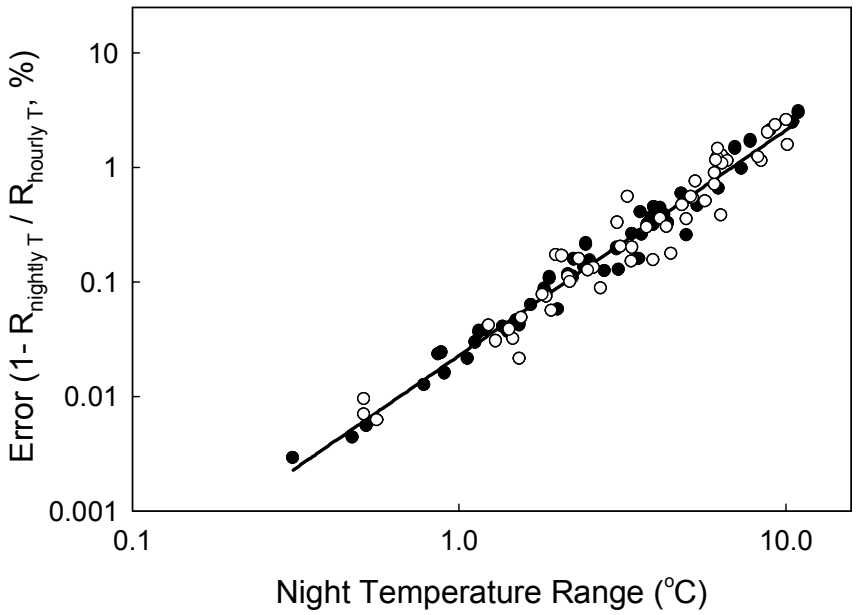
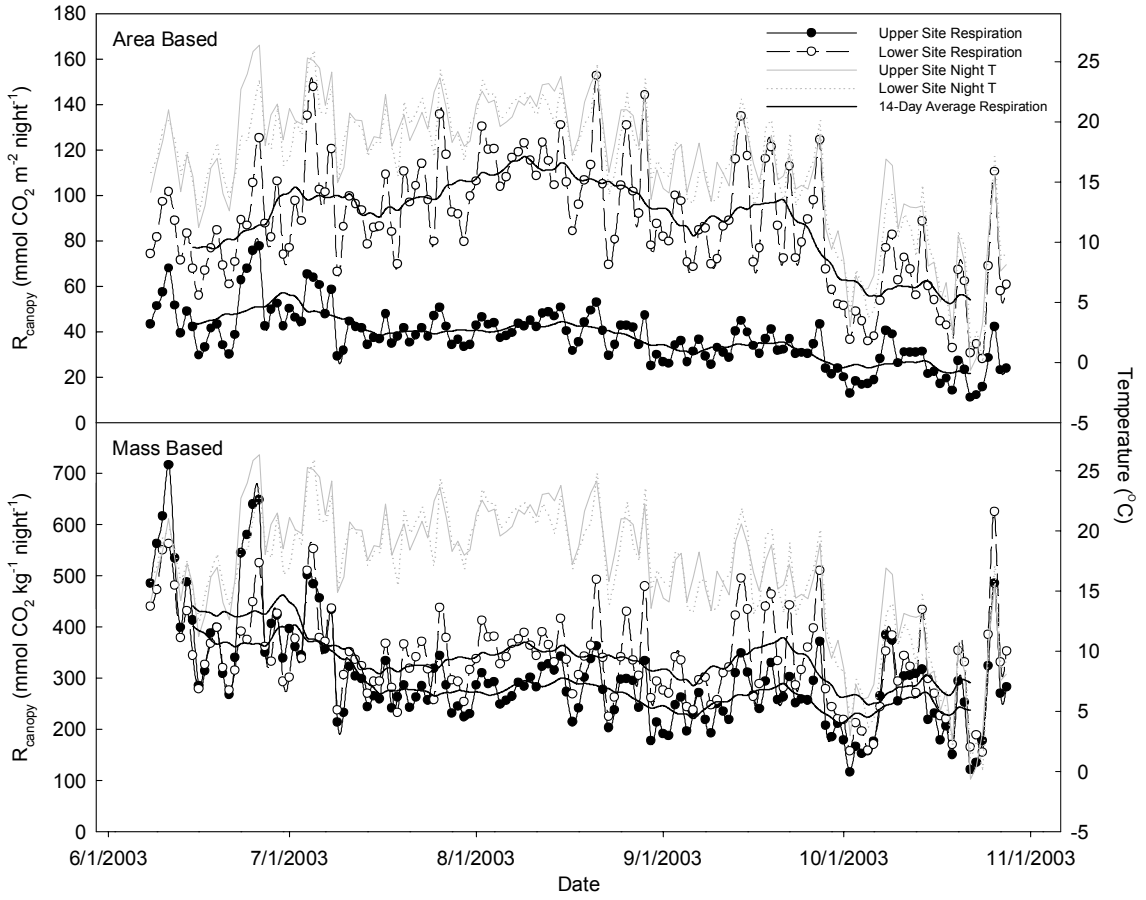

10
1
0.1
0.01
0.001
0.1
1.0
10.0
Error (1- R nightly T / R hourly T, %)
Night Temperature Range (°C)

Figure 4


Area Based
Mass Based
Upper Site Respiration
Lower Site Respiration
Upper Site Night T
Lower Site Night T
14-Day Average Respiration
R_canopy (mmol CO2 m-2 night-1)
R_canopy (mmol CO2 kg-1 night-1)
Temperature (°C)
Date
6/1/2003
7/1/2003
8/1/2003
9/1/2003
10/1/2003
11/1/2003

Chapter 3

Seasonal variation of temperature response of respiration in invasive Japanese barberry (*Berberis thunbergii*) and two co-occurring native understory shrubs in a northeastern US deciduous forest

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Abstract:

In the understory of a closed forest, plant growth is strongly limited by light availability, and early leafing, is proposed to be an important mechanism of plant invasion by providing a significant spring carbon "subsidy" when high light is available. However, studies on respiration, another equally important process determining plant net carbon gain, are rare in understory invasive species. In this study, the temperature response of leaf respiration and leaf properties were compared between invasive *Berberis thunbergii* (Japanese barberry), an early leafing understory shrub, and two native shrubs, *Kalmia latifolia* (mountain laurel), a board leaf evergreen and *Vaccinium corymobsum* (highbush blueberry), a late leafing deciduous species in an oak dominated deciduous forest in southern New York State. The seasonal pattern of the basal respiration rates (R_0) and E_0, a model variable related to the cumulative energy of activation of respiration, were significantly different among the three shrubs. In all three shrubs, we observed a species-specific negative correlation between R_0 and E_0, which if generalizable, can significantly simplify both the field measurement of respiratory temperature responses and subsequent modeling of carbon flux. On an area basis, all three shrubs showed significant correlation between respiration rate (R_{area}, 20 ºC) and leaf nitrogen (N_{area}). The relationship was attributed to the variation of both nitrogen concentration (N_{mass}) and leaf mass per area (LMA) in *B. thunbergii*, but to LMA only in *K. latifolia* and *V. corymbosum*. After scaling leaf respiration to canopy level throughout 2004, the annual canopy foliar carbon loss (R_c) per unit area leaf production of *K. latifolia* was 3.8 and 6.5 times that of *B. thunbergii* and *V. corymbosum* respectively. Dormant season respiration contributed (27%) of the annual R_c of *K. latifolia*. In the region of southern New York state, this

model predicts that the annual R_c has increased 12.9%, 10.3% and 8.9% respectively for *B. thunbergii*, *K. latifolia* and *V. corymbosum* since the early 20th century. The species-specific warming effect on annual (R_c) is mainly determined by E_0, but not by the seasonal pattern of warming or leaf phenology.

Introduction

Light strongly limits plant growth in the understory of closed forests where net carbon gain, defined as the difference between photosynthesis and respiration is low (Baars and Kelly 1996; Björkman 1972; Finzi and Canham 2000). In temperate deciduous forests dominated by summer- or raingreen trees, phenological niche separation from overstory trees is common in understory shrubs. For example, in these forests, understory species may show a complementary leaf phenology (*e.g.* earlier leafing than overstory trees) or be evergreen to capture light when canopy is open (Givnish 2002).

The rapid expansion and obvious competitive success of invasive species in the forest understory, indicates that these plants have a relative carbon balance advantage over the co-occurring natives. Early leafing, leading to a significant spring carbon subsidy by stimulating photosynthetic carbon gain during periods of high irradiance, has been proposed to be an important mechanism of invasion in temperate deciduous forests (Harrington et al. 1989; Myers and Anderson 2003; Zotz et al. 2000). However, plant carbon balance is simultaneously determined as the net equilibrium between carbon gain and carbon loss, and thus respiration needs to be considered when explaining the advantage of understory invasive plants. In general, plant respiration consumes 30% -- 70% of the carbon fixed through net photosynthesis (Amthor 1989; Ryan 1991). In light-limited environments, the net carbon gain may be predominately determined by respiration. For example, seedlings of shade tolerant woody plants tend to maintain low respiration rates and minimize carbon loss rather than maximizing growth potential by low-light-enhanced carbon gain (Walters and Reich 1999). For early leafing understory

invaders, since light acclimated leaves usually have high respiration rates (Griffin et al. 2001; Griffin et al. 2002; Turnbull et al. 2001; Turnbull et al. 2003), significant respiratory carbon loss may occur following canopy closure. On the other hand, compared with native evergreen species, the carbon balance of early leafing invaders are not likely to be favored by superior annual carbon gain (Xu et al. unpublished data), but may benefit from a lack of leaf respiration in the dormant season (late fall to early spring). However, comparative studies on respiratory properties and their reactions to environment conditions are rare between invasive species and the co-occurring natives in understory environments.

The temperature sensitivity of respiration suggest that respiration may be strongly influenced by anthropogenic climate change. There is a general consensus that global warming is apparent in the long-term climate records and the patterns are temporally dynamic (IPCC 1999). Over land, the annual minimum temperatures have increased at nearly twice the rate of maximum temperatures and warming is more significant at night and during the winter (Alward et al. 1999; Easterling et al. 1997; IPCC 1999). Furthermore, by the end of this century, warming is predicted to increase global temperatures by 1.4 to 5.8 °C (Hansen et al. 1999; IPCC 1999). Together these facts indicate that respiration, the dominant physiological process at night, will be more directly affected by warming than photosynthesis, particularly in evergreen plants. Therefore, warming can potentially lead to alterations in net primary productivity (Alward et al. 1999; Coughenour and Chen 1997; Myneni et al. 1997; Nemani et al.

2003) and the effect, if species-specific (Gunnarsson 2005), may change the current competitive continuum and facilitate further plant invasions.

In this study, we investigated the temperature response of leaf dark respiration of the early leafing invasive shrub *Berberis thunbergi*i, (Japanese barberry) and two co-occurring native shrubs, *Kalmia latifolia* (mountain laurel) a broad leaf evergreen and *Vaccinium corymbosum* (highbush blueberry) a late leafing decidous species throughout the 2004 growing season in Black Rock Forest, an oak dominated deciduous forest in Southern New York. We first measured the leaf respiratory temperature response and then scaled these leaf level results to address the annual canopy foliar carbon loss. Our goal is to examine whether invasive *B. thunbergii* shows lower carbon loss than the co-occuring natives and how significant winter warming affects the carbon loss pattern in these three species. We test the hypotheses that 1) *B. thunbergii* can downregulate respiration following canopy closure; 2) annual canopy foliar carbon loss of *B. thunbergii* is comparable or lower than *K. latifolia* and *V. corymbosum*; 3) *K. latifoliar* shows significant carbon loss during winter; and 4) the warming pattern in northeastern US leads to more significant annual carbon loss in *K. latifolia*.

Materials and Methods

Description of study site

The Black Rock Forest is a 1500 ha preserve in Hudson Highlands of southeastern New York State, locating at 41°24' N and 74°01' W with elevations ranging from 150 to 450m above sea level. The air temperature is strongly seasonal, with monthly average temperature ranges from -2.7°C in January to 23.4°C in July. The average annual precipitation is 1.2m (Black Rock Forest field station database). The forest is a *Quercus* dominated secondary growth forest that characterizes the northeastern United States. The most recent flora survey identified 729 vascular species of 117 families (Barringer and Clemants 2003). Among these, approximately 20% were introduced. The common shrubs in the forest understory include native *Gaylussacia baccata* L. (huckleberry), *Kalmia latifolia* L. (mountain laurel), *Rhododendron periclymenoides* L. (pink azalea), and *Vaccinium spp.* (blueberries) (W.S.F. Schuster, personal communication). Japanese barberry (*Berberis thunbergii*) is considered one of the most invasive species in the forest understory and produces leaves approximately one month earlier than native deciduous shrubs, and two to three weeks earlier than the overstory canopy (Sliander and Klepeis 1999; Xu et al., unpublished data). Meteorological conditions within the forest are obtained from several standard meteorological stations maintained by the Black Rock Forest staff.

Gas exchange measurements

Leaf level gas exchange measurements were made near Alec Meadow pond, an artificial reservoir located centrally in the Black Rock Forest (41° 24' N; 74° 00' W) and

surrounded by oak woods, where three individuals each of *B. thunbergii*, *K. latifolia* and *V. corymbosum*, were permanently tagged. All individuals were fully exposed in an open canopy during winter and spring, but shaded by the upper canopy during the majority of the summer and fall (Xu, personal observation). For each species, measurements were made on two top canopy leaves from different branches of each selected individual. During the 2004 growing season, leaf respiratory temperature responses were measured *in situ* during five periods: May 7th – 9th (day 128 – 130, BT & KL), June 15th – 16th (day 167 – 168, VC, BT & KL), August 23nd – 30th (day 236 – 243, VC, BT & KL), September 25th – 27th (day 269 – 271, VC, BT & KL), and November 17th – 18th (day 322 – 323, KL).

The 2004 leaf phenology of the study species and the overstory canopy has been previously reported (Xu et al, unpublished data). In general, the overstory canopy closed in mid May while *B. thunbergii* buds opened in late March and leaf development was completed in June. The buds of *V. corymbosum* and *K. latifolia* opened in early and mid May respectively. During May 7th – 9th, the leaves of *B. thunbergii* were not completely expanded and *K. latifolia* 2004 new leaves had not flushed. Therefore, at this time, measurements were made only on the most fully-expanded leaves of *B. thunbergii* and the 2003 overwintering leaves of *K. latifolia*. *V. corymbosum* was not measured since the bud just opened andleaves were obviously immature. In all other cases, measurements were made on intact, visually mature leaves (well expanded, with well-developed waxy cuticle, etc.) for all three species. Beginning on June 15th, measurements of *K. latifolia* were all made on newly grown leaves expanded in 2004.

Leaf respiratory temperature response was measured with an infrared gas analysis system (LI-6400, Li-Cor, Inc., Lincoln NE) equipped with CO_2 and temperature control modules. All measurements were made *in situ* on attached leaves between 10:30 AM and 2 hours before sunset. One leaf was covered by aluminum foil for at least one hour to allow dark adaptation. Then the block temperature of the leaf cuvette was controlled at 5 set points (ambient temperature ± 0/ 4/ 8 ºC) using the thermoelectric coolers, and the respiration rate and leaf temperature were measured. Between two temperature set points, the leaves were left for 8 – 10 minutes to stabilize the respiration rate before being recorded. The CO_2 partial pressure in the cuvette was maintained at 375 ppm. All measurements were recorded only when respiratory gas exchange had equilibrated (taken to be when the rate of CO_2 efflux was visually stable and the coefficient of variation for CO_2 partial pressure differential between the sample and reference was < 0.3%). The respiration rate is reported in area-, mass- and nitrogen- based units (R_{area}, R_{mass}, R_N).

Temperature response of respiration model fitting

The temperature response curves were analyzed using a modified Arrhenius equation described by Lloyd and Taylor (1994):

$$R = R_0 e^{\frac{E_0}{R_g}\left(\frac{1}{T_0} - \frac{1}{T_a}\right)} \quad (1)$$

where R_0 is the respiration rate at a base temperature T_0 (10 °C, 283 K in our study), T_a is the leaf temperature (K) when R is measured, R_g is the ideal gas constant (8.314 J mol^{-1} K^{-1}). Originally, this type of model was used to describe the temperature response of a

simple chemical reaction and E_0 is the energy of activation (kJ mol^{-1}). When applying the model to respiration, we thus simplify and treat the overall chemical processes of respiration as a single reaction. By doing so, E_0 is equivalent to the overall energy of activation, similar but not identical to the energy of activation for a single enzyme reaction and thus E_0 should simply be considered a temperature response variable. Previous studies indicated that E_0 appears constant over the physiological temperature range of temperate species (Lyons and Raison 1970). When using this model, the temperature response curve can be described by the intercept (base respiration rate), which is represented by the parameter R_0, while the curvature (sensitivity of respiratory temperature response) is represented by both R_0 and E_0. The model was fitted with SigmaPlot 2001 (SPSS Inc., Chicago, IL, USA). Besides R_0 (Respiration at 10 °C), respiration rate at 20 °C (R_{20}), and 7-day average night temperature bracketing the measurement period (R_{ave}), were also calculated.

The commonly used Q_{10}, which is a simple parameter to measure respiratory temperature response, can be linked to this model by:

$$Q_{10} = e^{\frac{E_0}{R_g}\left(\frac{1}{T_2}-\frac{1}{T_1}\right)} \quad (2) \text{ and,}$$

$$T_1 - T_2 = 10\ (^{\circ}C) \quad (3)$$

Clearly, as defined by this model, Q_{10} is temperature dependent (Atkin and Tjoelker 2003) and is determined by E_0 at a set temperature. In this study, a Q_{10} of 15 – 25ºC was calculated to facilitate comparison with other studies reporting only Q_{10} values.

Analysis of leaf properties

Following the photosynthetic measurements, the area of the measured leaf was determined using a leaf area meter (Li-3000, Li-cor Inc. Lincoln NE, USA) and then dried in 60°C oven for a minimum of 48 hrs. The dried leaf material was weighed and ground to fine powder for nitrogen analysis with a CHNS/O analyzer (2400 Series II, Perkin-Elmer, Boston, MA, USA). Leaf mass per area (LMA) was calculated from the leaf area and dry weight. For the leaf samples taken in May 2004, leaf mass was not measured, so the leaf LMA was surveyed in May and June 2005 on the same individual plants to get an approximate estimation. LMA measured in June 2005 and June 2006 displayed only 3% average difference, providing confidence in the estimation of LMA for May of 2004.

Statistical analysis

The seasonal effect on respiratory parameters (R_0 and E_0) were tested by ANOVA (Statistica, Statsoft Inc, Tulsa, OK, USA) and the means were compared amongst species/season throughout the growing season of 2004 with a simple t test (Excel, Microsoft, Seattle, WA, USA). Differences were considered significant if the probabilities were less than 0.05. In order to fulfill the assumptions of normality and homoscedasity, log-transformed data were used for t test and ANOVA. To address the relationships between respiration rate, or E_0 and leaf properties across the growing season, linear regressions and multi-variant regressions were run. In regression analysis, original data passed tests of normality and homoscedasity, so no data transform was made.

Modeling canopy foliar carbon loss

For interspecies comparison, R_c is calculated as canopy foliar carbon loss per unit leaf production. We assume that the leaf temperature is the same as the nighttime air temperature. R_c and accumulated function of R_c (F) throughout 2004 is calculated by:

$$R_{ci} = R_{0i} e^{\frac{E_{0i}}{R_g}\left(\frac{1}{T_0}-\frac{1}{T_i}\right)} \times LAI_i \times L_{ni} \quad (4)$$

and,

$$F(j) = \frac{\sum_{i=1}^{j} R_{ci}}{\sum R_{ci}} \times 100\% \quad (5)$$

in which R_{ci} is the instantaneous night respiration rate of the i^{th} day of the year, calculated by respiratory temperature response at the average night temperature of the i^{th} day (T_i). In chapter 2, we reported that ignoring the nighttime temperature fluctuation leads only to small error to the estimation of canopy respiration at Black Rock Forest (for 98% of the nights, temperature fluctuation < 15 ºC, and error < 5%, Xu & Grriffin unpublished data). R_{0i} and E_{0i} are the respiratory temperature response parameters on the i^{th} day of the year and they were assumed to change linearly between each measurement period. LAI_i is the leaf area index on the i^{th} day of the year, which is weighted by the maximum LAI contributed by the leaves produced in 2004 . For *B. thunbergii*, LAI was detailedly reported in appendix I (Xu et al, unpublished data). Since the LAI of *K. latifolia* and *V. corymbosum* was not surveyed in detail, we assumed a linear change between the dates of bud opening, canopy formation, and defoliation to estimate the approximate value of LAI_i. L_{ni} is the length of the i^{th} night. For *K. latifolia*, R_{ci} was respectively allocated into

2003 overwintering leaves and 2004 produced leaves. F(j) is the accumulated R_c from the 1^{st} to the j^{st} day of 2004, and is expressed as percentage of annual total R_c. Calculated R_c was expressed on an area and a mass based unit.

R_c was further modeled for the three shrub species to examine the response of R_c to measured warming during the 20^{th} century. The increment of average temperature of each season over the 20^{th} century was subtracted from the 2004 temperature records and we assume that this to be representative of the average temperatures in the early 20^{th} century. Then R_c was recalculated with these early 20^{th} century conditions and compared with the estimated R_c of 2004 to quantify the increment of annual/ seasonal R_c in the 20^{th} century. This estimation does not account for long-term thermal acclimation of respiration, which is likely to have occurred but impossible to predict based on our current understand in respiration, and thus should be considered maximum estimates to further constrain the actual response.

Results

Model fitting: temperature response of respiration

On an area basis, respiration of *B. thunbergii* was the most responsive to temperature in early May (steepest fitting curve/ highest slope, Figure 1a), when increasing the temperature from 10 ºC to 30 ºC increased the rate of respiration from 0.5 to 3.2 μmol m^{-2} s^{-1}. By contrast, R_{area} of *B. thunbergii* was the least responsive to temperature during mid June, but after that, the sensitivity increased throughout the growing season. The response to temperature of R_{mass} showed a similar seasonal pattern to that of R_{area}. For *K. latifolia*, R_{area} was highly responsive to temperature during early summer (May to June, Figure 1a, b) and late fall (late November, Figure 1e), but R_{mass} was not sensitive to seasonal variation in temperature as there was no significant difference in the shape of model fitting curves across the growing season. Respiration of *V. Corymbosum* generally showed a low sensitivity to the temperature throughout 2004 growing season, on either an area or a mass basis.

E_0

B. thunbergii and *V. corymbosum* displayed significant seasonal variation in E_0 (Table 1). For *B. thunbergii*, the lowest and highest E_0 respectively occurred in early May and mid June. For *V. corymbosum*, E_0 in late September was approximately only 50% of that in June and August. By contrast, there were no significant seasonal effects on E_0 in *K. latifolia* and the mean E_0 of each season was within the range 55 ± 9 kJ mol^{-1}. Due to the functional relationship between Q_{10} and E_0 (equation 2), the seasonal trend of Q_{10} was the same as E_0. *B. thunbergii* and *V. corymbosum* displayed large seasonal variation in Q_{10}

(15 – 25 ºC), 2.5 – 3.9 and 1.7 – 3.0 respectively. For *K. latifolia*, the average Q_{10} across the entire growing season of was 2.2 and the seasonal variation was not statistically significant.

Respiration rate

B. thunbergii had a strong seasonal trend in R_{area}, R_{mass} and R_N (at 10 ºC and 20 ºC). The respiration rate was the highest in early May but dropped to its lowest point in mid June, and then gradually increased during the growing season. Similarly, *K. latifolia* displayed high R_{area} early and late in the growing season. However, the seasonal trend in R_{mass} of *K. latifolia* was much smaller. *V. corymbosum* displayed a higher respiration rate at a set temperature (10 ºC or 20 ºC) during the late growing season (late September) than in summer, regardless of the unit of expression.

Respiration rates estimated at the average night temperatures (R_{ave}) corresponding to the measurement periods (7 days) shed light on the actual *in situ* respiration rates. For *B. thunbergii*, the seasonal trend of R_{ave} is similar to that of the respiration at a set temperature (*e.g.* at 20 ºC, Figure 2a, b, c). By comparison, R_{ave} of *K. latifolia* displayed a peak in mid June, when leaves produced in 2004 had just expanded. The seasonal effect on R_{ave} of *V. corymbosum* was not significant on an area (ANOVA, $F = 2.2$, $P = 0.14$) or on a mass basis (ANOVA, $F = 3.4$, $P = 0.06$), but was significant on a nitrogen basis (ANOVA, $F = 5.1$, $P = 0.02$).

Leaf properties

Among the three shrubs, *K. latifolia* had the thickest leaves (highest LMA, Figure 2g). The LMA of the 2003 overwintering leaves averaged 145 g m^{-2}, and the 2004 produced leaves initially had a lower LMA but generally increased throughout the growing season and reached similar thickness to the overwintering leaves by the end of the growing season. On average, *B. thunbergii* leaves were thicker in early May than in the rest of the growing season. *V. corymbosum* leaves were thinnest among the three shrubs and LMA did not vary seasonally (ANOVA, $F = 3.7$, $P = 0.61$).

On an area basis, leaf nitrogen (N_{area}) of *B. thunbergii*, *V. corymbosum*, and *K. latifolia* displayed a different seasonal response (Figure 2h). *B. thunbergii* had high N_{area} in early May (1.8 gN m^{-2}), but the values decreased by 50% in mid June and then remained low. *K. latifolia* had the highest N_{area} among the three shrubs. The 2003 overwintering leaves of *K. latifolia* maintained high N_{area} (1.7 gN m^{-2}) in early May while N_{area} of the 2004 produced leaves increased throughout the growing season from 1.1 gN m^{-2} in mid June to 2.2 gN m^{-2} in late November. By contrast, *V. corymbosum* had lower N_{area} than the other two shrubs (0.6 – 0.7 gN m^{-2}) and no significant seasonal variation. On a mass basis (N_{mass}), the seasonal responses of leaf nitrogen in *B. thunbergii* and *V. corymbosum* were similar to that of N_{area}. On the contrary, N_{mass} of *K. latifolia* was much lower than the other two shrubs (1.1% - 1.5%) and the seasonal response was nearly absent (Figure 2i), indicating that the seasonal variation of N_{area} was due mainly to variation in LMA.

Relationships between respiratory characteristics and leaf properties

All three shrubs displayed significant correlation between R_{area} (20 ºC) and N_{area}, and the relationship was the strongest in *B. thunbergii* (Figure 3a, $R^2 = 0.74$, $P < 0.0001$). However, The $R_{area} – N_{area}$ relationship was mediated by different leaf characteristics in the three shrubs. For *B. thunbergii*, $R_{area} – N_{area}$ relationship was attributed to the variation both in the leaf nitrogen concentration (N_{mass}, Figure 3b) and LMA (indicator of leaf thickness/ cell density, Figure 3c). By contrast, respiration – N_{area} relationships were mainly mediated by the change of LMA in *K. latifolia* and *V. corymabosum* (Figure 3b, c). When expressed on a mass basis, a significant relationship was found only in *B. thunbergii* (R_{mass} and N_{mass}, $R^2 = 0.35$, $P = 0.003$, data not shown).

There is a significant correlation between the two respiratory model parameters R_0 and E_0 (Figure 4, Table 2). The relationship is the strongest in *V. corymbosum* ($R^2 > 0.8$), followed by *B. thunbergii* ($R^2 \approx 0.7$), and the weakest in *K. latifolia* ($R^2 = 0.28 – 0.43$). The pattern is constant regardless of the expression of respiration. There is also similar significant relationship between R_0 and Q_{10} (15 ºC – 20 ºC, data not shown).

Photosynthesis to respiration ratio

We used photosynthetic data reported in appendix I (Xu et al., unpublished data) to calculate the ratio of saturating photosynthetic rate to respiration (A_{max}/ R, at 20 ºC) and the ratio of photosynthesis rate at *in situ* light levels to respiration at the 7-day average temperature bracketing the measurement period (A/R_{ave}) in the three shrubs (Figure 5). The A/R ratios displayed significant seasonal variation in all three shrubs. In general, a

peak of A_{max}/ R occurred after leaf maturation (mid June for *B. thunbergii* and *V. corymbosum*, early August for *K. latifolia*). On the other hand, A/ R_{ave} showed the highest value in early May and mid November, when the overstory canopy was open.

Canopy foliar carbon loss throughout growing season 2004

Per unit leaf area, the total annual R_c of *B. thunbergii*, *K. latifolia* and V. corymbosum in 2004 was respectively 1.7, 6.5 and 1.0 mol C m^{-2} leaf production. *K. latifolia* displayed 3.8 and 6.5 times carbon loss of *B. thunbergii* and *V. corymbosum* per unit area leaf production respectively, partly attributed to the longer leaf life span. Nightly average R_c (for the period when the leaves are present) of *B. thunbergii*, *K. latifolia* and *V. corymbosum* was 7.3, 17.9 and 5.3 mmol C $night^{-1}$ m^{-2} leaf production. If corrected by leaf biomass, the annual R_c of *B. thunbergii*, *K. latifolia* and *V. corymbosum* were respectively 33.9, 44.7 and 25.1 mol C kg^{-1} leaf production, showing much smaller inter-species difference.

B. thunbergii and *K. latifolia* displayed uneven seasonal distribution of canopy foliar carbon loss (R_c, Figure 6). *B. thunbergii* shows a respiration peak in early summer, which can be mainly attributed to the high base respiration rate (R_0). Approximately 39% of annual R_c occurred within the first 42 days after canopy establishment, (120 – 161 day) or only 18% of the growing season. In the evergreen *K. latifolia*, the 2003 overwintering leaves and 2004 produced leaves respectively contributed to 48% and 52% of the annual R_c. The peak of R_c occurred in June and July (158^{st} – 217^{th} day, or 16% of the year), when foliar respiration contributed approximately 35% of the annual R_c. The high carbon

loss in summer is due to the combined effect of high LAI (with both 2003 overwintering leaves and 2004 produced leaves), higher temperatures and peak base leaf respiration rates. In addition, considerable proportion of R_c (27%) occurring during the winter and early spring dormant season (day 1 – 87 and day 322 – 366) when no leaves were present on the deciduous shrubs. Since the temperature was low during this period, the high carbon loss can be attributed to the long night length and high R_0 caused by cold acclimation. By contrast, R_c of *V. corymbosum* in general displayed an even distribution across the 2004 growing season.

The average spring, summer, fall, and winter temperatures have increased by 1.05 ºC, 1.22 ºC, 0.63 ºC and 1.4ºC respectively in southern New York state during the 20th century (Rosenzweig and Solecki, 2001). Assuming a constant temperature response, we predict that in the past century, the annual R_c increased by 12.9%, 10.3%, and 8.9% in *B. thunbergii*, *V. corymbosum*, and *K. latifolia* respectively (Table 3). The predicted increment of R_c was the lowest in fall, due to the less significant warming during this season. For the evergreen *K. latifola*, the strongest increment of R_c occurs in winter, when the most significant warming occurs. However, winter R_c of *K. latifolia* increased only 11.5%, lower than the maximum seasonal R_c increment of *B. thunbergii* and *V. corymbosum*, which occurs in summer.

Discussion

Seasonal variation of respiration rate

The temperature response of leaf respiration is known to be a function of both temperature and the physiological history of the respiring biomass (Amthor 1989; Atkin et al. 2000). Leaf respiration can further be influenced by seasonal thermal acclimation, maintenance requirements and other non-maintenance physiological processes (*e.g.* growth, translocation, herbivore defense, Xu and Griffin, unpublished). In particular, the respiration rate of understory shrubs is subjected to the maintenance demands of the photosynthetic apparatus, which is known to acclimate to the seasonal light variation by adjusting the leaf nitrogen concentration and LMA (Xu et al., unpublished data). The seasonal variation of the area based respiration rate at a set temperature (e.g. 20 ºC, $R_{area\ 20}$) thus reflects the combined effect of these factors. *B. thunbergii* displayed very high R_{area} in early May because the leaves were simultaneously subjected to low temperature respiratory acclimation, active growth and high irradiance photosynthetic acclimation (Xu et al. unpublished data). In *K. latifolia*, the high $R_{area\ 20}$ in early May and late November was primarily caused by low temperature respiratory acclimation and high irradiance photosynthetic acclimation, while the high $R_{area\ 20}$ in mid June can be attributed principally to growth. On the other hand, the seasonal variation of nitrogen based *in situ* respiration rates (indicated by the respiration rate at the 7-day average nighttime temperature, $R_{N\ ave}$) excluded the effect of thermal acclimation, LMA and leaf nitrogen concentration (N_{mass}) and reflects the physiological demand for respiratory products. The $R_{N\ ave}$ peak of *B. thunbergii* and *K. latifolia* respectively in mid May and mid June can be attributed to leaf growth. Similarly, material translocation before defoliation can lead to

the increment of $R_{N\ ave}$ in *B. thunbergii* and *V. corymbosum* during late September. However, methods need to be developed to quantify the relative effects of thermal acclimation, LMA, nitrogen concentration and non-maintenance physiological processes on respiration.

