

characterizes the onset of Holocene warming in high-resolution isotope records from Greenland ice (20).

About three centuries after the initiation of Holocene warming, a $\delta^{18}\text{O}$ minimum in Greenland ice records (27). Although exact dating of the non-ice core plateaus is hampered by the occurrence of ^{14}C -age plateaus during the early Holocene, multiproxy analysis suggests that all reported events collectively reflect the Preboreal Oscillation (3). In the Borchert section, the reconstructed CO_2 values drop from ~ 340 to ~ 300 ppmv at this time (Fig. 1A). A relation between CO_2 dynamics and the Preboreal Oscillation had been suspected on the basis of an abrupt rise in the early Holocene $\Delta^{14}\text{C}$ curve inferred from German pine dendrochronology (3, 22), but this could not be confirmed by ice core data.

Our results falsify the concept of relatively stabilized Holocene CO_2 concentrations of 270 to 280 ppmv until the industrial revolution. SI-based CO_2 reconstructions may even suggest that, during the early Holocene, atmospheric CO_2 concentrations that were >300 ppmv could have been the rule rather than the exception (23).

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Contribution of Disturbance to Increasing Seasonal Amplitude of Atmospheric CO_2

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Recent increases in the seasonal amplitude of atmospheric carbon dioxide (CO_2) at high latitudes suggest a widespread biospheric response to high-latitude warming. The seasonal amplitude of net ecosystem carbon exchange by northern Siberian ecosystems is shown to be greater in disturbed than undisturbed sites, due to increased summer influx and increased winter efflux. Increased disturbance could therefore contribute significantly to the amplified seasonal cycle of atmospheric carbon dioxide at high latitudes. Warm temperatures reduced summer carbon influx, suggesting that high-latitude warming, if it occurred, would be unlikely to increase seasonal amplitude of carbon exchange.

Explaining recent changes in the global environment is a scientific challenge with important political and economic implications. Although increases in concentrations of greenhouse gases such as CO_2 and CH_4 have clear anthropogenic origins (1), the causes of the observed increased

seasonal amplitude of atmospheric CO_2 are less clear. The increased amplitude is most pronounced at arctic and subarctic CO_2 monitoring stations (2) and largely reflects terrestrial carbon exchange at high latitudes (3). Two hypotheses have been advanced to explain this pattern: (i) The recent increase in March–April temperatures in high-latitude continental regions of North America and Siberia (4) could advance snowmelt and increase the length of the growing season (2), causing an increase in productivity and net ecosystem carbon gain (5); or (ii) temperature-driven increases in summer carbon gain balanced by increased winter respiration could enhance the seasonal amplitude of atmospheric CO_2 .

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21. A distinctive cooling pulse is not reflected in the late Preboreal part of the pollen record of the Borchert section, suggesting that mild regional climatic conditions (18) prevented a detectable interruption in the forest development. Controversially, some authors (3) correlate the Preboreal Oscillation with the earlier Rammelsbeek phase. There is strong evidence, however, that the characteristic expansion of grasses during this interval (Fig. 1A) was related to relatively warm dry summers rather than to cooling (18).
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5 March 1999; accepted 17 May 1999

spheric CO₂ without a change in net annual carbon accumulation (6, 7). Either hypothesis would explain the increased seasonal amplitude of atmospheric CO₂ observed at high latitudes, but neither can readily account for annual variation in this amplitude. Between 1974 and 1989, 75% of the annual increases in mean annual air temperature coincided with decreases in CO₂ amplitude at the Barrow CO₂ monitoring station [calculated from figure 2b in (2)], consistent with observations of net summer CO₂ efflux in warm years in tundra (8) and boreal forest (9). An additional contribution to low CO₂ amplitude in warm years could come from the CO₂ released by combustion, if fires are more common in warm years (10). There is a positive correlation between surface temperature and the seasonal amplitude of atmospheric CO₂ in the following year (2). Lags in correlations of temperature with CO₂ amplitude are best explained as indirect temperature effects, mediated by changes in plant growth or nutrient supply rather than a direct enhancement of photosynthesis and CO₂ uptake (11). During the first half of the CO₂ record (1962 through 1979), CO₂ amplitude at Barrow increased without a corresponding increase in temperature (2). What can explain the general

increase in CO₂ amplitude for the entire record (1962 through 1994) if the temperature correlations are so complex?

