Response of C_3 and C_4 plants to middle-Holocene climatic variation near the prairie–forest ecotone of Minnesota

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Paleorecords of the middle Holocene (MH) from the North American mid-continent can offer insights into ecological responses to pervasive drought that may accompany future climatic warming. We analyzed MH sediments from West Olaf Lake (WOL) and Steel Lake (SL) in Minnesota to examine the effects of warm/dry climatic conditions on prairie–woodland ecosystems. Mineral composition and carbonate $\delta^{18}O$ were used to determine climatic variations, whereas pollen assemblages, charcoal $\delta^{13}C$, and charcoal accumulation rates were used to reconstruct vegetation composition, C_3 and C_4 plant abundance, and fire. The ratio of aragonite/calcite at WOL and $\delta^{18}O$ at SL suggest that pronounced droughts occurred during the MH but that drought severity decreased with time. From charcoal $\delta^{13}C$ data we estimated that the MH abundance of C_4 plants averaged 50% at WOL and 43% at SL. At WOL C_4 abundance was negatively correlated with aragonite/calcite, suggesting that severe moisture deficits suppressed C_4 plants in favor of weedy C_3 plants (e.g., *Ambrosia*). As climate ameliorated C_4 abundance increased (from ≈33 to 66%) at the expense of weedy species, enhancing fuel availability and fire occurrence. In contrast, farther east at SL where climate was cooler and wetter, C_4 abundance showed no correlation with $\delta^{18}O$-inferred aridity. Woody C_3 plants (e.g., *Quercus*) were more abundant, biomass flammability was lower, and fires were less important at SL than at WOL. Our results suggest that C_4 plants are adapted to warm/dry climatic conditions, but not to extreme droughts, and that the fire regime is controlled by biomass–climate interactions.

Pervasive drought, expected to be associated with climatic change in continental interiors, will have profound ecological, economic, and societal repercussions (1–4). Drought conditions are likely to alter ecosystem function by changing the relative abundance of plant functional groups (e.g., C_3 vs. C_4) in natural systems (5–7). For example, episodic droughts that occurred throughout the 20th century in the midwestern United States killed woody C_3 genera, such as *Quercus* (8–10), and favored better adapted C_3 and C_4 herbaceous species (9). General circulation models coupled with dynamic crop-growth models project that, in agricultural systems, such climatic conditions could significantly reduce both C_3 (e.g., wheat, *Triticum aestivum* L.) and C_4 (e.g., corn, *Zea mays* L.) cereal crop yields (11, 12), resulting in billions of dollars of economic loss (13, 14). Empirical evidence of the response of plant functional groups to climatic conditions characteristic of drought is therefore important for evaluating predictions of future change. However, such evidence is mostly limited to historical records (15, 16), which lack the full range of past drought variability (3), and to short-term experimental manipulations (17, 18), which lack a sufficient temporal dimension for understanding future vegetational response.

During the middle Holocene (MH), ≈8.0–4.0 thousand years (ka) B.P. (19), the midwestern United States experienced higher summer temperatures and lower annual precipitation than during the early or late Holocene (20, 21), with episodes of pronounced drought (1). Retrospective studies of this period provide an opportunity to examine vegetational dynamics in response to climatic change. To this end, numerous pollen profiles spanning the Holocene have been published from the midwestern United States, especially Minnesota (22, 23). However, a detailed understanding of the vegetational history of the MH remains limited because of similar pollen morphologies of the taxa within Poaceae (the grass family) and because of the likely underrepresentation of pollen from important taxa that are not wind-dispersed, such as insect-pollinated forbs.

On the basis of the distinct carbon-isotopic signatures of C_3 and C_4 plants (24), charcoal $\delta^{13}C$ was recently used by Clark *et al.* (25) to estimate the relative abundance of C_3 and C_4 plants across a transect of three sites from North Dakota, Minnesota, and Wisconsin. This study demonstrated for the first time that C_4 plant abundance increased during the MH, which is consistent with the generalization that C_4 plants are adapted to warm and moisture-limited habitats (26, 27). However, these charcoal $\delta^{13}C$ records did not have adequate temporal resolution (≈200 years between samples) to offer insights into the shorter-term dynamics of C_3 and C_4 response to climatic change within the MH. Furthermore, experimental and field results suggest that, although C_4 plants are adapted to warm and moisture-limited habitats, they are not suited to conditions characterized by severe drought (28, 29). To resolve the temporal and spatial details of C_3 and C_4 plant response, we analyzed climatic and vegetational proxies from the MH portion of well dated sediment cores from two west-central Minnesota lakes, with an average resolution of 58 years between charcoal $\delta^{13}C$ samples. Specifically, we tested the hypothesis that C_4 plants were more tolerant than C_3 plants of warm and dry climatic conditions within the MH.

