

# Compound-specific stable isotopes of organic compounds from lake sediments track recent environmental changes in an alpine ecosystem, Rocky Mountain National Park, Colorado

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## *Abstract*

Compound-specific nitrogen, carbon, and hydrogen isotope records from sediments of Sky Pond, an alpine lake in Rocky Mountain National Park (Colorado, United States of America), were used to evaluate factors contributing to changes in diatom assemblages and bulk organic nitrogen isotope records identified in lake sediments across Colorado, Wyoming, and southern Montana. Nitrogen isotopic records of purified algal chlorins indicate a substantial shift in nitrogen cycling in the region over the past ~60 yr. Temporal changes in the growth characteristics of algae, captured in carbon isotope records in and around Sky Pond, as well as a -60‰ excursion in the hydrogen isotope composition of algal-derived palmitic acid, are coincident with changes in nitrogen cycling. The confluence of these trends is attributed to an increase in biologically available nitrogenous compounds caused by an expansion of anthropogenic influences and temporal changes in catchment hydrology and nutrient delivery associated with meltwater dynamics.

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## *Acknowledgments*

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Dissolved inorganic nitrogen (DIN) from atmospheric deposition is an important part of the nutrient budget in unproductive lakes (Bergström et al. 2005). Variations in the concentration of nitrogenous compounds (N) in atmospheric inputs (including nitrate and ammonium), as well as changes in the amount of precipitation received, will alter N availability. Globally, agricultural practices and fossil fuel combustion have profoundly increased the biological availability of N compounds (Galloway 1998). Across Colorado's Front Range, the growth of cities and industrialized agriculture coincide with the passage from historical states of N limitation in lacustrine environments to frequent N saturation (Williams et al. 1996). Lake sediments from mountainous Colorado, Wyoming, and southern Montana record rapid increases in the abundance of mesotrophic diatoms over the past ~60 yr (Baron et al. 2000; Wolfe et al. 2003; Saros et al. 2005) concurrent with a

2‰ to 4‰ negative excursion in bulk nitrogen isotopes ( $\delta^{15}\text{N}_{\text{bulk}}$ ) and decreases in the ratio of atomic abundances of carbon and nitrogen (C:N) (Wolfe et al. 2003; Wolfe and Saros unpubl. data). These changes at Sky Pond and elsewhere (Vreca and Muri 2006) have been interpreted as an increase in the regional N availability associated with N enrichments from atmospheric deposition on relatively pristine catchments (Baron et al. 2000) (see Fig. 1). However, a variety of early diagenetic processes can overprint  $\delta^{15}\text{N}_{\text{bulk}}$  (Sachs and Repeta 1999) and C:N ratios (Meyers and Ishiwatari 1993), confounding interpretations of nitrogen cycling.

This study applies compound-specific isotopic measurements from lake sediments to further detail the character and causes of ecological change in Rocky Mountain National Park (RMNP). Compound-specific  $\delta^{15}\text{N}$  measurements of chlorins—chlorophyll *a* (Chl *a*) and its degradative products pheophytin *a* and pheophorbide *a*—are used to avert diagenetic concerns related to  $\delta^{15}\text{N}_{\text{bulk}}$  and assess the recent history of N cycling. Carbon isotopic compositions ( $\delta^{13}\text{C}$ ) of sedimentary *n*-alkanes and *n*-acids are used to detect changes in organic matter sources and organism growth characteristics; hydrogen isotopic compositions ( $\delta\text{D}$ ) of the same compounds are used to evaluate historical changes in the local hydrological cycle, which exerts important controls on N delivery in alpine ecosystems.

### Study site

Sediments for this study derive from Sky Pond, located above timberline at an elevation of 3,322 m in RMNP (Fig. 1). The lake is only accessible by hiking trail and is surrounded by alpine tundra comprised of shrub birch, prostrate limber pine, grasses, sedges, and various herbaceous plants. Isolated pockets of subalpine fir and Engelmann spruce are also present (Baron 1992). Bedrock and talus, often lichen covered, are the dominant substrates in the catchment. The lake occupies a cirque of last glacial age (Pinedale), formed in bedrock composed of Precambrian granites and biotitic gneiss (Baron 1992). Late-glacial moraines terminate at the lake and extend upslope to the southwest of the lake (Menounos and Reasoner 1997). A small glacier, heavily debris covered, drains into Sky Pond from the southwest (Fig. 1). Additional inflow is from snowmelt and shallow groundwater flow through talus.

