Biogeosciences

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 (^{14}C) 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

numerous cold geologic features in YNP emitting large quantities of CO_2 at very high concentrations (>99%) with very minimal traces of H_2S 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
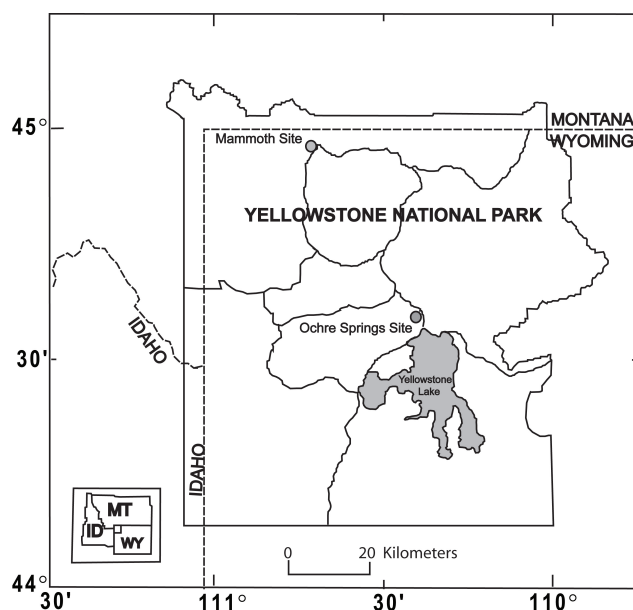

Fig. 1. Map of Yellowstone National Park showing the Ochre Spring and Mammoth Hot Springs sampling locations.

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 effect 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 CO_2 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 CO_2 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 ^{14}C content of leaves to estimate the assimilation-weighted CO_2 concentration exposure. The CO_2 at our sampling sites can only come from 2 sources i.e. atmosphere or the vent and therefore the sum of fractions of CO_2 fixed by plant coming from the vent and normal atmosphere should be equal to unity. Using mass balance approach, the measured ^{14}C abundance in plant material (M_p) will be equal to the sum of fractions coming from vent and atmosphere weighted by the abundance of ^{14}C in each of these gases (van Gardingen et al., 1995). CO_2 emitted from the vents originates from dissolution of carbonate sediments and therefore is highly depleted in [14]C (so called "dead CO_2") and ^{14}C 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 CO_2 concentration surrounding individual lodgepole pine and dalmation toadflax plants:

$$[CO_2]_p = [CO_2]_b (M_b/M_p) \tag{R1}$$

where $[CO_2]_b$ is the mean CO_2 concentration in the normal background atmosphere (taken to be 380 ppm). M_b and M_p are the ^{14}C abundances (reported as fraction of modern standard) in leaves growing at ambient CO_2 concentrations and higher CO_2 concentrations respectively.

Carbon isotope discrimination was determined from $\delta^{13}C$ of leaf biomass ($\delta^{13}C_l$) and $\delta^{13}C$ of air surrounding the plant ($\delta^{13}C_p$) from (Farquhar et al., 1989):

$$\Delta^{13}C = (\delta^{13}C_p - \delta^{13}C_l)/(1 + \delta^{13}C_l/1000) \tag{R2}$$

The $\delta^{13}C$ of CO_2 in air surrounding the plant ($\delta^{13}C_p$) will represent a mixture of $\delta^{13}C$ of CO_2 from the vent ($\delta^{13}C_v$) and background air ($\delta^{13}C_b$). CO_2 emitted from geologic vents at Mammoth Upper Terraces and Ochre Spring sites has $\delta^{13}C$ values of −3.9 and −3.2‰, respectively (Werner and Brantley, 2003). The $\delta^{13}C$ of background atmospheric CO_2 was assumed to be −8‰ (Keeling et al., 2001). The assimilation-weighted $\delta^{13}C$ value of CO_2 in the air surrounding individual plants was calculated using mass-balance:

$$\delta^{13}C_p = \delta^{13}C_b + f(\delta^{13}C_v - \delta^{13}C_b) \tag{R3}$$

where f is the fraction of CO_2 contribution from the vent. The fraction of CO_2 contributed by vent is calculated using mass balance approach:

$$f = (\Delta^{14}C_l - \Delta^{14}C_b)/(\Delta^{14}C_v - \Delta^{14}C_b) \tag{R4}$$

where $\Delta^{14}C$ is the relative deviation of ^{14}C activity of the sample from the oxalic Acid I standard in per mil values (after accounting for ^{14}C fractionation with a $\delta^{13}C$ correction). $\Delta^{14}C_l$ is the measured value of leaf samples, $\Delta^{14}C_b$ is the measured value for 2006 atmosphere at Pt. Barrow Alaska (50‰) and $\Delta^{14}C_v$ is the assumed value for CO_2 from vent (−1000‰).