Here we present a hypothesis that accounts for the long-term increase in CO₂ amplitude through time. We propose that increased ecological disturbance such as fire and grazing at high latitudes contributes to the increased seasonal amplitude of atmospheric CO₂ by causing species replacements that enhance both peak summer CO₂ uptake and winter CO₂ efflux. Most undisturbed ecosystems at high latitudes have abundant mosses, lichens, and evergreen trees or shrubs, which photosynthesize at low rates throughout the snow-free season (12). Mosses and the associated organic mat reduce soil thaw during summer because of their low thermal conductance, resulting in rapid freezing in autumn, thereby minimizing winter respiration (13). Even in deciduous Siberian larch forests, the understory is primarily mosses and evergreen shrubs.

After disturbance at high latitudes, there is a shift in dominance from evergreen plants to herbs, grasses, and deciduous woody species that produce leaves 2 to 4 weeks after snowmelt and shed leaves 2 to 4 weeks before autumn snow cover, shortening the season of photosyn-

thetic activity. These deciduous species have higher maximum rates of photosynthesis (14) and productivity (15) than evergreens and produce litter that decomposes readily (16), especially during the autumn and winter after it is produced (17). A decline in mosses augments summer soil heat flux and depth of thawed soil by a factor of 2 to 3 and delays winter freezing of soils (18). Deepening of the thawed soil exposes old organic matter to autumn decomposition (18, 19).

High-latitude disturbance has increased in recent decades. The annual area burned has increased by about a factor of 3 in boreal North America from 1.2×10^6 ha in the 1960s to 3.2×10^6 ha in the 1990s (20), with similar increases likely to have occurred in Russia (21). Fire causes a shift from mosses to grasses and deciduous shrubs (22). Recent warming in Alaska promoted outbreaks of spruce bark beetle by shortening its life cycle from 2 to 1 years (23), causing replacement of forest by grassland. Domestic reindeer have overgrazed 25% of northern Siberia, causing a replacement of lichens by grasses (24). Nitrogen deposition, acid rain, and atmospheric pollution also reduce mosses and increase grass dominance in northern Russia (24). Agriculture is a less extensive disturbance that also enhances both summer productivity and autumn-winter respiration. Thus, many types of disturbance have increased at high latitudes since the beginning of atmo-

Fig. 1. Seasonal pattern of daytime, nighttime, and average daily net carbon exchange at undisturbed and disturbed sites in northeast Siberia. Data shown are 2-week averages ($\pm$ SE, where errors are larger than symbols) of weekly measurements made in the forest tundra zone at five undisturbed sites and five disturbed sites; near-zero fluxes from January through April are not shown. Positive numbers are fluxes to the ecosystem. Also shown are averages of measurements made in arctic coastal tundra (Arc.) in four undisturbed sites and four disturbed sites. A repeated-measures analysis of variance showed disturbance effects on summer fluxes for daytime CO₂ influx ($P = 0.02, 0.01$, and 0.05 in 1995, 1996, and 1997, respectively), nighttime CO₂ efflux ($P = 0.004, 0.02$, and 0.11 in 1995, 1996, and 1997, respectively), and daily CO₂ influx ($P = 0.12, 0.02$, and 0.009 in 1995, 1996, and 1997, respectively).