Model parameters of respiratory response to temperature and thermal acclimation

In the modified Arrhenius equation we used, the response of respiration to leaf temperature is partially represented by the parameter E_0, which linearly determines ln R. On the other hand, R_0 determines not only the base respiration rate (intercept of modeled temperature response), but also affects the respiratory temperature response (slope of modeled temperature response). In this study, the three shrubs could be classified into two groups based on seasonal variation of E_0. *K. latifolia* had a generally constant E_0 throughout the year (55 ± 9 kJ mol^{-1}), and the variation in the respiratory temperature response appears to be mainly related to the significant variation of R_0 (Table 1). By contrast, *B. thunbergii* and *V. corymbosum* displayed significant seasonal variations in both E_0 and R_0 (Table 1). In previous studies, a relatively constant E_0 has been observed in *Quercus rubra* (Xu and Griffin, unpublished data), and several tree species in a New Zealand temperate rainforest (e.g. *Weinmannia racemosa*, Turnbull et al. 2005; Turnbull et al. 2003), while a variable E_0 has also been previously reported (Dungan et al. 2003; Griffin et al. 2002). Since E_0 is subjected to biochemical and physiological adjustments in respiration, linking plant physiological activities (e.g. leaf senescence, growth rate) and biochemical components to the variation of E_0 will help to explain in which species and in what condition these two response types of thermal acclimation tend to occur.

Temperature acclimation of respiration has been suggested to be of two theoretical types (Atkin and Tjoelker 2003). Type I acclimation is predominantly characterized by a change in Q_{10} (related to E_0 in our study) and is probably affected by substrate availability, adenylate restriction or both. By contrast, Type II acclimation is mainly associated with a change in R_0 and has been attributed to temperate mediated changes in respiratory capacity. In this study, we find a consistent relationship between E_0 (also Q_{10}) and R_0 in all three shrubs. Furthermore, this relationship was not influenced by either leaf nitrogen or LMA. The relationship indicates that the regulation of respiratory capacity and the limitation of substrates and/ or adenylates will interactively influence the respiratory temperature response model parameters. The $R_0 - E_0$ (or Q_{10}) relationship is further explained by the rationale shown in figure 7a. Compared with a cold acclimated leaf (curve a), a typical Type II warm acclimated leaf (curve b) will show a decreased R_0 and a constant E_0, indicating both a decreased respiratory capacity (*e.g.* decreased enzyme activity/ amount, or a change in enzyme proportions, Atkin et al. in press) and an increased substrate/ adenylate limitation (*e.g.* lower concentration of non-structural carbohydrates Farrar and Williams 1991; Oleksyn et al. 2000). In general, the respiration rate is mainly limited by overall respiratory capacity at low temperature, and by substrate/ adenylate in high temperature (Atkin et al. in press), so a decreased respiratory capacity will result in further limitations to the rate of respiration at higher temperatures. If substrate/ adenylate limitation does not change (curve c) or the regulation in the substrate availability and/ or adenylate restriction is not sufficient to maintain a constant E_0 (or Q_{10}, curve d), downregulation of respiratory capacity will lead to an increased E_0 (or Q_{10} in the

whole temperature regime). Previous studies have shown that growth-temperature-mediated changes in respiratory capacity could be attributed to the establishment of a new steady-state concentration of leaf soluble carbohydrates (Hurry et al. 1994; Mooney and Billings 1965; Wilson 1966), but there is no evidence that the new equilibrium status will always be complete enough to maintain a constant E_0 (or Q_{10}). Therefore, R_0 and E_0 will tend to show a negative correlation, unless the plants show very typical and complete Type I or Type II acclimation. It is not surprising that the $R_0 - E_0$ (or Q_{10}) relationship would be species-specific since the degree of respiratory thermal acclimation is highly variable in different species (Loveys et al. 2003). This $R_0 - E_0$ (or Q_{10}) relationship will improve our understanding of the thermal acclimation of the short-term respiratory temperature response and if generalizable, will simplify modeling efforts and field measurements.

A general illustration of $R_0 - E_0$ (or Q_{10}) relationship and its indications on the plant acclimation mode is shown in figure 7b. First, the slope of the R_0 *vs*. E_0 (or Q_{10}) regression line can quantify the relative involvement of Type I (line a) and Type II (line b) acclimation. A relatively flat regression line indicates a predominately Type II acclimation and *vise verse*. For example, *K. latifolia* in our study in general shows a Type II seasonal thermal acclimation while *V. corymbosum* showed more significant Type I acclimation. Second, the intercepts of the R_0 *vs.* E_0 (or Q_{10}) regression will give the upper limit of the varying range of R_0 (line e) and E_0 (or Q_{10}). Practically, the respiratory temperature response must fulfill the condition of $E_0 > 0$ (or $Q_{10} > 1$) and $R_0 > 0$. Thus, the $R_0 - E_0$ (or Q_{10}) relationship indicates that R_0 and E_0 (or Q_{10}) are not likely to exceed

the intercept values (for Q_{10}, should be the intercept on line Y = 1). Finally, for a particular species, if the $R_0 – Q_{10}$ (or E_0) relationship is determined, the respiratory temperature response model will be significantly simplified since one parameter can be omitted, and further simplifying field measurements of respiratory temperature response.

Respiration – leaf characteristic relationships and photosynthesis – respiration balance

Correlations among leaf respiration and leaf nitrogen have been reported in many studies (Engel et al. 2002; Griffin et al. 2001; Reich et al. 1998a; Reich et al. 1998b; Ryan 1995; Ryan et al. 1996; Tissue et al. 2002; Turnbull et al. 2003). It has been proposed that this relationship is derived from the more general relationship between nitrogen and protein concentration, which is linked to maintenance respiration (Ryan 1991; Vose and Ryan 2002). In our study, the seasonal variation of R_{area} is adjusted by both N_{mass} and LMA in *B. thunbergii* and the $R_{mass} – N_{mass}$ correlation further confirms that respiration is subjected to the adjustment of leaf nitrogen concentration. By contrast, in *K. latifolia*, the seasonal variation of R_{area} was is mediated by leaf ontogeny only (LMA), while the R_{area} – LMA correlation of *V. corymbosum* reflects a general inter-leaf relationship in this species, since the seasonal effect on LMA was not significant. This respiration – nitrogen relationship is very similar to the photosynthesis – nitrogen relationship reported in appendix I (Xu & Griffin unpublished data). The similarity indicates that the seasonal variation of respiration is closely related to the maintenance requirement of the photosynthetic apparatus in these shrubs.

It has been proposed that the photosynthesis/ respiration ratio (A/R) is insensitive to environmental conditions (*e.g*. temperature, Dewar et al. 1999), or between species with different growth rates (Loveys et al. 2003). However, we observed significant seasonal variation of A/R ratios in all three shrubs. In general, attributing to the high respiration rate related to leaf growth, A_{max}/ R was low during early leaf development (May for *B. thunbergii* and June for *K. latifolia*). The highest A_{max}/ R values occurred after leaf maturation, and declined throughout the growing season, probably due to the senescence and low light acclimation. On the other hand, A/ R_{ave} appears mainly determined by the *in situ* light level, showing the highest values when the overstory canopy was open (May and November). This result is similar to an observation in a New Zealand temperate rainforest along a long-term soil-development chronosequence, in which significantly lower leaf A_{max}/ R values were observed in the sites with N and P limitation (Turnbull et al. 2005). Even in Loveys et al. (2003) study, which found a general relationship between A_{max} and R across diverse species and growth temperatures, the A_{max}/ R values in each species/ treatment displayed large variance. Therefore, it appears that the balance between leaf R and A_{max} is mainly maintained across species, but for a particular species, A/ R value can be subjected to the leaf ontogeny and resource availability. Although respiration appears to be closely related to photosynthetic maintenance as discussed previously, other physiological processes that demand respiratory products (*e.g*. growth, translocation) or environmental factors (e.g. shade, nutrient limitation, herbivory) that limit photosynthesis can lead to variable A/ R throughout the growing season.

The effect of climate warming on canopy foliar carbon loss

The Black Rock Forest is located in a region of southern New York State that has experienced a significant warming during the last century and the most pronounced temperature changes have occurred in winter (Rosenzweig and Solecki 2001). Thus, if long-term thermal acclimation, which is impossible to predict based on our current understanding in respiration, is not accounted, it is reasonable to assume that the winter warming would have the most significant affect on the carbon balance of evergreen species that maintain overwintering leaves. In our study, the evergreen *K. latifolia* displayed much higher respiratory carbon loss per unit leaf production (area or mass) than *V. corymbosum* and *B. thunbergii*. Although the high carbon loss of *K. latifolia* may be balanced by a winter and spring carbon subsidy or lower leaf production, winter warming may stimulate dormant season respiration and lead to excessive carbon loss.

However, contrary to this prediction, the evergreen *K. latifolia* displayed the lowest warming induced increment in R_c among the three shrubs in the 20th century, despite the fact that more than 1/4 of the annual R_c of *K. latifolia* occurred during the dormant season (November 16th – March 27th). We attribute this interesting phenomenon to the relatively low E_0 of *K. latifolia* throughout the growing season. In our calculation, if *K. latifolia* is to match the warming induced increment in R_c of *V. corymbosum* (10.3 %) and *B. thunbergii* (12.9 %), a further winter warming of 1.1 ºC – 2.7 ºC was required (assuming the other seasons remain at the current warming levels), which is much higher than the observed winter warming in the past century. This observation indicates that the temporal warming pattern has limited influence on the warming induced R_c increment. Therefore,

it seems that the physiological properties, rather than phenological characteristics or seasonal warming pattern, are more important in determining the carbon balance of these shrubs in a warmer world, and more significant carbon losses tend to occur in species with a higher E_0. Since a warmer winter is also likely to alleviate cold induced photoinhibition of *K. latifolia*, we conclude that warming in the southern New York state region benefited the net carbon gain of *K. latifolia* relative to *B. thunbergii* and *V. corymbosum* and perhaps might limit the displacement of native shrubs by the introduced invasive species.

In summary, we observed that (1) in *B. thunbergii*, leaf nitrogen reallocation and thinner LMA significantly decreased maintenance demands and led to the downregulation of respiration after overstory canopy closure; (2) per unit area leaf production, B. thunbergii showed much less R_c than *K. latifolia* but higher than *V. corymbosum*; (3) 27% of annual R_c of *K. latifolia* occurred in the dormant season; and (4) the seasonal warming pattern in southern New York state has the least effect on annual R_c in evergreen *K. latifolia*. These results fully support our first hypothesis that *B. thunbergii* can downregulate respiration following canopy closure and the third hypotheses that *K. latifolia* shows significant carbon loss during winter. Our second hypothesis that, annual canopy foliar carbon loss of *B. thunbergii* is comparable or lower than *K. latifolia* and *V. corymbosum*, is only partially supported. The forth hypothesis that the warming pattern in northeastern US leads to a more significant annual carbon loss in the evergreen species *K. latifolia* is rejected. To explain the obvious competitive advantage of *B. thunbergii* and determine the role warming may play in its invasion in the understory of northeastern deciduous

forest, it is necessary to address leaf construction costs (Nagel and Griffin unpublished data), whole plant carbon budgets (Givnish 2002) and the warming response of other ecological properties (e.g. phenology). In summary, our study discovered a significant negative relationship between R_0 and E_0 (or Q_{10}) in the three shrubs, which, if generalizable, will simplify the model and measurement of respiratory temperature response, and elucidated that the warming effect on canopy foliar carbon loss is mainly determined by plant physiological properties, but not seasonal pattern of warming or phenology.

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Table 1. Model parameters of respiratory temperature response in three shrubs across the 2004 growing season. E_0 is a parameter equivalent to the energy of activation for respiration as an overall reaction, and is similar but not identical to the energy of activation for a single enzyme reaction. R_0 (on an area, a mass and a nitrogen basis) is the base respiration rate at 10ºC. Values shown are means (±SEM), where n=6. The values followed by the same letter are not significantly different at P=0.05 level (t test). The significance levels of ANOVA on seasonal effect are shown ("*" = significant at $P < 0.05$, "**" = significant at $P < 0.01$ and "***" = significant at $P < 0.001$). For statistical analysis, the data were log transformed to fulfill normality and homoscedasity.

Model Parameter	Measurement Period	*Vaccinium corymbosum*	*Berberis thunbergii*	*Kalmia latifolia*
E_0 (kJ mol^{-1})	05/7 – 05/09	--	66.3 (3.7)^{cd}	58.1 (2.5)^{de}
	06/15 – 06/16	73.6 (7.0)^{bcd}	97.4 (3.5)^{a}	54.7 (10.3)^{cdefg}
	08/23 – 08/30	77.0 (4.5)^{bc}	84.7 (5.3)^{ab}	63.9 (4.7)^{cd}
	09/25 – 09/27	38.3 (2.7)^{g}	79.4 (6.6)^{bc}	50.5 (2.5)^{ef}
	11/17 – 11/18	--	--	47.5 (2.9)^{f}
	ANOVA	F = 26.2 P < 0.0001***	F = 7.2 P = 0.002**	F = 1.9 P = 0.15 ns
R_0 (area, 10°C) (µmol m^{-2} s^{-1})	05/7 – 05/09	--	0.50 (0.07)^{ab}	0.35 (0.04)^{b}
	06/15 – 06/16	0.064 (0.01)^{gh}	0.04 (0.01)^{h}	0.32 (0.09)^{bcd}
	08/23 – 08/30	0.065 (0.01)^{gh}	0.10 (0.01)^{fg}	0.10 (0.02)^{efg}
	09/25 – 09/27	0.144 (0.01)^{de}	0.17 (0.04)^{cdef}	0.20 (0.02)^{c}
	11/17 – 11/18	--	--	0.57(0.05)^{a}
	ANOVA	F = 8.4 P = 0.004**	F = 30.7 P < 0.0001***	F = 12.3 P < 0.0001***
R_0 (mass, 10°C) (µmol kg^{-1} Biomass s^{-1})	05/7 – 05/09	--	7.74 (0.48)^{a}	2.42 (0.24)^{c}
	06/15 – 06/16	1.84 (0.38)^{cde}	0.90 (0.15)^{f}	3.51 (0.91)^{cd}
	08/23 – 08/30	1.61 (0.22)^{d}	2.16 (0.18)^{cd}	0.87 (0.16)^{ef}
	09/25 – 09/27	3.83 (0.25)^{b}	3.74 (1.20)^{bcd}	1.80 (0.14)^{d}
	11/17 – 11/18	--	--	3.89 (0.29)^{b}
	ANOVA	F = 10.9 P = 0.001**	F = 27.4 P < 0.0001***	F = 11.6 P < 0.0001***
R_0 (N, 10°C) (µmol g^{-1} N s^{-1})	05/7 – 05/09	--	0.28 (0.02)^{a}	0.20 (0.01)^{a}
	06/15 – 06/16	0.091 (0.02)^{e}	0.04 (0.01)^{f}	0.28 (0.08)^{ab}
	08/23 – 08/30	0.098 (0.01)^{de}	0.12 (0.01)^{de}	0.08 (0.01)^{cd}
	09/25 – 09/27	0.225 (0.01)^{cd}	0.22 (0.07)^{b}	0.12 (0.01)^{abcd}
	11/17 – 11/18	--	--	0.26 (0.02)^{abc}
	ANOVA	F = 12.0 P = 0.0006***	F = 27.4 P < 0.0001***	F = 9.44 P = 0.0001***

Table 2. The relationship between R_0 (x) and E_0 (y, kJ mol^{-1})

Unit of R_0	Regression Results	*Vaccinium corymbosum*	*Berberis thunbergii*	*Kalmia latifolia*
Area Based (μmol C m^{-2} s^{-1})	Slope	-398.9	-61.9	-34.2
	Y-intercept	99.2	94.7	65.5
	X-intercept	0.25	1.53	1.92
	R^2	0.81	0.67	0.29
	P	<0.0001	<0.0001	0.003
Mass Based (μmol C kg^{-1} Biomass s^{-1})	Slope	-16.8	-4.39	-5.77
	Y-intercept	103.7	98.0	68.9
	X-intercept	6.17	22.3	11.9
	R^2	0.92	0.70	0.43
	P	<0.0001	<0.0001	0.0002
Nitrogen Based (μmol C kg^{-2} N s^{-1})	Slope	-246.3	-71.8	-44.6
	Y-intercept	113.8	102.2	66.9
	X-intercept	0.46	1.42	1.50
	R^2	0.86	0.70	0.28
	P	<0.0001	<0.0001	0.004

Table 3. Increase of R_c based on the warming trend of the 20th century in southern New York state and the 2004 temperature record.

	Increase of Average Temperature in the 20th Century (ºC)	Increase of R_c in the 20th Century (%)		
		Vaccinium corymbosum	*Berberis thunbergii*	*Kalmia latifolia*
Annual	1.09	10.3	12.9	8.9
Spring	1.05	11.8	11.7	8.7
Summer	1.22	13.7	16.7	10.6
Fall	0.63	4.5	7.7	4.8
Winter	1.40	--	--	11.5

Figure Legends

Figure 1. Respiratory temperature response of three shrubs across the 2004 growing season (*B. thunbergii*: dotted line, *K. latifolia*: dash line, *V. corymbosum*: solid line). Data shown are modeled responses based on the mean parameters from 6 replicate response curves (individual curves are fitted by Equation 1). a – e, area based estimates, f – j, mass-based estimates.

Figure 2. Leaf respiration at 20 ºC and 7-day average temperature bracketing when the measurements were made, and leaf properties (*B. thunbergii*: ○ and dotted line, *K. Latifolia*: ▼ and dash line, *V. corymbosum*: ● and solid line). The values are mean ± standard error (SE, n=6).

Figure 3. Correlation between R_{area} (20 ºC) and leaf characteristics (N_{area}, N_{mass}, LMA). For correlations significant at $P<0.05$ level, correlation efficient (R^2), P values and linear regression lines are shown. The legends are the same as that described in figure 1 and 2.

Figure 4. Correlations between R_0 and E_0. The legends are the same as that described in figure 1 and 2. The statistics of regressions is listed in Table 2.

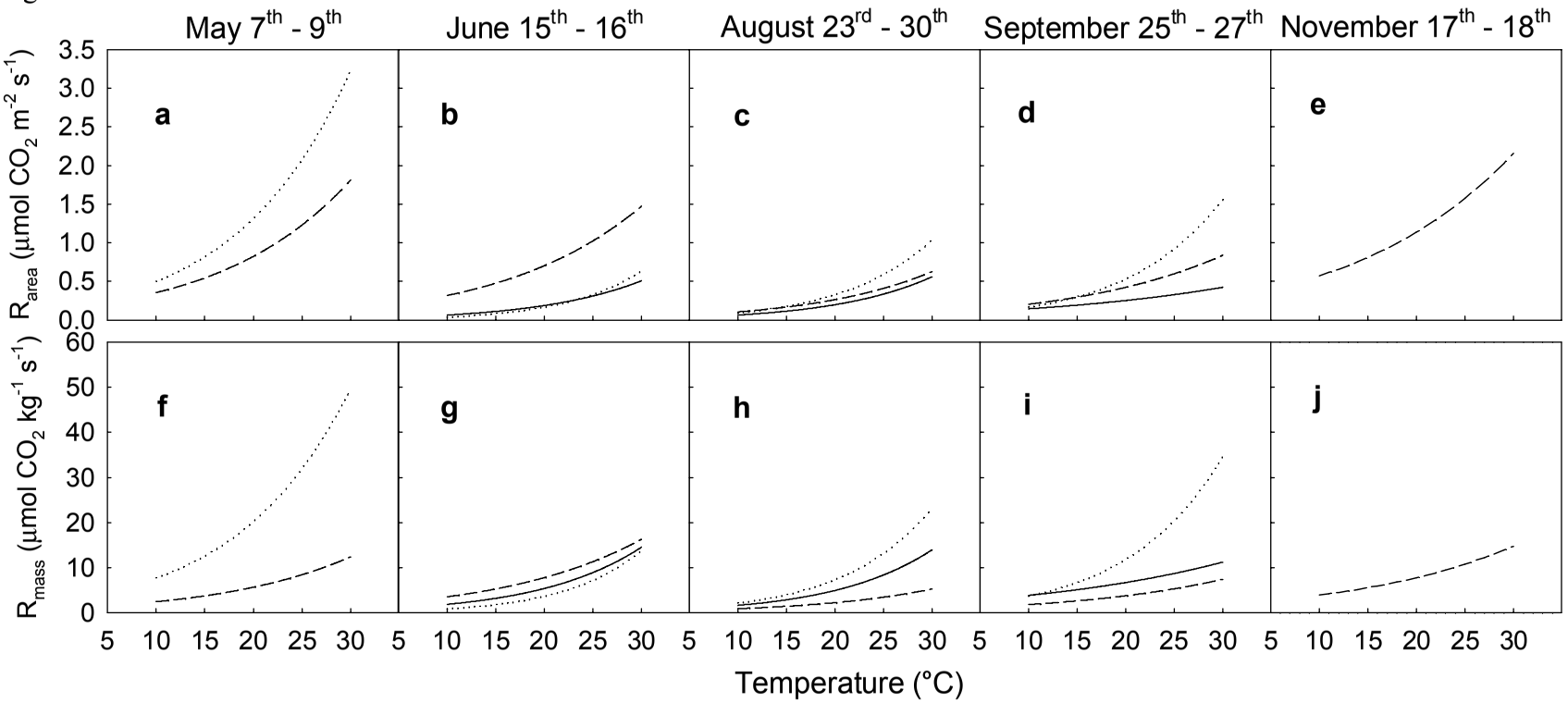
Figure 5. Seasonal variation of the ratio of maximum photosynthetic rate (at ambient $[CO_2]$ and saturating light level) to respiration rate (A_{max}/ R) at 20 ºC (a), and the ratio of

photosynthetic rate at in situ light level to respiration ratio at 7-day average (b). The legends are the same as that described in figure 2.

Figure 6. Canopy foliar carbon loss (R_c) of the three shrubs throughout the 2004 growing season. a) average night temperature of 2004; b) leaf area index of three shrubs (relative value to the maximum LAI); c) – e) canopy foliar carbon loss of R_c of the three shrubs; f) accumulated canopy foliar respiration throughout 2004 (% of annual total). For b and f, the legends are the same as that in figure 1.

Figure 7. Theoretical explanation of the $R_0 – E_0$ (or Q_{10}) relationship (a) and the identification of acclimation mode with $R_0 – E_0$ (or Q_{10}) relationship.

Figure 1.


May 7th - 9th
June 15th - 16th
August 23rd - 30th
September 25th - 27th
November 17th - 18th
a
b
c
d
e
f
g
h
i
j
Rarea (µmol CO2 m-2 s-1)
Rmass (µmol CO2 kg-1 s-1)
Temperature (°C)

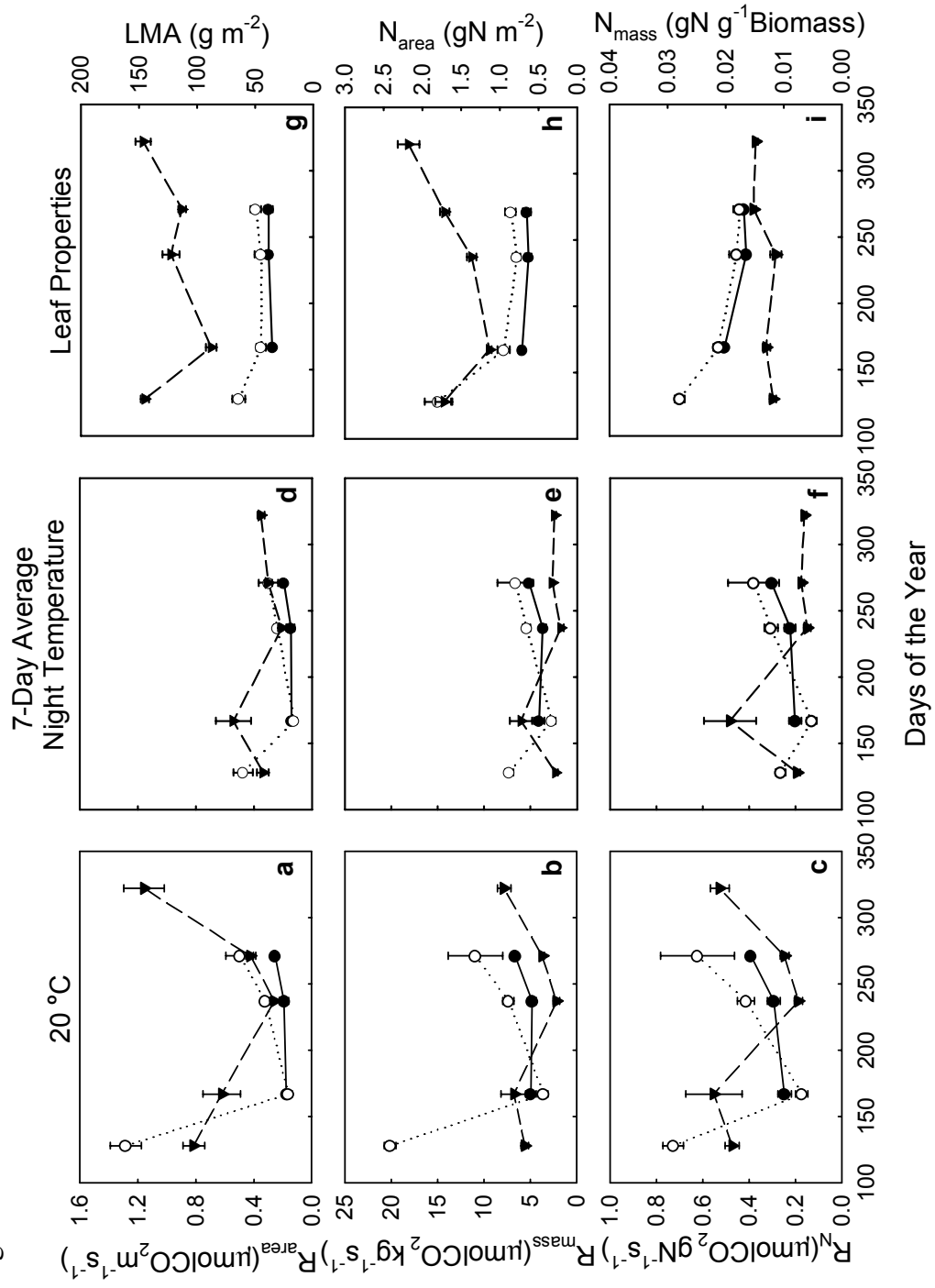

20 °C
7-Day Average
Night Temperature
Leaf Properties
LMA (g m-2)
Narea (gN m-2)
Nmass (gN g-1Biomass)
Raea(μmolCO2m-1s-1)
Rmass(μmolCO2 kg-1s-1)
RN(μmolCO2 gN-1s-1)
Days of the Year
a
b
c
d
e
f
g
h
i

Figure 2.

Figure 3.

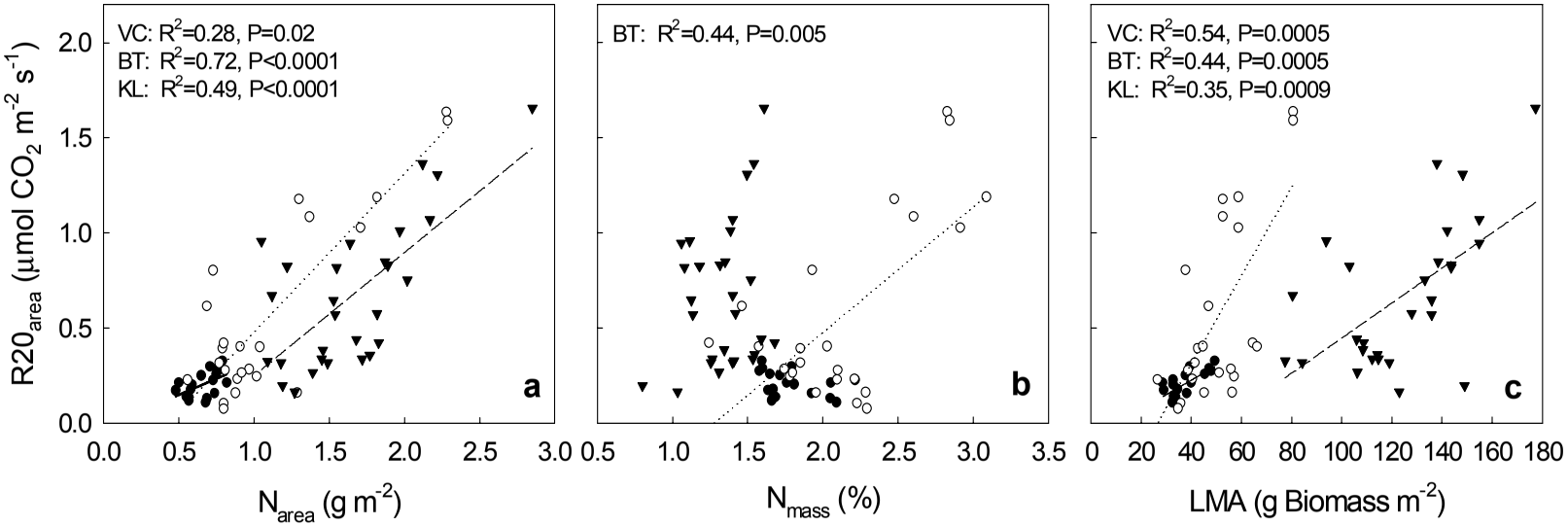

VC: R2=0.28, P=0.02
BT: R2=0.72, P<0.0001
KL: R2=0.49, P<0.0001
a
BT: R2=0.44, P=0.005
b
VC: R2=0.54, P=0.0005
BT: R2=0.44, P=0.0005
KL: R2=0.35, P=0.0009
c
R20area (µmol CO2 m-2 s-1)
Narea (g m-2)
Nmass (%)
LMA (g Biomass m-2)

Figure 4.

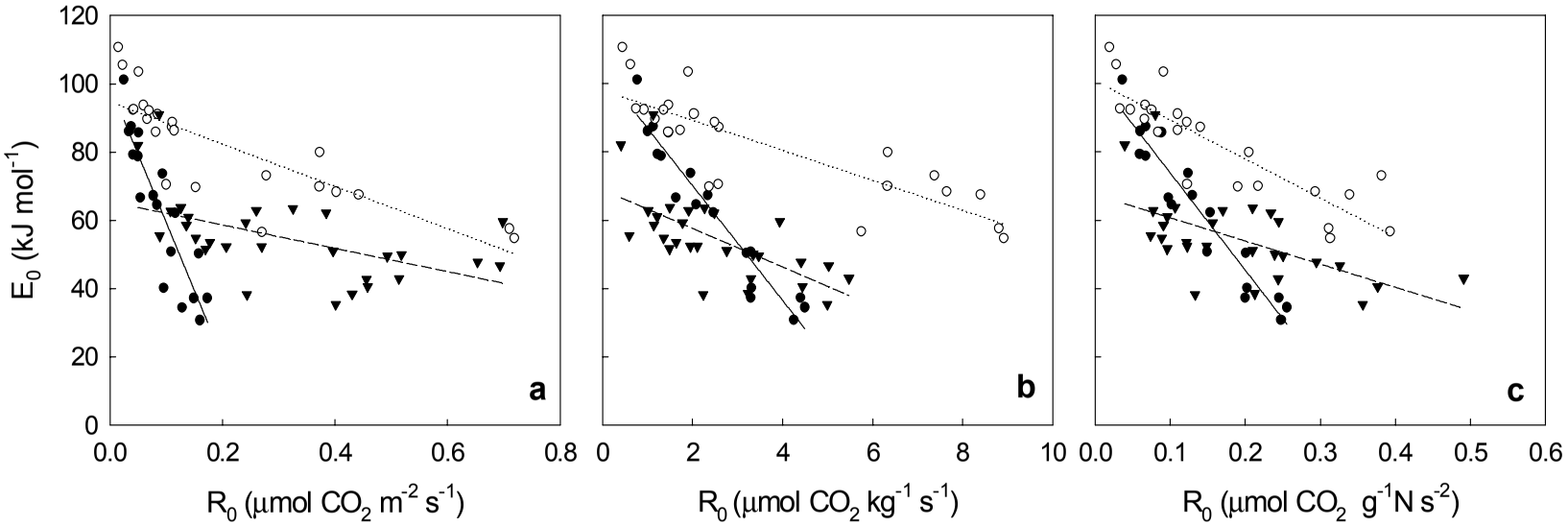

E0 (kJ mol-1)
120
100
80
60
40
20
0
0.0
0.2
0.4
0.6
0.8
a
R0 (µmol CO2 m-2 s-1)
0
2
4
6
8
10
b
R0 (µmol CO2 kg-1 s-1)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
c
R0 (µmol CO2 g-1N s-2)

Figure 5.