Study Sites

Our two sites, ≈120 km apart, straddle the pre-European-settlement prairie–forest border (Fig. 1*a*), which was controlled by interactions among climate, fire, soils, and topography (30). West Olaf Lake (WOL; 46° 37′ N, 96° 11′ W) has an area of 58 hectares, a maximum water depth of ≈18 m (Fig. 1*b*), and anoxic conditions below ≈9 m during the midsummer of 2002. At Detroit Lakes, MN, ≈40 km northeast of WOL, the mean annual temperature is 4.2°C, and the mean annual precipitation is 62 cm. Before European settlement, the vegetation near WOL was oak woodland and brushland dominated by *Quercus macrocarpa* Michx. (bur oak), *Quercus ellipsoidalis* E.J. Hill (pin oak), *Populus tremuloides* Michx. (quaking aspen), and *Corylus cornuta* Marsh. (beaked hazelnut), with upland tall-grass prairie openings (31, 32).

Steel Lake (SL; 46° 58′ N, 94° 41′ W) covers 23 hectares and has a maximum water depth of ≈21 m (Fig. 1*c*), with midsummer anoxia below ≈8 m in 2002. At Park Rapids, MN, ≈30 km west of
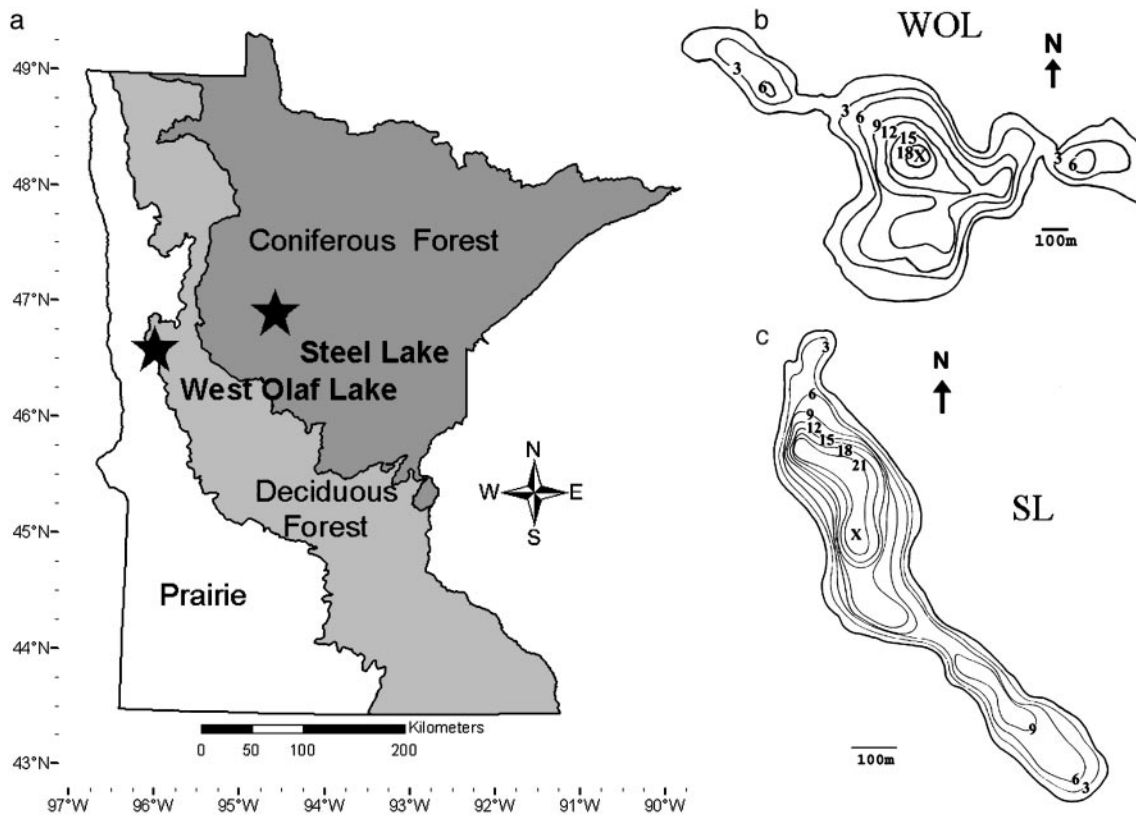

Fig. 1. (a) Location of study sites within the major vegetational zones of Minnesota. (b and c) Bathymetric maps (depth in meters) of WOL (b) and SL (c) with coring locations indicated by X.