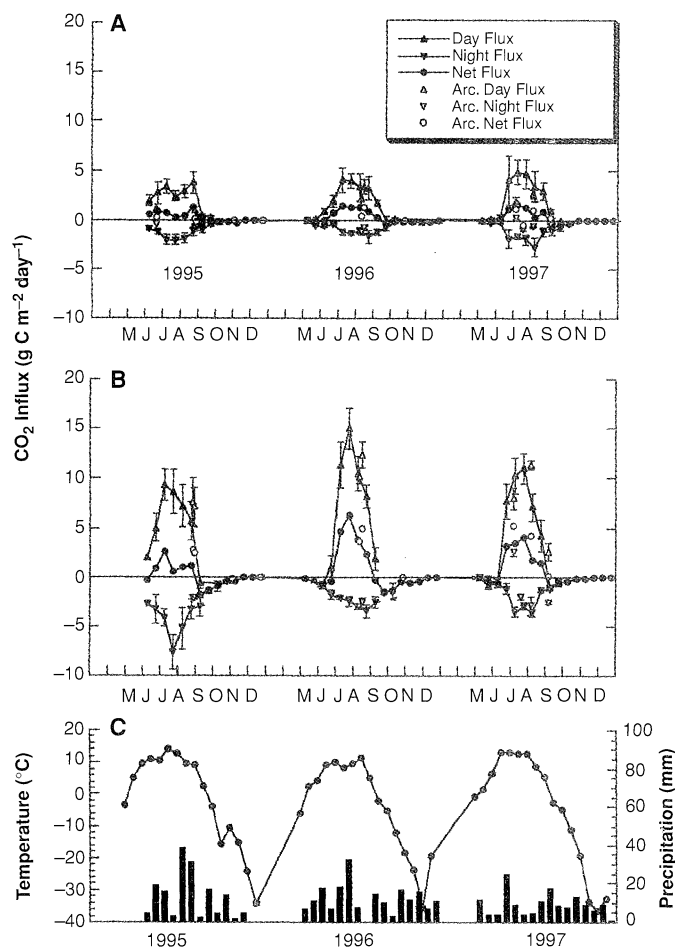


Table 1. Monthly integrated net CO₂ fluxes during summer from five disturbed (Dist.) and five undisturbed (Undist.) sites in forest tundra. Positive numbers are fluxes to the ecosystem.

Month	Integrated CO ₂ influx (g C m ⁻² month ⁻¹)		
	Undist. sites	Dist. sites	Increase due to disturbance
Year 1995			
May	-10	-31	-21
June	20	9	-11
July	15	48	33
August	27	34	7
Sept.-Dec.	-18	-81	-63
May-Dec. total	34	-21	
Year 1996			
May	-4	-12	-8
June	10	-18	-28
July	42	169	127
August	32	95	63
Sept.-Dec.	-15	-73	-58
May-Dec. total	65	161	
Year 1997			
May	-2	-9	-7
June	15	40	25
July	45	116	71
August	17	49	32
Sept.-Dec.	-23	-40	-17
May-Dec. total	52	156	

spheric CO₂ monitoring and should increase the amplitude of seasonal carbon exchange through high rates of summer carbon gain and enhanced winter respiration.

In contrast to the well-studied disturbance effects on species composition and associated changes in photosynthesis, the effects of disturbance on the seasonality of net ecosystem carbon exchange have not been documented. To test the effects of temperature and disturbance on carbon exchange, we measured the seasonal amplitude of carbon exchange over 3 years in five undisturbed sites and five disturbed sites in the forest tundra zone near Cherskii (Cherskiy) in northeast Siberia (69°N, 161°E). The undisturbed sites included two mature *Larix* forests (7), *Betula* shrub tundra, *Eriophorum vaginatum* tussock tundra, and a *Sphagnum* bog (25, 26). The disturbed sites were a 15-year-old post-fire *Larix* forest, a shrub tundra converted to grassland by grazing, a shrub tundra converted to grassland by ice scour during occasional spring floods, a bog dominated by *Eriophorum angustifolium* after a single passage of a tracked vehicle, and a grass-dominated plot in forest tundra after removal of the moss layer (7). We made similar measurements in four undisturbed sites, in three sites compacted by occasional passage of a tracked vehicle, and in one site disturbed by human trampling near Ambarchik on the arctic coast 130 km further north (70°N, 161°E). Mosses and dwarf evergreen shrubs were present in all undisturbed plots and absent from all disturbed plots. The previous river scouring occurred 2 years before the study. All other disturbances occurred at least 10 years before the study, allowing time for the observed species changes. We focused our sampling effort on true replication of sites and disturbance types and regular weekly sampling rather than on within-day variance in CO₂ flux, which in our earlier studies accounted for less than 10% of the variance in flux (26).

