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Authors: Koh, Seok Chan, Demmig-Adams, Barbara, and Adams, William W.

Novel Patterns of Seasonal Photosynthetic Acclimation, Including Interspecific Differences, in Conifers over an Altitudinal Gradient

Seok Chan Koh*

Barbara Demmig-Adams† and William W. Adams III†‡

*Department of Biology, Jeju National University, Jeju 690-756, Korea
†Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, Colorado 80309-0334, U.S.A.
‡Corresponding author: william.adams@colorado.edu

Abstract

Photosynthesis, as the basis of most food chains and a crucial global carbon sink, makes chief contributions to overall ecosystem carbon budgets, but specific responses of the plant component cannot be obtained from such budgets. To gain much-needed further information on possible interspecies differences in seasonal patterns of photosynthesis, capacities for light- and CO_2-saturated rates of oxygen evolution at 25°C (photosynthetic capacity) were determined during the summer-fall-winter transition for five conifer species over their natural distribution along a steep altitudinal gradient. Findings include (i) a transient upregulation of photosynthetic capacity during the summer-to-fall transition in all five conifer species that preceded the previously reported winter downregulation in conifers. However, there were (ii) interspecific differences in this response at the highest altitudes, with higher maximal photosynthetic capacities displayed by pine and spruce species compared to fir species. Lastly, the winter downregulation of photosynthetic capacity was not as complete in the present study (winter of 2006) as that which has been reported for previous winter seasons, which has implications for the winter survival strategy of conifers in response to global warming.

Introduction

Photosynthesis is important as the basis of almost all food chains and a crucial carbon sink for the entire planet. In recent years, research on evergreen forests has seen an increasing focus on the ecosystem carbon budget (sum of carbon gains by photosynthetic plants and algae plus losses from all organisms; Law et al., 2000a, 2000b; Huxman et al., 2003; U et al., 2004; Wang et al., 2004; Winner et al., 2004; Monson et al., 2005; Ponton et al., 2006; Sacks et al., 2006, 2007; Urban et al., 2007). Although this approach has provided important insights into the overall net response of these ecosystems to seasonal changes and year-to-year variation in environmental conditions, it does not provide critical information on the dynamics of the specific responses of the plant components independent of the consumers and on potential differences among the dominant plant species.

Intrinsic capacity for photosynthesis (maximal photosynthetic rate) is a measure of the investment on the part of plant, and changes in this investment occur in response to the environment as well as genetic factors. This information on photosynthetic capacities is cumbersome to come by, as it requires time-consuming characterizations of individual leaves/needles under conditions optimal for photosynthesis (e.g. saturating with respect to light, CO_2, and water vapor, and measured at a favorable temperature). However, it does provide an objective assessment of the intrinsic photosynthetic capacity that is not constrained by stomatal limitations due to short-term limited water availability or increased water vapor pressure deficit between the needles and the atmosphere (e.g. Bond and Kavanagh, 1999; Hubbard et al., 2001; Brodersen et al., 2006).

It has been reported that conifers downregulate photosynthesis in mid-winter compared to the spring through autumn seasons typified by conditions more favorable for growth (Adams et al., 2001, 2002, 2004, 2006; Öquist and Huner, 2003), while some herbaceous species instead exhibit elevated photosynthetic capacities during winter (Verhoeven et al., 1999; Adams et al., 2001, 2002, 2004, 2006), and some evergreen shrubs may exhibit little or no seasonal change (Adams et al., 2002; Zarter et al., 2006a). In addition to these general trends that focused on mid-winter versus mid-summer characterizations, detailed month-to-month analyses are now needed to understand the nature of these differences more fully. To more precisely understand the responses of different conifers to a range of environmental conditions as climate changes from summer through winter, we have examined the response of five conifer species to seasonal changes over the extent of their natural distribution along a steep altitudinal gradient (representing a strong variation of environmental parameters over short distance).

Methods

STUDY SITES

Populations of ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.), Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco), limber pine (*Pinus flexilis* James), Engelmann spruce (*Picea engelmannii* Parry ex. Engelm.), and subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) were studied at five sites on the eastern slope of the Rocky Mountains from 1800 m to near treeline (Table 1) from the summer of 2006 through the winter of 2007 (characterized six times, once every other month, from June 2006 through February 2007). All needles (2005 cohort) were sampled from the south-facing side of 6 trees of each species at each site where, during midday, they were exposed to direct sunlight. During a monthly characterization, some, but not all, of the needles came from the same trees that had been sampled during a previous characterization. During each month that

TABLE 1

Sites, latitude, longitude, altitude, and species studied at each site.