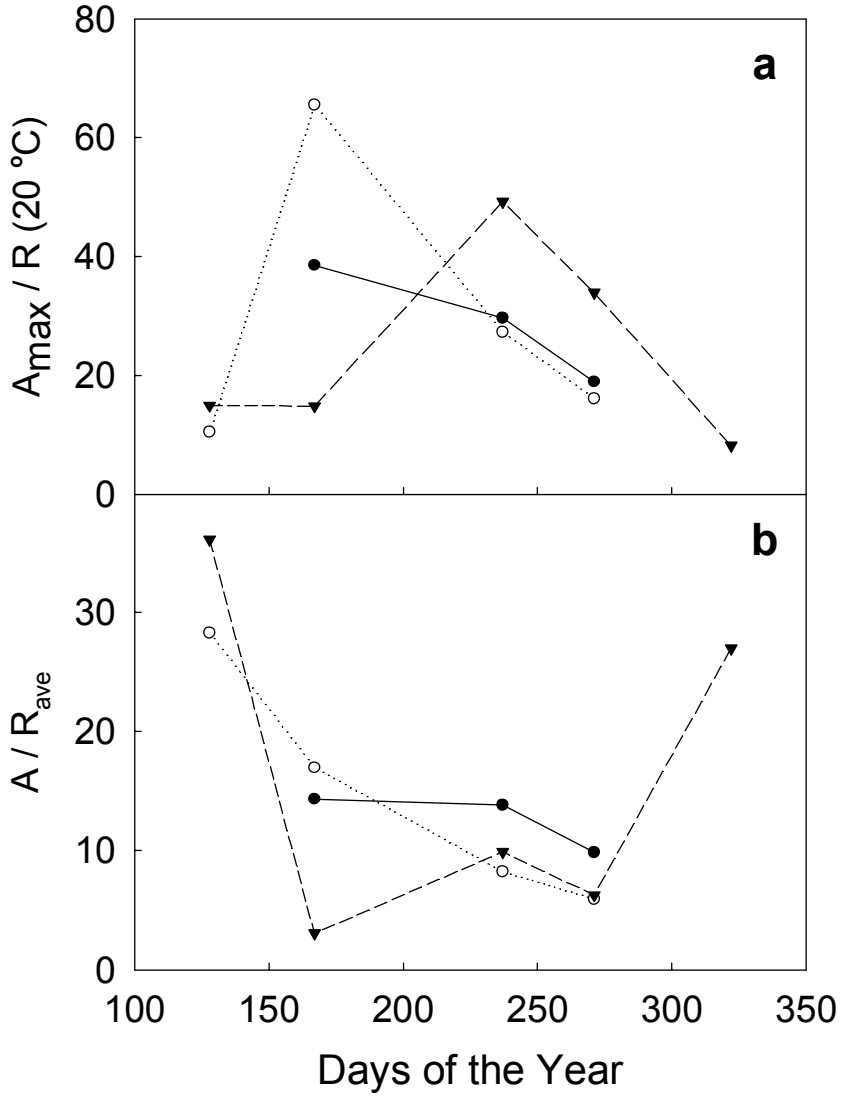

a
b
A_{max} / R (20 °C)
A / R_{ave}
Days of the Year
0
20
40
60
80
0
10
20
30
100
150
200
250
300
350

Figure 6.

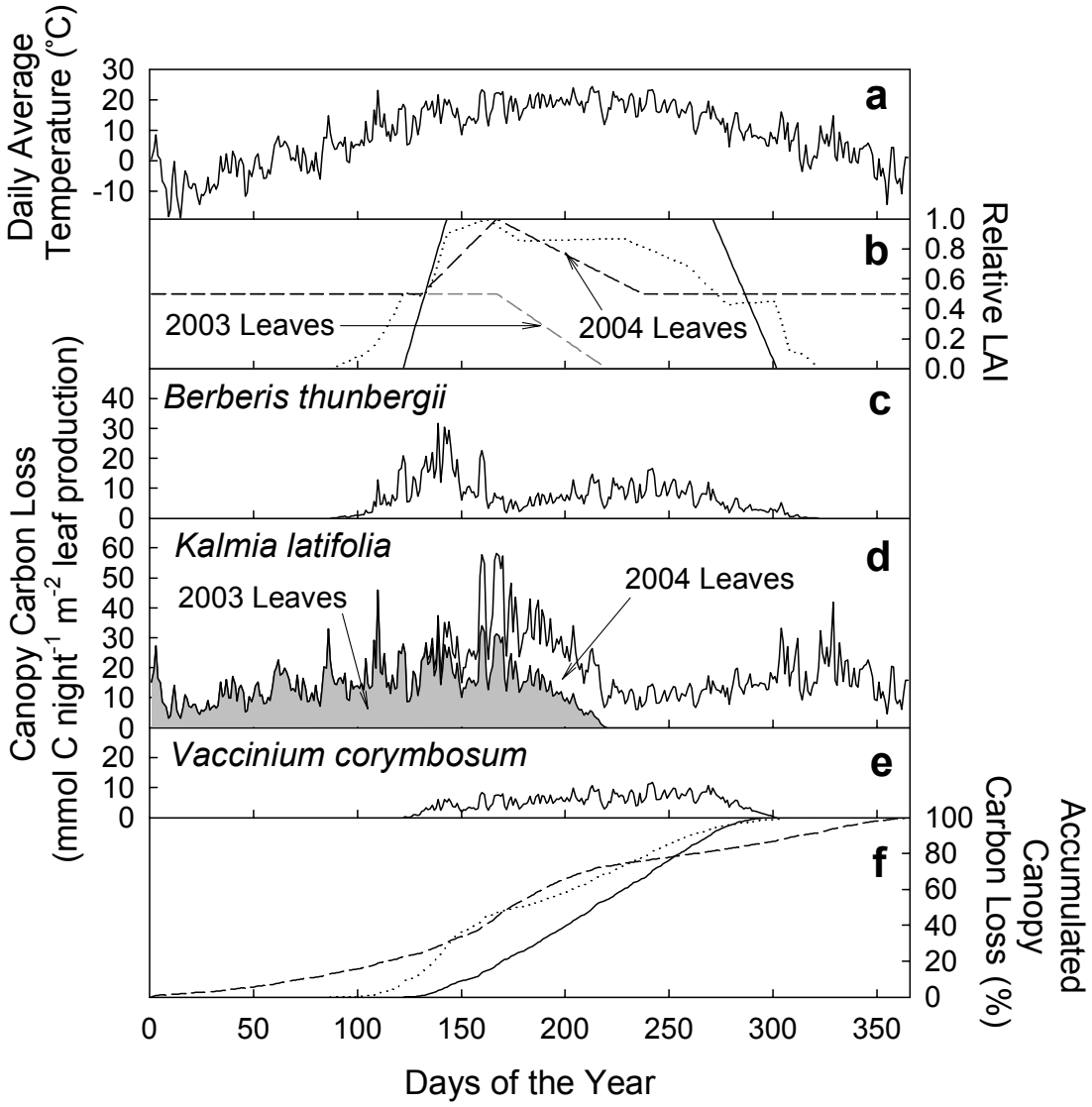
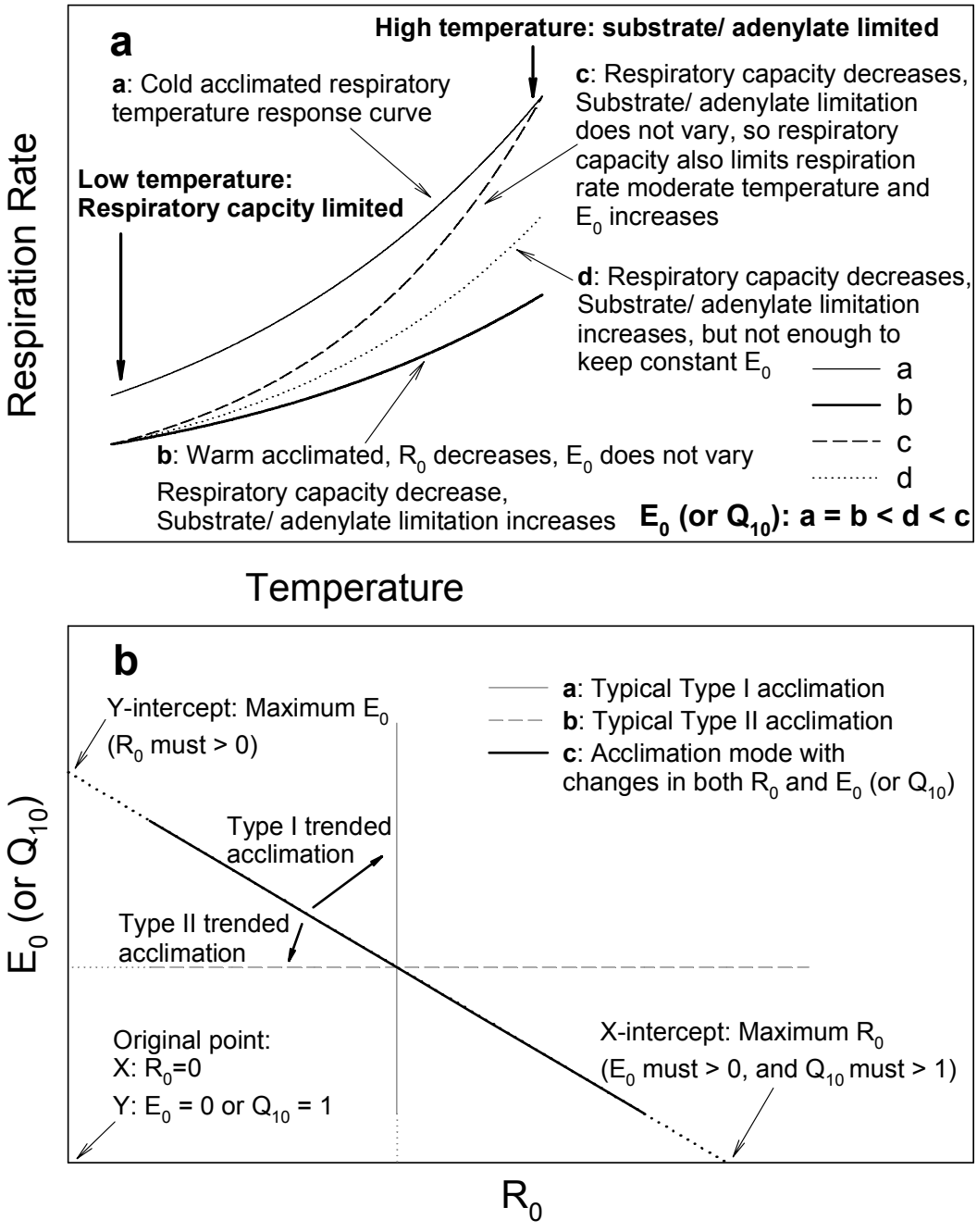

Daily Average Temperature (°C)
30
20
10
0
-10
a
b
1.0
0.8
0.6
0.4
0.2
0.0
Relative LAI
2003 Leaves
2004 Leaves
Canopy Carbon Loss (mmol C night-1 m-2 leaf production)
Berberis thunbergii
c
40
30
20
10
0
Kalmia latifolia
d
60
50
40
30
20
10
0
2003 Leaves
2004 Leaves
Vaccinium corymbosum
e
20
10
0
f
100
80
60
40
20
0
Accumulated Canopy Carbon Loss (%)
0
50
100
150
200
250
300
350
Days of the Year

Figure 7.


a
High temperature: substrate/ adenylate limited
a: Cold acclimated respiratory temperature response curve
c: Respiratory capacity decreases, Substrate/ adenylate limitation does not vary, so respiratory capacity also limits respiration rate moderate temperature and E0 increases
Low temperature: Respiratory capcity limited
d: Respiratory capacity decreases, Substrate/ adenylate limitation increases, but not enough to keep constant E0
a
b
c
d
b: Warm acclimated, R0 decreases, E0 does not vary
Respiratory capacity decrease, Substrate/ adenylate limitation increases
E0 (or Q10): a = b < d < c
Respiration Rate
Temperature
b
a: Typical Type I acclimation
b: Typical Type II acclimation
c: Acclimation mode with changes in both R0 and E0 (or Q10)
Y-intercept: Maximum E0
(R0 must > 0)
Type I trended acclimation
Type II trended acclimation
Original point:
X: R0=0
Y: E0 = 0 or Q10 = 1
X-intercept: Maximum R0
(E0 must > 0, and Q10 must > 1)
E0 (or Q10)
R0

Chapter 4:

Leaf Respiratory CO_2 is ^{13}C-enriched relative to leaf organic components in five species of C_3 Plants

CHENGYUAN XU, GUANGHUI LIN, KEVIN L. GRIFFIN AND

RAYMOND N. SAMBROTTO

Abstracts

We compared the carbon isotope ratios of leaf respiratory CO_2 ($\delta^{13}C_R$) and leaf organic components (soluble sugar, water soluble fraction, starch, protein and bulk organic matter) in 5 C_3 plants grown in a greenhouse and inside Biosphere 2. One species, *Populus deltoides*, was grown under 3 different CO_2 concentrations. The Keeling plot approach was applied to the leaf scale to measure leaf $\delta^{13}C_R$ and these results were compared with the $\delta^{13}C$ of leaf organic components. In all cases, leaf respiratory CO_2 was more ^{13}C-enriched than leaf organic components. The amount of ^{13}C enrichment displayed a significant species-specific pattern, but the effect of CO_2 treatment was not significant on *P. deltoides*. In C_3 plant leaves, ^{13}C-enriched respiratory CO_2 appears widespread. Among currently hypothesized mechanisms contributing to this phenomenon, non-statistical carbon isotope distribution within the sugar substrates seems most likely. Caution should be taken when attempting to predict the $\delta^{13}C$ of leaf respiratory CO_2 at the ecosystem scale by upscaling the relationship between leaf $\delta^{13}C_R$ and $\delta^{13}C$ of leaf organic components.

Introduction

It is well known that carbon isotope discrimination takes place during plant photosynthetic CO_2 fixation, resulting in all higher plants being depleted in ^{13}C in organic carbon relative to atmospheric CO_2. The models of ^{13}C fractionation in photosynthesis have been well established (Farquhar, O'leary & Berry, 1982). In contrast, studies on the carbon isotope ratio of CO_2 generated by dark respiration ($\delta^{13}C_R$) are limited. Although a possible isotope effect during dark respiration might significantly influence the carbon isotope signature of plants and other components of an ecosystem, fewer studies have focused on determining the magnitude of this potential effect and the results appear contradictory (O'leary, 1981; Lin & Ehleringer, 1997; Duranceau *et al.*, 1999; Duranceau, Ghashghaie & Brugnoli, 2001).

Studies on the carbon isotopic effects during respiration trace back half a century. Historically, carbon isotope discrimination during respiration was considered to be negeligible (O'leary, 1981; Farquhar *et al.*, 1982; Farquhar, Ehleringer & Hubick, 1989; Flanagan & Ehleringer, 1998). Early experimental studies observed that $\delta^{13}C_R$ is very close (approximately ±1‰) to bulk carbon in some geminating crop seedlings (Baertschi, 1953; Smith, 1971). More recently, Lin and Ehleringer (1997) cultured mesophyll protoplasts of bean and corn leaves with carbohydrates of known isotopic ratios as the only carbon source and found no significant differences between $\delta^{13}C_R$ and $\delta^{13}C$ of the substrate in either species.

Still, other studies suggest that respiratory CO_2 of plants can be remarkably ^{13}C-enriched or ^{13}C depleted (4-5‰ more positive or -4-8‰ more negative) in comparison with whole plant or leaf $\delta^{13}C$ (Smith, 1971; Troughton, Card & Hendy, 1974). Recently, Duranceau *et al.* (1999., 2001) and Ghashghaie *et al.* (2001) compared $\delta^{13}C$ of leaf respiration and leaf organic components in beans, tobacco, and sunflower. They report a 3-6‰ ^{13}C-enrichment in respiratory CO_2 compared to sucrose, the assumed substrate of dark respiration. Although this hypothesis was not tested in other species, they concluded that carbon isotope fractionation during dark respiration was widespread in C_3 plants. In C_4 plants, Henderson, Voncaemmerer and Farquhar (1992) found that the $\delta^{13}C$ of dry matter was more negative than that predicted by the discrimination occurring during CO_2 uptake and partly attribute the difference to an isotope effect during dark respiration. Furthermore, $\delta^{13}C_R$ can change daily or seasonally (Park & Epstein, 1961; Jacobson *et al.*, 1970; Damesin & Lelarge, 2003) and can be influenced by environmental or physiological factors (temperature, respiratory quotient etc., Tcherkez *et al.*, 2003).

Despite the growing contradictory evidence, the assumption that carbon fractionation in dark respiration is negligible is widely applied in ecological and physiological studies (Flanagan & Ehleringer, 1998; Yakir & Sternberg, 2000; Ehleringer *et al.*, 2002). At the ecosystem scale, the concept of ecosystem ^{13}C discrimination ($\Delta^{13}C_e=(\delta^{13}C_{trop}-\delta^{13}C_R)/(1+\delta^{13}C_R)$, or $\Delta^{13}C_e=(\delta^{13}C_{atm}-\delta^{13}C_R)$), has recently been used to partition NEE (Net Ecosystem Exchange) into photosynthesynthetic and respiratory components (Bowling, Tans & Monson, 2001), by assuming that the $\delta^{13}C_R$ should reflect the ^{13}C signature of total organic carbon in the ecosystem (Buchmann *et al.*, 1997a; Yakir &

Sternberg, 2000). Likewise, the $\delta^{13}C$ of organic carbon in leaf, soil, and litter were used to estimate the $\delta^{13}C_R$ generated by each components (Lin *et al.*, 1999; Lin *et al.*, 2001). If the $\delta^{13}C_R$ does not correctly reflect the $\delta^{13}C$ of the respiration substrate pool, the conclusions of these studies will need to be reconsidered and modified accordingly.

Additional uncertainties regarding the use of $\delta^{13}C_R$ as a tool for understanding ecosystem scale processess arise from several other factors. For example, initial studies focused on seedlings or tubers and subsequent leaf scale studies were conducted in only a few crop species. In addition, plant materials were subjected to a CO_2-free environment in all previous studies, which may itself influence leaf $\delta^{13}C_R$ (O'leary, 1981). Clearly, much more detailed information on the species effects, and ultimately the mechanisms are needed to gain insight into ecosystem level processes. In this study, we applied a Keeling plot approach on the leaf scale to measure leaf $\delta^{13}C_R$ (Fessenden & Ehleringer, 2003) and studied its relationship with $\delta^{13}C$ of leaf soluble sugar, water soluble fraction, starch, protein, and bulk organic carbon of five C_3 plants. Our primary goal was to test the hypothesis (1) that $\delta^{13}C_R$ will be the same as major substrates (assumed to be soluble sugar), an assumption that underlies many current ecological studies, neglecting the carbon isotope effect in respiration. If hypothesis (1) is rejected, we further hypothesize (2) the differences between $\delta^{13}C_R$ and $\delta^{13}C$ leaf organic components is not species-specific and (3) will not be significantly influenced by growth CO_2 level (in *Populus deltoides*).

Materials and Methods

Plant Materials

We studied five C_3 plants, which are among the most abundant species in the Tropical Rain Forest (TRF) and Intensive Forest Biome (IFB) of Biosphere 2 (a 1.29 hector glass enclosed research facility in Oracle, Arizona). This leaf scale study was also designed to provide background information for further investigation on the respiratory isotope effect at mesocosom scale within Biosphere 2. Among 4 tropical species we studied, *Musa paradisiacal* (tree), *Coffea arabica* (shrub), and *Epipremumn pinnatum* (vine) were grown in a greenhouse (the demonstrate lab or DL), while *Clitoria racemosa* (tree) was grown in theTRF biome. *Populus deltoides* (IFB monoculture), a temperate tree species, was grown in 3 CO_2 concentrations: close to ambient in the DL, 800 ppm in the IFB mid bay (MB) and 1200 ppm in west bay (WB). In the IFB, tank supplied CO_2 with a very low $\delta^{13}C$ (about -28‰) was used to maintain elevated CO_2 concentrations. All plants grew under a natural photoperiod and night time temperatures of 23 to 28 °C depending on the location.

Air Sampling

All air samples were collected between 20:00 to 00:30, when plants were in natural darkness. One to several healthy, intact, visually mature (well expanded and with developed cuticle) leaves were sealed in an opaque respiration chamber (modified from a mylar balloon) with a small fan to ensure air mixing. The chamber was connected to a closed loop gas exchange system including a pump, a CO_2 infrared gas analyzer (LI-6200, Licor, Inc., Lincoln, USA), a desiccant tube containing magnesium perchlorate and

six 100 ml flasks (Fig. 1a). The entire system was 10 – 15 liters to hold the largest leaf (*Epipremumn pinnatum*) in our study and was checked for leakage prior to each sampling by exhaling on all connections. The airflow rate was approximately 1 liter per minute. Ambient air was pumped through the entire system before closure and then allowed to circulate for 10 – 15 minutes to ensure adequate mixing prior to sampling. The air samples were collected in sequence by closing both stopcocks on a flask for each 15 – 20 ppm CO_2 increment. Humidity was not strictly controled in our study.

We compared our leaf-level Keeling plot method with a traditional CO_2 free chamber connected to the Li-6200 photosynthesis system and found that two methods yielded similar results in leaf $\delta^{13}C_R$ (±0.5%) when the incubation chamber was well sealed. However, the incubation method often gave much more scatter results with same plant leaf than leaf–level Keeling plot approach.

Leaf Sampling and Chemical Extraction

After air sampling, half of the leaf material contained within the cuvette was immediately frozen in liquid nitrogen and then stored in -20°C freezer for subsequent extraction of carbohydrate and protein. The remaining leaf material was dried in a 60°C oven for carbon isotope analysis of bulk leaf organic matter.

A subsample of 0.1-1g of leaf material was used for soluble sugar and starch extraction. For each 0.1g of sample, 1 ml of deionized water was added and the mixture was ground in a chilled mortar and pestle. The resulting extract was kept at 0°C for 20 minutes before

centrifugation at 12,000g for 10 minutes. The supernatant containing the soluble fraction was then boiled for 3 minutes and centrifuged again as described above (Duranceau *et al.*, 1999). The water soluble fraction was then mixed with Dowex-50 (H^+) and Dowex-1 (Cl^-) resins in sequence to remove amino acids and organic acids respectively. The eluate has been showen to have a carbon isotope composition representative of leaf soluble sugars (Brugnoli *et al.*, 1988). The pellets were washed in ethanol (80% v:v) at 80 ºC to eliminate chlorophyll and then suspended twice in 6 mol/L HCl at 5 °C (1 hr each) to solubilize the starch. After adding methanol (4x by volumn), the supernanent was kept at 5°C overnight and starch precipited was desiccated in a freeze dryer (Damesin & Lelarge, 2003, with a few modifications). Proteins were extracted by boiling the supernanent of grounded leaf tissue (in 2% NaCl; 10,000g 15 min,) for 30 minutes (Jacobson *et al.*, 1970). The precipitant was dried overnight in a desiccator at room temperature. All products from these extractions were kept at -20ºC until carbon isotope analyses were performed. According to the references mentioned above, fractionation of carbon isotopes did not occur during the extraction processes.

Carbon Isotope Analysis

The carbon isotope ratios in delta notation were expressed as $\delta^{13}C$ (‰) = $[R_{sample} / R_{standard}-1] \times 1000$, where R is the molar ratio of ^{13}C / ^{12}C. $\delta^{13}C_R$ was measured in an Isochrom isotope-ratio mass spectrometer (Fison Instrument Inc., Manchester, UK) at Biosphere 2 Center (B2C). $\delta^{13}C$ of the leaf organic components was analyzed either at B2C or with an Europa 20/20 continuous flow (CF) isotope ratio mass spectrometer (IRMS) coupled with an ANCA NT combustion system at Lamont-Doherty Earth

Observatory (PDZ-Europa, Cheshire, UK). NIST sucrose was used as the standard for inter-machine calibration. All $\delta^{13}C$ values are expressed relative to Pee Dee Belemnite (PDB).

The mixing model of (Keeling, 1958, 1961) was used to calculate the isotope ratio of CO_2 respired by a leaf:

$$\delta^{13}C_{cham} = [CO_{2\,atm}] \times (\delta^{13}C_{atm} - \delta^{13}C_R)/[CO_{2\,cham}] + \delta^{13}C_R$$

where $[CO_2]$ is the concentration of CO_2 and δ is the stable isotope ratio of CO_2. The subscripts cham, atm and R represent the air within the chamber, the air in experimental atmosphere and respiratory CO_2, respectively. Geometric mean regressions were used to establish the linear relationship between $\delta^{13}C_{cham}$ and $1 / [CO_{2\,cham}]$ (Pataki *et al.*, 2003) and the intercept at the Y axis is the $\delta^{13}C$ value of leaf respiratory CO_2 (Fig 1b).

Statistical Analysis

A one-way analysis of variance (ANOVA) was used to test the species effects on the possible differences of $\delta^{13}C$ between the respiratory CO_2 and leaf organic components. Effects were considered to be significant at the 0.05 probability level. In addition, A student's t-test was used for multiple comparisons among *P. deltioides* grown in three CO_2 concentrations to evaluate the effects of CO_2 treatments.

Linear regressions were used to analyze the relationship between $\delta^{13}C_R$ and $\delta^{13}C$ of the leaf organic components of all samples or averages of each species/treatment combination. Data from similar leaf-level studies (Duranceau *et al.*, 1999; Duranceau *et*

al., 2001; Ghashghaie *et al.*, 2001; Tcherkez *et al.*, 2003), which had 3 C_3 crop species in 3 sets of environmental or genetic treatments, were included in the regression. We assume that the average $\delta^{13}C$ of all sugars and water soluble materials (soluble sugar and organic acids) analyzed in those studies are equivalent to the "soluble sugar" and "water soluble fraction" in our study.

Results

The isotopic signatures of the measured pools exhibited a similar pattern for all five species and the three CO_2 treatments for *P. Deltoides*. In each case, leaf $\delta^{13}C_R$ was the most positive, followed by the $\delta^{13}C$ of starch (except *M. paradisiacal*), while the $\delta^{13}C$ of the bulk organic matter was the lightest (Fig. 2). The amount of ^{13}C-enrichment in leaf $\delta^{13}C_R$ was 3.5 – 5.9 ‰ relative to soluble sugar (Table 1), the assumed major substrate for dark respiration. Compared with the water soluble fraction, starch, and bulk organic matter, the amount of ^{13}C enrichment in respiratory CO_2 was 2.7 – 5.2‰, 1.4 – 4.2‰, and 4.1 – 6.9‰, respectively, depending on species (Table 1). During the sampling period, leaves released less than 0.001g carbon, which should not influence the $\delta^{13}C$ of remaining organic component pools significantly.

The amount of ^{13}C enrichment in respiratory CO_2 relative to soluble sugar, water soluble fraction and starch each had a significant species effect (Table 1). This effect was apparent but not so significant in the bulk leaf organic matter (P=0.066). The effect of CO_2 treatment in *P. deltoides* was not significant. Although leaf respiratory ^{13}C-enrichment in the DL was smaller than that in the IFB in average, the differences were not statistically significant (Table 1), partly due to the large variation of leaf $\delta^{13}C_R$ in the MB and WB of the IFB.

The correlation between leaf $\delta^{13}C_R$ and $\delta^{13}C$ of the leaf organic components was highly significant ($P<0.01$). On average across all species and treatments, the leaf respiratory CO_2 was 3.8‰ to 5.8‰ more positive than the four leaf organic components (Fig. 3) and

in all cases, the slope of regression line was close to 1 (F test to compare actual slope and 1, P=0.08 to 0.98).

Discussion

Our results obtained by the leaf scale Keeling plot method are comparable to previous studies using a CO_2 free respiration chamber (Park & Epstein, 1961; Jacobson *et al.*, 1970; Smith, 1971; Duranceau *et al.*, 1999; Ghashghaie *et al.*, 2001; Damesin & Lelarge, 2003; Tcherkez *et al.*, 2003). O'leary (1981) pointed out that the ambient CO_2 concentration would influence stomatal conductance and the extent of anapleurotic respiratory CO_2 refixation. However, no experimental study to date has evaluated the influence of a CO_2-free environment on $\delta^{13}C$ of leaf respiratory CO_2. The "leaf Keeling plot" approach we used here provides another means to measure $\delta^{13}C$ of respiratory CO_2 in C_3 plants under CO_2 concentrations close to natural conditions (not more than 150 ppm above ambient levels) and on plants grown in field. In addition, the smaller air sampling flask (100 cc) used is signficantly more convenient for field measurement in remote areas. However, because $\delta^{13}C$ of C_4 respiratory CO_2 is close to surrounding ambient CO_2 (-7‰ to -15‰ vs -8‰) and the change in the $\delta^{13}C$ within the respiration chamber CO_2 is not large enough (Pataki *et al.*, 2003), the leaf-scale Keeling plot method may not apply directly to C_4 plants.

In all 5 C_3 species we studied, respiratory CO_2 was more ^{13}C-enriched than the corresponding leaf organic components; the degree of ^{13}C enrichnment in respiratory CO_2 is species specific but not significantly affected by growth CO_2 concentration. The results rejected our hypothesis (1) and (2), but support hypothesis (3). Compared with soluble sugar, the assumed substrate of dark respiration, leaf $\delta^{13}C_R$ is 3.5‰ to 5.9‰ more positive, which is consistent with the observation of Ghashghaie *et al.* (2001) and

Duranceau *et al.* (1999, 2001) in crop plants. Therefore, we conclude that a 3‰ to 6‰ ^{13}C-enrichment relative to soluble sugar is widespread in leaf respiratory CO_2 of C_3 plants and there is a sigificant species effect. The growth CO_2 concentration did not influence the pattern of ^{13}C enrichment in *P. detoides*. However, *P. deltoides* grown in 800 ppm and 1200 ppm CO_2 concentration in Biosphere 2 showed large variation in the amount of ^{13}C enrichment. We attribute the deviation to the variable CO_2 environment in IFB, which had diurnal fluctuation of CO_2 concentration as much as 300 ppm and varible tank CO_2 injections while maintaining the set concentration. The variable atmospheric CO_2 isotope signature within this portion of Bioshere 2 could have led to the isotopic heterogeneity in the substrate pool of leaf respiration, increasing the variation on $\delta^{13}C$ of leaf respiratory CO_2.

Tcherkez *et al.* (2003) concluded that leaf $\delta^{13}C_R$ in C_3 plants is determined by (1) the carbon source used for respiration, (2) possible isotope effects of respiratory enzymes, and (3) non-statistical distribution of ^{13}C in glucose. It is difficult to justify that an isotopically heavier respiratory substrate was used to any significant extent in addition to the pools measured here (particularly in light of the good correlations between putative substrates and $\delta^{13}C_R$; Fig. 3). Also, previous studies on mesophyll protoplasts (Lin & Ehleringer, 1997) indicated that fractionation likely does not occur in the main stream of respiratory enzyme reactions (glycolysis and TCA cycle). Instead, our synthesis of current results suggest that the non-statistical distribution of ^{13}C in sugars (Rossmann *et al.*, 1991) is the most reasonable explanation for ^{13}C-enriched respiratory CO_2. In dark respiration, the C-3 and C-4 of carbon atoms of glucose are ^{13}C-enriched (-20.9‰ in

average), and are released early in glycolysis. The other four carbon atoms are isotopically lighter (-27.1‰ in average) and can enter secondary metabolisms through the TCA cycle. Thus a greater contribution of the C-3 and C-4 carbon atoms to respired CO_2 would result in the isotopically heavier $\delta^{13}C_R$ we observed. Based on the results of Rossmann *et al.* (1991), we estimate that a 3‰ enrichment in ^{13}C of respiratory CO_2 requires that 82% of the respired carbon be drived from C-3 and C-4. Although this number is possible, it is critical to conduct a more complete carbon budget for the entire leaf in future studies. If the non-statistical distribution of carbon atoms in sugar is the preferred explanation for ^{13}C-enriched respiratory CO_2, the overall leaf carbon budget will significantly influence $\delta^{13}C_R$. It could be predicted that the amount of ^{13}C-enrichment in respiratory CO_2 will be smaller when a higher proportion of photosynthates are used for dark respiration (higher R/A, assuming complete oxidation glucose and a small constant export of carbon to secondary chemical pathways). This view is supported by the work of (Duranceau *et al.*, 2001; Ghashghaie *et al.*, 2001), who found that in the cytoplasmic male sterile (CMS) mutant of *Nicotiana sylvestris* Spegazz and drought treated *Helianthus annuus*, which has higher R/A, the amount of ^{13}C enrichment in respiratory CO_2 is smaller. Short periods of high temperature treatment, which increased plant respiration, also decreased the $\delta^{13}C$ value of respiratory CO_2 in French bean (Tcherkez *et al.*, 2003). Therefore, the R/A – ^{13}C-enrichment relationship, and environmental factors which may influence plant carbon budget (temperature, light, nitrogen, etc.) deserve further study. Alternatively, culturing protoplast in substrates labeled with ^{13}C on certain carbon atoms can directly illustrate the origin of respiratory CO_2 as shown in Lin & Ehleringer (1997) .

The concept of discrimination, defined as $\Delta = (\delta_{source}-\delta_{product})/\delta_{source}-1$, applies to reactions with distinguishable source and product. However, in dark respiration, diverse substrates can be oxidized and a variety of compounds can be produced. Generation of CO_2 is only one branch of the overall metabolic network and it is not clear how an enzyme isotope effect (e.g. pyruvate dehydrogenase, Deniro & Epstein, 1977) may have influence the overall $\delta^{13}C_R$. Therefore we suggest that more suitable terminology is needed the ^{13}C enrichment of leaf respiratory CO_2 in C_3 plants.