SL, mean annual temperature and precipitation are 4.6°C and 67 cm, respectively. Before European settlement, the vegetation near SL was coniferous–hardwood forest consisting of *Pinus strobus* L. (white pine), *P. resinosa* Aiton (red pine), *P. banksiana* Lamb. (jack pine), *Betula papyrifera* Marsh. (paper birch), and *Populus tremuloides* Michx. (31, 32).

Materials and Methods

Stratigraphically overlapping sediment cores were obtained from the deepest part of each lake with a modified Livingstone piston sampler (33). Sediment subsamples were sieved with distilled water to isolate terrestrial plant macrofossils for ^{14}C dating. Macrofossils were treated with an acid–base–acid protocol and submitted to Lawrence Livermore National Laboratory for accelerator-mass-spectrometry ^{14}C dating. Age–depth models were developed based on seven ^{14}C dates from WOL and 12 ^{14}C dates from SL (Table 1 and Fig. 2) that were converted to calibrated years with CALIB 4.3 (34) using the atmospheric decadal calibration data set (35) (http://depts.washington.edu/qil/calib/calib.html).

Sediment mineral composition was determined by x-ray diffraction with a Scintag θ–θ diffractometer following conventional procedures (36). Minerals were quantified as the dry weight percentage of total major identified minerals, which included aragonite, calcite, dolomite, feldspars, and quartz (37). We used the ratio of aragonite/calcite to infer climatic change at WOL; this proxy was not applicable at SL because of the absence of aragonite in its sediment. We did not conduct $\delta^{18}O$ analysis at WOL because of the presence of aragonite, which has a fractionation factor different from calcite and therefore makes it difficult to derive climatic inferences from $\delta^{18}O$.

Carbonate $\delta^{18}O$ was analyzed at SL because the mineralogical profile was dominated by calcite with no significant stratigraphic change in the composition of carbonate mineral types. $\delta^{18}O$ was determined by reacting sediment with ultra-pure phosphoric acid at 70°C in an automated Kiel device interfaced with an isotope–ratio mass spectrometer (IRMS; Finnigan MAT 252). The instrumental standard error for $\delta^{18}O$ analysis was 0.1‰.

Macroscopic charcoal particles were concentrated from subsamples of 3-cm^3 sediment from WOL and 8-cm^3 sediment from SL by disaggregating the sediment with 10% KOH and 10% HCl and washing it through a 180-μm sieve. The sediment subsamples from SL were of a greater volume than those from WOL to obtain an adequate number of charcoal particles for $\delta^{13}C$ analysis. Charcoal particles were identified and counted at ×30 magnification and converted to charcoal accumulation rates (CHAR) following Long *et al.* (38). For charcoal $\delta^{13}C$ analysis, a minimum of 35 pieces of randomly selected charcoal particles of generally similar size were analyzed per sample. Samples were combusted in an elemental analyzer (Carlo Erba NC2500) interfaced with an IRMS (Finnigan MAT 252). The instrumental standard error for charcoal $\delta^{13}C$ analysis was ±0.1‰. Replicate samples were analyzed throughout each core for ≈10% of the samples to determine the reproducibility of charcoal $\delta^{13}C$ signatures, which revealed an average error of ±2‰.

As the average isotopic composition of C$_3$ and C$_4$ plants ($-27‰$ and $-13‰$, respectively) (39) is unaffected by charring (40), we used these values as end members in a mixing model described by Clark *et al.* (25) to estimate the relative abundance of C$_3$ and C$_4$ plants on the landscape from charcoal $\delta^{13}C$ values. Our estimates of C$_3$ and C$_4$ proportions are not restricted to grasses because potential charcoal sources include other C$_3$ plants (trees, shrubs, and other herbaceous species). We assume that the charcoal was produced from terrestrial vegetation. Littoral and wetland vegetation in this region rarely burn, and today they cover only a small fraction of each lake's watershed.