Collection site	Altitude (m)	Species
Gregory Canyon, south-facing slope 39°59.875′N, 105°17.969′W	1852	Ponderosa pine and Douglas fir
Sugarloaf Road, near base of Sugarloaf Mountain 40°01.177′N, 105°24.772′W	2456	Ponderosa pine and Douglas fir
Low hill beside Ridge Road 39°58.422′N, 105°29.786′W	2570	Limber pine
Ridgetop near Ward 40°02.911′N, 105°31.535′W	2949	Ponderosa pine, Douglas fir, subalpine fir, Engelmann spruce, and limber pine
Niwot Ridge, just below treeline 40°02.268′N, 105°34.167′W	3325	Subalpine fir, Engelmann spruce, and limber pine

needles were examined, they were collected and characterized over a period of approximately 12 days.

ENVIRONMENTAL PARAMETERS

The daily minimum and maximum temperatures and precipitation records were obtained from two databases, one corresponding to the lowest altitude site (from the National Atmospheric and Oceanic Administration <http://www.cdc.noaa.gov/Boulder/data.daily.html>) and the other from the C-1 meteorological station at the University of Colorado Mountain Research Station at 3022 m <http://culter.colorado.edu/NWT/data/datmanaccess.html>.

PHOTOSYNTHETIC OXYGEN EXCHANGE

Measurements of photosynthetic oxygen exchange were conducted in a leaf disc oxygen electrode chamber (Model LD-2, equipped with an LS-2 halogen light source, Hansatech, King's Lynn, Norfolk, U.K.) at room temperature in 5% CO_2, 21% O_2, with N_2 for the balance. For all dates, needles were collected in the morning and kept in moist paper towels in darkness before insertion into the electrode. Prior to insertion, needles were aligned on gas-permeable tape and total area determined using a leaf area meter (AM100; Analytical Development Company, Hoddesdon, Hertfordshire, U.K.). Time between transfer from field conditions to laboratory conditions and measurements ranged from 90 minutes to 12 hours. Based on previous characterizations of conifer needles, there is no significant change in photosynthetic capacity when needles are maintained in this manner (Zarter et al., 2006b, 2006c). Rates of steady-state photosynthetic oxygen evolution (photosynthetic capacity) were determined under saturating light (1800 to 2400 μmol photons m^{-2} s^{-1}) and CO_2 (5%) with saturating water vapor, and rates of respiration (oxygen uptake) were measured upon return to darkness, the sum of which yielded gross photosynthesis (light- and CO_2-saturated photosynthetic capacity).

STATISTICAL ANALYSES

For comparisons between two means, a Student's *t*-test was performed. To test for significant differences among means, analysis of variance was applied followed by a Tukey-Kramer comparison for honestly significant differences (JMP Statistical Software; SAS Institute, Cary, North Carolina).

Results

PEAK LEVELS OF PHOTOSYNTHETIC CAPACITY DURING THE FAVORABLE SEASON

Photosynthetic capacities exhibited rather similar profiles for all five species over the summer-fall-winter seasonal progression at moderately high altitudes up to about 2600 m (2949 m for Engelmann spruce). At these moderately high altitudes, photosynthetic capacities peaked in the fall (typically around October) and reached rather similar maximal values of around 25–30 μmol O_2 m^{-2} s^{-1} (Figs. 1A–1E). Even at the highest altitudes, peak values for photosynthetic capacities in the fall were in this same range (of 25–30 μmol O_2 m^{-2} s^{-1}) for the two fir species (Figs. 1D, 1E). However, in contrast to these trends for photosynthetic capacities in the two firs, the two pines as well as the spruce species exhibited significantly higher capacities (mean of 43 versus 24 μmol O_2 m^{-2} s^{-1}; $p < 0.0001$) at the *highest* altitudes where these peak capacities were furthermore reached a little earlier in the season, i.e. in August to September (Figs. 1A–1C). The response to altitude thus clearly varied among species or groups of species. It is particularly noteworthy that limber pine, Engelmann spruce, and subalpine fir—as the dominant species at very high altitudes—showed such different responses, with subalpine fir failing to exhibit the high peak capacities displayed by limber pine and Engelmann spruce at the highest altitude.