The strong correlation between leaf $\delta^{13}C_R$ and $\delta^{13}C$ of leaf organic components suggest that C_3 plants may share similar mechanisms of ^{13}C-enriched respiratory CO_2 generation and that the amount of ^{13}C enrichment is limited within a narrow range. The pattern suggests that it is possible to predict canopy leaf $\delta^{13}C_R$ at the ecosystem level by combining $\delta^{13}C$ of plant organic components and the average amount of ^{13}C enrichment of respiratory CO_2. For example, if the dominant tree species in a forest follow such patterns, we may assume that on average, leaf respiratory CO_2 is 3.9‰ more positive than soluble sugar or 5.8‰ more positive than bulk organic matter of the canopy leaves. Studies on $\delta^{13}C_R$ and $\delta^{13}C$ of organic components in more diverse species, and the effect of environmental factors (temperature, moisture, etc.) on the amount of ^{13}C enrichment in respiratory CO_2 are required to establish the empirical model for ecosystem scale application.

To this end, we surveyed 59 ecosystem level Keeling plot studies in C_3 forests of North and South America (Flanagan *et al.*, 1996; Buchmann *et al.*, 1997a; Buchmann, Kao & Ehleringer, 1997b; Buchmann, Hinckley & Ehleringer, 1998; Flanagan, Kubien & Ehleringer, 1999; Bowling *et al.*, 2002; Fessenden & Ehleringer, 2002; Ometto *et al.*, 2002) to investigate the correlation between ecosystem $\delta^{13}C_R$ and $\delta^{13}C$ of high canopy foliage carbon, surface soil organic carbon and soil respiration. The results of the survey were organized into 3 clusters: tropical, temperate and boreal and were shown in Fig. 4. We observed obvious latitudinal influence on the correlation between ecosystem $\delta^{13}C_R$ and $\delta^{13}C$ of leaf organic carbon (Fig 4). In boreal forests, ecosystem $\delta^{13}C_R$ and leaf organic carbon were comparable to each other. In tropic forest, however, ecosystem respiration became more positive than leaf organic carbon. We did not observe latitudinal pattern in the correlation between $\delta^{13}C_R$ and $\delta^{13}C$ of surface soil organic matter (SOM) or soil respiration, partly due to fewer available data (especially in the tropical zone). In most cases, soil respiratory CO_2 was more positive than ecosystem respiration, and the $\delta^{13}C$ of surface SOM proved to be a poor indicator of ecosystem respiration.

The above survey results also indicated that it may be over-simplistic to predict vegetation $\delta^{13}C_R$ simply by assuming that it is several per mill more positive than leaf organic components. First, soil respiration is more positive than ecosystem respiration in most cases, which, in contrast to our leaf level observations, indicates relatively ^{13}C-depleted vegetation respiration (Fig. 4) and soil respiration is usually a much larger component of ecosystem respration than foliar respiration (Law *et al.*, 2001). In that case, 3-6‰ ^{13}C-enrichment in leaf $\delta^{13}C_R$ seem too high to apply directly to the ecosystem

level. Secondly, the correlation pattern between $\delta^{13}C_R$ and $\delta^{13}C$ of leaf organic carbon changes with latitude (Fig. 4), which indicates that the degree of ^{13}C enrichment in leaf respiratory CO_2 is different among vegetation types. The species included in both previous studies and the current study on ^{13}C-enriched respiratory CO_2 all originate from tropical and temperate areas, where ecosystem respiration shows more significant ^{13}C enrichment relative to foliage $\delta^{13}C$ than in boreal forest. It is possible that in boreal forests, ^{13}C enrichment in leaf respiratory CO_2 is less significant, which requires further studies. Finally, it is important to consider the scale difference when applying leaf-level results to ecosystem processes. Respiratory CO_2 collected on the leaf level is generated in a very short time period (hours) and the substrate composition is less heterogeneous. However, on the ecosystem level, photo-assimilated carbon on short timescales would release to atmosphere over longer timescales as a "lagged and prolonged" flux (Fig 5). Ecosystem respiratory CO_2 sampled is temporally heterogeneous in its origin, composed of carbon pools assimilated at different times, whose $\delta^{13}C$ are subject to the corresponding environmental conditions or secondary metabolic isotope effects. Therefore, the carbon isotopic origin can be much more complex than that at the leaf scale. More studies on $\delta^{13}C_R$ in other plant tissues, like the trunk and roots (Ekblad & Högberg, 2001) and their contribution to total plant respiration are required to scale leaf or plant level results to the ecosystem. Further studies on the isotope effects in litter and soil organic carbon respiration (Santruckova, Bird & Lloyd, 2000) are also important to understand $\delta^{13}C$ of CO_2 generated by these "old" carbon pools. On this regard, direct comparison of $\delta^{13}C$ of assimilated and respired CO_2 at the ecosystem level, at both short and long timescales will be critical when evaluating the influence of temporally

heterogeneous CO_2 on the carbon isotope composition of ecosystem respiratory CO_2. Thus, special cautions must be considered if one attempts to predict $\delta^{13}C$ value of leaf respiratiory CO_2 at ecosystem scale by integraating the average ^{13}C-enrichment in respiratory CO_2 and the leaf organic component signatures of dominant species.

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Table 1. The amount of ^{13}C enrichment in respiratory CO_2 (‰) in comparison with leaf organic components, shown by mean ± SEM (n = 3 – 6). One way ANOVA results of the species effect of ambient grown plants are listed in the last row ($P<0.05$ are considered significant). In *P. deltoides*, ^{13}C enrichnments in respiratory CO_2, relative to all organic components are not significantly influenced by the growth [CO_2] (multicomparison with student's t-tests, P = 0.10 ~ 0.87)

Species	Soluble Sugar	Water Soluble Fraction	Starch	Protein	Organic Matter
P. deltoides Bartr. (Ambient CO_2)	3.83±0.37	3.81±0.52	2.32±0.37	3.30±0.98	4.27±0.53
P. deltoides Bartr. (800 ppm)	5.24±1.68	4.90±1.73	4.39±1.37	--	6.46±1.73
P. deltoides Bartr. (1200 ppm)	4.62±0.86	4.25±1.24	3.70±1.80	--	6.83±1.8
M. paradisiaca	5.86±0.16	3.71±0.29	4.06±0.46	--	5.55±0.47
C. arabica	5.12±0.67	5.11±0.68	2.77±0.52	--	6.14±0.57
E. pinnatum	3.74±0.51	2.67±0.16	1.91±0.83	2.88±.33	4.05±0.55
C. racemosa	3.40±0.39	3.49±0.46	1.28±0.24	--	5.16±0.36
ANOVA of Species Effect	P = 0.008	P=0.049	P=0.020	--	P=0.066

Figure Legends

Figure 1. Leaf scale Keeling plot appratus and result. a: the diagram of sampling apparatus used in this study. b: the result of one *Musa paradisiacal* leaf. (R^2=0.9986 y=5822.9x-23.76, geometric mean regression). The scale of x axis (0.0023-0.0018) is equavalent to [CO_2] of 435-555 ppm. In all measurements, R^2 is greater than 0.95 and in most cases close to 0.99.

Figure 2. Leaf $\delta^{13}C_R$ and leaf organic components in 5 species: a, *Epipremumn pinnatum*; b, *Musa paradisiacal*; c, *Populus deltoides* Bartr (in 3 growth [CO_2]); d, *Coffea arabica*; e, *Clitoria racemosa*. Values are mean±SEM among leaves(n=3-6)..

Figure 3. Relationship between leaf $\delta^{13}C_R$ (y) and $\delta^{13}C$ of leaf organic components (a, soluble sugar; b, water soluble fraction; c, starch; d, organic matter). Data from previous similar studies at leaf scale are also included. R^2 of the regression line (in dashes) and the mean of ^{13}C-enrichment are shown. For all organic components compared, the slopes of linear regression are close to 1 (solid line, P = 0.08 – 0.96). Each point respresents a data set from a single species or treatment, shown as mean ±SEM among leaves.

Figure 4. Correlation of $\delta^{13}C$ between ecosystem respiration and leaf organic carbon (solid circle; a, tropic; c, temperate; e, polar) or surface soil organic carbon (b, tropic; d, temperate; f, polar; solid diamond, depth<10cm) and soil respiration (open diamond). The line shown in each chart is 1:1 line.

Figure 5. A conceptual model showing the “temporal heterogeneity of ecosystem respiration”. Empty parts with bold border describe the fate of atmosphere CO_2 fixed at time A and the arrows symbolize the carbon flux in atmosphere-plant-soil system. Shaded parts with dashed borders are simplified description of the same process beginning at different time (B and C). Darkened oval with bold border shows the temporally heterogeneous origin of ecosystem respiration.

Figure 1.

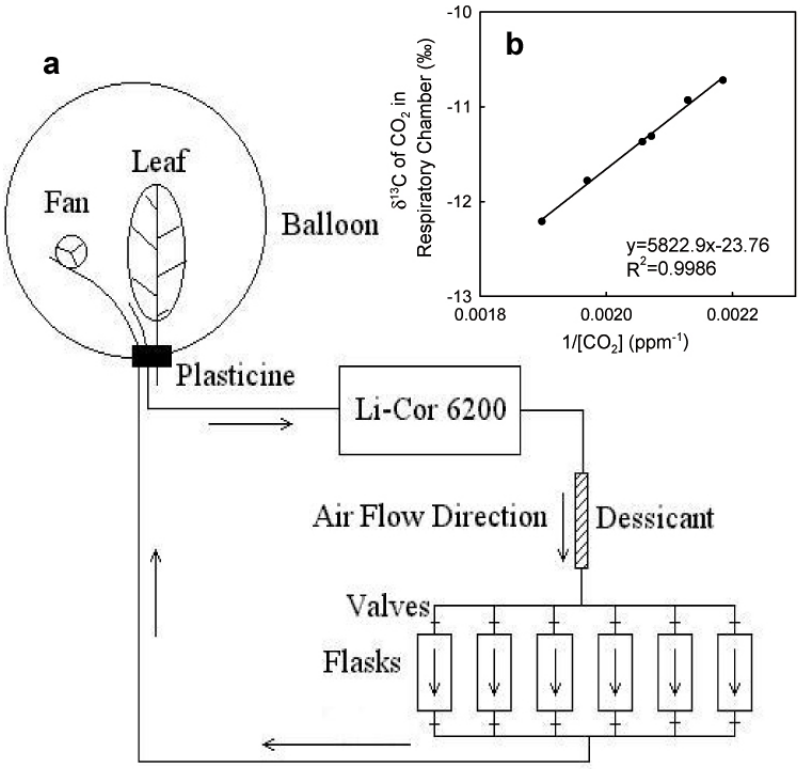

a
Leaf
Fan
Balloon
Plasticine
Li-Cor 6200
Air Flow Direction
Dessicant
Valves
Flasks
b
δ13C of CO2 in Respiratory Chamber (‰)
-10
-11
-12
-13
0.0018
0.0020
0.0022
1/[CO2] (ppm-1)
y=5822.9x-23.76
R2=0.9986

Figure 2.

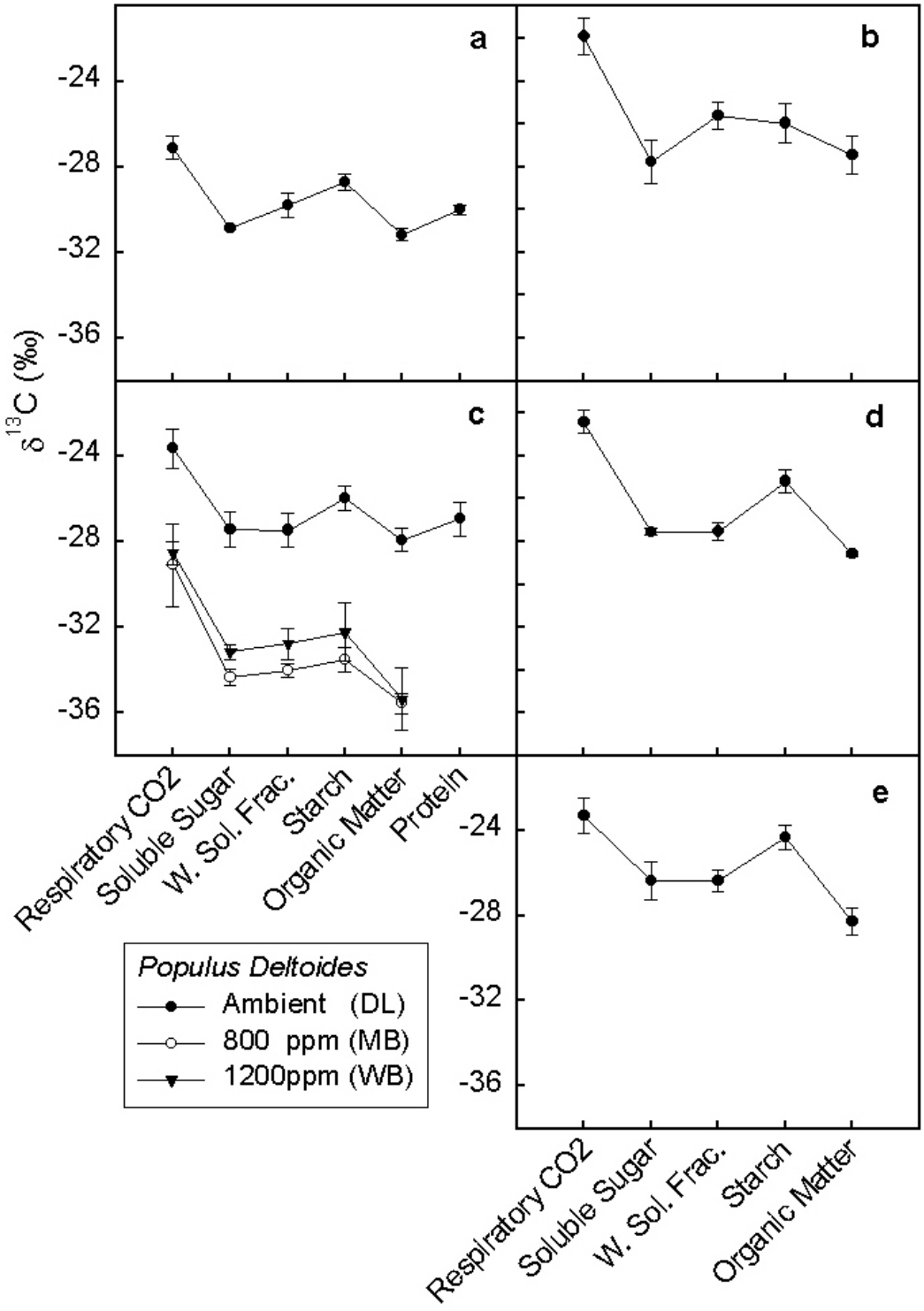

a
b
c
d
e
-24
-28
-32
-36
δ13C (‰)
Respiratory CO2
Soluble Sugar
W. Sol. Frac.
Starch
Organic Matter
Protein
Populus Deltoides
Ambient (DL)
800 ppm (MB)
1200ppm (WB)

Figure 3.

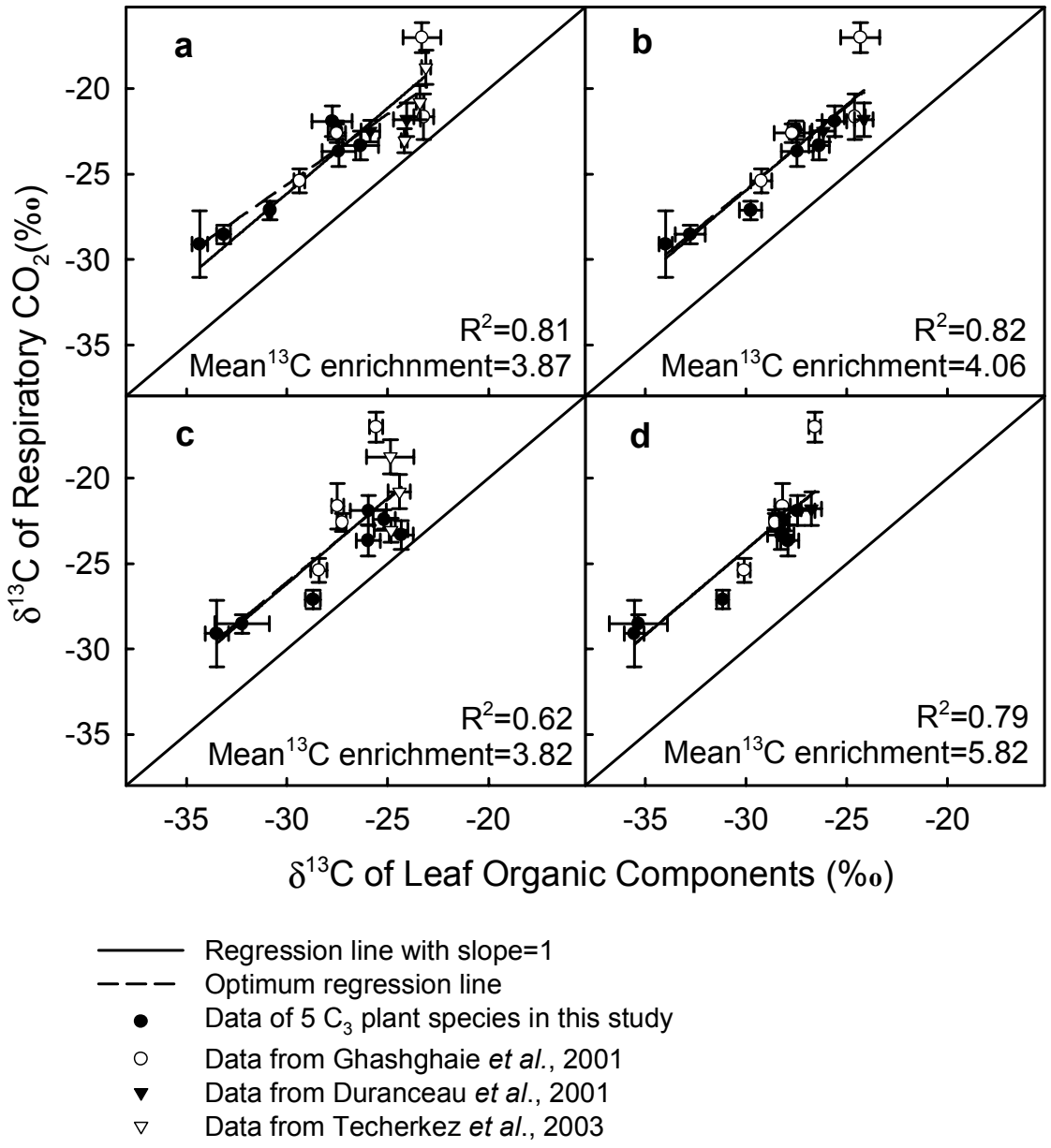
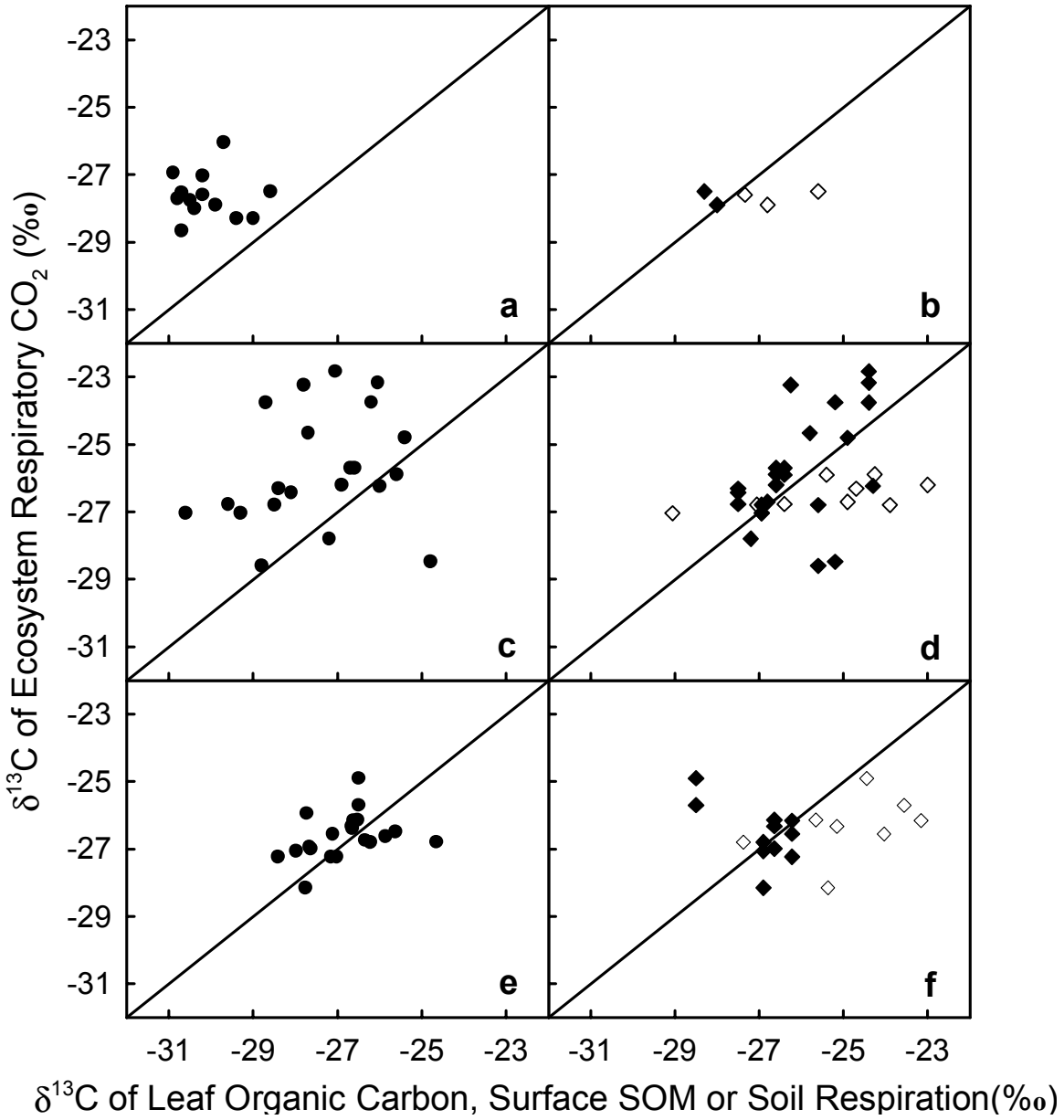

a
b
c
d
R2=0.81
Mean13C enrichnment=3.87
R2=0.82
Mean13C enrichment=4.06
R2=0.62
Mean13C enrichment=3.82
R2=0.79
Mean13C enrichment=5.82
δ13C of Respiratory CO2(‰)
δ13C of Leaf Organic Components (‰)
-20
-25
-30
-35
Regression line with slope=1
Optimum regression line
Data of 5 C3 plant species in this study
Data from Ghashghaie et al., 2001
Data from Duranceau et al., 2001
Data from Techerkez et al., 2003

Figure 4.


-23
-25
-27
-29
-31
a
b
c
d
e
f
-31
-29
-27
-25
-23
δ13C of Ecosystem Respiratory CO2 (‰)
δ13C of Leaf Organic Carbon, Surface SOM or Soil Respiration(‰)

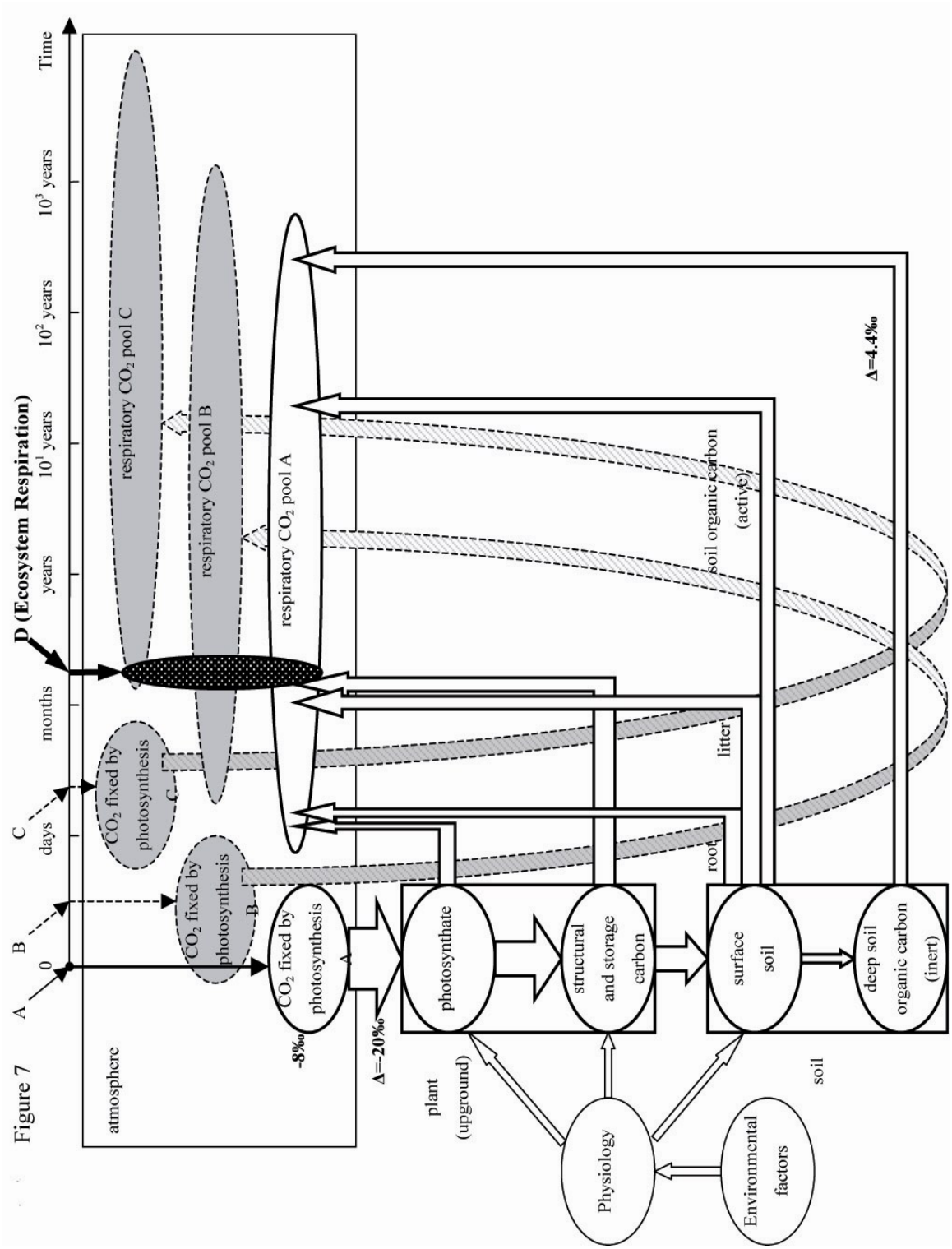


Figure 7
A
B
C
D (Ecosystem Respiration)
0
days
months
years
10^1 years
10^2 years
10^3 years
Time
atmosphere
CO_2 fixed by photosynthesis C
CO_2 fixed by photosynthesis B
CO_2 fixed by photosynthesis A
respiratory CO_2 pool C
respiratory CO_2 pool B
respiratory CO_2 pool A
-8‰
Δ=-20‰
Δ=4.4‰
photosynthate
structural and storage carbon
litter
root
surface soil
soil organic carbon (active)
deep soil organic carbon (inert)
plant (upground)
soil
Physiology
Environmental factors

Conclusions

In this thesis, leaf respiratory properties were upscaled to interpret the ecological processes at the canopy and ecosystem level. In general, I addressed three major questions: (1) How is the leaf respiratory temperature response of *Q. rubra* affected by season, site water availability and canopy height and how does the heterogeneity in leaf respiratory temperature response affect our estimation of canopy foliar carbon loss? (2) How does the seasonal trend of photosynthesis and respiration influence the leaf carbon balance of invasive *B. thunbergii* and co-occurring native shrubs, and would this trend facilitate the invasion of *B. thunbergii* in the understory of a closed forest? (3) Does the stable carbon isotope ratio of leaf respiratory CO_2 correctly reflect $\delta^{13}C$ of the respiration substrate pool? If not, how will the respiratory carbon isotopic effect influence $\delta^{13}C$ of ecosystem respiration? The first question was addressed in chapters 1 & 2 and the second question was address in chapter 3, in an oak dominated northeastern deciduous forest in southern New York State (Black Rock Forest). The third question was studied in controlled environment of greenhouse and Biosphere 2 in Arizona (chapter 4).

Seasonal variation of leaf respiration and leaf properties in *Q. rubra* (Chapter 1)

The respiration of *Q. rubra* leaves were influenced by season, canopy height and site water availability. At a set temperature, leaf respiration rates were higher in both the early and late growing season than in the mid growing season. Upper canopy leaves generally had higher respiration rates than lower canopy leaves. At the drier site, a more significant seasonal pattern in respiration was observed, while at the more mesic site, a stronger

canopy position effect was detected. Leaf respiration of *Q. rubra* was mainly determined by R_0, the base respiration rate, while E_0, a model variable related to the over-all energy of activation of respiration, only varied slightly (52 ± 5 kJ mol^{-1} K^{-1}), and was not influenced by season, site, or canopy position. Leaf properties (specific leaf area, nitrogen, soluble sugars) also varied across the seasons, sites and canopy positions. Leaf nitrogen and reducing monose were significantly correlated to the leaf respiration rate. After isolating single factors (season, site, canopy position), reducing monose could partially explain the seasonality in respiration, and leaf nitrogen (N_{area}) was well correlated to canopy position effect.

These results suggest that the temporal and spatial heterogeneities of respiration need to be considered in ecosystem models, but significant simplifications may be made when modeling *Q. rubra* by assuming a constant temperature coefficient (E_0) or predicting the base respiration rate (R_0) from well understood leaf properties.

Scaling leaf respiration of *Q. rubra* to canopy level (Chapter 2)

Neglecting the season, site or canopy position effects on leaf respiration results in considerable error (up to 130%) in the estimation of stand canopy foliar carbon loss, but assuming a constant E_0 or constant night temperature (average nighttime temperature) results in only a small error (< 5%). I classify the five simplifications into three types. First, the canopy effect on respiration, which is mediated by leaf photosynthetic light acclimation, cannot be neglected in the models since the light gradient in canopy is almost impossible to avoid and a important driver of physiological activity. Second, the

environmental conditions of season or site, and the variation of E_0 have an ecosystem-/ species- specific influence on leaf respiration, so their applicability depends on the particular system studied. Third, assuming a constant night temperature (average night temperature) will not result in an unacceptable error in the estimation of canopy foliar carbon loss. Using a simplified model, I estimated that, from June 8th to October 28th of 2003, the canopy foliar carbon loss of a virtual *Q. rubra* monoculture was 5.4 mol m^{-2} ground and 12.6 mol m^{-2} ground at the drier and mesic sites respectively.

Seasonal variation of leaf respiration in invasive *B. thunbergii* and the co-occurring native shrubs (Chapter 3)

The seasonal pattern of respiratory temperature response (indicated by R_0 and E_0), were significantly different among *B. thunbergii*, *K. latifolia* and *V. corymbosum*. However, a negative correlation between R_0 and E_0 was observed in all three species, which if generalizable, can significantly simplify both the modeling of respiratory temperature responses and field measurements. On an area basis, all three shrubs showed significant correlation between respiration rate (R_{area}, 20 ºC) and leaf nitrogen (N_{area}). The relationship was attributed to the variation of both nitrogen concentration (N_{mass}) and leaf mass per area (LMA) in *B. thunbergii*, but to LMA only in *K. latifolia* and *V. corymbosum*.

The annual canopy foliar carbon loss per unit area leaf production of *K. latifolia* was much higher than in *B. thunbergii* and *V. corymbosum*. Despite the fact that the most pronounced warming occurred in winter in the southern New York state, the evergreen *K.*

latifolia, displayed the lowest warming induced increment in R_c among the three shrubs. This is regardless of the fact that more than one quarter of the annual canopy foliar carbon loss occurred in dormant season (November 16th – March 27th) in this species. Overall, the species-specific warming effect on annual canopy foliar carbon loss was mainly determined by respiratory properties, not the seasonal pattern of warming or phenology.

$\delta^{13}C$ of respiratory CO_2 in five C_3 plants (Chapter 4)

In all five C_3 plants I studied, leaf respiratory CO_2 was always more ^{13}C-enriched than leaf organic components (soluble sugar, water soluble fraction, starch, protein and bulk organic matter). The amount of ^{13}C enrichment displayed a significant species-specific pattern, but the effect of CO_2 treatment (in *Populus deltoids*) was not significant. Therefore, ^{13}C-enriched respiratory CO_2 appears widespread in C_3 plant leaves. However, there is a constant correlation between leaf $\delta^{13}C_R$ and $\delta^{13}C$ of any particular leaf organic components (with slope close to 1). On average, the leaf respiratory CO_2 was 3.8‰ to 5.8‰ more positive than the four leaf organic components.

Among currently hypothesized mechanisms contributing to this phenomenon, non-statistical carbon isotope distribution within the sugar substrates seems most likely. The ecosystem respiration has complex origins with significant "temporal heterogeneity". Therefore, caution should be taken when attempting to predict the $\delta^{13}C$ of leaf respiratory CO_2 at the ecosystem scale by upscaling the relationship between leaf $\delta^{13}C_R$ and $\delta^{13}C$ of leaf organic components.