Daytime carbon gain in undisturbed ecosystems in the forest tundra zone was compensated by a similar seasonal pattern of nighttime CO₂ efflux (Fig. 1). Consequently, the average net daily flux was relatively small throughout the summer (Table 1). Disturbed sites differed from undisturbed sites during summer in having 2.1 to 2.5 times the daytime CO₂ influx, 1.8 to 2.6 times the nighttime CO₂ efflux, generally 1.6 to 3.0 times the average daily CO₂ influx, and a 6-week shorter season of positive daytime carbon gain. Winter respiration in disturbed sites was 1.7 to 4.9 times that in undisturbed sites (Table 1). Thus, the seasonal amplitude of CO₂ exchange (integrated summer uptake minus integrated winter efflux) in disturbed sites was 2.3 to 3.6 times that in undisturbed sites (Fig. 1 and Table 1). In disturbed and undisturbed forested sites we measured the understory only and therefore underestimated whole-system carbon uptake. The difference between disturbed and undisturbed sites would be larger than we esti-

mated if we had included the trees, because the trees are more productive in disturbed than in undisturbed sites. Over the 3 years of measurement, disturbed sites did not differ consistently from undisturbed sites in CO₂ source-sink strength during the May to December period (Table 1).

The summer of 1995 was 2.1°C warmer with 45% less precipitation during June and July compared with 1996, with 1997 being intermediate (Fig. 1). Total summer carbon gain was greatest in the cool, wet year (1996) at our sites (Fig. 1 and Table 1). Thus, in northern Siberia it is unlikely that increased summer warming would directly enhance summer net CO₂ uptake, although it might enhance early summer uptake through earlier snow-melt and greenup (2, 5). The disturbed sites differed more in annual carbon exchange between warm and cold years (182 g of C per square meter per year) than did the undisturbed sites (31 g C m⁻² year⁻¹), suggesting that disturbance increased the sensitivity of carbon exchange to temperature.

Daytime CO₂ influx, nighttime CO₂ efflux, and average daily carbon gain during summer at the cold arctic coastal site were similar to values in forest tundra (Fig. 1), despite an 8°C cooler summer temperature on the coast. As in forest tundra, net carbon gain in summer at the arctic site was greater in disturbed than in undisturbed sites.

Our results show that disturbance increased the seasonal amplitude of net carbon exchange and had a larger effect on this seasonal amplitude than did either interannual or geographic differences in growing-season temperature. Is this increased seasonal amplitude of carbon exchange caused by disturbance large enough to be significant to the atmosphere? If the fluxes that we observed in 12 sites in two climate zones over 3 years are representative of the effect of disturbance on high-latitude carbon flux, and if the increase in area burned in North America is representative of the circumpolar zone, disturbance would have increased the seasonal amplitude of high-latitude carbon flux by about 15% since the 1960s (27), a value similar to that observed in the atmospheric record (2, 3). This rough calculation shows that disturbance could contribute substantially to the observed increase in seasonal amplitude of atmospheric CO₂. However, an accurate assessment of the contribution of disturbance to the atmospheric record requires improved information on changes in disturbance and recovery rates.