Table 1. ¹⁴C dates from WOL and SL

Lab number	Depth, cm*	¹⁴C date, year B.P.	Calibrated age†	Material
WOL				
CAMS-85892	2,554–2,562	3,110 ± 80	3,315 (3,076–3,540)	Charcoal
CAMS-85893	2,712–2,720	3,345 ± 40	3,577 (3,472–3,662)	Charcoal
CAMS-85225	2,902–2,910	4,080 ± 70	4,595 (4,419–4,822)	Charcoal
CAMS-85894	3,032–3,040	4,260 ± 45	4,832 (4,626–4,964)	Charcoal
CAMS-85895	3,265–3,271	5,875 ± 45	6,692 (6,554–6,845)	Charcoal
CAMS-87119	3,350–3,356	6,680 ± 50	7,539 (7,422–7,656)	Charcoal
CAMS-29670	3,455	8,140 ± 70	9,107 (8,782–9,398)	Wood
SL				
CAMS-71312	2,448	2,920 ± 90	3,071 (2,849–3,335)	One *Alnus* seed
CAMS-70220	2,556	4,100 ± 90	4,620 (4,411–4,836)	Leaf fragments
CAMS-70221	2,586	4,290 ± 40	4,853 (4,731–4,968)	Wood
CAMS-69532	2,586	4,510 ± 100	5,150 (4,862–5,454)	One *Betula* seed
CAMS-70222	2,632	4,840 ± 210	5,559 (4,970–5,994)	Wood
CAMS-66722	2,672.5	5,280 ± 40	6,063 (5,935–6,173)	Leaf fragments
CAMS-68539	2,742	5,830 ± 40	6,642 (6,501–6,732)	One bud
CAMS-68540	2,753	6,170 ± 50	7,076 (6,909–7,227)	Wood
CAMS-70223	2,798	6,890 ± 90	7,729 (7,580–7,926)	Charcoal, leaf fragment
CAMS-70224	2,858	7,880 ± 70	8,719 (8,539–8,991)‡	One seed bract
CAMS-71313	2,866	8,160 ± 50	9,115 (9,008–9,271)‡	Two Cyperaceae seeds
CAMS-71314	2,888	7,210 ± 60	8,016 (7,876–8,164)	One Cyperaceae seed
CAMS-70225	2,890	7,240 ± 60	8,060 (7,949–8,169)	One seed bract
CAMS-70226	2,983	8,580 ± 130	9,607 (9,272–9,924)	Three *Betula* seeds

*Depth from lake water surface.
†Calibration was performed by using CALIB 4.3 with the median value chosen as the calibrated age. The 2σ range is in parentheses.
‡Dates from a turbidite layer are excluded from the age–depth model following Wright *et al.* (51).

Subsamples of 1-cm³ sediment were prepared for pollen analysis following standard methods (41), with *Lycopodium* spore tablets added to determine pollen concentrations. At least 300 pollen grains were counted per sample at ×400 magnification. Pollen percentages were based on the sum of arboreal and nonarboreal pollen types, excluding spores and aquatics. Pollen accumulation rates show similar patterns as pollen percentages and are therefore not presented.
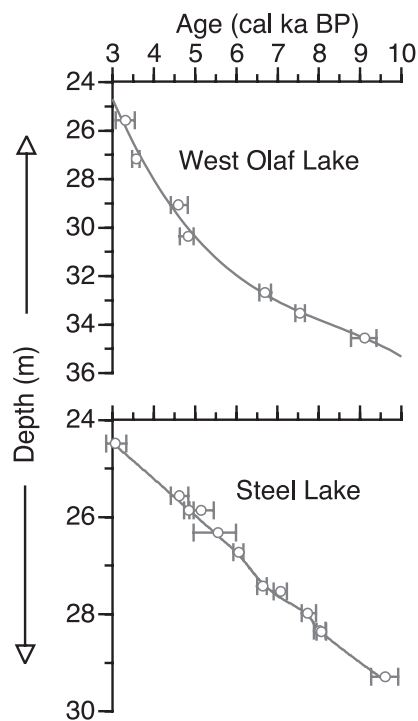
Results and Interpretations

Climatic Variation Within the MH. We combine the records of aragonite/calcite at WOL and $\delta^{18}O$ at SL to infer the temporal and spatial patterns of the regional climate during the MH (Fig. 3). Aragonite and calcite in the MH sediments of WOL are most likely endogenic because their stratigraphic patterns differ greatly from those of detrital minerals, such as quartz and feldspars (J.T., unpublished data). Aragonite is a polymorph of calcite that precipitates preferentially over calcite as lake-water Mg/Ca molar ratios increase to >7 (42). Today, aquifers in the WOL region have Mg/Ca ratios <1 (www.pca.state.mn.us/water/groundwater/gwmap/gwbaseline.html), suggesting that the presence of aragonite during the MH was likely due to concentration within the lake, controlled by water balance related to temperature and/or aridity, rather than by the input of high Mg/Ca ground water.