Phenology and photosynthesis of understory shrubs (Appendix 1), and growth response of alligator weed in a simulated power plant flue gas (Appendix 2)

I found a clear temporal photosynthetic niche separation in *B. thunbergii*, *K. latifolia* and *V. corymbosum*. *B. thunbergii* leafed out approximately one month earlier than *V. corymbosum* and the canopy developed approximately two weeks prior to the overstory trees, so it tends to utilize high irradiance in spring. By contrast, *K. latifolia*, the evergreen, tends to utilize high irradiance in fall and following spring, while *V. corymbosum* generally does not experience high irradiance environment and adapts well to the low irradiance understory. Furthermore, in *B. thunbergii*, light acclimation of photosynthetic capacity was mediated by adjustment in both LMA and N_{mass}, which is much weaker in *K. latifolia* and *V. corymbosum*. Therefore, *B. thunbergii*'s apparent success over the co-occurring natives seems related to a significant spring carbon subsidy and the acclimation to varying irradiance through active nitrogen allocation and leaf morphology modification. In the northeastern US deciduous forest, pronounced winter warming and nitrogen deposition may facilitate the carbon gain of *B. thunbergii* over the natives.

Ultra high [CO_2] in power plant flue gas can significantly enhance the growth of alligator weed. When the acidic components of the flue gas were excluded, the biomass yield of *A. philoxeroides* saturated near 2000 ppm [CO_2] and resulted in 107% enhancement relative to plants in an ambient control. The growth enhancement in aboveground biomass was maintained at 5000 ppm [CO_2] and declined only when atmospheric [CO_2] was above

1%. However, the acidic components in the flue gas significantly offset the observed CO_2 growth enhancement. The demonstrated CO_2-enhanced biomass accumulation rate, if sustainable, would scale to 47 – 66 Mg ha^{-1} yr^{-1}, a rate comparable to the highest yields reported in other biofuel projects. Thus, flue-gas-fed greenhouse bio–carbon–sequestration systems can potentially serve to offset the carbon released from fossil fuel emissions more economically viable than current biofuel programs. More effort is warranted to identify or engineer ultrahigh [CO_2]-/ pollutant-tolerant species for the system.

In summary, this thesis elucidated four novel points that significantly improve our understanding in leaf respiratory properties and their effects on large scale ecological processes. First, I found a species-specific negative correlation between base respiration rate (R_0) and temperature response coefficient (E_0 or Q_{10}), which if can be generalized, will significantly simplify the respiratory temperature model and field measurements. Second, I demonstrated that ignoring temporal and spatial heterogeneity of respiratory temperature response, which is very common in natural ecosystems, can potentially cause large error in the estimation of canopy foliar carbon loss. Thus, simplifications in model parameterization need to be done carefully. Third, my study indicated that warming-induced respiratory carbon loss is mainly determined by the respiratory properties of plants, but not plant phenology or temporal patterns of warming. Finally, I draw the conclusion that ^{13}C enriched respiratory CO_2 is widespread in C_3 plant leaves so caution should be taken in ecosystem level studies to assume that there is no carbon isotope effect during dark respiration. I suggest future research needs to focus on 1) surveying diverse

species to examine the generality of the $R_0 – E_0$ (or Q_{10}) relationship; 2) integrating leaf photosynthesis and respiration to address the whole plant carbon balance and net primary productivity at the ecosystem level, and to evaluate the warming effect on whole plant/ ecosystem carbon balance; and 4) introducing algorithms describing carbon isotope effects of dark respiration to ecosystem models. These efforts will lead to a more accurate prediction on the plant carbon balance and ecosystem carbon budgets in light of global climate change.

Appendix 1:

Temporal photosynthetic niche separation of invasive Japanese barberry (*Berberis thunbergii*) and two co-occurring native understory shrubs in a northeastern US deciduous forest

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Abstract:

Temporal photosynthetic niche separation can be an important mechanism for the invasion of the forest understory by early leafing species. We compared the phenology and photosynthetic characteristics of Japanese barberry (*Berberis thunbergii*), an early leafing invasive shrub, and two co-occurring native species, *Kalmia latifolia* (mountain laurel), an broad leaf evergreen, and *Vaccinium corymbosum* (highbush blueberry), a late leafing deciduous species, throughout the 2004 growing season. *B. thunbergii* leafed out approximately one month earlier than *V. corymbosum* and the canopy developed approximately two weeks prior to the overstory trees. The photosynthetic capacity (characterized by V_{cmax} and J_{max}) of *B. thunbergii* was the highest in spring when the overstory canopy was open, and declined with canopy closure. The 2003 overwintering leaves of *K. latifolia* displayed high V_{cmax} and J_{max} in spring 2004. In the new leaves of *K. latifolia*, produced in 2004, the photosynthetic capacity gradually increased to a peak in mid September, and then showed signs of downregulated in late November. *V. corymbosum*, by contrast, maintained low V_{cmax} and J_{max} throughout the growing season, showing typical shade adaptation. In *B. thunbergii*, light acclimation of V_{cmax} and J_{max} was mediated by adjustment in both LMA and N_{mass}. Similar, but weaker N_{mass}/ LMA adjustment was also found in *K. latifolia*, but not in *V. corymbosum*. These results indicate clear temporal photosynthetic niche separation in these three shrubs. *B. thunbergii* and *K. latifolia* tend to utilize high irradiance in spring, and high irradiance in fall and following spring respectively. By contrast, *V. corymbosum* generally does not experience high irradiance environment and adapts well to the low irradiance understory. *B. thunbergii*'s apparent success over the co-occurring natives seems related to a

significant spring carbon subsidy and acclimation to varying irradiance through active nitrogen allocation and leaf morphology modification. In the northeastern US deciduous forest, pronounced winter warming and nitrogen deposition may facilitate the carbon gain of *B. thunbergii* over the natives and may further contribute to its invasion of the forest understory.

Introduction

Biological invasions have been greatly facilitated by global transportation and have become an important component of human-mediated global change (Cohen and Carlton 1998; Mooney and Hobbs 2000). Once prolific populations are established, invasive plants can change the structure and/or function of native ecosystems (Otto et al. 1999; Tilman 1999; Wyckoff and Webb 1996), and threaten global biodiversity (Chapin et al. 2000; Dukes and Mooney 1999; Vitousek et al. 1996). Although competitive advantage of invasive plants appears obvious, recent comparative studies summarized that, in fact, relative performances of invasive plants to the co-occurring natives are highly condition- or context-dependent (Daehler 2003). In general, invasive species show the largest advantage over the natives in environments with high resource availabilities (nutrient, water, light etc.) or high levels of disturbance, which are commonly associated with human activities.

Although undisturbed, low-resource habitats are less likely to be invaded (Cassidy et al. 2004; Hutchinson and Vankat 1997; Mazia et al. 2001; Totland et al. 2005), a number of invasive plants can successfully establish and dominate the understory of closed forests (Harrington et al. 1989a; Luken et al. 1997; Meekins and McCarthy 2001; Sliander and Klepeis 1999; Webb and Kaunzinger 1993), which have relatively low light and nutrient levels (Denslow et al. 1998; Parsons et al. 1994). An important mechanism of invasion in the forest understory lies in temporal photosynthetic niche separation from co-occurring native trees, shrubs and herbs (Harrington et al. 1989a; Myers and Anderson 2003; Zotz et al. 2000). Many successful understory invaders leaf out earlier than their native

counterparts and have extended leaf longevity (Harrington et al. 1989a; Harrington et al. 1989b; Myers et al. 2005; Nelson et al. 1982; Schierenbeck and Marshall 1993; Zotz et al. 2000). Some invaders can produce sun leaves in early spring and shade leaves following canopy closure (Myers and Anderson 2003; Myers et al. 2005). These phenological characteristics can result in a carbon subsidy in the spring and/or fall, which is particularly important given the high understory light availability at these times (contributing as much as 27%-35% of the annual carbon gain, Harrington et al. 1989a). However, many pervious studies have been based on instantaneous gas exchange measurements (*e.g*. maximum photosynthetic rates in saturating light or the *in situ* photosynthetic light response curves), limiting the mechanistic interpretation of these studies (*but see* Rothstein and Zak 2001). In early leafing understory invaders, we have very little knowledge regarding the physiological acclimation of photosynthesis to the dynamic light regime during the early and/or late growing season.

Berberis thunbergii (Japanese barberry, BT) is an understory shrub that successfully invades the deciduous forests of the eastern United States. Currently, *B. thunbergii* can be found within the interior of many protected forest areas (Ehrenfeld 1997; Hunter and Mattice 2002) and may change the component of native plants, soil properties, microbial community structure and functions (Ehrenfeld et al. 2001; Kourtev et al. 2002; Kourtev et al. 2003; Kourtev et al. 1998). Various factors may facilitate the invasion of *B. thunbergii* in the understory, such as ability to exclude native competitors, low herbivore pressure, shade tolerance, vigorous growth and fecundity (Sliander and Klepeis 1999). *B. thunbergii* leafs out approximately one month before the overstory trees and most native

shrubs (Sliander and Klepeis 1999), so it can potentially utilize the higher irradiance in spring to gain a carbon subsidy, which may significantly promote growth and reproduction.

In this study, we compared the phenology and photosynthesis of *B. thunbergii* with two other common native shrubs (mountain laurel, *Kalmia latifolia*, KT, evergreen; and high bush blueberry, *Vaccinium corymbosum*, VC, late leafing deciduous) in a deciduous forest in Southeastern New York State throughout the 2004 growing season. The three shrubs have similar regional distribution in the northeastern states (USDA plants database, http://plants.usda.gov/). Our primary goal was to examine if *B. thunbergii* gains a spring carbon subsidy by temporal photosynthetic niche separation from the native trees and understory shrubs. In order to gain a mechanistic understanding of the regulation of the photosynthetic capacity, we characterized V_{cmax} (maximum carboxylation rate of Rubisco) and J_{max} (RuBP regeneration capacity mediated by maximum electron transport rate). Furthermore, we analyzed the relationships between photosynthetic characteristics and leaf properties to investigate how photosynthesis acclimates to the dynamic seasonal light regime. We hypothesized that 1) the photosynthetic capacity of *B. thunbergii* is higher in spring than in summer and fall, 2) *B. thunbergii* has higher photosynthetic capacity than the co-occurring natives throughout the growing season, and 3) the leaf photosynthetic capacity of *B. thunbergii* is strongly correlated to both leaf nitrogen concentration (N_{mass}) and leaf mass per unit area (LMA). Through this case study of *B. thunbergii*, we hope to shed light on the physiological mechanisms used by understory invaders to temporally separate its photosynthetic niche from the co-occurring natives. In

addition, a mechanistic understanding will help to evaluate the invasiveness of other early leafing species in the northeastern US within the framework of regional environment change (warming, nitrogen deposition etc.).

Materials and Methods

Description of study site

The Black Rock Forest is a 1500 ha preserve in Hudson Highlands of Southeastern New York State, locating at 41°24' N and 74°01' W with elevations ranging from 150 to 450m above sea level. The air temperature is strongly seasonal, with monthly average temperature ranges from -2.7°C in January to 23.4°C in July. The average annual precipitation is 1.2m (Black Rock Forest field station database). The forest is a *Quercus* dominated secondary growth forest that characterizes the northeastern United States. The most recent flora survey was carried out during 1990 – 1998, and identified 729 vascular species of 117 families (Barringer and Clemants 2003). Among these, approximately 20% were introduced and several of these were considered to be invasive. Japanese barberry (*Berberis thunbergii*) is one of the most critical invasive species in the forest understory, which widely invades roadside and previously cut areas. Common co-occurring native shrubs include *Gaylussacia baccata* L. (huckleberry), *Kalmia latifolia* L. (mountain laurel), *Rhododendron periclymenoides* L. (pink azalea), and *Vaccinium spp.* (blueberries) (Schuster, personal communication). Meteorological conditions within the forest are obtained from several standard meteorological stations run by the Black Rock Forest staff.

Leaf phenology and ontology survey

Throughout the growing season of 2004, leaf phenology and ontogeny of *B. thunbergii* was regularly surveyed at five sites with dense, continuous *B. thunbergii* cover in BRF. The openness of the *B. thunbergii* canopy was estimated optically using a canopy

analyzer (Li-2000, Licor Inc., Lincoln, NE, USA). In each site, five branches were randomly collected and from each branch, the length of the three largest leaves was measured to track the leaf ontogeny. The upper canopy openness was determined by hemispheric photography (Gap Light Analysis, Simon Frazer Univ. BC, Canada & Institute of ecosystem studies, NY, USA). The leafing phenology of *V. corymbosum* and *K. latifolia* was similarly observed.

Gas exchange measurements

During the 2004 growing season, leaf-level gas exchange measurements were made *in situ* during five periods: May 6th – 8th (day 127 – 129, BT & KL), June 12th – 13th (day 164 – 165, BB, JB & ML), August 23nd – 25th (day 236 – 238, VC, BT & KL), September 24th – 25th (day 268 – 269, VC, BT & KL), and November 16th – 17th (day 321, KL). Measurements were made near Alec Meadow pond, an artificial reservoir located centrally in the Black Rock Forest (41° 24' N; 74° 00' W) and surrounded by oak woods (Nagel and Griffin, unpublished), where three individuals, respectively of *B. thunbergii*, *K. latifolia* and *V. corymbosum*, were permanently tagged. All individuals were fully exposed in an open canopy during winter and spring, but shaded by the upper canopy during the majority of the summer and fall (Xu, personal observation). For each species, measurements were made on two top canopy leaves from different branches of each selected individual. During May 6th – 8th, measurements were made only on the most fully-expanded leaves of *B. thunbergii* and the 2003 overwintering leaves of *K. latifolia*. *V. corymbosum* was not measured at this time since its buds had just opened and the leaves were obviously immature. In all other cases, measurements were made on

intact, visually mature leaves (well expanded, with well-developed waxy cuticle, etc.) for all three species. Beginning on June 12th, measurements of *K. latifolia* were all made on leaves produced in 2004 .

Leaf photosynthetic characteristics were measured with a portable photosynthesis system (Li-Cor 6400, Lincoln, NE, USA) equipped with CO_2 and temperature control modules. A steady-state responses of photosynthesis (A) to internal leaf CO_2 partial pressure (A-C_i Curve) was generated for each selected leaf. External CO_2 partial pressure (C_a) was set to 10 or 11 levels between 5 and 200 Pa. At each C_a set point, photosynthetic parameters were recorded when gas exchange had equilibrated (taken to be when the coefficient variation for C_a between the sample and reference analyzer was below 1%), which typically took 1-2 minutes to achieve. A constant, saturating photosynthetic photon flux density (1500 μmol m^{-2} s^{-2} PPFD) was supplied by blue-red light emitting diodes mounted above the leaf cuvette. After generating the A-C_i curve, the leaf was stabilized in 37.5 Pa C_a (ambient CO_2 concentration) and moderately high light level (600 μmol m^{-2} s^{-2} PPFD) for 8 – 10 minutes to minimize the effect of the changing C_a on stomata. Then, the leaf was exposed to 37.5 Pa C_a and a photosynthetic response to light (an A-Q curve) was measured. The steady-state photosynthesis was recorded at 10-11 steps from 10 μmol m^{-2} s^{-2} PPFD to saturating light level (800 – 1500 μmol m^{-2} s^{-2} PFD, depending on species and season). During all measurements, leaf temperature was maintained at 20 ºC using thermoelectric coolers. Due to the seasonal variation of humidity and temperature manipulation in cuvette, the leaf water vapor pressure deficit (VpdL) varied across the growing season, 1.7 ± 0.3, 1.2 ± 0.2, 0.9 ± 0.3, 0.7 ± 0.2 and 1.3 ± 0.2 kPa (mean ±

standard deviation across the five sampling times respectively). All measurements were made on clear, warm days, between 9:30 AM to 2 hours before sunset.

The A-Q curves were analyzed by the software Photosynthesis Assistant (Dundee Scientific, Scotland, UK). The maximum quantum yield (QY_{max}) was calculated based on the light-limited portion of A-Q curve. To exclude the influence of C_i on QY_{max}, the photosynthetic response to light were corrected to C_i of 30.0 Pa (Singsaas et al. 2001). The convexity, light saturation point and maximum photosynthetic rates at ambient [CO_2] and saturating light (A_{max}) measured in the A-Q curves were strongly affected by leaf stomatal conductance, which is subjected to cuvette environmental manipulation (mainly VpdL). Therefore, we have used a mechanistic model calculated estimation to compare A_{max} among species and season (see below).

With the same software, A-C_i curves were analyzed to calculate the parameters potentially limiting to photosynthesis: maximum carboxylation rate of Rubisco (V_{cmax}) and RuBP regeneration capacity mediated by maximum electron transport rate (J_{max}). The calculations were made based on the biochemical model described by Farquhar et al. (1980). The measured stomatal conductance at ambient CO_2 and saturating light (g_{smax}) in A-Q curve was corrected for the influence of VpdL by an empirical model,

$g_s = -m + b \times \ln VpdL$ (Oren et al. 1999),

to obtain an estimation of average g_{smax} at 7-day average air Vpd bracketing the measurement periods (assuming the leaf temperature is the same as the air). Then, A_{max} (expressed on an area and a mass basis) was calculated for comparison among the species

and the season. The photosynthetic nitrogen use efficiency ($PNUE_{max}$) at A_{max} was calculated as the photosynthetic rate at per gram leaf nitrogen. To estimate the general effect of g_{smax} on A_{max}, relative stomatal limitation of photosynthesis (ls) was calculated as,

$ls = (A_0 - A_{max}) / A_0$ (Farquhar and Sharkey 1982),

where A_0 is a photosynthetic rate which would occur if stomatal resistance to CO_2 diffusion is zero. A_0 used here was the light saturating photosynthetic rate at 37.5 Pa C_i.

Leaf analysis and chlorophyll fluorescence survey

Following the photosynthetic measurements, the area of the measured leaf was determined using a leaf area meter (Li-3000, Li-cor Inc. Lincoln NE, USA) and then dried in 60°C oven for a minimum of 48 hrs. The dried leaf material was weighed and ground to fine powder for nitrogen analysis with a CHNS/O analyzer (2400 Series II, Perkin-Elmer, Boston, MA, USA). Leaf mass per area (LMA) was calculated from the leaf area and dry weight. For the leaf samples taken in May 2004, leaf mass was not measured, so the leaf LMA was surveyed in May and June 2005 on the same plant individuals to get an approximate estimation. LMA measured in June 2005 and June 2006 displayed only a 3% average difference, indicating the estimation is appropriate for *B. thunbergii* leaves in May 2004.

To investigate the possible leaf photo- inhibition/ protection in shrub leaves, F_v/F_m was surveyed with a chlorophyll fluorometer (FMS2, Hansatech, Norfolk UK) on 6 leaves for each of the plant individuals on which gas exchange measurements were made. The

leaves surveyed were dark adapted *in situ* for a minimum of 20 minutes and chlorophyll fluorescence measurements were made.

Statistical analysis

The seasonal effect on most variables were tested by ANOVA (Statistica, Statsoft Inc, Tulsa, OK, USA) and the means were compared amongst species/season throughout the growing season of 2004 with a simple t test (Excel, Microsoft, Seattle, WA, USA). Since the data of F_v/F_m did not match with the assumption of a normal distribution, nonparametric methods applicable to samples with unknown distribution (Kruskal-Wallis ANOVA test and Mann-Whitney U test), were used for analysis (Statistica, Statsoft Inc, Tulsa, OK, USA). Differences were considered significant if the probabilities were less than 0.05. The relationships between photosynthetic characteristics, or between photosynthetic parameters and leaf properties were analyzed using linear regression.

Results

Leaf ontology and phenology

The leaf phenology of *B. thunbergii* was several weeks in advance of the co-occuring native shrubs and upper canopy trees (Figure 1). *B. thunbergii* had two major leaf flushes, in early spring and early summer respectively. The existing buds on old stems of *B thunbergii* opened in late March of 2004 (~90 day) and the canopy was well established by late April (~120 day). In contrast, the overstory tree canopy did not close until mid May (~135 day), lagging the *B. thunbergii* approximately 2 weeks. The 2004 new branches of *B. thunbergii* began elongating in early May (~125 day). The second leaf flush during mid May was mainly from these new branches and significantly increased the LAI of the *B. thunbergii* canopy over a two week period. The *B. thunbergii* leaves from the first flush gradually expanded throughout spring and early summer, while the leaves of the second flush expanded very quickly during the first two weeks after they appeared in mid May. Leaves of both flushes were fully expanded by early June (~160 day) and did not show apparent morphological difference (e.g. LMA, leaf length). By comparison, the two co-occurring native shrubs had only one major leaf flush. The buds of deciduous *V. corymbosum* opened approximately one month after *B. thunbergii* (~120 day). For the evergreen *K. latifolia*, the old leaves (produced in 2003) did not show senescence till mid June (~165 day) and new 2004 leaves flushed in mid May (~130 day). The decrement of LAI in *B. thunbergii* canopy during mid August (~230 day) to early October (~280 day) indicates that the first flushed leaves might gradually shed during the growing season. However, the defoliation of *B. thunbergii* in late fall (300 – 320 day) was generally synchronous to that of the upper canopy trees and *V. corymbosum*.

Photosynthetic characteristics

Photosynthetic characteristics differed significantly between species and across the growing season for most variables (Figure 2). Overall, J_{max} and V_{cmax}, which regulate the maximum photosynthetic capacity in optimum CO_2 and light conditions, displayed a similar response (Figure 2a, b). The highest V_{cmax} and J_{max} of *B. thunbergii* occurred in early May (128 day), when the upper canopy was still open, and declined throughout the growing season. Similarly, the 2003 overwintering leaves of *K. latifolia* also showed high V_{cmax} and J_{max} in early May. In the 2004 produced *K. latifolia* leaves, V_{cmax} and J_{max} increased gradually throughout the growing season and reached their peak by the end of September (271 day), but the values dropped again in late November (322 day). By contrast, V_{cmax} and J_{max} in *V. corymbosum* were significantly lower than in the other two shrubs and displayed a much smaller seasonal variation, declining slightly from mid June (167 day) to late September. Moreover, *B. thunbergii* had a higher J_{max} to V_{cmax} ratio than *V. corymbosum* and *K. latifolia* (Figure 2c). Although the J_{max}/V_{cmax} of *B. thunbergii* and *V. corymbosum* displayed an increasing trend throughout the growing season, the deviation was large and the seasonal effect was not statistically significant. By contrast, J_{max}/V_{cmax} of *K. latifolia* varied seasonally and the highest value (~2.6) occurred both in early May and late September.

B. thunbergii and *K. latifolia* displayed low F_v/F_m (< 0.8) in early May and late November (Figure 2f), when high light from open upper canopy might cause some degree of photo- inhibition/ protection in photosynthetic system II (PSII). The three shrubs

displayed low QY_{max} values in general (0.04 – 0.07, Figure 2g). The seasonal variation of QY_{max} was not significant for *B. thunbergii* and *V. corymbosum*, but *K. latifoliar* showed significantly lower QY_{max} in May and November, which can also be attributed to photo-inhibition/ protection in high light environment.

The response of A_{max} on an area basis was similar to that of V_{cmax} and J_{max} throughout the year (Figure 2f). On a mass basis, the seasonal variation of A_{max} was largely absent for *B. thunbergii* and *K. latifolia*. Throughout the growing season, *B. thunbergii* displayed highest mass based A_{max} among three species (Figure 2g). The lowest $PNUE_{max}$ occurred in early and late growing season for all threes species. In general, leaves of *B. thunbergii* had higher $PNUE_{max}$ than *K. latifolia* and *V. corymbosum*, especially during the mid growing season (Figure 2h).

The seasonal patterns in g_{smax} were similar in *B. thunbergii* and *K. latifolia* from May to September: g_{smax} declined slightly from May to June, but then increased to a peak in September. In November, g_{smax} of *K. latifolia* declined again. The seasonal effect on g_{smax} was not significant in *V. corymbosum* (Figure 2i). The stomatal limitation on A_{max} decreased throughout the growing season (Figure 2j) in all three shrubs. Overall, *B. thunbergii* showed the highest g_{smax} and lowest ls among the three shrubs.

Leaf characteristics

On an area basis, leaf nitrogen (N_{area}) of *B. thunbergii*, *V. corymbosum*, and *K. latifolia* displayed different pattern of seasonal response (Figure 3a). *B. thunbergii* had very high

N_{area} in early May, but the values reduced significantly in mid June and then continued to gradually decline during the growing season. The 2003 overwintering leaves of *K. latifolia* maintained high N_{area} in early May while the 2004 produced leaf accumulated nitrogen throughout the growing season. By contrast, N_{area} of *V. corymbosum* was lower than in the other two shrubs and the seasonal variation was generally small. On a mass basis, seasonal responses of leaf nitrogen (N_{mass}) in *B. thunbergii* and *V. corymbosum* were similar to that of N_{area}. By comparison, N_{mass} of *K. latifolia* was much lower than the other two shrubs and the seasonal variation was small (Figure 3b), indicating that the large seasonal variation of N_{area} was mainly determined by the change in leaf thickness. Among the three shrubs, *K. latifolia* had much thicker leaf (higher LMA) than *B. thunbergii* and *V. corymbosum* and displayed significant seasonal variation (Figure 3c). On average, *B. thunbergii* leaves were thickest in early May, but the overall seasonal effect was only marginally significant (P=0.051). *V. corymbosum* leaves were thinnest and LMA did not vary seasonally.

Relationships between photosynthetic parameters and leaf characteristics

The relationships between V_{cmax} and leaf nitrogen/LMA were species-specific (Figure 4). *B. thunbergii* leaves showed significant relationships between V_{cmax} and N_{area}/ N_{mass}/ LMA (R^2=0.52 – 0.76, Figure 4a, b, c). Although there also was a strong $V_{cmax} - N_{area}$ relationship in *K. latifolia* (R^2=0.57, Figure 4a), the $V_{cmax} - N_{mass}$/ LMA relationships were much weaker in this speices (R^2=0.20 – 0.26, Figure 4b, c). Furthermore, *K. latifolia* leaves in late November had high nitrogen/ LMA but a relatively low V_{cmax} (marked in dark grey, Figure 4) and thus, were these points were excluded from the

analysis of the $V_{cmax} - N_{area}/ N_{mass}/$ LMA relationships. *V. corymbosum* displayed only a general positive $V_{cmax} - N_{area}$ relationship (R^2=0.39, Figure 4a). In general, the relationships of J_{max} or A_{max} to leaf characteristics were similar to that of V_{cmax}. However, *V. corymbosum* showed a marginally significant $J_{max} - N_{mass}$ (R^2= 0.20, P = 0.04, Figure 4e).

In *K. latifolia*, we found that $PNUE_{max}$ was significantly correlated to F_v/F_m (R^2=0.96, Figure 5a) and QY_{max} (R^2=0.89, Figure 5b). These relationships indicate that photo-inhibition/ protection can lead to low photosynthetic nitrogen use efficiency in spring and late fall for this evergreen species.

Discussion

Temporal photosynthetic niche separation

B. thunbergii, *K. latifolia* and *V. corymbosum* show clear temporal photosynthetic niche separation (highlighted by V_{cmax}, J_{max} and A_{max}, Figure 2a, b, f). We calculated leaf photosynthetic rates of the three shrubs in the prevailing light regime across the growing season for a conceptual comparison (Figure 6). In general, the photosynthetic rates were comparable between the three species during any particular period of the growing season (assuming the leaves are present), but the temporal patterns of carbon gain were drastically different. *B. thunbergii* tends to utilize high irradiance during the spring when the overstory canopy is open, showing a peak of photosynthetic capacity/ rate before upper canopy closure. In summer, leaves of *B. thunbergii* acclimate well to low light and downregulate the photosynthetic capacity. *K. latifolia*, on the other hand, produces new leaves after the overstory canopy has closed and then builds up the photosynthetic capacity of these leaves during the remainder of the summer, thereby pre-acclimating to the coming high irradiance environment after the overstory defoliates. The major carbon gain in this species may occur in late fall, and the following spring. In winter, the photosynthetic apparatus appears photo- inhibited/ protected but photosynthetic capacity recovers in the spring. The area based photosynthetic capacity/ rate of *K. latifolia* is comparable or higher than *B. thunbergii*. *V. corymbosum* by constrast, showed photosynthetic characteristics of a typical shade species and mainly utilizes low light in the understory. Among the three shrubs, *V. corymbosum* has the lowest V_{cmax}, J_{max} (Figure 2a, b) and respiration rates (Xu, unpublished data).

We estimate that, at leaf level, the spring carbon subsidy of *B. thunbergii* during the two weeks in late April – early May, before upper canopy closure, can contribute up to one third of the total annual carbon gain. The propitiation of spring carbon subsidy is similar to that in two exotic shrubs, *Rhamnus cathartica* (35%) and *Lonicera X bella* (29%), observed in mid-west (Harrington et al. 1989a). Compared with *V. corymbosum*, the early leafing phenology of *B. thunbergii* may result in 50% higher leaf level carbon gain annually. *K. latifolia* has comparable or even higher leaf level carbon gain in the open canopy than *B. thunbergii*, but the cost of producing *K. latifolia* leaves is much higher (Nagel and Griffin unpublished data). This is further demonstrated in the present study, as *B. thunbergii* has a higher A_{max} when expressed on a biomass basis, (Figure 2g) and higher $PNUE_{max}$ (Figure 2h) than *K. latifolia* and *V. corymbosum*. In summary, *B. thunbergii* possesses a greater capacity to gain carbon per unit investment in leaf tissue/ photosynthetic apparatus than the other two species, and thus gains further advantage through photosynthetic niche separation.

Regulation of photosynthetic characteristics

Stomatal conductance usually imposes the largest resistance to diffusion of CO_2 and leads to a C_i limitation on photosynthesis (Farquhar and Sharkey 1982). *B. thunbergii* displayed highest g_{smax}, and thus the lowest stomatal limitation on photosynthesis among the three shrubs (Figure 2i, j). Overall, the estimated stomatal limitation on photosynthesis of *B. thunbergii* was slightly lower than for *K. latifolia* in early May and was approximately 6 to 8 % lower than *K. latifolia* and *V. corymbosum* throughout the summer. Stomatal limitation of photosynthesis in evergreen herbs has been previously

observed and it was proposed that g_{smax} may be regulated by photosynthetic capacity to maintain a constant C_i (Yoshie and Yoshida 1987). However, in our study, all three shrubs displayed the highest stomatal limitation on photosynthesis in the early growing season, when photosynthetic capacity was high yet stomatal conductance was low. The estimated stomatal limitation declined throughout the year and in these shrubs, greater stomatal conductance during the most humid part of the growing season to enhance carbon gain.

In addition to decreased stomatal limitation of photosynthesis, *B. Thunbergii* also more dynamically adjusted leaf nitrogen investment in the photosynthetic apparatus relative to the other two native shrubs. Furthermore, in this study, we found a positive correlation between photosynthetic capacity (V_{cmax}, J_{max}, and A_{max}) and N_{area} in all three shrubs. Since light is a critical limiting factor of photosynthesis in understory shrubs, these area based relationships directly reflect the association between light use and nitrogen investment in the photosynthetic apparatus. However, the investment of photosynthetic apparatus per unit leaf area can be adjusted by both nitrogen concentration (N_{mass}) and/or leaf thickness/ mesophyll density as indicated by the LMA. *B. thunbergii* displayed highly significant correlations (Figure 4b, c, e, f, h, i) between photosynthetic capacity and N_{mass} and LMA, highlighting that photosynthetic capacity is subject to adjustments in both N_{mass}/ LMA. In particular, *B. thunbergii* shows a greater range of N_{mass} than the two co-occurring natives, which is not likely to be simply related to environmental N availability across the growing season because there is no homogenous seasonal trend in the three shrubs. The phenomenon indicates that nitrogen allocation may play more

important role to adjust photosynthetic capacity seasonally in *B. thunbergii* or *B. thunbergii* can compete for N more effectively in early growing season. By comparison, the correlation between photosynthetic capacity and N_{mass}/ LMA is much weaker in *K. latifolia* (excluding November leaves), and only marginally significant in *V. corymbosum*. Since *V. corymbosum* leaves do not typically experience a high irradiance environment, they are more typical of shade adapted leaves and show little seasonal adjustments of photosynthetic capacity by N_{mass} and/ or LMA.

Declined F_v / F_m and QY_{max} in November indicate that significant photoprotection may be important to overwintering leaves of *K. latifolia*. In winter, the dark reactions of photosynthesis would be limited by temperature and a considerable part of the absorbed light energy can not be used. In response, plants typically downregulate photosynthesis to protect the photosynthetic apparatus from photodamage (*reviewed by* Öquist and Huner 2003). Previous studies have identified two major protective strategies in evergreen plants: heat dissipation of light energy mediated by the xanthophyll cycle and degradation of proteins responsible for generating high-energy electrons (*reviewed by* Adams et al. 2004). The former is very common in evergreen species, while the latter was found in fewer species and can occur when a shortened photoperiod is combined with moderately low day/night temperature (15/10 ºC). In *K. latifolia*, high leaf nitrogen in late November may indicate that the photosynthetic apparatus was preserved, but partially non-functional since A_{max} was downregulated by approximately a third. Thus, in the late fall high irradiance environment, photoprotection significantly decreased the photosynthetic nitrogen use efficiency ($PNUE_{max}$, Figure 5). Furthermore, the decreased J_{max} to V_{cmax}

ratio in November indicates decreased electron transport capacity, which may also be related to the degradation of PSII proteins. Further investigation is required to confirm the precise mechanism responsible. This photoprotection may limit the carbon gain of For *K. latifolia* in winter.

