Carbon and oxygen isotope analysis of leaf biomass reveals contrasting photosynthetic responses to elevated CO$_2$ near geologic vents in Yellowstone National Park

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Abstract. In this study we explore the use of natural CO$_2$ emissions in Yellowstone National Park (YNP) in Wyoming, USA to study responses of natural vegetation to elevated CO$_2$ levels. Radiocarbon (14C) analysis of leaf biomass from a conifer (Pinus contortus; lodgepole pine) and an invasive, non-native herb (Linaria dalmatica; Dalmation toadflax) was used to trace the inputs of vent CO$_2$ and quantify assimilation-weighted CO$_2$ concentrations experienced by individual plants near vents and in comparable locations with no geologic CO$_2$ exposure. The carbon and oxygen isotopic composition and nitrogen percent of leaf biomass from the same plants was used to investigate photosynthetic responses of these plants to naturally elevated atmospheric CO$_2$ concentrations. The coupled shifts in carbon and oxygen isotope values suggest that dalmation toadflax responded to elevated CO$_2$ exposure by increasing stomatal conductance with no change in photosynthetic capacity and lodgepole pine apparently responded by decreasing stomatal conductance and photosynthetic capacity. Lodgepole pine saplings exposed to elevated levels of CO$_2$ likewise had reduced leaf nitrogen concentrations compared to plants with no enhanced CO$_2$ exposure, further suggesting widespread and dominant conifer down-regulated photosynthetic capacity under elevated CO$_2$ levels near geologic vents.

1 Introduction

Effects of elevated CO$_2$ on plant photosynthesis and growth have been intensively studied because of the prominent role terrestrial vegetation plays in the global carbon cycle. Unresolved is whether and to what degree photosynthesis will be stimulated and how stomatal conductance and carboxylation and photochemical capacities are likely to react to globally increased CO$_2$ levels (Ainsworth and Rogers, 2007). Free-Air CO$_2$ Enrichment (FACE) is the preferred approach for studying effects of elevated CO$_2$ on natural ecosystems under realistic soil conditions and climate. However, cost constraints and technical difficulties have severely limited application of FACE technology across different ecosystems and climate. Further due to cost constraints most FACE experiments are short term providing only limited opportunities to study feedbacks on community structure and soil biogeochemistry. Geologic CO$_2$ emissions at the terrestrial surface offer an alternative low cost approach for studying elevated CO$_2$ effects on plant communities growing in natural environments (Bettaraini et al., 1995; Miglietta et al., 1998; Tognetti et al., 2000). Although natural CO$_2$ gradients established near geologic CO$_2$ vents can be confounded with other environmental factors, observations from such systems provide an opportunity to examine effects of long-term exposure to elevated CO$_2$ to compare against results from short-term Free-Air CO$_2$ Enrichment (FACE) experiments.

In this study we explore the use of natural CO$_2$ emissions in Yellowstone National Park (YNP) in Wyoming, USA to study responses of a native conifer (Pinus contortus; Lodgepole pine) and an invasive, non-native herb (Linaria dalmatica; Dalmation toadflax) to elevated CO$_2$ levels. There are

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numerous cold geologic features in YNP emitting large quantities of CO$_2$ at very high concentrations (>99%) with very minimal traces of H$_2$S and CH$_4$ and the native vegetation has been exposed to elevated levels of CO$_2$ at these sites for generations. The CO$_2$ emitted from geologic vents in YNP has no $^{14}$C, whereas background air has distinguishable and stable levels of $^{14}$CO$_2$ incorporated into plant biomass during photosynthesis. Hence, radiocarbon ($^{14}$C) analysis of leaf biomass was used to trace the inputs of vent CO$_2$ and quantify assimilation-weighted CO$_2$ concentrations experienced by individual plants near vents and in comparable locations with no geologic CO$_2$ exposure.