Calcite $\delta^{18}O$ in lake sediment is determined by moisture source, atmospheric and water temperatures, evaporation, and precipitation seasonality. A change in moisture source is unlikely (43), and the fractionation effect of atmospheric temperature prevails over that of water temperature (44). We do not have sufficient information to tease apart the relative importance of the other factors. However, the similarity in the general stratigraphic trends of $\delta^{18}O$ and aragonite/calcite implies common climatic controls. Distinguishing between the effects of temperature and aridity is difficult, but both factors were likely important during the MH. This inference is supported by quantitative climatic reconstructions in the region, including pollen evidence of warm and dry conditions (21) and diatom evidence of saline water associated with low effective moisture (45).

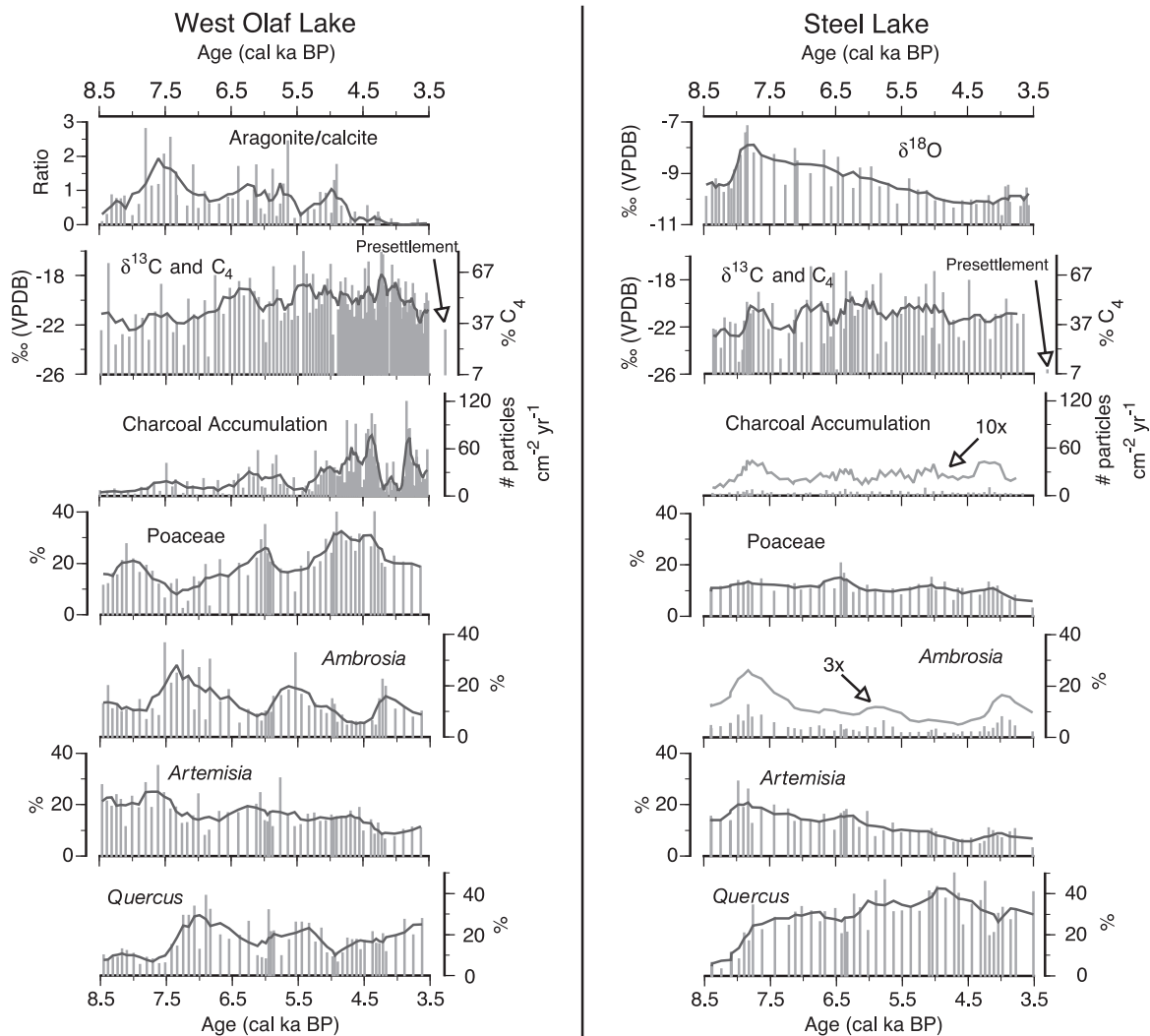Within the MH, aragonite/calcite at WOL reaches peak values

Fig. 2. Age–depth models for sediment cores from WOL and SL with depth from the water surface. Error bars represent the 2σ probability of calibrated age ranges from CALIB 4.3. The WOL dates are fit with a third-order polynomial, and the SL dates are fit with a locally weighted polynomial regression (59, 60).