Photosynthetic acclimation to the prevailing light regime

There are a diversity of physiological mechanisms for photosynthetic acclimation to varying light levels, including producing new leaves tuned to the prevalent light conditions (Langenheim et al. 1984; Popma and Bongers 1988; Popma and Bongers 1991), modifying leaf morphology (Oguchi et al. 2003; Oguchi et al. 2005; Rothstein and Zak 2001), redistributing nitrogen within and between the leaves (Avalos and Mulkey 1999; Brooks et al. 1994; Brooks et al. 1996), reallocating nitrogen among light harvesting machinery, the main carboxylating protein (Rubisco) and proteins associated to bioenergetics (Frak et al. 2001; Muller et al. 2005). For species with only a single leaf flush, mature leaves developed in a particular light regime usually are not morphologically plastic. In these species, Oguchi et al. (2003, 2005) proposed two alternative strategies of light acclimation and termed these “optimistic” or “pessimistic”. The so-called “optimistic” species builds leaves with much physically open space along the mesophyll cell surfaces (usually a thicker leaf) so that the photosynthetic apparatus can be expanded for high light acclimation. This strategy pre-acclimates the leaf to a future high irradiance environment, but the investment and maintenance cost in biomass are higher. By contrast, species defined as “pessimistic” have thinner, shade adapted leaves and reduced costs. Species with this strategy have a limited potential to increase

the photosynthetic apparatus even if the ambient irradiance is increased. In our study, *K. latifolia* would be characterized as an "optimistic" species, which continues to build photosynthetic capacity throughout leaf ontogeny. Although N_{mass} and LMA both affect V_{cmax} and J_{max}, the relationships are not strong (particularly for V_{cmax}, Figure 6b, c, e, f), perhaps reflecting a limited range of variation in N_{mass} and LMA. By contrast, *V. coryobosum* would be characterized as a "pessimistic" species with the buds opening late and the leaves maturing quickly. *V. coryobosum* possesses thin leaves with low V_{cmax}, J_{max} (Figure 2a, b), nitrogen (Figure 3a, b) and respiration rates (Xu et al., unpublished data).

Light acclimation in *B. thunbergii* is more complex than in the other two species. First, *B. thunbergii* has two leaf flushes respectively acclimated to the prevalent high- and low-irradiance environment. The first flush of leaves displayed sun-leaf characteristics when the upper canopy was open, and leaves from the second flush had more shade-leaf characteristics. Second, the prolonged two-month leaf ontogeny of the first leaf flush makes it morphologically plastic. In early stage, leaves had a higher LMA characteristic of high irradiance acclimation; following canopy closure the leaves were thinner, indicating better acclimation to shade conditions. Many previous studies have associated the thickening of mature leaves with shade to sun acclimation (Bauer and Thoni 1988; Kamaluddin and Grace 1992; Muller et al. 2005; Oguchi et al. 2003; Oguchi et al. 2005; Terashima et al. 2001), but the reverse, a decrement of LMA in conjunction with a sun to shade acclimation has rarely been observed (*e.g.* Rothstein and Zak 2001). Finally, leaf nitrogen of *B. thunbergii* seemed to be redistributed from late May to early June. During

that period, the LAI of *B. thunbergii* nearly doubled but the leaf nitrogen concentration (N_{mass}) decreased approximately 50%. Following canopy closure, the first leaf flush continues expanding while a second leaf flush expands promptly, and the reallocation of photosynthetic apparatus within or between leaves may occur during this time. Thus, the phenological characteristic favor *B. thunbergii* by providing both a spring carbon subsidy and low maintenance cost following canopy closure by maintaining a dynamic response to the ambient light conditions.

Indications of the invasion mechanism of Japanese barberry

Daehler (2003) summarized that alien invaders are not statistically more likely to outperform co-occurring natives and the relative performance often depends on the specific growing conditions. Thus, simply comparing V_{cmax}, J_{max}, or the *in situ* photosynthetic rate between *B. thunbergii, K. latifolia* and *V. corymbosum* during one particular time of the year may lead to biased conclusion (Figure 2, 6). Thus, our study sheds light on a more comprehensive understanding of the photosynthetic niche separation and photosynthetic acclimation of *B. thunbergii,* by examining these characteristics seasonally. Furthermore, these findings suggest that environmental change in northeastern United States may potentially facilitate the invasion of *B. thunbergii* into forests' understories.

The spring carbon subsidy of *B. thunbergii* may be enhanced by winter warming trends in the northeastern United States. In this region, the annual temperature warmed up 1.0 °C on average during the twentieth century, while the winter temperature increased by 1.6

°C (Wolfe et al. 2005). Since we find that a spring carbon subsidy significantly contributes to annual carbon gain of *B. thunbergii*, warming prior to typical growing season may benefit the growth and fecundity of *B. thunbergii* more than that of the late leafing natives (*e.g. V. corymbosum*).

Another regional change that may be promoting the invasion of *B. thunbergii* is increasing nitrogen deposition. In a relatively undisturbed forest understory, nitrogen availability can be the primary limitation of *B. thunbergii* (Cassidy et al. 2004; Harrington et al. 2004). There is evidence that nutrient shortages prevent acclimation to high light environments by limiting leaf nitrogen redistribution and increasing the leaf susceptibility to high light photoinhibitory damage (Grassi et al. 2001). However, anthropogenic nitrogen emission had been increasing since the preindustrial era in the northeastern United States (Aber et al. 2003; Driscoll et al. 2003; Galloway et al. 1984; Holland et al. 2005) and has significantly altered forest nitrogen budgets (Aber et al. 2003). In BRF, there is evidence that the biota removes a large fraction of fixed nitrogen influx from the precipication (Simpson, 1997). The photosynthetic capacity and light acclimation of *B. thunbergii* is critically driven by nitrogen reallocation following the prevailing light regime. The significant $A_{max} - N_{mass}$ relationship and wide N_{mass} range (Figure 4h) indicate that *B. thunbergii* has a greater capacity to turn increases in N into photosynthetic machinery per unit leaf investment than the native shrubs. By contrast, natives like *K. latifolia* and *V. corymbosum*, whose photosynthetic acclimation are less significantly influenced by leaf nitrogen concentration (N_{mass}), have to invest more biomass in leaf to turn additional N into photosynthetic capacity. Thus, they are perhaps

not as likely to gain a significant benefit in leaf carbon gain as a result of increased regional N deposition.

In summary, we found that *B. thunbergii* has much higher photosynthetic capacity in early May than during the remainder of the growing season, which leads to significant spring carbon subsidy. Furthermore, *B. thunbergii* displayed stronger correlations between photosynthetic capacity (V_{cmax}, J_{max}) and leaf properties (N_{mass}, LMA) than the native shrubs, indicating more active adjustment of the photosynthetic apparatus. However, across the growing season, the photosynthetic capacity of *B. thunbergii* was comparable or lower than that of *K. latifolia*, and all three shrubs show similar *in situ* photosynthetic rates at any particular time of the year (when all leaves are present). These results support our first hypothesis that *B. thunbergii* displays a higher photosynthetic capacity in spring than in summer and fall, and the third hypothesis that the photosynthetic capacity of *B. thunbergii* is correlated to N_{mass} and LMA, but reject the second hypothesis that *B. thunbergii* has higher photosynthetic capacity than co-occuring native shrubs across the growing season. Therefore, significant spring carbon subsidy, and effective acclimation to the dynamic light regime, but not superior physiological performance, may be partially responsible for the invasion of *B. thunbergii* in the forest understory. The knowledge gained in our study may more widely apply to invasive understory plants and can improve alien species management. For example, regional climate change may need to be considered in alien species auditing process. In regions showing significant winter warming, special cautions need to be taken on alien species with early phenology. Furthermore, if it is required to eliminate the existing early leafing

invaders locally in the forest, control efforts (i. e. herbicide application) in the spring may be more effective than that in the other periods of the year (Sliander and Klepeis 1999). Thus, further studies are warranted to consider the effects of regional environmental change on the invasion in closed forest and related management policy needs to be enacted.

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Figure Legends

Figure 1. Annual temperature variation and leaf phenology of the upper canopy and Japanese barberry in 2004. a) Daily average temperature of 2004 (days when gas exchange measurements were made are marked); b) leaf ontogeny of *B. thunbergii*; and c) leaf area index (LAI, relative value) of *B. thunbergii* (○) , and the upper canopy trees (●). The values are mean ± standard error (SE, n = 5 ~ 6). The intiataion of leaf development of *V. corymbosum* and *K. latifolia* are also marked.

Figure 2. Seasonal variation of photosynthetic characteristics of *B. thunbergii* (BT: ○ , dotted line), *V. corymbosum* (VC: ● , solid line), and *K. Latifolia* (KL: ▼ , dash line).

Values are mean ± SE (n=6). Points marked with the same letter are not significantly different at P=0.05 level (t test and Mann-Whitney U test). The P values of ANOVA of seasonal effect are listed on the upper right corner and are underlined if $P < 0.05$. For t test and ANOVA results, different letter fonts are used to identify the species (normal font, VC; *Italic font, BT*; **Black font, KL**).

Figure 3. Season variation of leaf nitrogen and leaf mass per area (LMA) of *B. thunbergii*, *V. corymbosum*, and *K. Latifolia*. Mean values ± SE (n=6). The values marked with the same letter are not significantly different at the $P < 0.05$ level (t test). The ANOVA results of the seasonal effect are listed in the lower right corner. The legends are the same as in figure 2.

Figure 4. The maximum carboxylation rate (V_{cmax}; a, b, c), maximum electron transport rate (J_{max}; d, e, f), and A_{max} of *B. thunbergii*, *V. corymbosum*, and *K. latifolia* determined at 20 ºC as a function of nitrogen (area and mass based) and leaf mass per area (LMA). For correlations significant at $P<0.05$ level, correlation efficient (R^2), P values and linear regression lines are shown. For *K. latifolia*, the November leaves (dark grayed, ▼) are not included in the linear regression analysis. The other legends are the same as in figure 2.

Figure 5. Relationships between $PNUE_{max}$ and a) F_v/F_m or b) QY_{max} in *K. latifolia*. The correlation efficient (R^2), P values, and linear regression lines are shown. The legends are the same as in figure 2.

Figure 6. Conceptual explanation of the temporally photosynthetic niche separation of *B. thunbergii*, *V. corymbosum*, and *K. Latifolia*. a) 7-day average temperature (daily maximum, average and minimum, grey solid and dotted lines), 7-day average vapor pressure deficit (solid line) and upper canopy LAI (relative value, thick solid line); b) to d) photosynthesis rate (20 ºC) of *B. thunbergii*, *V. corymbosum*, and *K. Latifolia* in the prevailing light regime calculated according to A-Ci model (20ºC, ambient light levels in early spring, summer and late fall were assumed to be 1500, 50 and 800 μmol PPFD m^{-2} s^{-1}). The period between leafing and defoliation is marked by dark bar on x-axis and the light acclimation patterns throughout 2004 are also briefly explained. The legends are the same as in figure 2. For in 5c, the dark grey symbol (▼) marks a potential short photosynthetic peak in late fall after upper canopy defoliation.

Figure 1.

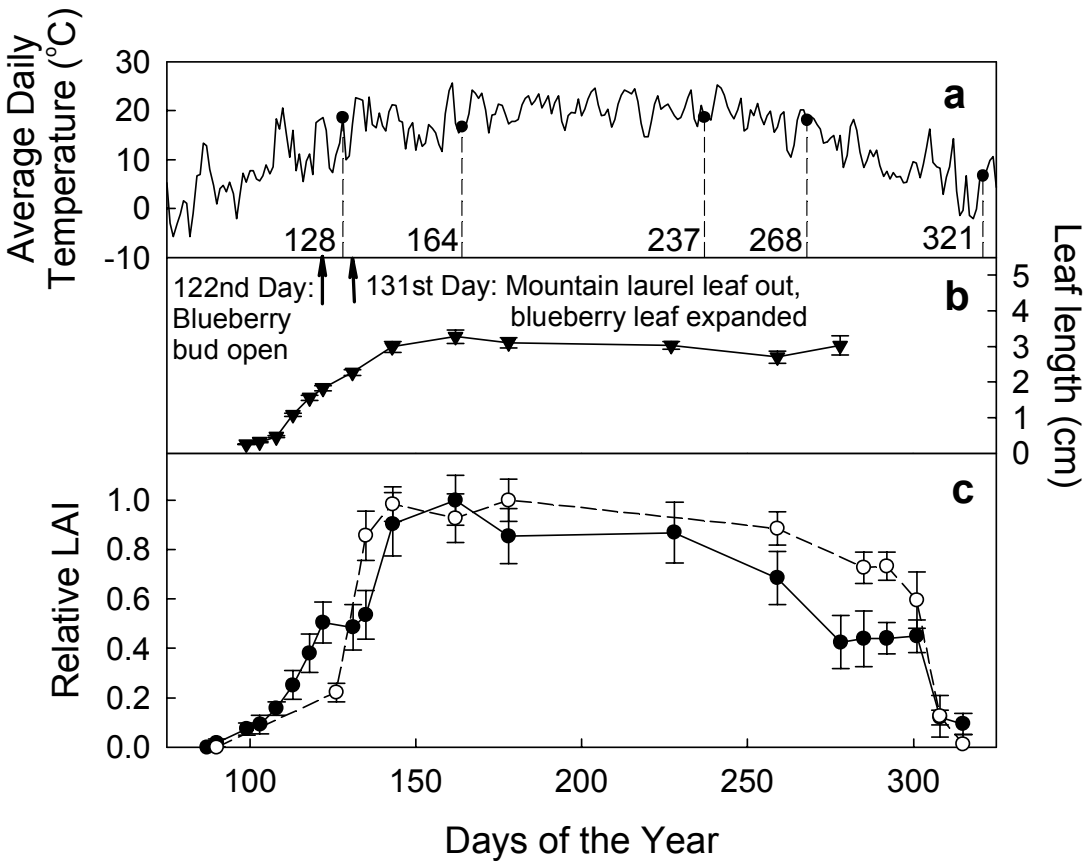

Average Daily Temperature (°C)
30
20
10
0
-10
a
128
164
237
268
321
122nd Day: Blueberry bud open
131st Day: Mountain laurel leaf out, blueberry leaf expanded
b
Leaf length (cm)
5
4
3
2
1
0
Relative LAI
1.0
0.8
0.6
0.4
0.2
0.0
c
100
150
200
250
300
Days of the Year

Figure 2.

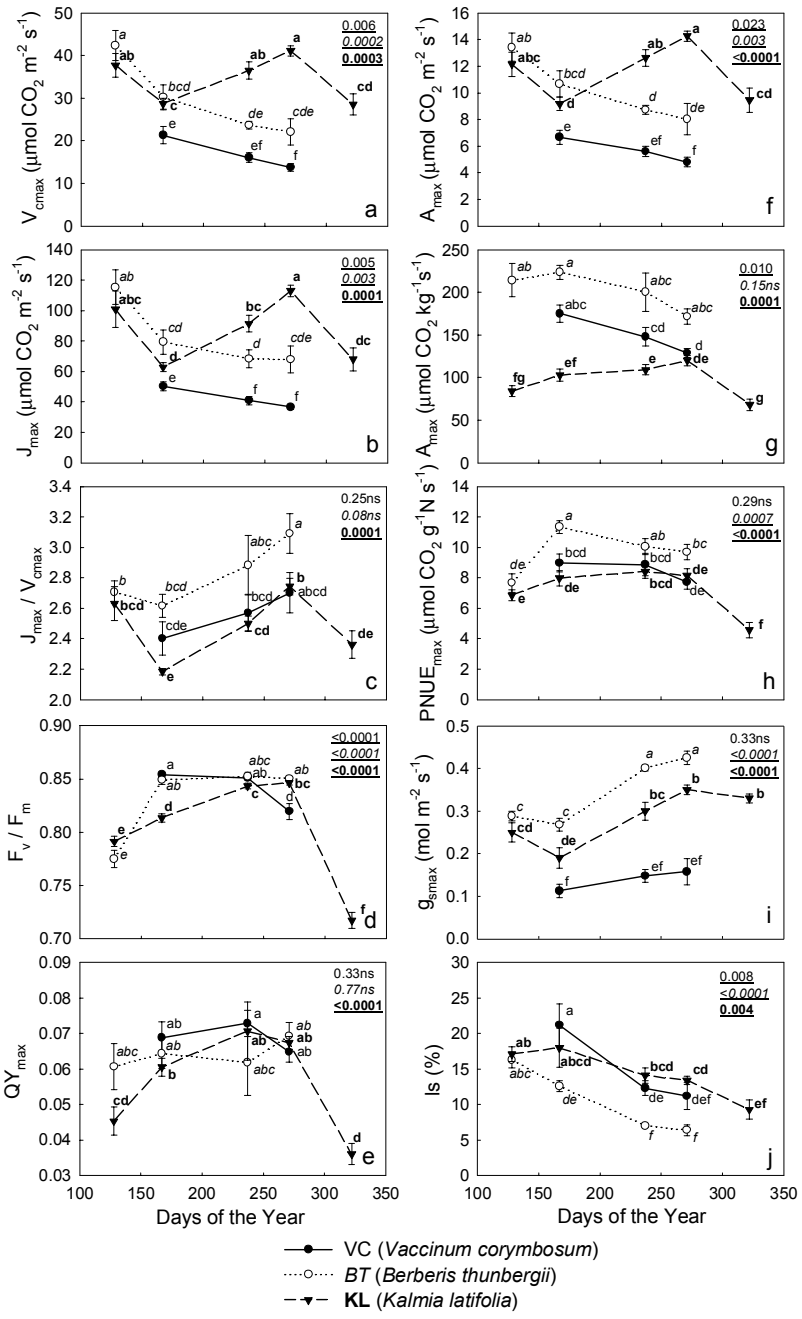

a
b
c
d
e
f
g
h
i
j
Days of the Year
Days of the Year
VC (Vaccinum corymbosum)
BT (Berberis thunbergii)
KL (Kalmia latifolia)

Figure 3.

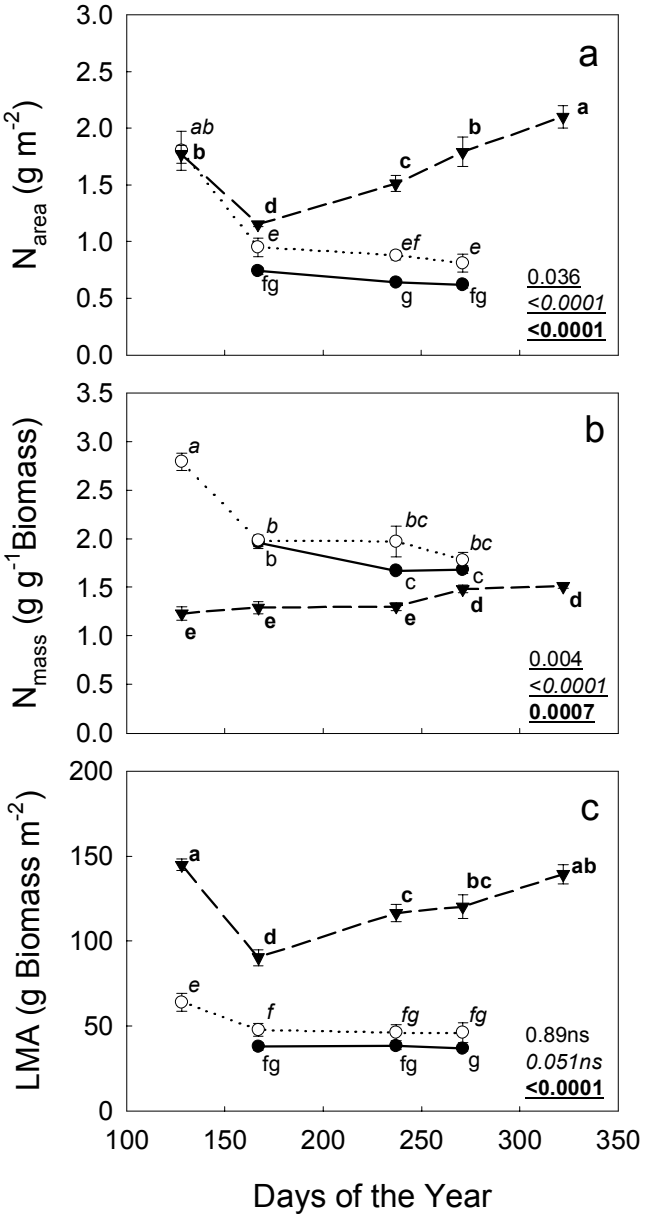

a
b
c
N_{area} (g m^{-2})
N_{mass} (g g^{-1}Biomass)
LMA (g Biomass m^{-2})
Days of the Year
0.036
<0.0001
<0.0001
0.004
<0.0001
0.0007
0.89ns
0.051ns
<0.0001

Figure 4.

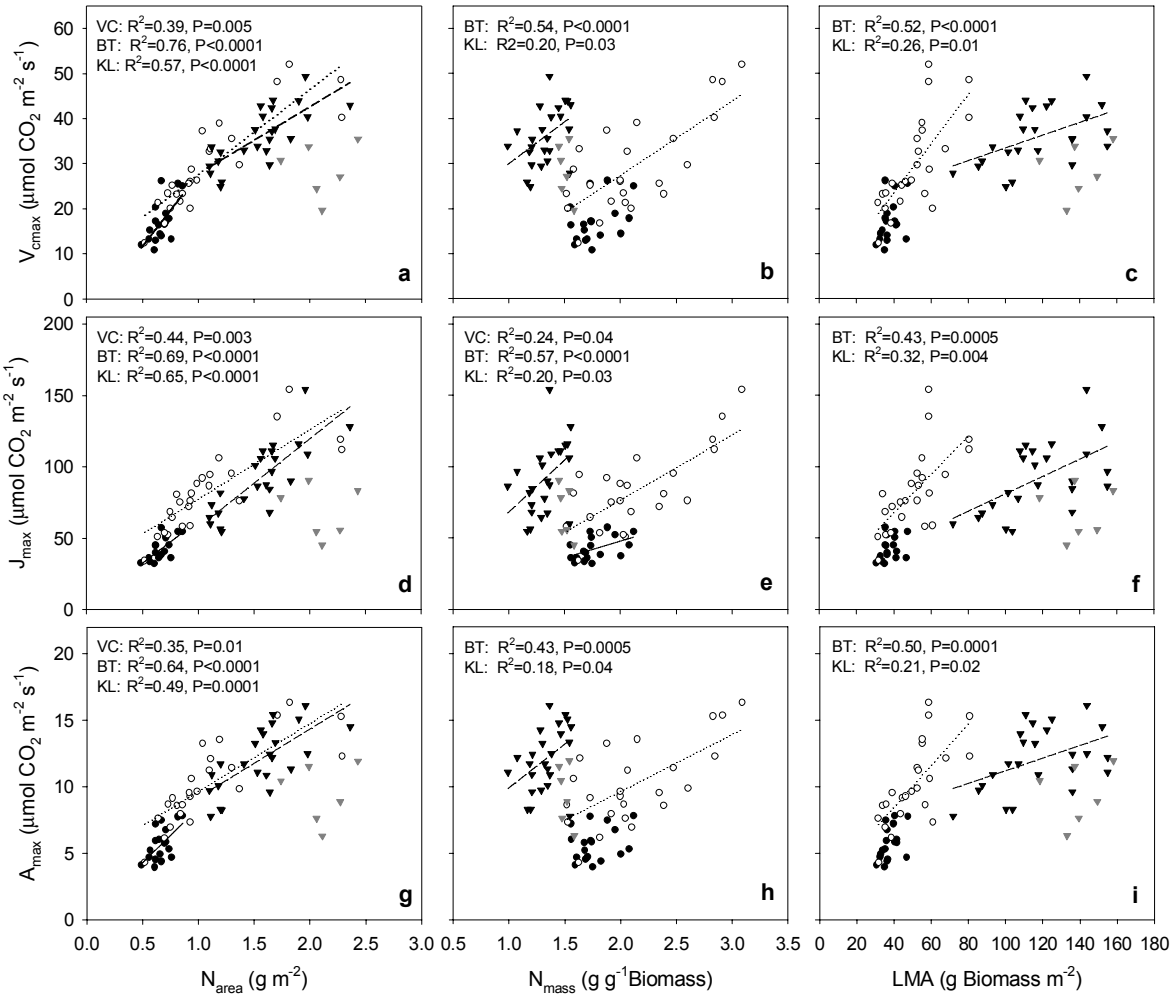

VC: R2=0.39, P=0.005
BT: R2=0.76, P<0.0001
KL: R2=0.57, P<0.0001
a
BT: R2=0.54, P<0.0001
KL: R2=0.20, P=0.03
b
BT: R2=0.52, P<0.0001
KL: R2=0.26, P=0.01
c
VC: R2=0.44, P=0.003
BT: R2=0.69, P<0.0001
KL: R2=0.65, P<0.0001
d
VC: R2=0.24, P=0.04
BT: R2=0.57, P<0.0001
KL: R2=0.20, P=0.03
e
BT: R2=0.43, P=0.0005
KL: R2=0.32, P=0.004
f
VC: R2=0.35, P=0.01
BT: R2=0.64, P<0.0001
KL: R2=0.49, P=0.0001
g
BT: R2=0.43, P=0.0005
KL: R2=0.18, P=0.04
h
BT: R2=0.50, P=0.0001
KL: R2=0.21, P=0.02
i
Vcmax (µmol CO2 m-2 s-1)
Jmax (µmol CO2 m-2 s-1)
Amax (µmol CO2 m-2 s-1)
Narea (g m-2)
Nmass (g g-1Biomass)
LMA (g Biomass m-2)

Figure 5.

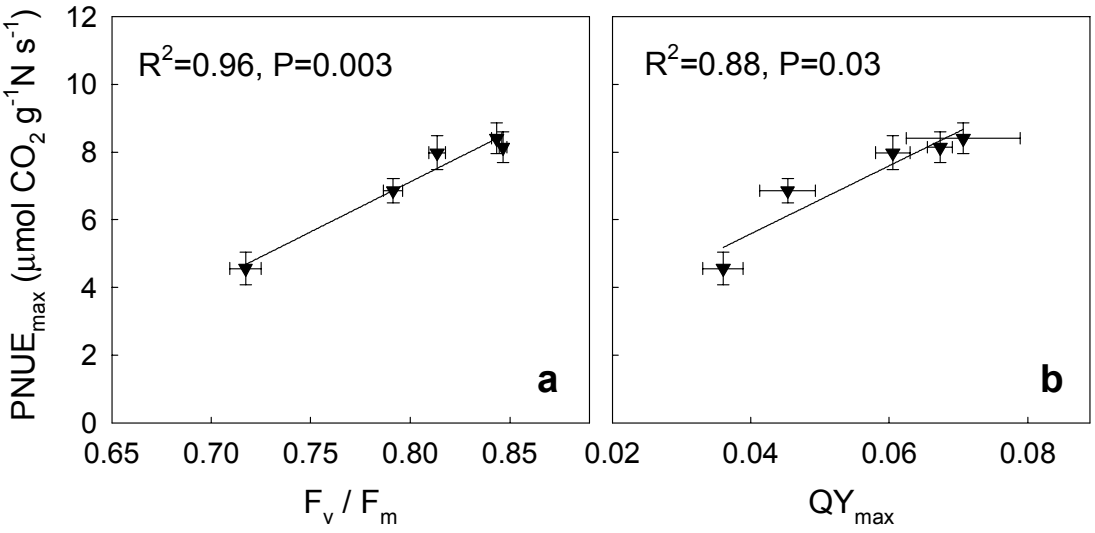
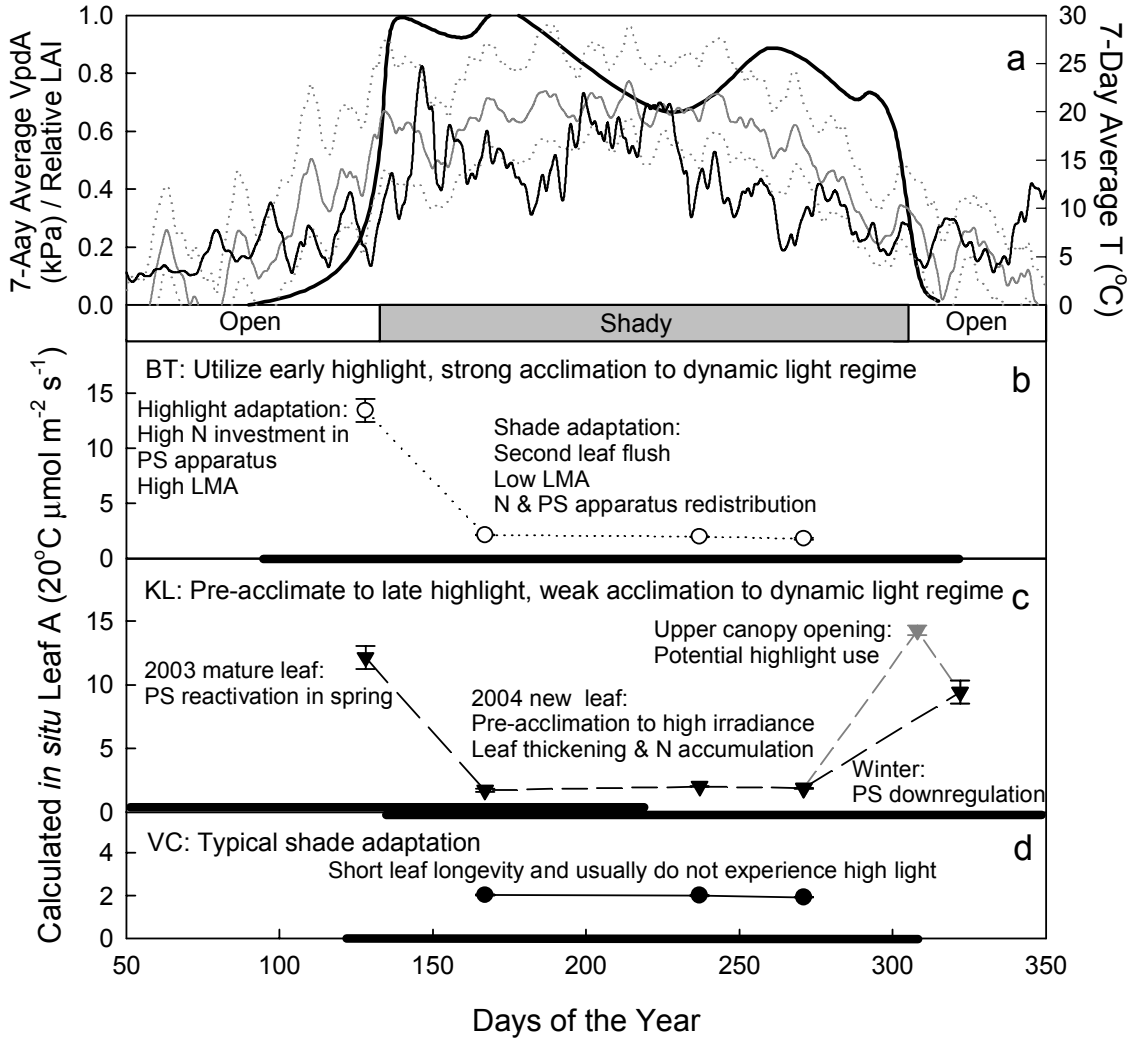

PNUE$_{max}$ (µmol CO$_2$ g^{-1}N s^{-1})
12
10
8
6
4
2
0
R^2=0.96, P=0.003
a
0.65
0.70
0.75
0.80
0.85
F$_v$ / F$_m$
R^2=0.88, P=0.03
b
0.02
0.04
0.06
0.08
QY$_{max}$

Figure 6.


7-Aay Average VpdA (kPa) / Relative LAI
7-Day Average T (°C)
a
Open
Shady
Open
BT: Utilize early highlight, strong acclimation to dynamic light regime
b
Highlight adaptation:
High N investment in
PS apparatus
High LMA
Shade adaptation:
Second leaf flush
Low LMA
N & PS apparatus redistribution
KL: Pre-acclimate to late highlight, weak acclimation to dynamic light regime
c
Upper canopy opening:
Potential highlight use
2003 mature leaf:
PS reactivation in spring
2004 new leaf:
Pre-acclimation to high irradiance
Leaf thickening & N accumulation
Winter:
PS downregulation
VC: Typical shade adaptation
d
Short leaf longevity and usually do not experience high light
Calculated in situ Leaf A (20°C µmol m-2 s-1)
Days of the Year

Appendix 2:

The use of alligator weed (*Alternanthera philoxeroides*) to remove CO_2 from a simulated power plant flue gas

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Abstract

Fossil fuel CO_2 emissions have significantly altered the chemical composition of the atmosphere, leading to an ongoing search for mitigation strategies. Unfortunately, the development of an economically viable carbon sequestration technology is progressing slowly and, although biomass can potentially substitute for fossil fuel as a renewable energy source, the current cost of biomass production, especially in terms of land use, is prohibitively large. A potential solution to this problem is to establish energy-farming greenhouses within the "buffer zone" surrounding modern power plants and to use flue gas of power plants as a CO_2 source to enhance biomass yield.