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25. We established permanent chamber bases in all sites with various chamber areas and replication. Among the undisturbed forest-tundra sites there were one 0.09-m² plot and one 6.25-m² plot in the forests, one 6.25-m² plot in shrub tundra, one 6.25-m² plot in tussock tundra, and two 0.2-m² plots in the *Sphagnum* bog. In the disturbed sites there were two 0.2-m² plots in the post-fire forest, two 0.2-m² plots in the previously grazed grassland, two 0.2-m² plots in the ice-scoured grassland, one 0.2-m² plot in the bog compacted by the tracked vehicle, and one 0.09-m² plot in the grass-dominated forest-tundra site. We used the mean flux among replicate chambers in a given site in comparisons between undisturbed and disturbed ecosystems to avoid pseudoreplication [S. H. Hurlbert, *Ecology* **54**, 187 (1984)]. On the arctic coast, there were four 0.09-m² plots in both undisturbed and disturbed tundra. There was no consistent difference in flux between large and small chambers at the same site, so differences in chamber size are unlikely to explain site differences in fluxes. Air temperature (at 1.5 m) and precipitation were measured at government weather stations within 5 km of each site.
26. At each forest-tundra site we measured CO₂ flux once per week from June 1995 through December 1997, with occasional measurements made each summer at the arctic site. On each measurement date, fluxes were measured once in daytime (1100 to 1400 hours) and once at nighttime (2300 to 0200 hours) and averaged to estimate daily net flux. Daily estimates of net flux based on measurements made at 3-hour intervals, once per week, over 5 years did not differ from daily estimates based on only midday and midnight measurements (7), so in the present study we used two measurements per day. Variation in flux among vegetation microsites and among weeks accounted for >90% of the variation in summer CO₂ flux in the earlier study (7), so in the present study we focused our attention on capturing variation among sites and among weeks rather

er than within days. Volumes of the Plexiglas (acrylic plastic) measurement chambers were 0.02 m³ for 0.09-m² plots and 0.1 m³ for 0.2-m² plots. The 4.4-m³ chambers for the 6.25-m² plots had permanent walls and a temporary plastic roof. CO₂ measurements were made with a LICOR 6200 portable infrared gas analyzer (7, 8). Measurement duration averaged 60 s, with a chamber effect on temperature (<2°C) only during periods of direct solar radiation. Because of the small magnitude of the temperature change, the small effect of temperature on flux (see results), and a study design that measured fluxes from disturbed and undisturbed sites under similar conditions, any biases due to chamber effects are probably small. Fluxes measured with an aerodynamic method, based on the vertical profile of

CO₂ concentration and wind speed [J. L. Monteith and M. H. Unsworth, *Principles of Environmental Physics* (Hodder and Stoughton, London, 1990)], gave identical seasonal amplitudes of net CO₂ flux to chamber measurements (S. A. Zimov *et al.*, data not shown), validating our chamber technique. In winter, we used a water jacket to stabilize the temperature of the gas analyzer.

27. We estimate the area-weighted average high-latitude carbon uptake (U) as $U = A_d(F_d) + (1 - A_d)(F_u)$, where $A_d = A_b(T)$. F_d is the average summer carbon uptake by disturbed ecosystems (186.7 g C m⁻² year⁻¹; Table 1); F_u is the average summer carbon uptake by undisturbed ecosystems (74.3 g C m⁻² year⁻¹); A_b is the proportion of the North American boreal zone burned annually [0.21% in 1960s, 0.57%

in 1990s (20)]; and T is 30 years, the time a disturbed site is dominated by early successional vegetation (21). U increased 15% from the 1960s to the 1990s. The average circumpolar changes in disturbance (A_b) and recovery rates (T) are unknown, so this calculation is only illustrative.

28. We thank I. Fung, J. Gruenzweig, J. Harte, A. McGuire, and J. Randerson for critical review of the manuscript. This research collaboration was funded by the Russian Fund for Fundamental Research, the International Science Foundation, the U.S. Department of Energy, and the Arctic System Science (ARCSS), the Long-Term Ecological Research (LTER), and the Terrestrial Ecosystems and Global Change (TECO) Programs of NSF.

15 December 1998; accepted 4 May 1999

Xyloglucan Fucosyltransferase, an Enzyme Involved in Plant Cell Wall Biosynthesis

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Cell walls are crucial for development, signal transduction, and disease resistance in plants. Cell walls are made of cellulose, hemicelluloses, and pectins. Xyloglucan (XG), the principal load-bearing hemicellulose of dicotyledonous plants, has a terminal fucosyl residue. A 60-kilodalton fucosyltransferase (FTase) that adds this residue was purified from pea epicotyls. Peptide sequence information from the pea FTase allowed the cloning of a homologous gene, *AtFT1*, from *Arabidopsis*. Antibodies raised against recombinant AtFTase immunoprecipitate FTase enzyme activity from solubilized *Arabidopsis* membrane proteins, and *AtFT1* expressed in mammalian COS cells results in the presence of XG FTase activity in these cells.