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Fig. 3. Data used for climatic interpretation (aragonite/calcite ratios and δ^{18}O), charcoal δ^{13}C and %C_4, charcoal accumulation rates, and Poaceae, *Ambrosia*, *Artemisia*, and *Quercus* pollen abundance at WOL and SL. Bars represent raw data, and black curves are five-sample moving averages of the raw data. Gray curves are exaggerations of the five-sample moving averages.

around 7.6 ka BP, suggesting maximum warmth and aridity at that time. As the MH progressed, variable and decreasing aragonite/calcite ratios suggest fluctuating but generally lower temperatures and greater effective moisture (Fig. 3). Similar to aragonite/calcite, δ^{18}O at SL reaches its peak value ($-7.9‰$) at the beginning of the MH and then displays a decreasing trend, which also suggests that conditions were warmest and driest at the beginning of the MH (Fig. 3).

In contrast to the similarity of general temporal trends, the spatial difference in effective moisture was likely large between the two sites. The presence of aragonite at WOL and its absence at SL indicate that lake-water Mg/Ca ratios were greater in WOL than in SL, suggesting greater moisture deficits at WOL throughout the MH. Alternatively, the presence of aragonite at WOL and not at SL could be caused by individual lake interactions with ground water (46). However, because the basin of WOL is substantially larger than that of SL, SL should be more susceptible to changes in moisture balance, with a greater tendency for aragonite precipitation. Greater moisture deficits at WOL than at SL are not unexpected, because annual precipitation minus evaporation decreases westward across Minnesota today (47) and during the Holocene (48). The inferred large difference in moisture between our two sites implies steep climatic gradients during the MH.

Plant Communities, C_3/C_4 Abundance, and Fire. *Ambrosia*, *Artemisia*, Poaceae, and *Quercus* were the most abundant pollen types at both WOL and SL during the MH. However, as with climate, distinct differences existed in community composition and structure between these sites. The combination of high *Ambrosia*, a weedy C_3 taxon that invades disturbed areas, and *Artemisia*, a noninvasive C_3 taxon that includes herbs and shrubs, along with moderately low Poaceae pollen at WOL during the early MH (Fig. 3) suggests a highly disturbed and patchy mid- to short-grass prairie (49, 50). *Quercus* was likely restricted to small groves near streams and lakes. As the MH progressed, the pollen assemblages at WOL became more typical of tall-grass prairie; grass pollen abundance gradually increased from 8% to 33% at the expense of *Ambrosia*, which decreased from 28% to 5%, and *Artemisia*, which decreased from 25% to 9%. In comparison with the pollen assemblages at WOL, at SL *Ambrosia* and Poaceae were less abundant, *Quercus* was more widespread, and *Artemisia* was slightly less abundant (Fig. 3), as is characteristic of an oak–prairie savanna or parkland (51). As the MH progressed, *Quercus* pollen increased in abundance from 14% to 43%, *Ambrosia* decreased from 9% to 2%, *Artemisia* decreased from 20% to 6%, and Poaceae showed little change, ranging between 15% and 9%.
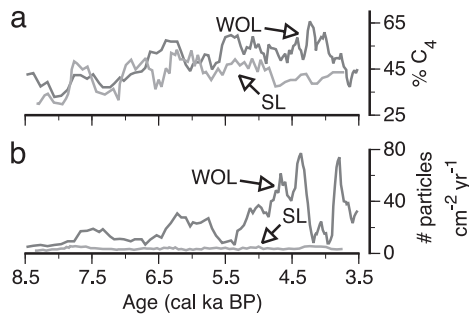
Fig. 4. Comparison of C_4 abundance (*a*) and charcoal accumulation rates (*b*) at WOL (black curves) and SL (gray curves).

The MH plant communities inferred from pollen agree with previous studies from the regions near WOL (50) and SL (25). However, our charcoal $\delta^{13}C$ results provide information on C_4 dynamics during the MH that is unavailable from pollen assemblages alone. MH charcoal $\delta^{13}C$ ranges from -16 to $-25‰$ at WOL and from -17 to $-26‰$ at SL. The $\delta^{13}C$-based estimates of C_4 plant abundance for the entire MH averaged 50% and 43% at WOL and SL, respectively. The largest differences in C_4 abundance between our sites occurred after ≈5.7 ka BP, when charcoal $\delta^{13}C$ averages $-19‰$ at WOL and $-21‰$ at SL, equivalent to 57 and 43% contribution of C_4 plants to sediment charcoal, respectively (Fig. 4*a*). In comparison, the $\delta^{13}C$ of single charcoal samples from WOL and SL representing presettlement vegetation were $-22‰$ and $-26‰$, respectively, which is equivalent to 36% and 7% C_4 (Fig. 3). These values of C_4 abundance are much lower than their respective MH C_4 abundance estimates of 50% and 43%, potentially reflecting regional forest development, which occurred under the mesic conditions of the late Holocene (51).