In this study, *Alternanthera philoxeriodes* was used as a model species to explore the growth response of plants to a simulated flue gas gradient in small, custom-made growth chambers. When the acidic components of the flue gas were excluded, the biomass yield of *A. philoxeroides* saturated near 2000 ppm [CO_2] and resulted in 107% enhancement relative to plants in an ambient control. The more numerous and denser starch deposits observed in leaf cells also indicated more active carbon sequestration in the elevated-CO_2 environment. Furthermore, the growth enhancement in aboveground biomass was maintained at 5000 ppm [CO_2] and declined only when atmospheric [CO_2] was above 1%. Although the acidic components in the flue gas significantly offset the observed CO_2 growth enhancement, the aboveground biomass yield still increased considerably when the pollution level was moderate (1000 ppm [CO_2], 0.8 ppm [NO_2] & 0.09 ppm [SO_2]).

The demonstrated CO_2-enhanced biomass accumulation rate, if sustainable, would scale to 47 – 66 Mg ha^{-1} yr^{-1}, a rate comparable to the highest yields reported in other biofuel projects, and this could still be a conservative estimation. The negative effect of acidic pollutants in the flue gas may be overcome either by diluting the gas to a safe level or by removing the pollutants. Further research is warranted to identify or engineer ultrahigh [CO_2]-/ pollutant-tolerant species for flue-gas-fed greenhouse bio–carbon–sequestration systems, which can potentially serve both to offset the carbon released from fossil fuel emissions and to provide clean biomass energy.

Introduction

The extensive burning of fossil fuels has raised the atmospheric CO_2 concentration ($[CO_2]$) from 270 ppm in the pre-industrial era to today's 372 ppm, the highest level in the past 26 million years (Pearson and Palmer 2000). In the next 100-150 years, atmospheric $[CO_2]$ may reach 700 ppm (Houghton et al. 1990). The potential threat posed by elevated-$[CO_2]$-led climate change (i.e. global warming) and increasing demand for energy has stimulated the search for carbon sequestration technologies and renewable energy sources (McKendry 2002). However, according to the United States Department of Energy's office of Fossil Energy Carbon Capture Research program, existing CO_2 capture technologies cost approximately 150 US$ to fix one ton of carbon and is considered prohibitive for large-scale implementation. (CO_2 Capture Project, ID DE-FC26-01NT41145, Office of Fossil Fuel, U.S. Department of Energy, USA)

Biomass is a renewable energy source and currently supplies 10-14% of energy globally (McKendry 2002). Since CO_2 emitted by biomass combustion is approximately equal to the amount taken up by plant growth, the use of biomass energy does not result in the accumulation of CO_2 in the atmosphere. Therefore, the substitution of biomass for fossil fuels can contribute to reducing anthropogenic CO_2 emissions. Biofuel programs in the US and Britain since the 1980s have concentrated on varieties of perennial C_3 and C_4 grasses, which yield between 1-35 metric tons ha^{-1} yr^{-1} (Lewandowski et al. 2003). However, due to the low energy-conversion efficiency of photosynthesis (~ 1% of the sunlight) and high costs to establish, produce and harvest crops, economically viable bio-

energy farming has not yet been developed. Furthermore, to significantly offset anthropogenic emissions, the land required may be prohibitively large (Nonhebel 2005).

Experiments in controlled environments indicate that elevated [CO_2] can increase photosynthetic carbon fixation and biomass accumulation in C_3 plants (Curtis and Wang 1998). In natural ecosystems, elevated atmospheric [CO_2] is also believed to stimulate the growth of forests and thereby the rate of carbon sequestration (Ciais et al. 1995, Fan et al. 1998, Myneni et al. 2001). Plant responses to elevated [CO_2] have been applied to greenhouse production techniques, where CO_2 gas is widely used as a fertilizer to increase crop yield (Stanhill and Enoch 1999).

Although both plant carbon uptake in elevated [CO_2] and biofuel development are active research topics, combined work in these two closely related fields has been limited. The flue gas from power plants, which contains high concentration of CO_2, is a potential source to supply energy-farming greenhouses, which could be built on the large "buffer zones" owned by most modern power plants. Compared with traditional biomass projects or greenhouse agriculture, this system features the use of waste (e.g. CO_2 production from fossil fuel and potentially heat as well) and existing unused land to create efficient carbon-sequestration systems (elevated [CO_2] and greenhouse conditions). Furthermore, the flue-gas-fed greenhouse system could be coupled with other environmental management projects; for example, eutrophic industrial wastewater could be introduced as a nutrient source and water supply, also helping purify the wastewater in the process. By integrating the benefits of stimulating biofuel production, offsetting fossil fuel

combustion, and other possible environmentally favorable functions, a system could be created that has the potential to establish a more cost-effective, commercially-viable carbon sequestration system.

However, power plant flue gas also contains other potentially damaging components. For example, the flue gas component from a typical power plant can be 71.5% [N_2], 19% [H_2O], 9.5% [CO_2], 300 ppm [SO_2], 150 ppm [NO_2] and 750 ppm [HCl] (Beaumont, unpublished data). In a related study, we modeled the effect of a newly designed condensing heat exchanger to clean the flue gas (Castaldi & Beaumont, unpublished data). The result indicated that 95% of the SO_2 could be scrubbed in the exhaust stack, and during the subsequent condensation process, most of the HCl (99.9%) and some NO_2 (~10%) would be removed. Finally, properly-treated flue gas would contain 12% [CO_2], 130 ppm [NO_2] and 15 ppm [SO_2]. However, before this gas can be introduced into a greenhouse, further cleaning, cooling and dilution with fresh air may be required. Determining the ideal treatment requires knowledge of the optimal photosynthetic responses of the species under cultivation to elevated [CO_2] and the co-occurring contaminants in flue gas.

Currently, most research in CO_2 mitigation strategies tends to focus on plant responses to projected future atmospheric [CO_2], which is unlikely to exceed 700 ppm during this century, and therefore experimental [CO_2] treatments typically range from ambient (350 – 370 ppm) to twice ambient (700 – 740 ppm) (Curtis and Wang 1998). Previous studies, however, indicate that photosynthetic rates may not saturate until [CO_2] reaches much

higher concentrations, between 1000 and 1500 ppm (reviewed in Reuveni and Bugbee 1997). There is limited research to suggest that higher [CO_2] (up to 1%) may be harmful to plants (Wheeler et al. 1993, Wheeler et al. 1994, Grotenhuis et al. 1997, Grotenhuis and Bugbee 1997), indicating that care will be needed to identify a [CO_2] that is optimal for carbon sequestration..

One potential problem with using flue gas as a CO_2 source is that other components of this gas, particularly acidic pollutants like SO_2 and NO_2, could be detrimental to plant growth and limit carbon sequestration. These acidic pollutants are known to cause phytotoxic reactions (e.g. acidification of leaves, reduction of photosynthetic pigments, inhibition of physiological processes, and alteration of enzyme activities) in many plant species (Darrall 1986, 1989, Saxe 1991, Okpodu et al. 1996, Verma and Agrawal 1996, Agrawal and Verma 1997). However, at lower levels, the effect of these pollutants on plant growth and biomass accumulation may be minimal (Okano et al. 1985, Kosobryukhov and Mudrik 1997, Van Der Kooij et al. 1997, Qiao and Murray 1998). In fact, under certain conditions such as nutrient limitation, these components of the flue gas may even improve plant growth (Okano et al. 1985, Murray et al. 1992, Jensen and Pilegaard 1993, Murray et al. 1994, Pandey and Agrawal 1994). In addition, some studies indicate that the adverse effects of acidic pollutants could be offset by elevated [CO_2] (1000-1200 ppm) or elevated nutrient supply (Carlson 1983, Idso and Idso 1994, Lee et al. 1997, Verma et al. 2000, Agrawal and Deepak 2003). Although the effects of specific individual pollutants have been widely studied, the combined effects of elevated [CO_2] and acidic pollutants found in flue gas are not known.

In this study, *Alternanthera philoxeriodes*, a fast growing C_3 weed, was used as a model species to establish the response of plant growth to a simulated flue gas. First, we grew this weed in [CO_2] up to 3000 ppm to determine the CO_2 saturation point, the potential maximum growth, the rate of carbon sequestration, and relative biomass/nitrogen allocation patterns. Second, the plants were grown in 5000 ppm to 2% [CO_2] to assess whether very high [CO_2] might reduce aboveground plant growth. Third, plants were grown in a dilution gradient of simulated flue gas (containing both CO_2 and acidic pollutants) to test whether the acidic components of the gas would negatively affect aboveground growth or whether, alternatively, the CO_2 would compensate for the phytotoxicity. We expected that (1) biomass accumulation would saturate between 1000 – 2000 ppm, and (2) that [CO_2] above 3000 ppm or the presence of acidic pollutants would cause negative effects on the growth and/or physiology of *A. philoxeroides*. Our goal is to establish a model of plant growth patterns in diluted flue gas, which can then be used to select species with maximum biofuel yield for use in a bio–energy farming system.

Materials & Methods

CO_2 / Pollutants exposure system:

In order to fully replicate the [CO_2] treatments, 12 small custom-built growth chambers (Figure 1), were placed in a single large environmentally controlled plant growth chamber (E15, Conviron, Winnipeg, Canada). Each small chamber was constructed from a translucent plastic tub, measuring approximately 33 cm L x 20 cm W x 27cm H (Sterilite Industries, Townsend MA, USA). Each contained a 12 V dc fan to insure adequate air circulation (FBA06T12H, Matsushita Electric, Tokyo, Japan). Air was injected behind the fan, which was lined on three sides by a tinfoil shroud to prevent the plant material from growing into and stalling the fan blades. The growth chamber functioned as a flow-through system with the exiting air passing through a 5/16" bulkhead fitting on the wall opposite the fan and the air inlet. A closed cell neoprene foam gasket was placed around the entire perimeter of each growth chamber which was then sealed on the top with clear a 1/16" plastic sheet (Lucite-ES, Lucite International Inc., Southampton, UK) with unbiased transmittance to the light wavelength of 400 – 700 nm (data not shown). The top was firmly held in place with eight to ten small bulldog clips. In addition, a gallium arsenide photodiode (Hamamatsu Photonics , Hamamatsu City, Japan) and a type T thermocouple was placed inside each growth chamber and was attached to a data logger (CR23x, Campbell Scientific, Logan UT, USA) to continuously monitor light level at the top of the chamber and chamber temperature. Light level above each growth chamber was read daily by a silicon light sensor and light meter (Li-250, Licor Inc, Lincoln NE, USA). The lower (sand-filled) portions of the growth chambers were wrapped with aluminum foil to discourage the growth of algae in the substrate.

In order to create the [CO_2] treatments, a custom mixing system was constructed (Figure 1) consisting of two air streams that could then be blended together to make a range of treatments. The first air stream was ambient (outside) air pumped into the lab at a rate of approximately 20 L min^{-1} using a diaphragm pump (400-1901, Barnant Co., Barrington, IL, USA) and supplied to the custom mixing manifold consisting of 12 rotometers (MMA-21, Dwyer Instruments, Michigan City, IN, USA). The second air stream was used to supply the CO_2 and acidic pollution through a series of dilutions. For example, in the first experiment, approximately 20 L min^{-1} of 3000 ppmv CO_2 air was created by adding an appropriate amount of pure CO_2 via an electronic mass flow controller (Sierra side trak 830, Sierra Instruments, Monterey, CA, USA) to CO_2 free air (outside air, treated by molecular sieves to remove CO_2). This elevated CO_2 air was supplied to an additional 12 rotometers in the custom mixing manifold. Each growth chamber was supplied with a total flow of 2 L min^{-1} through two inlet ports behind the fan connected to the mixing manifold. To compensate for plant growth, the relative amounts of ambient vs. elevated CO_2 air was adjusted daily (for experiment 1) or additional CO_2 was supplied by another electronic mass flow controller (Sierra side trak 830, Sierra Instruments, Monterey, CA, USA) to elevate the CO_2 concentration in the diluting air (experiment 2 and 3). This approach resulted in the same elevated nighttime [CO_2] as is typical in greenhouse systems due to respiration and lack of photosynthetic activity.

The [CO_2] levels were continually monitored using four models of cross-calibrated infrared gas analyzers (IRGA, Li-6262, Li-7500, Li-820, Li-6251, LiCor Inc., Lincoln

NE, USA, seven analyzers in all; the choice of analyzer depended on the CO_2 set point in the particular growth chamber since different analyzers have different sensitivity ranges). During each experiment, gas analyzers were attached to the outlet ports of four randomly selected growth chambers (one from each treatment, Figure 2). These analyzers were kept outside the large environmentally controlled plant growth chamber. To avoid condensation from the saturated airflow, the air was dried prior to entering the IRGA, first using condensation traps placed with in a 5 ºC water bath or refrigerator and then using drierite desiccant tubes. This system continuously monitored the [CO_2] within the four growth chambers and the data were automatically recorded on a PC equipped with a USB datalogger (PMD-1208LS, Measurement computing Co., Middleboro, MA, USA). In addition, the [CO_2] within each growth chamber was manually monitored and adjusted daily, either by using the system described above or by temporarily connecting the output of each growth chamber (sequentially) to a common IRGA (Li-6400, LiCor Inc., Lincoln, NE, USA).

To mimic a diluted flue gas in experiment 3, a NO_2-CO_2 mixture (1038 ppm [NO_2] balanced by CO_2, Matheson Tri-gas, Montgomeryville, PA, USA) was used to supply CO_2. Therefore, the NO_2 concentration could be estimated from the measured CO_2 concentration. A second tank containing a SO_2-N_2 mixture tank (2996 ppm [SO_2] balanced by N_2, Matheson Tri-gas, Montgomeryville, PA) was used to supply SO_2 through a separate electronic mass flow controller (Smart Trek 100, Sierra Instrument, Monterey, CA, USA) and the concentration in the main air stream was automatically monitored with a SO_2 analyzer (450C, Thermo Electric, Franklin, MA, USA). The final

composition of the gas mix added to the growth chambers was 3000 ppm [CO_2], 3.2 ppm [NO_2] and 0.35 ppm [SO_2], comparable to that of diluted flue gas.

The CO_2 control system effectively created a stable [CO_2] gradient among the treatments in all three experiments. For example, in experiment 1, the average daily manually recorded CO_2 concentrations (during the day) in 4 treatments were 342 ± 9, 939 ± 20, 1927 ± 14, and 3031 ± 141 ppm (mean ± standard error).

General Growth Conditions:

Clones of *A. philoxeriodes* collected from the Southeastern United States were grown for the experiment. In each growth chamber, the plants were grown in 3.8 kg of tap-water-saturated sand (pH 7). The wet sand matrix was then covered by 1.9 kg of gravel to impede the light penetration and to prevent algae growth on the wet sand surface. Slow releasing fertilizer (approximately 15ml or 16g of Osmocote, Scotts, Marysville, OH, USA) , provided most required macro nutrients, and Hoagland's solution (250 ml, strengthened with NH_4NO_3 to 12mM total N) was added at the beginning of each experiment to insure adequate nutrient supply. The humidity in the growth chambers was close to saturation as condensation typically appeared. Water was supplied weekly (usually 120 ml per growth chamber) or whenever condensation was absent from the growth chambers up on daily observation. The photoperiod in the large environmentally controlled plant growth chamber was set at 18 hours of day and 6 hours of night.

The photosynthetically activate radiation (PAR) level ranged from 600-650 μmol PPFD above the growth chambers and 470-540 μmol PPFD within the growth chambers (Figure 2). The differences in PAR among the treatments were not significant. For example, in the first experiment, the light level above the growth chambers was 627 – 658 μmol PPFD for each treatment on average (within treatment standard error < 13 μmol PPFD; between treatments $P > 0.12$, Mann-Whitney U-test). The temperature of the growth chamber was set at a constant 17 °C. The actual day/night temperatures in the growth chambers were 25.5/18 °C, with inter-growth-chamber variation of ± 1.5 / ± 1.0 °C (Figure 2). The day/night temperature difference was 7 to 9 ºC in all growth chambers.

Experiment Protocols:

Experiment 1: Since the first experiment was designed to determine the CO_2 saturation point of growth and the potential maximum yield in our experimental conditions, twelve stem cuttings (with 4-7 nodes each, no leaves or buds) were grown in each growth chamber to insure complete use of the available space. These cuttings were from 12 different clones and for each clone, the stem cuttings were distributed into 12 growth chambers as evenly as possible. Four treatments, ~ 350 (ambient), 1000, 2000 and 3000 ppm [CO_2], were randomly assigned to the 12 grow chambers creating three replicates of each treatment. All plants were started from cuttings on March 12th, 2005; CO_2 fumigation commenced on March 14th, and all treatments were then allowed to grow undisturbed for 21 days.

Experiment 2: Three stem cuttings of one clone from Texas (with 3-4 nodes each, no leaves or buds) were grown in each growth chamber. Since the goal was to test the aboveground growth response to high [CO_2], the plants were grown in the same [CO_2] gradients as experiment 1 (ambient, 1000 ppm, 2000 ppm, and 3000 ppm.) for 16 days in order to establish the root system. Aboveground biomass was removed 3 days before the high [CO_2] treatments were begun. After 16 days, ultrahigh [CO_2] treatments began: 1000 ppm was increased to 5000 ppm, 2000 ppm to 1.2% and 3000 ppm to 1.9%. Then plants were grown for an additional 19 days in the high [CO_2] treatments. The experiment began on June 13th , 2005 and the high CO_2 fumigation was begun on June 29th.

Experiment 3: Three stem cuttings from the same clone used in experiment 2 (with 3 nodes each, no leaves or buds) were grown in each growth chamber. Before fumigation with the simulated flue gas began, the plants were grown in the same [CO_2] gradient as experiment 1 for 10 days to establish the root system. Considering the possible serious negative effect of pollutions on the new buds, and relatively slow bud germination in this experiment, above ground parts were not removed before fumigation began. The plants were grown on July 19th, 2005 and fumigation pollution exposure started on July 29th. Four treatments in this experiment were, ambient, 1000 ppm [CO_2], 0.8 ppm [NO_2] & 0.09 ppm [SO_2]; 2000 ppm [CO_2], 1.9 ppm [NO_2] & 0.22 ppm [SO_2], and 3000 ppm [CO_2], 3.1 ppm [NO_2] & 0.35 ppm [SO_2] (pollutant concentrations were calculated, not measured value). These treatments mimic the pollution components in flue gas diluted 45, 75, and 200 times (respectively 1000 ppm [CO_2], 0.7ppm [NO_2] & 0.08 ppm [SO_2]; 2000 ppm [CO_2], 2 ppm [NO_2] & 0.23 ppm [SO_2]; and 3000 ppm [CO_2], 2.9 ppm [NO_2]

& 0.33 ppm [SO_2]). For safety, the air from the growth chambers was passed through activated carbon to remove the acidic components before being vented from the system.

The number of viable nodes and the length of the surviving original stems were recorded. The differences in the number of nodes and the total length of the original stems were not significant among treatments in all three experiments. For example, in the first experiment, there were on average 61 – 64 nodes per growth chamber (within treatment standard error < 2.6, between treatments $P > 0.38$, Mann-Whitney U-test) and 272 – 282 cm of total stem length for each treatment (within treatment standard error < 11cm, between treatments $P > 0.13$, Mann-Whitney U-test). The initial biomass was not used as covariant because the inter-treatment differences were very small and related studies indicate that initial cutting size would not affect the new growth yield (Geng et al. unpubished data).

When harvesting, all plants were separated into leaves, stems and roots. The tissues were dried at 80 °C for at least 48 hours before being weighed. Carbon and nitrogen concentrations were measured by a CHNS/O analyzer (2400 Series II, Perkin-Elmer, Boston, MA, USA). Biomass accumulation was measured in newly grown tissues only (not including the original stem cutting). In the first experiment, the light use efficiency (LUE) was calculated as:

LUE = Total biomass accumulation / [Total PAR (PPFD)×Theoretical maximum quantum yield (0.125)×12 (Dolton, for carbon) / Biomass carbon %].

Starch accumulation at the cellular level was observed in experiment 1. One leaf (5^{th} leaf on one new bud), respectively, from the ambient and elevated [CO_2] (2000 and 3000 ppm) growth chambers was sampled for transmission electronic microscope (TEM) observation. Portions of the leaf blade were cut into 3 mm wide strips and fixed in 3% TEM-grade glutaraldehyde prepared in cold 0.2 M phosphate buffer (pH = 7.2), post-fixed in 2% osmium tetroxide in the same phosphate buffer, rinsed in distilled water, dehydrated in an acetone series and embedded in TAAB epoxy resin. Ultrathin sections were obtained with a Porter-Blum MT-2 ultramicrotome fitted with a diamond knife, collected on 200 mesh copper grids, post-stained with Reynold's lead citrate, and viewed with a Philips TEM 201 transmission electron microscope operated at 60 kV accelerating voltage (TEM 201, Philips Electronics, Einthoven, Netherlands). Digital images of the negatives were prepared using a Polaroid high-resolution scanner (PrintScan 4000, Polaroid Co., Waltham, MA, USA) and composed as a plate using Photoshop (Adobe Systems Inc, San Jose, CA, USA).

Statistical Analysis

In our three experiments, there was no solid basis to determine whether the variables fit a particular distribution pattern. Therefore, a nonparametric method of Mann-Whitney U-test, which is applicable to samples with unknown distribution, was used. The variables derived from each treatment were compared to one another, and the inter-treatment differences were tested at the 0.05 level.

Results

Experiment 1: CO_2 Saturation Point:

Biomass accumulation in *A. philoxeroides* was significantly stimulated in growth chambers with elevated [CO_2] (Figure 3A). Compared with the ambient control, total biomass accumulation increased 65 % in the 1000 ppm [CO_2] treatment, 100% at the 2000 ppm [CO_2] level, but with no further increase at 3000 ppm [CO_2]. The LUE in plant biomass is about 12% of the total PAR. The increase in aboveground biomass accumulation was even larger (80% at 1000 ppm to 120% at 3000 ppm, Figure 5A). The pattern of carbon sequestration was similar to the pattern of biomass accumulation as the percent carbon of total biomass was not affected by [CO_2] (37% -- 39%). The cellular-level structural observations were consistent with the biomass data—more starch deposits were observed in leaves from the 3000 and 2000 ppm treatments and the starch grains were clearly denser (Figure 4).

The percent of carbon in the resulting biomass was nearly constant in all tissues across all [CO_2] treatments (data not shown). Elevated [CO_2] did not have a clear effect on N% and C:N in root and stem. However, in leaf tissue, N% was negatively correlated, and the C:N ratio was positively correlated, to the growth [CO_2] (Table 1). *A. philoxeroides* allocated 50-60% of biomass to the leaves, and the allocation pattern was relatively constant across all [CO_2] treatments (Figure 3B).

Experiment 2 & 3: The Effects of Excessive CO_2 and $CO_2 – NO_2 – SO_2$ on Aboveground Biomass Yield

Compared to the ambient control, 5000 ppm [CO_2] treatment increased the above ground biomass yield of *A. philoxeroides* by 110%. The yield enhancement was comparable to that in experiment 1, indicating that no suppression of growth occurred at this [CO_2] level. However, the degree of enhancement declined when the atmospheric [CO_2] level exceeded 1%. When plants were grown in 1.9% [CO_2], the average aboveground biomass dropped below the level of the ambient control. In experiments 1 and 2, the average aboveground biomass accumulations were similar, approximately 6 grams in the ambient control plants and reached a maximum of 13 grams (in elevated [CO_2] treatment) in approximately 20 days (Figure 5A). Considering that significantly fewer stem cuttings were used in experiment 2, the similar yields indicate that *A. philoxeroides* may have approached the maximum yield possible under the experimental conditions.

In experiment 3, the addition of acidic pollutants significantly offset the growth enhancement of elevated [CO_2] (Figure 5A). Compared with the ambient control, aboveground biomass yield in the 1000 ppm [CO_2], 0.8 ppm [NO_2] & 0.09 ppm [SO_2] treatment growth chamber increased by 55%. This enhancement, however, is lower than that observed in the 1000 ppm [CO_2] treatment (without pollutants) in experiment 1 (80%). The aboveground biomass yield dropped back to the ambient control level in the other two treatments with higher concentrations of pollutants (2000 ppm [CO_2], 1.9 ppm [NO_2] & 0.22 ppm [SO_2] and 3000 ppm [CO_2], 3.1 ppm [NO_2] & 0.35 ppm [SO_2]), indicating that [CO_2] enhancement was unable to compensate for the strong negative effect of these high concentrations of acidic pollutants.

Integrating the results of the three experiments reveals a bell-shaped CO_2 response curve for the aboveground biomass yield of *A. philoxeroides* (Figure 5B). Elevated [CO_2] significantly increased aboveground biomass yield, but saturated near 2000 ppm with more than 100% enhancement. This effect was maintained through [CO_2] as high as 5000 ppm and then declined when the [CO_2] exceeded 1%. Even the introduction of a low level of acidic gaseous pollutants (200 times dilution from original flue gas) diminished the [CO_2] enhancement effect. However, the aboveground biomass yield of *A. philoxeroides* was still considerably higher than the ambient control until the pollution level was increased to 2000 ppm [CO_2], 2.1 ppm [NO_2] & 0.22 ppm [SO_2], or an amount equivalent to 75 times diluted flue gas (Figure 5B).

Exposure to CO_2 and acidic gases did not have a large effect on the percentage of carbon in the aboveground biomass which varied between 38% and 41% and did not show any clear trend along the treatment gradient. Therefore, aboveground carbon accumulation showed the same pattern as aboveground biomass (data not shown), and the C:N ratio in aboveground biomass was mainly determined by the nitrogen content (data not shown). The C:N ratio of the plant material increased from 5.6 to 7.0 between the ambient and the 5000 ppm [CO_2] treatment and did not change when the CO_2 was increased to 1.2 %. At 2% CO_2 the C/N ratio dropped to 6.5. In experiment 3, despite the addition of pollutants, the C:N ratio response pattern did not differ significantly from that in pollutant-free treatments in experiment 1, increasing from 5.6 (ambient control) to 7.0 (3000 ppm [CO_2], 3.1 ppm [NO_2] & 0.35 ppm [SO_2]). Therefore, the C:N ratio response pattern seems correlated to growth [CO_2], regardless of the presence of pollutants.

Discussion

This study was designed to acquire a better understanding of some basic C_3 plant response to growth in a simulated flue gas and of the possible physiological controls of plant production in a flue-gas-fed bio-carbon-sequestration system. For the model species studied, *A. philoxeroides*, three main conclusions can be drawn. First, elevated [CO_2] in flue-gas-fed systems can significantly enhance biomass yield and the effect can be maintained over a large range of CO_2 concentrations (2000 – 5000 ppm). Second, the shape of the growth – CO_2 response curve indicates that the growth can be harmed with very high CO_2 (above 1%) and that the flue gas needs to be properly diluted to reach the optimum CO_2 range (2000 – 5000 ppm in this case). Thirdly, acidic components of the flue gas may cause significant damage to plants and thus need to be removed from the air stream or alternatively pollutant-tolerant species will need to be identified or engineered. Although it is expected that the growth response in actual diluted flue gas will be species specific, we believe the pattern observed in our study is likely to be general and qualitatively applicable to many C_3 species (see also Wheeler et al. 1994, Grotenhuis et al. 1997, Grotenhuis and Bugbee 1997, Reuveni and Bugbee 1997).

The Effects of [CO_2] Enhancement on Plant Biomass Accumulation

Previous studies have shown that moderately elevated CO_2 (700-1000 ppm) can stimulate the growth of plants and that the optimal effect occurs between 1000 – 1500 ppm (Drake et al. 1997, Reuveni and Bugbee 1997, Curtis and Wang 1998, Long et al. 2004). Nevertheless, our study demonstrated that the high [CO_2] growth enhancement is significant in *A. philoxeroides*. At the plant level, we found elevated [CO_2] enhanced

aboveground and total biomass accumulation / carbon sequestration by over 100%. At the cellular level, more numerous and denser starch mass deposits were observed in the protoplasts of leaves grown in 3000 ppm. Furthermore, the positive effect on aboveground biomass yield can be maintained up to 5000 ppm [CO_2]. Although there is abundant evidence that in the long term, plant photosynthesis acclimates to elevated [CO_2] and offsets the growth enhancement (see review Drake et al. 1997, Long et al. 2004), the acclimation will not be likely to reduce the [CO_2] enhancement of biomass yield in the flue–gas–fed greenhouse system, because the biomass can be regularly harvested and reestablished.

Because the day length used in our experiments is longer than most natural settings, we used LUE to compare the biomass accumulation rate in our experiment and previous field data. In our experiments, up to 12% of PAR was fixed into the plant biomass. Scaling up this light use efficiency to the community level in major habitats of *A. philoxeroides* (0º – 40º N/S, Holm et al. 1997, annual average PAR 29 – 41.5 mol PPFD day^{-1}, calculated by Gap Light Analysis, Simon Frazer Univ. BC, Canada & Institute of ecosystem studies, NY, USA), the maximum biomass accumulation rate in saturating [CO_2] in our study is equivalent to 47 – 66 Mg ha^{-1} yr^{-1}. This is comparable to some of the most productive monoculture ecosystems in the world (Piedade et al. 1991, Jones and Muthuri 1997) and higher than the productivity in most biofuel programs in the US and UK (McKendry 2002, Lewandowski et al. 2003). In comparison, the maximum reported total biomass of *A. philoxeroides* grown in the field is approximately 32 Mg ha^{-1} in an Australian marsh

(Julien et al. 1992). Therefore, compared with field yield, the [CO_2] enhancement of biomass accumulation in our study is very significant.

We believe that the current biomass accumulation rate is a conservative estimation and has potential to be improved in an optimized greenhouse system. In our experiments, both space and LUE may have been major limitations to the accumulation of biomass. First, due to the limitation of the growth chamber size, the plants were harvested only after 21 days of growth. However, the growth trajectory of *A. philoxeriodes* indicates possible exponential growth if the growth period could be extended (Geng *et al.*, unpublished data). In a growth experiment over a longer period of 81-days, the biomass accumulation of individuals increased 8-10 times with only four times longer growth period (from 21st to 81st days), and the average growth rate from these experiments is twice the rate measured in our experiment. Furthermore, the average biomass accumulation rate between the 61st and 81st day was 3 (for a 30% sunlight treatment) to 9 times (full sunlight treatment) higher than the growth rate measured between the 1st and 21st day of that experiment. Second, the light use efficiency in our experiment may be an underestimation. In the first half of the growth period, when the buds and leaves were in development, the leaf area index in the growth chambers was so low that very little light was actually used. Therefore, the maximum LUE of *A. philoxeriodes* in our experimental condition should be much higher than 12%, and a doubling (~25%) of LUE seems reasonable. In practice, light is most likely to be the final limiting factor of growth in flue-gas-fed greenhouse system. In the regions between 0º and 40º N/S, total PAR is equal to 400 to 560 Mg ha^{-1} yr^{-1} biomass productivity. After discounting unavoidable loss

(20% light loss in greenhouse, 30% respiration) and unharvestable underground allocation (30%), theoretically the net harvestable PAR (80%×70%×70%=39%) may be between 160 and 220 Mg biomass ha^{-1} yr^{-1}. Thus, there is theoretically substantial room for improving the biomass yield; even a 2-4 fold increase is possible.

Insufficient nutrients could also limit the growth of plants in elevated atmospheric [CO_2]. However, nutrient limitation was not a likely factor in our experiments. For example, in the highest yield growth chamber, nitrogen in plant tissue was about 1.2g, only about 50% of the nitrogen in the Osmocote (mostly released by the end of the experiment, as indicated by the empty fertilizer pellet shells). On the other hand, if nitrogen becomes the limiting factor in a fast growing treatment, the C:N ratio should increase accordingly. However, we did not find any such correlation between the C:N ratio and the growth rate. In contrast, C:N ratio seems to be determined by growth [CO_2]. Since other elements were added in proportion to nitrogen, deficiency in other nutrients is not likely to have been a limiting factor on biomass accumulation in our study.

Detrimental Effects of Ultrahigh CO_2 and Acidic Pollutants

Previous studies on the growth response to ultrahigh [CO_2] (> 2000 ppm) are limited (Wheeler et al. 1994, Grotenhuis et al. 1997, Grotenhuis and Bugbee 1997, Reuveni and Bugbee 1997, Tisserat and Vaughn 2003). In general, ultrahigh CO_2 concentrations have been reported to reduce the seed yield but do not have significant negative effects on vegetative growth. For example, in a greenhouse experiment, seed yield of wheat grown in 1000 ppm, 2000 ppm, 3000 ppm, and 1% [CO_2] was respectively about 10%, 130%,

100% and 90% of that grown in ambient control. In contrast, vegetative biomass peaked at 2000 ppm (about 30 – 40% increase) but only a slight decline occurred in higher [CO_2] up to 1%. The CO_2 response curve of total biomass was also bell shaped, peaking at 1000 – 2000 ppm [CO_2] and dropping to the level close to ambient control by 1% [CO_2]. Therefore, the response curve of growth to [CO_2] generated in our experiments is consistent with earlier studies, but with different CO_2 saturation and damage points. Such response pattern in a wide [CO_2] range suggests that proper dilution of the flue gas (regardless of the effect of other acidic pollutants, diluted from 45 to 200 times in our experiment) will be required. However, the actual CO_2 set point in a greenhouse would involve tradeoffs among the effects of [CO_2] on yield, heat dissipation, humidity control and ventilation costs. For practical consideration, if acidic pollutants are removed, growing plants in the flue–gas–fed system with higher [CO_2] may provide three advantages. First, the cost of diluting flue gas can be significantly lowered. Second, ultrahigh [CO_2] may reduce pest damage in the greenhouse system by inhibiting the activity of herbivorous insects and/or reducing the nutrient value of the plant tissue (Nicolas and Sillans 1989, Grodzinski et al. 1999). Finally, a higher C:N ratio of plants grown in ultrahigh CO_2 indicates higher nitrogen use efficiency. Therefore, the mechanism causing detrimental effects in ultrahigh [CO_2] merits further investigation and can perhaps provide guidance in finding ultrahigh-[CO_2]-tolerant species.