Changes in plant photosynthesis associated with increased atmospheric CO$_2$ concentration can be detected in the $^{13}$C and $^{18}$O isotope composition of leaves (Farquhar et al., 1989; Ehleringer et al., 1993; Scheidegger et al., 2000; Barbour, 2007). The combined $^{13}$C and $^{18}$O isotopic composition of leaves can be used as long-term integrators of plant response to elevated CO$_2$ conditions (Grams et al., 2007). Increases in leaf carbon isotope discrimination can be a result of either (1) a proportional increase in stomatal conductance relative to photosynthetic CO$_2$ demand by chloroplasts or (2) a proportional decrease in photosynthetic CO$_2$ demand by chloroplasts relative to stomatal conductance (Scheidegger et al., 2000). Measurements of the carbon and oxygen isotopic composition of leaf organic matter can be used to investigate independent effects of photosynthetic capacity and stomatal conductance on the ratio of internal to ambient CO$_2$ composition ($c_i/c_a$) and hence $\Delta^{13}$C (Scheidegger et al., 2000; Barbour, 2007; Grams et al., 2007). The combined assessment of $\delta^{13}$C and $\delta^{18}$O and N content of collected leaf samples helped us to investigate photosynthetic responses of both plant species to naturally elevated atmospheric CO$_2$ concentrations.

## 2 Methods

### 2.1 Leaf sampling and determination of CO$_2$ exposure

Foliage samples were collected from lodgepole pine saplings at Ochre Springs and from adult plants of the invasive, non-native forb dalmation toadflax at Mammoth Upper Terraces in July 2006 (Fig. 1). Plants were sampled from near low-temperature CO$_2$ vents ("elevated" CO$_2$ sites) and from nearby sites where CO$_2$ exposure from vents was minimal ("control" CO$_2$ sites). Sampling locations were selected based on observed atmospheric CO$_2$ concentration values measured with a portable infra-red gas analyzer (IRGA; LI 6200, LiCor Inc., Lincoln NE, USA) and from site surveys (Tereck, 2004). Instantaneous CO$_2$ concentrations within plant canopies measured with the IRGA were elevated (400–600 µL/L) above background levels near the CO$_2$ vents and were near background levels (378 µL/L) at comparable locations distant from vents. The control CO$_2$ site at Ochre Springs (44°37′1″ N; −110°26′12″ W) was located approximately 1600 m from the elevated CO$_2$ site (44°36′45″ N; −110°24′52″ W). At Mammoth Upper Terraces the control site (44°57′42″ N; −110°42′51″ W) and elevated CO$_2$ site (44°57′45″ N; −110°42′54″ W) were at distance of approximately 200 m from each other. Soil temperature variations can potentially affect the photosynthesis and water relations in different plant communities (Schwarz et al., 1997; Dang and Cheng, 2004; Zhang and Dang, 2005). Therefore, soil temperatures were measured at all sampling sites and we noted that soil temperatures at 10 cm depth were similar (between 20 and 25°C at midday) at both control and elevated CO$_2$ sites.

Five individuals of lodgepole pine were haphazardly selected for isotope sampling at each of the control and elevated CO$_2$ sites in the Ochre Springs area. Isotopic composition of leaves might be effected by increased hydraulic resistance resulting from increased xylem path lengths in taller trees and longer branches (Warren and Adams, 2000) and the effective CO$_2$ exposure could also vary considerably with canopy height. To reduce this variability in this study needles were sampled (from the 2006 cohort) from branches of same length at the middle crown position from 0.5 to 1 m tall trees. The light environment of leaves can also potentially affect their carbon isotope signature (Lockheart et al., 1997; Grams et al., 2007). Therefore, we sampled needles only from tips of south facing branches of trees exposed to similar microhabitat conditions within the forest stand. Leaves of dalmation toadflax at Mammoth Upper Terraces were collected from 5 plants growing near a point CO$_2$ emission source and from 4 toadflax plants growing in a nearby site.
where atmospheric CO₂ levels were similar to background levels. At both the elevated and control site leaves were sampled from 4 to 5 plants growing at a distance of 3–4 m from each other along a 15 m transect. Leaves (10–15) were collected from 4 different stems on each plant avoiding the small leaves subtending flowers.

Quantitative exposure to CO₂ from the geologic emissions integrated over the growth period of leaf development and maturation is difficult to assess solely from short-term IRGA measurements of air in the plant canopy. Therefore, we used the 14C content of leaves to estimate the assimilation-weighted CO₂ concentration exposure. The CO₂ at our sampling sites can only come from 2 sources i.e. atmosphere or the vent and therefore the sum of fractions of CO₂ fixed by plant coming from vent and normal atmosphere should be equal to unity. Using mass balance approach, the measured 14C abundance in plant material (Mₚ) will be equal to the sum of fractions coming from vent and atmosphere weighted by the abundance of 14C in each of these gases (van Gardingen et al., 1995). CO₂ emitted from the vents originates from dissolution of carbonate sediments and therefore is highly depleted in 14C (so called “dead CO₂”) and 14C contribution from vent can be taken as 0 (Bettarini et al., 1995; van Gardingen et al., 1995). A simple mass-balance approach was then used to calculate the average CO₂ concentration surrounding individual lodgepole pine and dalmation toad-flax plants:

\[ [\text{CO}_2]_p = [\text{CO}_2]_b (M_b / M_p) \]  

where \([\text{CO}_2]_b\) is the mean CO₂ concentration in the normal background atmosphere (taken to be 380 ppm). \(M_b\) and \(M_p\) are the 14C abundances (reported as fraction of modern standard) in leaves growing at ambient CO₂ concentrations and higher CO₂ concentrations respectively. Carbon isotope discrimination was determined from δ¹³C of leaf biomass (δ¹³C_l) and δ¹³C of air surrounding the plant (δ¹³C_p) from (Farquhar et al., 1989):

\[ \Delta^{13}C = (\delta^{13}C_p - \delta^{13}C_l)/(1 + \delta^{13}C_l/1000) \]  

The δ¹³C of CO₂ in air surrounding the plant (δ¹³C_p) will represent a mixture of δ¹³C of CO₂ from the vent (δ¹³C_v) and background air (δ¹³C_b). CO₂ emitted from geologic vents at Mammoth Upper Terraces and Ochre Spring sites has δ¹³C values of −3.9 and −3.2‰, respectively (Werner and Brantly, 2003). The δ¹³C of background atmospheric CO₂ was assumed to be −8‰ (Keeling et al., 2001). The assimilation-weighted δ¹³C value of CO₂ in the air surrounding individual plants was calculated using mass-balance:

\[ \delta^{13}C_p = \delta^{13}C_b + f \left( \delta^{13}C_v - \delta^{13}C_b \right) \]  

where \(f\) is the fraction of CO₂ contribution from the vent. The fraction of CO₂ contributed by vent is calculated using mass balance approach:

\[ f = (\Delta^{14}C_l - \Delta^{14}C_b) / (\Delta^{14}C_v - \Delta^{14}C_b) \]  

where Δ¹⁴C is the relative deviation of ¹⁴C activity of the sample from the oxalic Acid I standard in per mil values (after accounting for ¹⁴C fractionation with a δ¹³C correction). Δ¹⁴C_v is the measured value of leaf samples, Δ¹⁴C_b is the measured value for 2006 atmosphere at Pt. Barrow Alaska (50‰) and Δ¹⁴C_v is the assumed value for CO₂ from vent (−1000‰).

The calculated carbon isotope discrimination was then used to estimate the influence of elevated CO₂ concentration on the ratio of leaf intercellular to ambient CO₂ concentration (c_i/c_a) using the simplified model of Farquhar et al. (1989) as:

\[ c_i/c_a = (\Delta^{13}C - a)/(b - a) \]  

where \(a\) is the carbon isotope discrimination associated with CO₂ diffusion through air (−4.4‰), and \(b\) is the net discrimination associated with CO₂ fixation in C3 plants (−27‰).

2.2 Leaf isotopic and N content analyses

Whole leaf samples of dalmation toadflax and lodgepole pine needles from the 2006 growth year were dried and ground in a mill to a consistent particle size. Stable isotope analysis was carried out at the University of Wyoming Stable Isotope Facility (http://www.uwyo.edu/sif/). The δ¹³C and N% values were determined by continuous flow EA-IRMS using a Costech 4010 element analyzer online with a Finnigan Delta XP isotope ratio mass spectrometer. The δ¹⁸O composition of bulk leaf material was measured by online pyrolysis to CO using a Finnigan TC/EA coupled to the Finnigan Delta Plus XP isotope ratio mass spectrometer. Measurement precision was assessed by replicate analysis of samples and internal lab standards and was better than ±0.1‰ for δ¹³C and ±0.25‰ for δ¹⁸O. The δ¹³C and δ¹⁸O values are reported in per mil (‰) relative to V-PDB and V-SMOW, respectively. Radiocarbon analysis was performed at the W.M. Keck AMS Laboratory at the University of California, Irvine. Radiocarbon concentrations are reported as fractions of the Modern Standard and Δ¹⁴C is reported following conventions of Stuiver and Polach (1977).