Century-scale fluctuations in charcoal $\delta^{13}C$ of up to $7‰$ at WOL and $8‰$ at SL during the MH suggest large variations in the relative abundance of C_3 and C_4 plants (Fig. 3). High sample-to-sample variation may also result partially from the $\pm2‰$ error associated with our charcoal-$\delta^{13}C$ analysis; thus, we focus on the long-term trends. The $\delta^{13}C$-estimated average abundance of C_4 plants at WOL ranges from a low of 33% at 8.0 ka BP to a high of 66% at 4.2 ka BP, with an overall increasing trend from the early to late MH. In conjunction with the overall positive relationship between charcoal $\delta^{13}C$ and Poaceae pollen interpolated to a common sampling resolution ($r = 0.49$, $P < 0.0001$, $n = 60$), this trend suggests that C_4 grasses became more abundant as the MH progressed. Before European settlement near WOL, *Andropogon*, *Bouteloua*, *Panicum*, and *Sorghastrum* were the dominant C_4 grasses, and these genera were likely among the C_4 grass genera present during the late MH. Unlike WOL, no strong trend exists in C_4 abundance from the early to late MH at SL, and the average C_4 plant abundance there ranges from 30% to 53%. However, at SL, larger fluctuations in C_4 abundance occurred during the early MH than during the late MH.

CHAR also exhibit distinct differences between our two sites (Fig. 4*b*). At WOL, CHAR range from 6 to 77 particles of charcoal $cm^{-2} \cdot yr^{-1}$ and generally increase from the early to late MH, with the greatest increase after ≈5.2 ka BP, followed by a sharp decrease at 4.3 ka BP (Fig. 3). The increase in CHAR, particularly between ≈5.2 and 4.3 ka BP, suggests an increase in fire importance and/or the amount of biomass on the landscape during the later part of the MH at WOL. At SL, CHAR vary between 2 and 5 particles of charcoal $cm^{-2} \cdot yr^{-1}$ with no strong temporal trend, suggesting little change in the fire regime during the MH (Fig. 3). Furthermore, CHAR are much lower than at WOL, suggesting that fire was relatively unimportant and/or that fire did not consume as much biomass at SL.
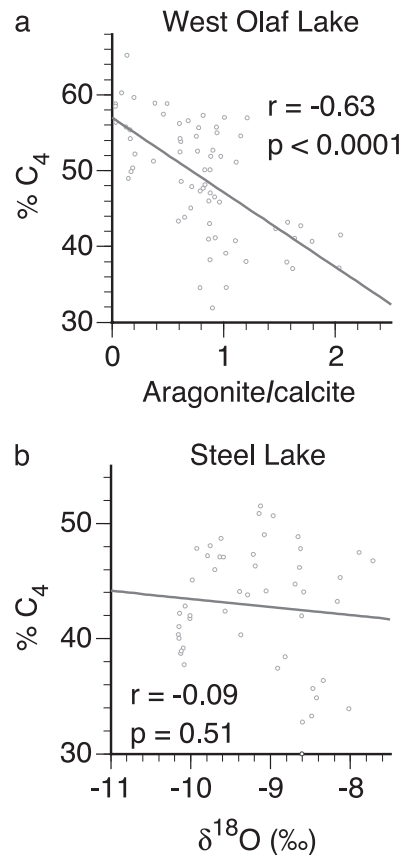

Fig. 5. Correlation between aragonite/calcite and C_4 abundance at WOL (*a*) and $\delta^{18}O$ and C_4 abundance at SL (*b*). The five-sample moving averages of each time series were resampled to obtain the same resolution for correlation.

Discussion

Two lines of evidence support the hypothesis that C_4 plants were more tolerant of warm and dry MH climatic conditions than C_3 plants. First, at WOL, where moisture deficits were greater than at SL throughout the MH, C_4 plants were more abundant than at SL after ≈5.7 ka BP. Second, consistent with Clark *et al.* (25), C_4 plants were more abundant during the warm and dry MH than during the mesic period before European settlement, although our data from the latter period are limited.