In most multicellular organisms, cells are embedded in a complex extracellular matrix that keeps them together and influences the shape, development, and polarity of the cells they contact. Animal cells have such an extracellular matrix at their surface, but plants possess a distinct wall that encloses every cell. Many important differences between plants and animals with respect to nutrition, growth, reproduction, and defense mechanisms can be traced to the plant cell wall (1). Cell wall extensibility is a major determinant of plant growth (2). The biosynthesis of plant cell walls is very tightly regulated. Although an individual plant cell may expand its volume by nearly 20,000 times,

its cell wall must maintain a uniform thickness and structure to prevent hemorrhaging of the cell through local defects (2). However, despite extensive descriptions of the chemical and physical structure of the plant cell wall, very little is known about its biosynthesis. One gene encoding a cell wall-synthesizing enzyme, cellulose synthase, has been cloned (3).

The flexible primary walls of young plant cells are mainly composed of cellulose microfibrils and matrix polysaccharides. Matrix polysaccharides include hemicelluloses that bind tightly but noncovalently to cellulose microfibrils, cross-linking them into a complex network. The hemicellulose xyloglucan (XG) makes up approximately 20% of the total cell wall in dicot and nongraminaceous monocot plants and forms a load-bearing network by associating to the surfaces of surrounding cellulose microfibrils through hydrogen bonds (4, 5). XG contains a β -1,4-glucan backbone decorated with side chains of xylose alone; xylose and galactose; and xylose, galactose, and fucose. The presence or absence of the terminal fucose residue may have structural and biological significance. Some models suggest that the presence or absence of this fucose residue will determine whether the xyloglucan conformation is planar and thus better able to bind to

cellulose (6), though contradicting evidence has been described (7). XG networks may be modified by XG endotransglycosylase (XET), an enzyme that cleaves and rejoins adjacent XG chains. A recombinant XET demonstrated different activity rates for fucosylated versus nonfucosylated XG oligosaccharide acceptors, indicating that the fucosylation state may affect XET modification of the cell wall (8). In addition, oligosaccharides consisting of an XG nonasaccharide prevent auxin-promoted elongation of pea stems if these oligosaccharides contain fucose but not if they lack fucose (9). Thus, it is possible that XG fragments act as signaling molecules *in vivo*.

Most matrix polysaccharides are branched molecules modified by various sugars. These modifications are important because they allow heterogeneity in the shape of matrix polysaccharides and in the patterns of cross-links, resulting in a dynamic and porous cell wall. These polysaccharide modifications occur via glycosyltransferase reactions, many of which occur in the Golgi complex (10). Attempts to clone plant glycosyltransferases using sequences derived from bacterial or mammalian transferases have been unsuccessful (11). This is not entirely unexpected, for although Golgi glycosyltransferases often have similar general structural features, they rarely share extensive sequence similarity (12).

The terminal fucosyl residue on XG side chains is added by a fucosyltransferase (FTase). We purified enough of this FTase from pea epicotyls to determine partial amino acid sequences from the enzyme. Microsomes were prepared from the pea epicotyls, carbonate-washed to enrich for membrane proteins (13), and solubilized with nonionic detergent such as Triton X-100. A specific assay for this enzyme was developed using tamarind or nasturtium seed storage XG, which lack fucosyl residues, as acceptor molecules and radiolabeled guanosine diphosphate (GDP)-fucose as a donor (14, 15). GDP-agarose affinity chromatography, size exclusion chromatography, and anion exchange chromatography were used in conjunction with FTase activity assays to purify and detect the enzyme (Fig. 1) (16). It was possible to purify XG FTase 1400-fold after size exclusion chromatography, resulting in a total of 50 μ g of

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