This finding of highest capacities in some of the species occurring at the highest altitudes is remarkable, considering that these highest altitudes ("C1 site") represent considerably lower monthly mean temperatures compared with the moderately high altitudes ("Boulder") (Fig. 2) as well as lower mean maximum and minimum temperatures (Fig. 2). In August and September, when peak levels of photosynthetic capacity were observed in ponderosa pine, limber pine, and Engelmann spruce at the highest altitudes, monthly mean temperatures at these altitudes were already declining rapidly (to values of 12.3°C in August and 6.5°C in September). In contrast to temperature, precipitation was equally high or higher at the highest altitudes compared to moderately high altitudes (Fig. 3). For a few months before the onset of characterizations, and throughout the study period, there was consistently equal or greater monthly mean precipitation at the highest altitudes (Fig. 3). The total precipitation at the lower altitude (490.5 mm; Boulder) was only 79.6% of that at the higher altitude (616.0 mm; C1) from January through September of 2006 (when the photosynthetic capacities peaked in the trees growing at higher altitude) (Fig. 1).
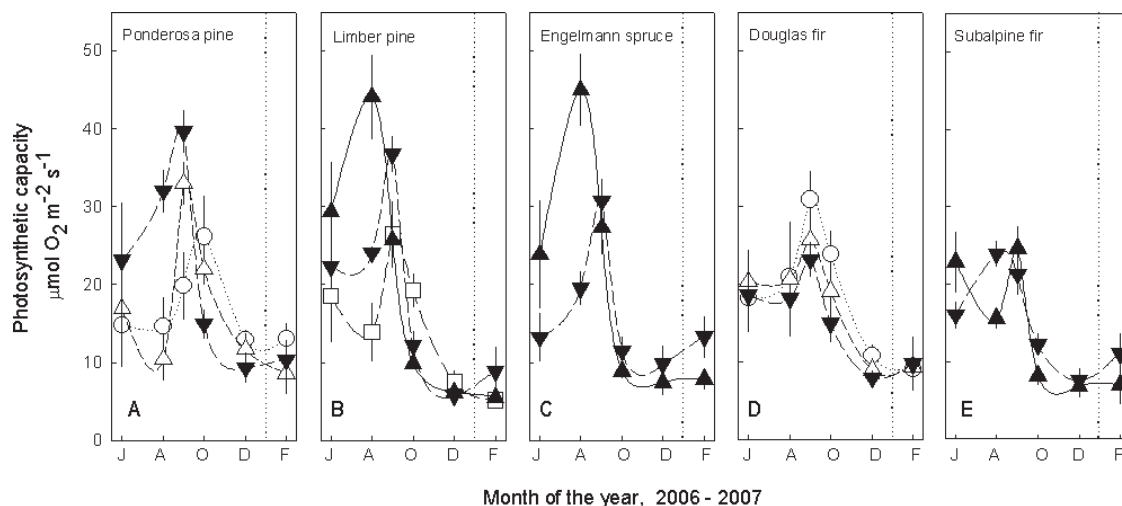
FIGURE 1. Changes in photosynthetic capacity (light- and CO_2-saturated rates of oxygen evolution determined at $25°C$) from June 2006 through February 2007 (characterized six times, once every other month) in ponderosa pine (A), limber pine (B), Engelmann spruce (C), Douglas fir (D), and subalpine fir (E) growing at different altitudes from the foothills to near treeline across the Front Range of the Rocky Mountains in Colorado. The vertical dotted line delineates the transition from 2006 to 2007. Means $\pm$ standard deviations are depicted ($n = 5$ to 6 different trees). The different symbols represent the following altitudes: open circles ($\cdots\bigcirc\cdots$) = 1852 m; open triangles ($---\triangle---$) = 2456 m; open squares ($---\square---$) = 2576 m; upside-down closed triangles ($---\blacktriangledown---$) = 2949 m; and closed triangles ($——\blacktriangle——$) = 3325 m. Significant differences between means from trees at different altitudes were observed on the following dates for the following species: (A) ponderosa pine—between 2949 m versus the two sites at 1852 and 2456 m in August ($p < 0.001$); between the two sites at 2949 and 2456 m versus 1852 m in September ($p < 0.05$); between the two sites at 1852 and 2456 m versus 2949 m in October ($p < 0.05$); (B) limber pine—between 3325 m versus the two sites at 2949 and 2576 m in both August and September ($p < 0.001$ and 0.05, respectively); between 2576 m versus the two sites at 2949 and 3325 m in October ($p < 0.01$); (C) Engelmann spruce—between 3325 versus 2949 m in August ($p < 0.0001$); (D) Douglas fir—between 1852 m versus 2949 m in both September and October ($p < 0.05$); (E) subalpine fir—between 2949 m versus 3325 m in both August and October ($p < 0.001$ and 0.05, respectively). For further information on the field sites, see Table 1.