However, patterns of C_3 and C_4 plant response at our sites also differ from the predictions of the hypothesis that C_4 plants were more tolerant of MH climatic conditions than C_3 plants. For example, at WOL, the aragonite/calcite ratio and C_4 abundance display generally opposite stratigraphic trends during the MH (Fig. 3). When interpolated to a common sampling resolution, these two time series have a significant negative relationship ($r = -0.63$, $P < 0.0001$, $n = 67$) (Fig. 5*a*). Thus, C_4 plants did not generally dominate in the warmest and driest early part of the MH at WOL. Rather, such climatic conditions appeared to have created bare ground, which favored the establishment of weedy C_3 species, such as *Ambrosia*, over C_4 grasses, as suggested by the overall positive relationship between average *Ambrosia* pollen and aragonite/calcite data interpolated to a common sampling resolution ($r = 0.45$, $P < 0.0001$, $n = 52$). As MH temperatures became cooler and aridity decreased, C_4 grasses increased in abundance.

This counterintuitive C_4 plant response to warm and dry climatic conditions is not restricted to the early MH. During the severe drought of 1930s Dust Bowl period, weedy C_3 species colonized bare ground formerly dominated by C_4 grasses in prairies of the midwestern United States (52). Furthermore, today C_4 productivity

declines relative to C_3 productivity westward beyond the prairie–forest ecotone (53) with the decline of mean annual precipitation, most of which is summer precipitation (5–7). Thus, although C_4 grasses are adapted to warm and semiarid regions and have various mechanisms to cope with drought (54, 55), they require adequate warm-season moisture (6, 7, 56). Drought-like conditions characterized by high interannual moisture variability at WOL during the early MH could have greatly reduced the proportion of C_4 plants relative to C_3 weedy species (49). We cannot tease apart the specific climatic factors that resulted in the severe early MH droughts using our climatic proxies. Regardless, an increase in overall moisture availability during the later MH appears to have favored C_4 plants relative to C_3 plants.

Farther east, at SL, greater effective moisture throughout the MH relative to WOL caused woody C_3 species to be more abundant at SL compared to WOL. C_4 plants were likely present in the uplands near SL, as they were at Deming Lake ≈40 km northwest of SL (25). At SL, C_4 abundance and $\delta^{18}O$ are not correlated during the MH (r = −0.09, P = 0.51, n = 48) (Fig. 5b), suggesting that C_4 abundance did not respond to the trend toward cooler and moister conditions. Thus, as the result of overall cooler and moister conditions at SL, climatic variation within the MH exerted a weaker control on the abundance of C_4 plants relative to C_3 plants at SL.

Our data also suggest that fuel dynamics and climate interacted to control the fire regimes at our sites. At WOL, the extremely warm and dry conditions of the early MH likely limited productivity and the accumulation of flammable fuels, as can occur in semiarid grasslands today (57). During the late MH, the cooler and wetter climatic conditions resulted in increased productivity and C_4 grasses, which enhanced biomass accumulation and changed the vegetation structure at WOL, as inferred from our pollen and charcoal $\delta^{13}C$ data. Thus, fire was a direct consequence of changes in fuel conditions in response to climatic variation; the warmest and driest conditions of the early MH did not result in maximum burning because of biomass limitation (1, 25). Unlike those at WOL, MH fires at SL were likely never biomass-limited, and the fire regime did not show a large response to increased effective moisture from the early to late MH. A plausible explanation for the much lower CHAR at SL than at WOL is that woody species with relatively low flammability, such as *Quercus*, were more abundant at SL throughout the MH. The lower biomass flammability along with cooler and moister conditions at SL caused fires to be less frequent and prevented a discernible change in burning at this site throughout the MH. Thus, fires responded to fuel and climatic conditions, and they did not appear to have been the primary driver of vegetation change at either of our sites, which is in agreement with other studies from the region (25, 58).

This study provides a detailed temporal and spatial record of C_3 and C_4 dynamics during the MH in the mid-continent of North America. Our data, together with other recent results (1, 25), demonstrate the complex responses of vegetation to climatic change in this region. Our results confirm that C_4 plants appear to be adapted to warm and arid environments. However, this relationship may be reversed under climatic conditions characterized by severe moisture deficits, which compromise the advantage of C_4 grasses over C_3 weedy species (28, 29). Furthermore, these results illustrate that the fire regimes of grassland–woodland ecosystems are determined by the interactions of biomass, fuel flammability, and climatic change. Documenting these complex relationships in paleorecords is important for anticipating how these ecosystems may respond to future climatic changes in the continental interiors of North America.

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