As we have seen, the use of flue gas as a CO_2 source for greenhouses presents challenges since, in addition to CO_2, flue gas also contains high concentration of acidic air pollutants, mainly SO_2 and NO_2 (12% [CO_2], 15 ppm [SO_2] and 130 ppm [NO_2] after

proper treatment, but before dilution). Our experiment illustrated that this level of acidic pollutants can significantly offset the growth enhancement from elevated [CO_2]. However, this problem can potentially be resolved. For example, the flue gas can be diluted to optimize the [CO_2] enhancement effect and minimize the deleterious effects of the acidic components. Previous studies (Carlson 1983, Idso and Idso 1994, Lee et al. 1997, Agrawal and Deepak 2003) found that elevated [CO_2] could compensate for the damage caused by acidic pollutants, mainly by inducing closure of the stomata. In our experiments, the aboveground biomass yield of plants grown in 1000 ppm CO_2 , 0.8 ppm NO_2 & 0.09 ppm SO_2 (comparable to 200 times diluted flue gas) still resulted in considerably higher biomass accumulation than in the ambient control treatment. Alternatively, the acidic components could be scrubbed from the air stream, perhaps with water. With further basification of this water, it may be possible to convert these pollutants to nutrient sources necessary for the growth of the plants and thereby decrease fertilizer cost.

Finding ultrahigh-[CO_2]-/pollutant-tolerant species is another potential means of minimizing the negative effects in a flue-gas-fed bio-carbon-sequestration system. It is widely known that the susceptibility to particular pollutants is species and genotype specific (Morikawa et al. 1998). It may therefore be possible to find pollutant-tolerant, fast-growing plants to use in a flue-gas-fed system. Clearly, many species would need to be screened, and a mechanistic understanding of the possible growth responses of various plant species in a flue-gas-fed greenhouse system is needed. Species selection is a longer-term question, and many more physiological properties need to be considered. The

selection standards for the biofuel crop grown in a flue-gas-supplied-greenhouse can be significantly different from that for crops grown in an open field. The main questions regard biomass yield maximization in elevated [CO_2], tolerance to acidic pollutants in the flue gas, light use efficiency, and biofuel quality. Due to the nature of the manipulated greenhouse environment, some physiological properties such as temperature adaptation and acclimation to high [CO_2] require less attention. Furthermore, the need for additional information on other physiological properties depends on the specific greenhouse system. We suggest further that screening work in a high-PAR greenhouse fed with actual power plant flue gas needs to be carried out. In order for this mode of carbon sequestration to be practical, the commercial value of the biomass and the cost of establishing, maintaining, and harvesting plants will all need to be evaluated. In the long term, it is possible that artificial selection and genetic modification (e.g. transgenic plants, Morikawa et al. 2003) can also be used to create the genetic plant lines appropriate for the system.

Conclusion

In summary, the biomass yield of *A. philoxeroides* saturated at 2000 ppm [CO_2] and resulted in plants 107% larger than ambient control plants. The more numerous, denser starch deposits observed leaf cells of *A. philoxeroides* also indicate that plants can sequester more carbon in the elevated-[CO_2] environment. High [CO_2] treatments approaching 3000 ppm did not change the biomass allocation pattern or nitrogen content in roots and stems, and leaf nitrogen showed a negative correlation to growth [CO_2]. The growth enhancement in aboveground biomass was maintained up to 5000 ppm [CO_2] and began to decline only when atmospheric [CO_2] was above 1%. Aboveground biomass dropped to less than the ambient control when atmospheric [CO_2] approached 2%. The growth enhancement by elevated [CO_2] was significantly offset by acidic components of the flue gas, but aboveground biomass yield still increased considerably when the pollution level was moderate (1000 ppm [CO_2] – 0.8 ppm [NO_2], 0.09 ppm [SO_2]). The C:N ratio of aboveground biomass increased with elevated growth [CO_2], but the response curve flattened at 1%, regardless of the presence of acidic pollutants. These results indicate that growth [CO2], rather than growth rate, determined the C:N ratio in *A. philoxeroides*, and nutrients are not likely to have limited plant growth in our experiment.

If the biomass accumulation rate in our experiment could be maintained and scaled up, it would be equivalent to 47 – 66 Mg ha^{-1} yr^{-1}, comparable to the highest yield of current biofuel projects. We believe this is still a conservative estimation since space limitation and low light use efficiency can be largely eliminated in a greenhouse setting. The adverse effect of acidic pollutants in flue gas can be overcome by removing the pollutants

or by diluting the gas to a safe level (diluted approximately 200 times by ambient air). More effort is warranted to select ultrahigh [CO_2]-/pollution-tolerant species/lines. It may also be possible to create more appropriate species for a flue-gas-fed greenhouse bio–carbon–sequestration system through genetic engineering once tolerant species are located and the physiology of this tolerance has been investigated. This technology has the potential to help solve two important problems at once, sequestering the carbon from fossil fuel emissions and providing clean biomass energy.

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Table 1. Nitrogen percentage and carbon to nitrogen ratio of *A. philoxeriodes* grown in different [CO_2] treatments. Values shown are means (± standard error of the mean, SEM), where n=3. The values not sharing any superscript letters indicate significant difference ($P<0.05$) between the [CO_2] treatments (Mann – Whitney U-test).

	CO_2 Concentration	Root	Stem	Leaf	Total
N%	Ambient	3.28(0.17)^{a}	5.68(0.11)^{b}	7.33(0.05)^{b}	5.77(0.13)^{b}
	1000ppm	3.29(0.12)^{a}	5.86(0.09)^{b}	6.89(0.17)^{a}	5.70(0.10)b
	2000ppm	3.16(0.24)^{a}	5.53(0.21)^{ab}	6.42(0.13)^{a}	5.19(0.24)^{a}
	3000ppm	3.37(0.16)^{a}	5.39(0.03)^{a}	6.38(0.16)^{a}	5.33(0.04)^{a}
C:N (w/w)	Ambient	9.52(0.17)^{a}	6.37(0.16)^{a}	5.57(0.07)^{a}	6.42(0.12)^{a}
	1000ppm	10.26(0.57)^{ab}	6.18(0.13)^{a}	6.09(0.17)^{b}	6.79(0.16)^{a}
	2000ppm	10.99(0.45)^{ab}	6.77(0.26)^{ab}	6.50(0.13)^{b}	7.46(0.20)^{b}
	3000ppm	10.43(0.62)^{b}	7.04(0.14)^{b}	6.57(0.21)^{b}	7.36(0.21)^{b}

Figure Legend

Figure 1. The plant growth system. A) the photograph of a custom built small growth chamber; B) diagram of the CO_2 control system.

Figure 2. Growth conditions in the small growth chambers. The figure shows a one-week record of A) [CO_2] control in 4 continuously monitored growth chambers in experiment 1; B) temperature and C) light in all growth chambers in experiment 2. The occasional declines in [CO_2] were caused by tube blockage due to condensation. Decreases in light levels during the day were caused by manual CO_2 measurements and regulation.

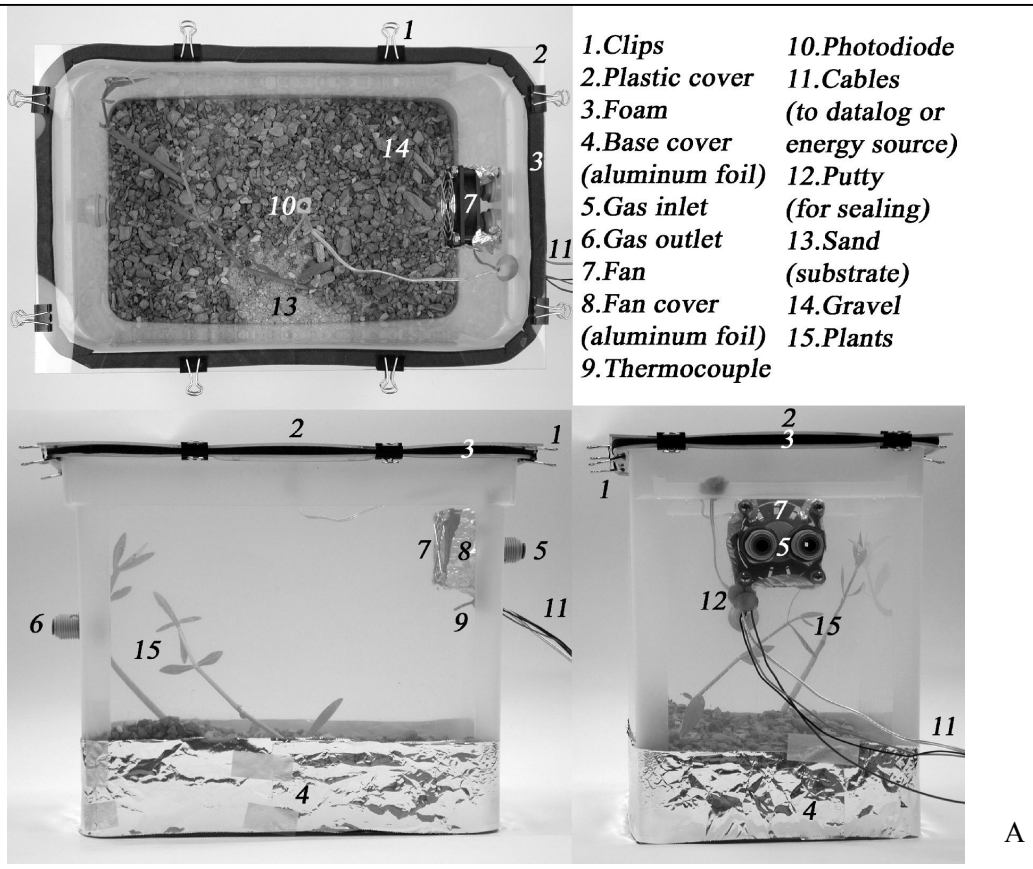
Figure 3. Growth and allocation of *A. philoxeroides* in experiment 1 (ambient to 3000 ppm [CO_2] treatments). A) New biomass accumulation and carbon sequestration. Values are means (± SEM), where n = 3. The vertical bars not sharing any letters (normal font, biomass; **black font**, carbon) show significant difference ($P < 0.05$) between the [CO_2] treatments (Mann-Whitney U-test). Biomass allocation in root, stem and leaf. The stacking bars not sharing any letters (normal font, leaf; *Italic font*, stem; **black font**, root) show significant difference ($P < 0.05$) between the [CO_2] treatments (Mann-Whitney U-test).

Figure 4. Cell structure of the leaf tissues of *A. philoxeroides*. A) Ambient and B) 3000 ppm CO_2. The arrows point to masses of starch deposits.

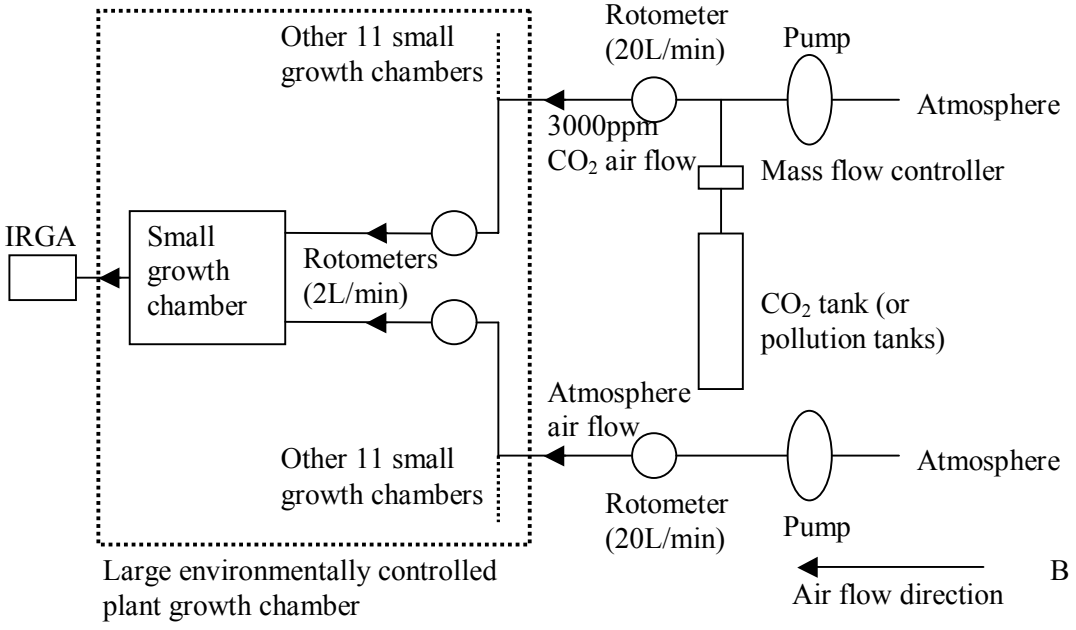
Figure 5. Aboveground biomass growth response to [CO_2] and [CO_2]/pollution. A) Aboveground biomass accumulation in three experiments.Values are means (± SEM), where n = 3. The points not sharing any letters (normal font, experiment 1; **black font,** experiment 2; *Italic font,* experiment 3) show significant difference ($P < 0.05$) between the [CO_2] treatments in each experiment (Mann-Whitney U-test). The differences between ambient controls in three experiments are not significant at $P = 0.05$ level (underline letters, Mann-Whitney U-test). B) Relative growth to ambient control (mean to mean) in each experiment.

Figure 6. Aboveground carbon to nitrogen ratio. Values are means (± SEM), where n=3. The points not sharing any letters (normal font, experiment 1; **black font,** experiment 2; *Italic font,* experiment 3) show significant difference ($P < 0.05$) between the [CO_2] treatments in each experiment (Mann-Whitney U-test). The differences between ambient controls in three experiments are not significant at $P = 0.05$ level (underline letters, Student's t test multi-comparison).

Figure 1


1.Clips
2.Plastic cover
3.Foam
4.Base cover (aluminum foil)
5.Gas inlet
6.Gas outlet
7.Fan
8.Fan cover (aluminum foil)
9.Thermocouple
10.Photodiode
11.Cables (to datalog or energy source)
12.Putty (for sealing)
13.Sand (substrate)
14.Gravel
15.Plants

A


Other 11 small growth chambers
Rotometer (20L/min)
Pump
Atmosphere
3000ppm CO_2 air flow
Mass flow controller
IRGA
Small growth chamber
Rotometers (2L/min)
CO_2 tank (or pollution tanks)
Atmosphere air flow
Other 11 small growth chambers
Atmosphere
Rotometer (20L/min)
Pump
Large environmentally controlled plant growth chamber
Air flow direction

B

Figure 2.

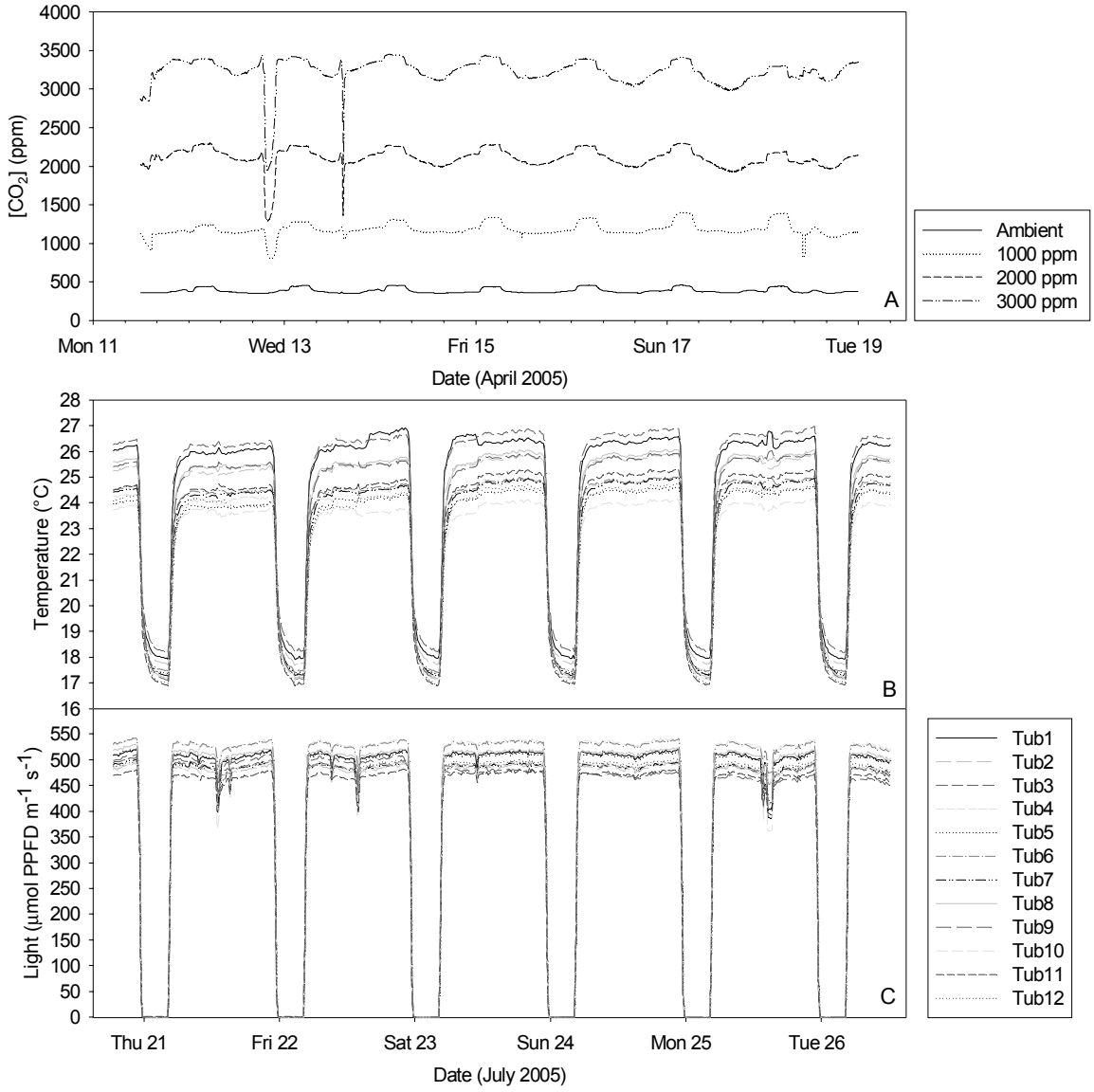

4000
3500
3000
2500
2000
1500
1000
500
0
[CO2] (ppm)
A
Ambient
1000 ppm
2000 ppm
3000 ppm
Mon 11
Wed 13
Fri 15
Sun 17
Tue 19
Date (April 2005)
28
27
26
25
24
23
22
21
20
19
18
17
16
Temperature (°C)
B
550
500
450
400
350
300
250
200
150
100
50
0
Light (µmol PPFD m-1 s-1)
C
Tub1
Tub2
Tub3
Tub4
Tub5
Tub6
Tub7
Tub8
Tub9
Tub10
Tub11
Tub12
Thu 21
Fri 22
Sat 23
Sun 24
Mon 25
Tue 26
Date (July 2005)

Figure 3.

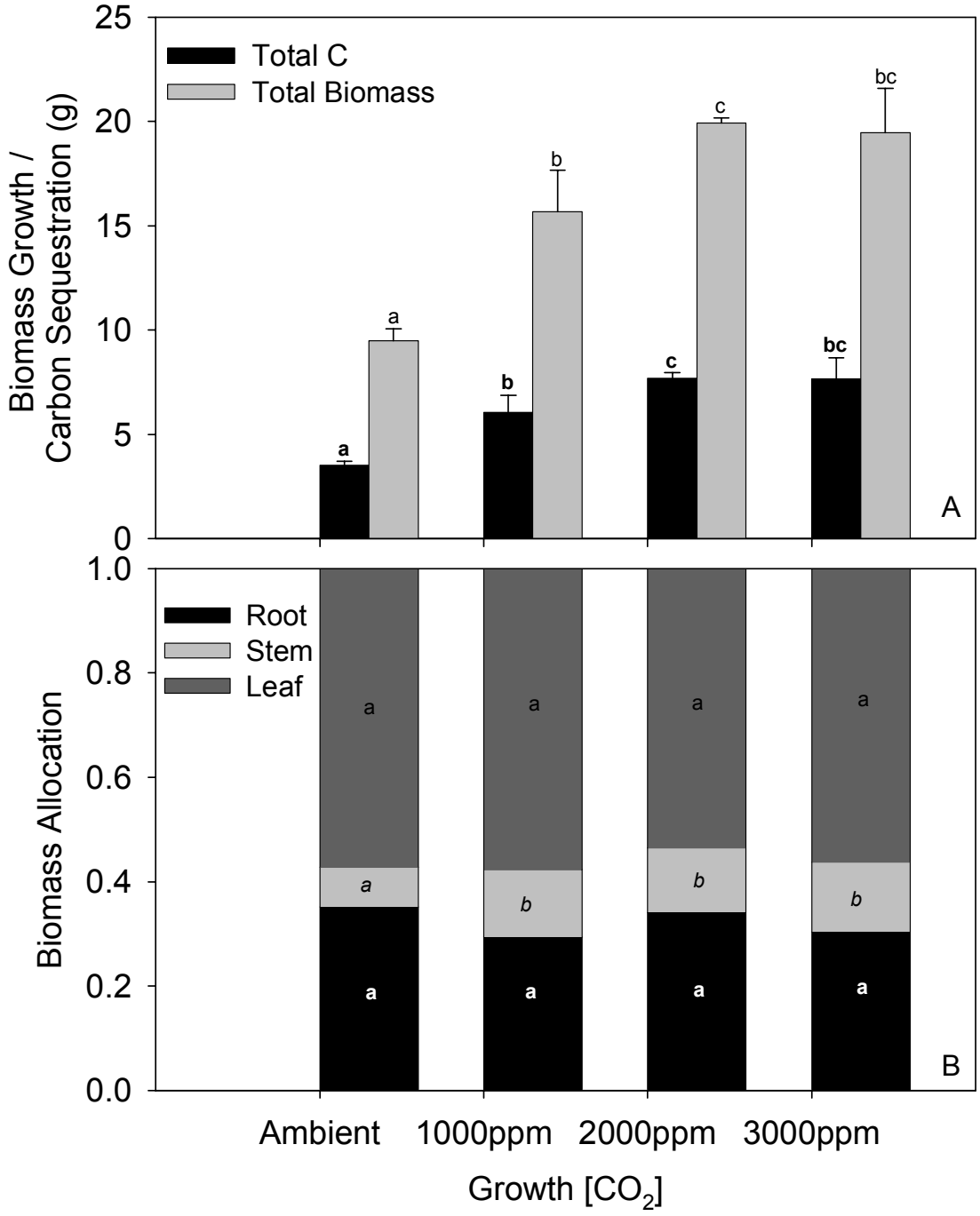

Biomass Growth / Carbon Sequestration (g)
Total C
Total Biomass
25
20
15
10
5
0
a
a
b
b
c
c
bc
bc
A
Biomass Allocation
Root
Stem
Leaf
1.0
0.8
0.6
0.4
0.2
0.0
a
a
a
a
a
b
b
b
a
a
a
a
B
Ambient
1000ppm
2000ppm
3000ppm
Growth [CO2]

Figure 4.

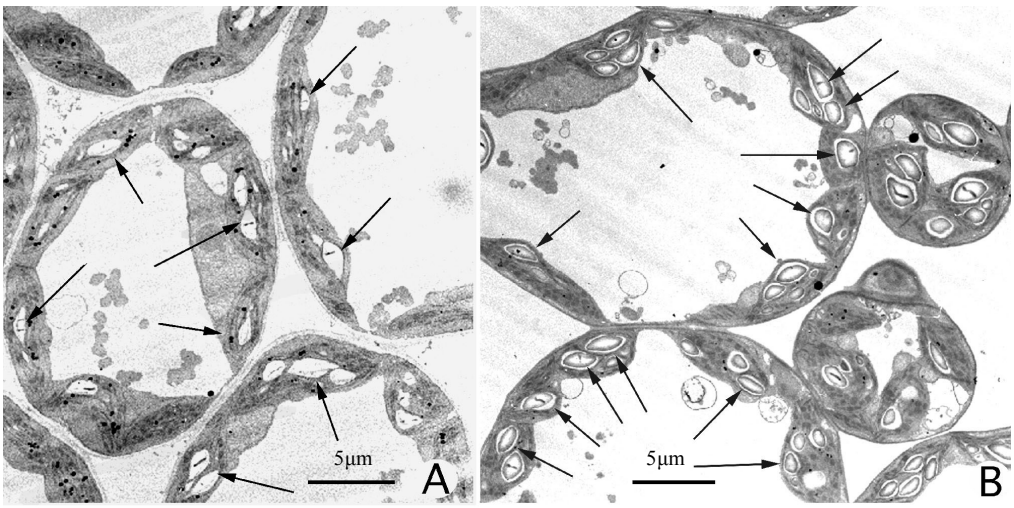


Figure 5.

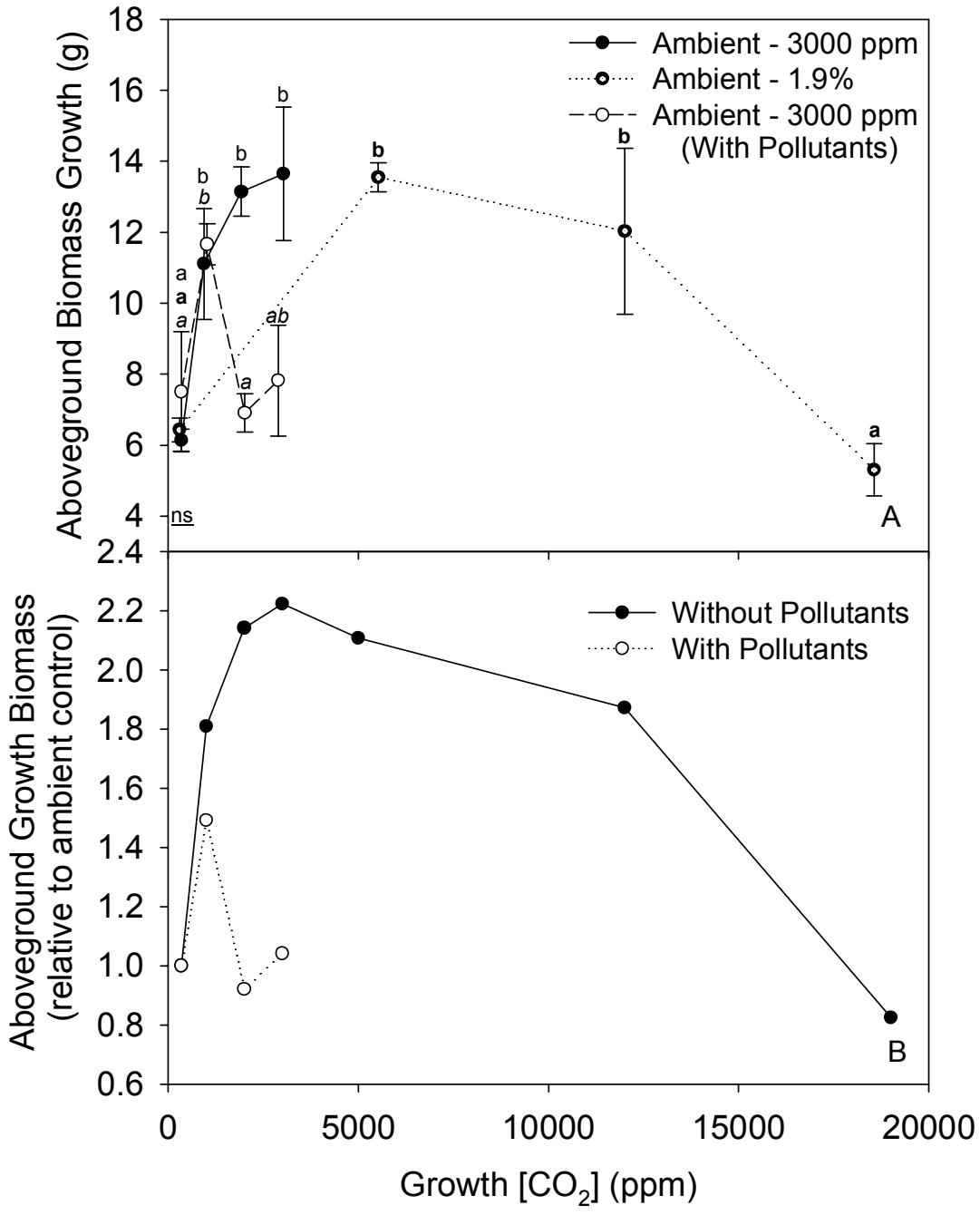
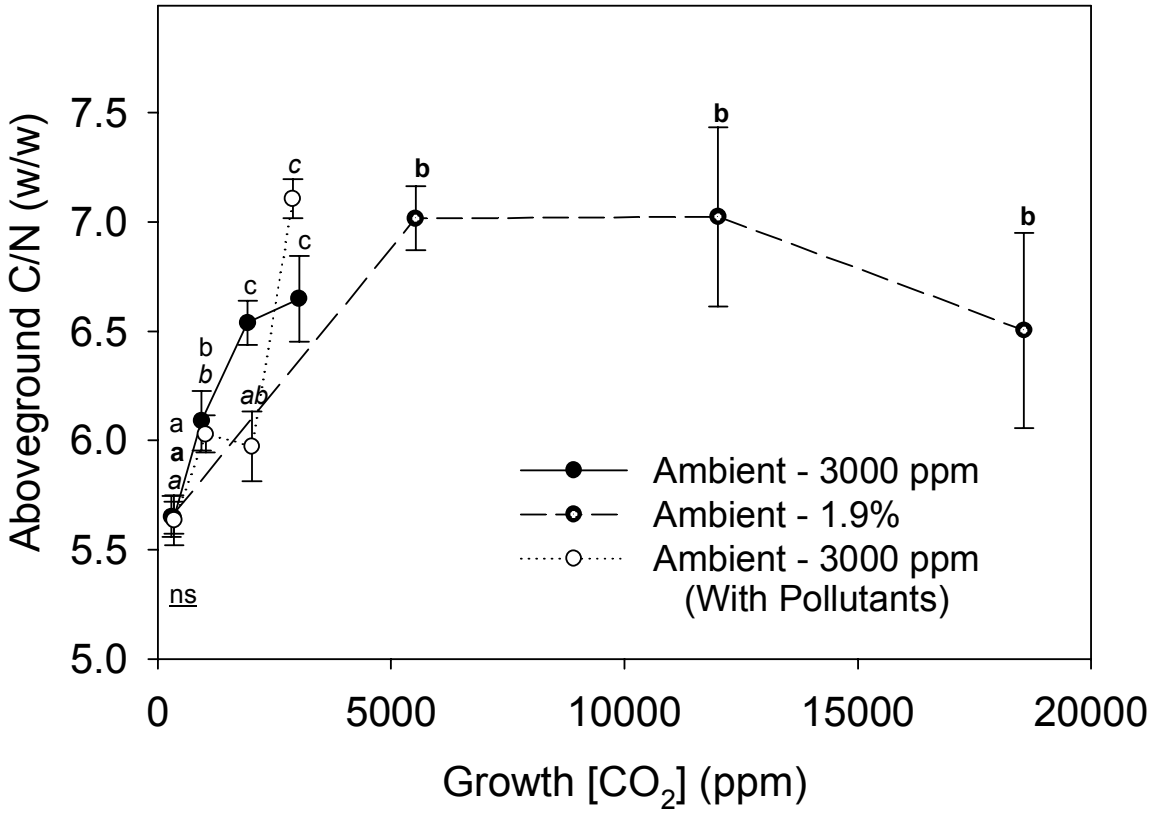

Aboveground Biomass Growth (g)
Ambient - 3000 ppm
Ambient - 1.9%
Ambient - 3000 ppm
(With Pollutants)
ns
A
Aboveground Growth Biomass
(relative to ambient control)
Without Pollutants
With Pollutants
B
Growth [CO2] (ppm)

Figure 6.


Aboveground C/N (w/w)
7.5
7.0
6.5
6.0
5.5
5.0
0
5000
10000
15000
20000
Growth [CO2] (ppm)
Ambient - 3000 ppm
Ambient - 1.9%
Ambient - 3000 ppm
(With Pollutants)
ns