## ACCLIMATION OF THE POTENTIAL FOR PHOTOSYNTHESIS DURING THE WINTER SEASON

In all five conifer species, maximal possible photosynthetic capacities (in leaves warmed to $25°C$) declined strongly during the winter season to levels around 10 $\mu mol\ O_2\ m^{-2}\ s^{-1}$ (Fig. 1). While these trends were rather uniform overall, limber pine showed the relatively most pronounced drop in photosynthetic capacity (Fig. 1B). It is noteworthy that none of the five conifer species exhibited a complete downregulation of photosynthetic capacity to undetectable rates (undetectable levels of photosynthetic capacity had been reported in mid-winter for some conifers in previous years; Zarter et al., 2006b, 2006c).

Furthermore, the remaining photosynthetic capacities (in leaves warmed to $25°C$) were similar at the highest versus moderately high altitudes (Fig. 1), despite the fact that the monthly mean temperatures (Fig. 2) as well as the monthly mean maximum and minimum temperatures (Fig. 2) were all below freezing at the highest altitudes in mid-winter, whereas all
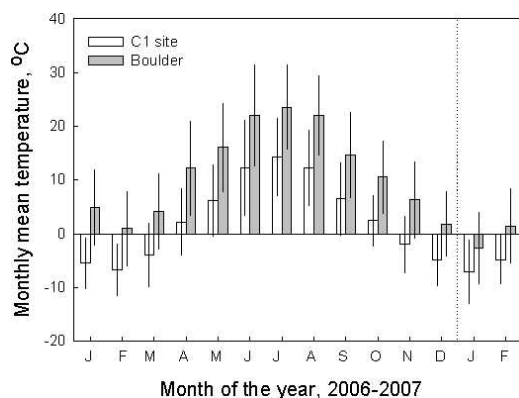
FIGURE 2. Monthly mean, and mean minimum and mean maximum (upper and lower points on each vertical line, respectively) temperatures from Boulder, Colorado (approximate altitude of 1700 m; <http://www.cdc.noaa.gov/Boulder/data.daily.html>; hatched columns) and from the C-1 meteorological station at the University of Colorado Mountain Research Station at 3022 m <http://culter.colorado.edu/NWT/data/datmanaccess.html> (open columns) from January 2006 through February 2007. The vertical dotted line delineates the transition from 2006 to 2007.
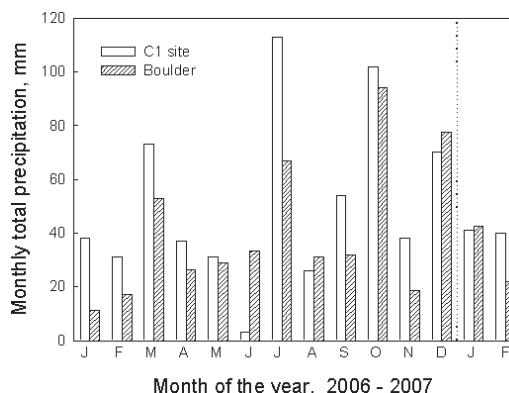
FIGURE 3. Monthly precipitation totals from Boulder, Colorado (approximate altitude of 1700 m; <http://www.cdc.noaa.gov/Boulder/data.daily.html>; hatched columns) and from the C-1 meteorological station at the University of Colorado Mountain Research Station at 3022 m <http://culter.colorado.edu/NWT/data/datmanaccess.html> (open columns) from January 2006 through February 2007. The vertical dotted line delineates the transition from 2006 to 2007.

temperatures hovered around the freezing point at the moderately high altitudes (Fig. 2).