3 Results and discussion

The assimilation-weighted CO₂ exposure of a leaf calculated from radiocarbon values of leaves Eq. (1) ranged from 379–381 (control site) to 401–607 (elevated site) ppm in dalmation toadflax from the Mammoth Upper Terraces site and from 378–383 (control site) to 419–482 ppm (elevated site) in lodgepole pine at the Ochre Springs site. Changes in leaf δ¹³C values in response to variations in environmental conditions integrate changes in the relative demand of CO₂ by chloroplasts during photosynthesis and in the supply of CO₂ associated with stomatal conductance in plants (Farquhar et al., 1989; Ehleringer et al., 1993). We
found that $\Delta^{13}C$ was significantly positively correlated with assimilation-weighted CO$_2$ exposure levels (Fig. 2) for both dalmation toadflax and lodgepole pine (dalmation toadflax, $r^2=0.77$, $P < 0.002$; lodgepole pine, $r^2=0.85$, $P < 0.001$) indicating that elevated CO$_2$ levels caused an increase in leaf $c_i/c_a$. The observed increase in $\Delta^{13}C$ values reflect a 13% and 17.5% increase in leaf $c_i/c_a$ in the case of toadflax and lodgepole pine samples, respectively.

We observed a 2.4‰ decrease in $\delta^{18}O$ of leaf biomass with increasing CO$_2$ exposure in dalmation toadflax and a 2.5‰ increase in $\delta^{18}O$ of leaf biomass along the gradient of increasing CO$_2$ exposure in lodgepole pine (Fig. 3a and b). Because of close proximity, we assumed that the oxygen isotopic composition of soil water and atmospheric vapor was similar for plants growing within control or elevated CO$_2$ sites, and the conceptual model proposed by Grams et al. (2007) can be used to understand components of the photosynthetic response of the two species. This model infers the changes in stomatal conductance, $c_i/c_a$ and photosynthetic capacity of plants using a dual carbon and oxygen isotope approach. This model is an expansion of the conceptual model proposed by Scheidegger et al. (2000) which estimates changes in leaf-to-air vapor pressure deficit from the $\delta^{18}O$ of leaf biomass for prediction of changes that would have likely occurred in stomatal conductance. The model of Grams et al. (2007) infers stomatal conductance changes directly from $\delta^{18}O$ of leaf cellulose bypassing the previously used relative humidity/$\delta^{18}O$ relationship in the model of Scheidegger et al. (2000). Grams et al. (2007) did not find a significant correlation between $\delta^{18}O$ of leaf biomass and leaf cellulose in their study and therefore used the $\delta^{18}O$ of leaf cellulose as the input parameter in their model. However, a reliable correlation between $\delta^{18}O$ of leaf biomass and cellulose has been observed in several herbaceous and woody plants (Barbour and Farquhar 2000; Barbour et al., 2001). We feel justified in using the $\delta^{18}O$ of leaf biomass instead of $\delta^{18}O$ of leaf cellulose as an input parameter in the conceptual model of Grams et al. (2007).

In case of dalmation toadflax there is an increase in $\Delta^{13}C$ accompanied by a decrease in $\delta^{18}O$ of leaf biomass associated with increasing CO$_2$ exposure (Fig. 3a). The decrease in $\delta^{18}O$ of leaf biomass is likely a result of an increase in stomatal conductance. This is because higher stomatal conductance results in a higher rate of transpiration which cools the leaf surface and reduces the leaf intercellular vapor pressure resulting in lower $\delta^{18}O$ enrichment at evaporating sites. Further, due to increased transpiration, the convective flux of unenriched source water to evaporating sites increases, thereby reducing the enrichment of bulk leaf water (Farquhar and Lloyd 1993). This $\delta^{18}O$ enrichment signal of leaf water is transferred to the bulk leaf organic matter by isotope
exchange reactions. Using the dual isotope model of Grams et al. (2007) the increase in $c_i/c_a$ can then be inferred to be a result of increased stomatal conductance accompanied by no response of photosynthetic capacity. Our results do not fit predictions from FACE sites which generally suggest increasing exposure to CO$_2$ results in an average 22% decrease in stomatal conductance across all plant species (reviewed in Ainsworth and Rogers, 2007). However, preliminary studies in a CO$_2$ enrichment experiment (www.phace.us) near Cheyenne, Wyoming USA (Williams, unpublished data) also confirm that dalmation toadflax shows about a 6‰ ($n=2$–3) decline in leaf biomass $\delta^{18}$O values under elevated CO$_2$ conditions.