The calculated carbon isotope discrimination was then used to estimate the influence of elevated CO_2 concentration on the ratio of leaf intercellular to ambient CO_2 concentration (c_i/c_a) using the simplified model of Farquhar et al. (1989) as:

$$c_i/c_a = (\Delta^{13}C - a)/(b - a) \tag{R5}$$

where a is the carbon isotope discrimination associated with CO_2 diffusion through air (−4.4‰), and b is the net discrimination associated with CO_2 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 $\delta^{13}C$ and N% values were determined by continuous flow EA-IRMS using a Costech 4010 element analyzer online with a Finnigan Delta plus XP isotope ratio mass spectrometer. The $\delta^{18}O$ 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 $\delta^{13}C$ and ±0.25‰ for $\delta^{18}O$. The $\delta^{13}C$ and $\delta^{18}O$ 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 $\Delta^{14}C$ is reported following conventions of Stuiver and Polach (1977).

3 Results and discussion

The assimilation-weighted CO_2 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 $\delta^{13}C$ values in response to variations in environmental conditions integrate changes in the relative demand of CO_2 by chloroplasts during photosynthesis and in the supply of CO_2 associated with stomatal conductance in plants (Farquhar et al., 1989; Ehleringer et al., 1993). We
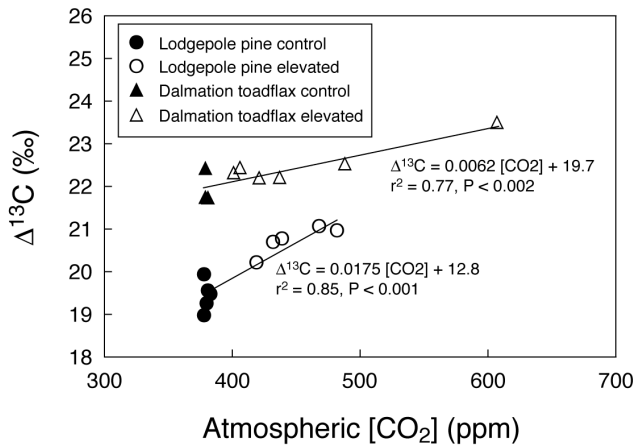
Fig. 2. Correlation between carbon isotope discrimination ($\Delta^{13}C$) and average flux-weighted CO_2 in the leaves of dalmation toadflax (open and filled triangles) and lodgepole pine (open and filled circles) were exposed to. The flux-weighted CO_2 exposure was calculated from the radiocarbon or ^{14}C content of leaves using Eq. (1).

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
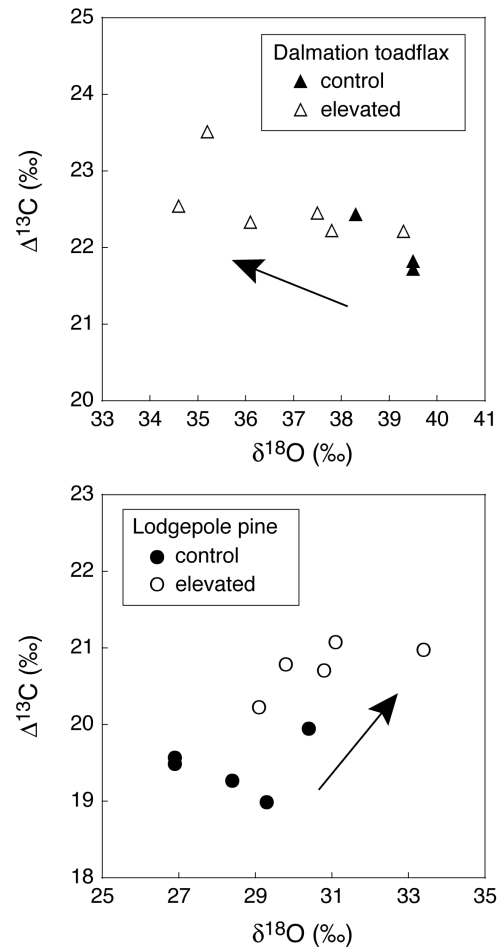

Fig. 3. Correlation between ^{13}C discrimination ($\Delta^{13}C$) and the $\delta^{18}O$ of bulk leaf material in **(a)** dalmation toadflax and **(b)** lodgepole pine. The filled and open symbols represent data from the control and elevated sites respectively.

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 lodegepole 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 C3 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).

Fig. 4. Nitrogen concentrations of leaf biomass show different relationships over gradients of CO_2 exposure between dalmation toadflax (open and filled triangles) lodgepole pine (open and filled circles). Only the lodgepole pine data was used for the regression statistics.

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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