Discussion

Measurements of photosynthetic activity most often focus on the actual rates of photosynthesis, mostly as carbon exchange, under ambient conditions, providing a measure of the carbon gain of the plant (Graves, 1990; Bond and Kavanagh, 1999; Hubbard et al., 2001; Brodersen et al., 2006). In contrast, maximal photosynthetic capacity (light and CO_2-saturated) is a measure of the physiological potential for photosynthesis. This capacity has been found to vary on the time scale of days, e.g. in response to the opportunity for growth. Environmental conditions favorable for growth (and/or carbon storage) typically induce increases in the maximal capacity for photosynthesis, which represents an investment of the plant in this opportunity (Adams et al., 2002; Zarter et al., 2006c). Conversely, environmental conditions adverse to growth typically induce decreases in maximal photosynthetic capacity (Verhoeven et al., 1999; Adams et al., 2001, 2002, 2004, 2006; Zarter et al., 2006b, 2006c). Furthermore, endogenous/developmental factors, such as age and/or status of a leaf as a "source" or "sink" organ, also affect the maximal photosynthetic capacity maintained by a leaf or needle (Krapp and Stitt, 1995; Layne and Flore, 1995; Koch, 1996). Maintaining a high maximal capacity for photosynthesis is a considerable investment for the plant, due to the cost of maintaining the protein components of the photosynthetic apparatus. Both environmental and developmental factors have been shown to exert gene regulatory control over the synthesis of photosynthetic proteins—and thereby the size and capacity of the photosynthetic apparatus—in response to the demand for photosynthate from the rest of the plant (Krapp and Stitt, 1995; Paul and Driscoll, 1997).

Based on preliminary findings, our group had previously speculated (Zarter et al., 2006b, 2006c) that the maximal photosynthetic capacities of conifers growing at high altitudes may differ for pines versus firs, with higher maximal capacities in pines. In examining this possibility in the present study, this hypothesis was confirmed, i.e. the two pines (as well as a spruce) had higher maximal capacities at high altitude than the two firs (Fig. 1). It is perhaps also noteworthy that the maximal photosynthetic capacities of the two pines and Engelmann spruce growing at the highest altitude during the autumn (Figs. 1A–1C) in the present study were also significantly greater than those observed in *Pinus cembra* and *Picea abies* growing at 1700 m in the Alps (Stecher et al., 1999) and those observed in *Pinus sylvestris* growing in the boreal forest of Siberia (Ensminger et al., 2004), with maximal photosynthetic capacities of approximately 20 μmol O_2 m^{-2} s^{-1} in all three of the latter. Moreover, the present, more comprehensive study also clearly shows that the photosynthetic capacities were remarkably similar for all five conifer species at moderately high altitude. These new findings indicate that some, but not all, conifer species take advantage of whatever opportunity exists at the highest altitudes. Such an opportunity may be the higher level of precipitation at the highest altitudes (cf. Fig. 3), but responses may also be related to temperatures.

Temperatures are clearly *lower* throughout all seasons at the *highest* altitude compared with the moderately high altitudes (Fig. 2). While it is unlikely that these lower temperatures at the highest altitudes would offer any opportunity for greater net growth, it must, however, be noted that some plant species do acclimate to *lower* temperatures by *increasing* photosynthetic capacities (for reviews, see Adams et al., 2001, 2002, 2004, 2006;

Öquist and Huner, 2003). On one hand, this acclimatory upregulation of photosynthetic capacity presumably represents an investment to counteract the lowering of enzyme activity at lower temperature by increasing the overall capacity of photosynthesis/size of the photosynthetic apparatus. It is, furthermore, also possible that an increased photosynthetic capacity in response to lower ambient temperatures allows a greater fraction of carbon acquisition to be compressed into a narrower window of time, with the relatively warmest temperatures, over midday under natural conditions, thus also favoring substantial carbon export before temperatures drop overnight (cf. Adams et al., 2002).