Several studies have shown that soil water savings induced by decreased stomatal conductance under elevated CO$_2$ conditions is primarily observed when moisture is limiting (Field et al., 1997; Wullschleger et al., 2002). However, when soil water availability is high several plant species show higher stomatal conductance and are insensitive to elevated CO$_2$ conditions (Mooney et al., 1983; Knapp et al., 1996; Schomp, 2007). We hypothesize that since dalmation toadflax maintains deeper maximal rooting depth and greater root mass density in deep soil layers compared to other co-existing native species it has access to surplus moisture stored in deeper soil layers under elevated CO$_2$ conditions (Schomp, 2007). Due to this more favorable plant water status it shows higher stomatal conductance under elevated CO$_2$ conditions.

A strikingly different pattern was observed in lodgepole pine. Here an increase in $\Delta^{13}$C was accompanied by an increase in $\delta^{18}$O of leaf biomass with increasing CO$_2$ exposure (Fig. 3b). The increase in $\delta^{18}$O of leaf biomass is likely a result of decreased stomatal conductance. The decreased stomatal conductance should result in a decrease in $c_i/c_a$ if photosynthetic CO$_2$ demand is unchanged. However, we observed an increase in $c_i/c_a$ in lodgepole pine exposed to elevated CO$_2$ levels likely due to decreased photosynthetic capacity. The N concentration of plant leaves is positively correlated with photosynthetic capacity (Field and Mooney, 1986), as most of the N in leaves is used for synthesis of photosynthetic enzymes, and in particular Rubisco (Shangguan et al., 2000). The N concentration of lodgepole pine decreases with an increase in atmospheric CO$_2$ concentration ($r^2=0.47$; $P<0.03$) (Fig. 4). This decrease in leaf N% at elevated CO$_2$ might be an additional indicator of decreased photosynthetic capacity in lodgepole pine at elevated CO$_2$ levels. No correlation was observed between leaf N and $\Delta^{13}$C in dalmation toadflax (Fig. 4). Therefore we can infer that photosynthetic capacity of lodgepole pine decreases under higher CO$_2$ concentrations. Our result again contradicts the results obtained from FACE sites which frequently show that elevated CO$_2$ stimulates photosynthesis in C$_3$ plants, with trees showing the largest net increase in photosynthesis with increasing CO$_2$ exposure (reviewed in Nowak et al., 2004; Ainsworth and Long, 2005).

Acclimation or down regulation of photosynthesis is likely to occur in ecological situations where nutrient conditions are low or nitrogen is limiting (Stitt and Krapp, 1999; Sinclair et al., 2000). Nonetheless, even if down regulation is observed photosynthesis is often stimulated in long-term experiments even after 7–10 years of FACE (Nowak et al., 2004). The sampled lodgepole pine stand in this study most likely has been exposed to elevated CO$_2$ levels for centuries. We hypothesize that decreased photosynthetic capacity observed in our case could be due to severe reduction in plant available nitrogen in this forest stand due to phenomenon known as progressive nitrogen limitation (PNL). PNL develops because elevated CO$_2$ leads to N immobilization by plants and microbes which deplete soils of N, causing slower rates of N mineralization progressively reducing the mineral N available for plant uptake in the long term (Gill et al., 2002; Zak et al., 2000; Luo et al., 2004).

In this study, the combined use of $^{14}$C, $\delta^{13}$C and $\delta^{18}$O values of leaf biomass provided useful insight into the photosynthetic response of a native conifer and a non-native forb to elevated CO$_2$ conditions in Yellowstone National Park. The non-native forb, dalmation toadflax, responded by increasing stomatal conductance with no change in photosynthetic capacity, and the native lodgepole pine by decreasing stomatal conductance and photosynthetic capacity under elevated atmospheric CO$_2$. The results from our study are in contrast to those from studies conducted in various FACE sites, emphasizing the importance of long-term, high resolution ecosystem-scale CO$_2$ experiments to understand responses of natural ecosystems to globally increasing CO$_2$ levels. However, the isotopic information provides indirect assessment of the long term integrated photosynthetic response of plant leaves to elevated CO$_2$. We suggest that in
such studies direct measurement of leaf gas exchange rates, stomatal conductance and the response of photosynthesis to leaf internal CO$_{2}$ concentration (A-ci curves) should also be used in conjunction to isotopes to determine direct short-term responses of these plants to elevated CO$_{2}$.

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