The plant species previously reported to exhibit an acclimatory *upregulation* of photosynthesis in response to decreased temperatures are all relatively short-lived, rapidly growing, herbaceous species, such as spinach (Holaday et al., 1992; Adams et al., 1995; Martindale and Leegood, 1997), winter cereals (Hurry et al., 1995a, 1995b, 1995c), *Arabidopsis* (Strand et al., 1997, 1999), and *Malva neglecta* (Verhoeven et al., 1999; Adams et al., 2001, 2002, 2004, 2006). It had furthermore previously been concluded that long-lived, xerophytic shrubs (Adams et al., 2001, 2002) and evergreen trees (Stecher et al., 1999; Adams et al., 2001, 2002, 2004, 2006; Öquist and Huner, 2003; Ensminger et al., 2004) typically show the opposite response, i.e. a *downregulation* of photosynthetic capacity in response to seasonally cold temperatures. However, the detailed month-by-month analysis presented in the present study reveals a more complex pattern: (i) declining temperatures in the fall were *initially* correlated with *increases* in maximal photosynthetic capacity, followed later by (ii) strong *decreases* in capacity *in full winter* when freezing events became common. The evergreen trees characterized in the present study thus showed the *same* principal response as many mesic annuals/biennials, i.e. photosynthetic upregulation—*as long as* the declining temperatures remained in a range that should likely permit further net carbon gain.

The most marked difference (between conifers and herbaceous species) was thus seen in mid-winter between the evergreen trees characterized here, showing photosynthetic downregulation, and previously characterized mesic annuals/biennials exhibiting photosynthetic upregulation even in mid-winter (albeit in the more moderate habitats to which these mesic species are confined). Another comparison can be made between the evergreen conifers characterized here and a previously characterized evergreen shrub, *Arctostaphylos uva-ursi* (bearberry or kinnikinnick), also growing over the whole range from moderately high to very high altitudes (Zarter et al., 2006a). While *A. uva-ursi* exhibited photosynthetic downregulation in mid-winter at the highest altitudes, it showed a markedly different response at moderately high altitude—no downregulation at all (Zarter et al., 2006a)—from that of the conifers in the present study. It thus appears that all conifers may have a uniform and relatively strong intrinsic propensity for photosynthetic downregulation in mid-winter. However, the present study also indicates that there are pronounced differences among conifer species, apparently specifically between pine/spruce versus fir, with only pine/spruce upregulating photosynthetic capacity in early fall to peak levels (the relatively high levels of 40–45 μmol O_2 m^{-1} s^{-2}) specifically at the highest altitude. Future studies are needed to explore possible causes for these apparent species-dependent differences in photosynthetic response.

Another, more speculative observation from the present study was that the mid-winter downregulation of photosynthetic capacity at the highest altitudes was not complete in the current study (December mean temperature of $-4.9°C$), and was thus less pronounced than what had been reported in previous winters of 2001–2002 (December mean temperature of $-5.6°C$) and 2002–2003 (December mean temperature of $-5.2°C$) (Zarter et al.,

2006b, 2006c). This mirrors a similar change reported previously for ponderosa pine growing at a more moderate altitude of 1900 m, which exhibited winter downregulation of photosynthetic capacity in 1996 (Verhoeven et al., 1999) and 1998 (Adams et al., 2002), but no downregulation during the winter of 2003 (Zarter et al., 2006b). We speculate that the continuing global warming trend may be involved in causing photosynthetic capacities in conifers at the highest altitudes to remain at measurable levels (of up to 10 μmol O_2 m^{-1} s^{-2})—as opposed to the photosynthetic apparatus being shut down completely. It is presently unknown whether this residual activity is a positive asset for plants, permitting photosynthetic activity (if only to photosynthetically generate some ATP to meet metabolic costs) during the harsh winter conditions at the highest altitudes, or whether an "incomplete shutdown" of the photosynthetic apparatus may, conversely, present any dangers/vulnerabilities in mid-winter (from the formation of reactive oxygen as a byproduct of photochemical reactions; see Adams et al., 2004, 2006). Future studies should address general trends for increased photosynthetic capacity in what previously were the most forbidding habitats, at e.g. extreme altitude, as well as species-specific differences in acclimation among evergreen conifer species. It is not even inconceivable that global warming will lead to an increase in the annual sequestration of carbon by such forests due to some net CO_2 uptake during the winter and an earlier and more rapid reactivation to maximal photosynthetic capacity during the spring.

Acknowledgments

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