Sky Pond is a dilute and oligotrophic lake (Table 1). However, DIN concentrations are relatively elevated (typically 15 to 20  $\mu\text{mol L}^{-1}$ ), implying some external contribution from atmospheric subsidies. Owing to low attendant total phosphorus concentrations, it is likely that Sky Pond and many other lakes in the region receive more atmospheric N than can be assimilated (Campbell et al. 2000). Historically, N limitation, or nitrogen–phosphorus (N–P) colimitation, was more widespread locally (Morris and Lewis 1988). At present, primary production in Sky Pond is dominated by the diatom *Asterionella formosa*, which blooms during spring snowmelt, and by the cyanobacterium *Oscillatoria limnetica* from summer to fall (McKnight et al. 1990).

Prevailing westerly winds dominate regional circulation and deliver moisture from the Pacific Ocean during winter months. However, episodic upslope easterly flow is common in spring and summer and occasional during the rest of the year. This secondary synoptic type is largely responsible for the delivery of nitrogenous pollution to alpine areas adjacent to the Front Range urban corridor (Williams et al. 1996). Chemical trends in precipitation (Heuer et al. 2000), surface waters (Baron et al. 2000), conifer biomass (Rueth and Baron 2002), and soil (Rueth and Baron 2002) all reflect greater concentrations of DIN on the east slope of the Continental Divide relative to the west. At Sky Pond, annual precipitation averages 110 cm, with 65% to 80% delivered as snow between November and May and the remainder supplied by summer thunderstorms (Baron 1992). Snowmelt occurs between April and July and accounts for 80% of runoff in the catchment. Baseline flow is maintained by snow and glacial melt, whereas summer storms can produce episodic discharge spikes (Clow et al. 2003). Water in the lake has an estimated residence time of ~39 d during snowmelt and ~105 d for the rest of the year (Baron 1992).

Sky Pond received 64,000 brook trout in six separate stocking events between 1931 and 1939 and maintains a reproducing population. The influence of fish on biogeochemical cycling is deemed minimal in the context of the present study because stocking records do not match the timing of changes in diatoms and stable isotopes. Further, both stocked and fishless lakes in RMNP record similarly synchronous ecological changes over recent decades (Wolfe et al. 2003).

### Methods and materials

*Coring and bulk sediment measurements*—Two sediment cores were taken from near the deepest point in Sky Pond on 13 March 2004. A modified Kajak-Brinkhurst device (Glew 1989) was deployed through 1.2 m of lake ice to collect cores of 7.62-cm internal diameter. Cores were extruded on site in 0.5-cm increments. These were kept cold during transport and then frozen at  $-20^\circ\text{C}$ . Both cores were thawed and subsampled for bulk analyses after drying at  $60^\circ\text{C}$ . Analysis of  $\delta^{15}\text{N}_{\text{bulk}}$ ,  $\delta^{13}\text{C}_{\text{bulk}}$ , %N, and %C was performed on 9 to 12 mg of sediment in tin capsules using a Costec ECS 4010 with a reduction column at  $650^\circ\text{C}$  and under standard conditions, measured against the house standard. A second split of dried sediment was acidified with 3 nmol  $\text{L}^{-1}$  HCl prior to drying and analyzed for %C and  $\delta^{13}\text{C}_{\text{org}}$  to confirm that the sediment contained negligible carbonate content. Subsamples of sediment (200 mg) were prepared for diatom analysis by oxidizing organic matter in 30%  $\text{H}_2\text{O}_2$  prior to dilution and mounting of siliceous residues in Naphrax® medium. Detailed diatom analysis was not performed on these two cores. The frequency (%) of *A. formosa* was determined and expressed relative to counts of 400 to 500 total valves. The correlation of *A. formosa* trends between the 2004 cores and a previously well-dated core (Wolfe et al. 2001) was used to determine the chronology of the cores used in this study, given that the initial rise of *A. formosa* is observed in

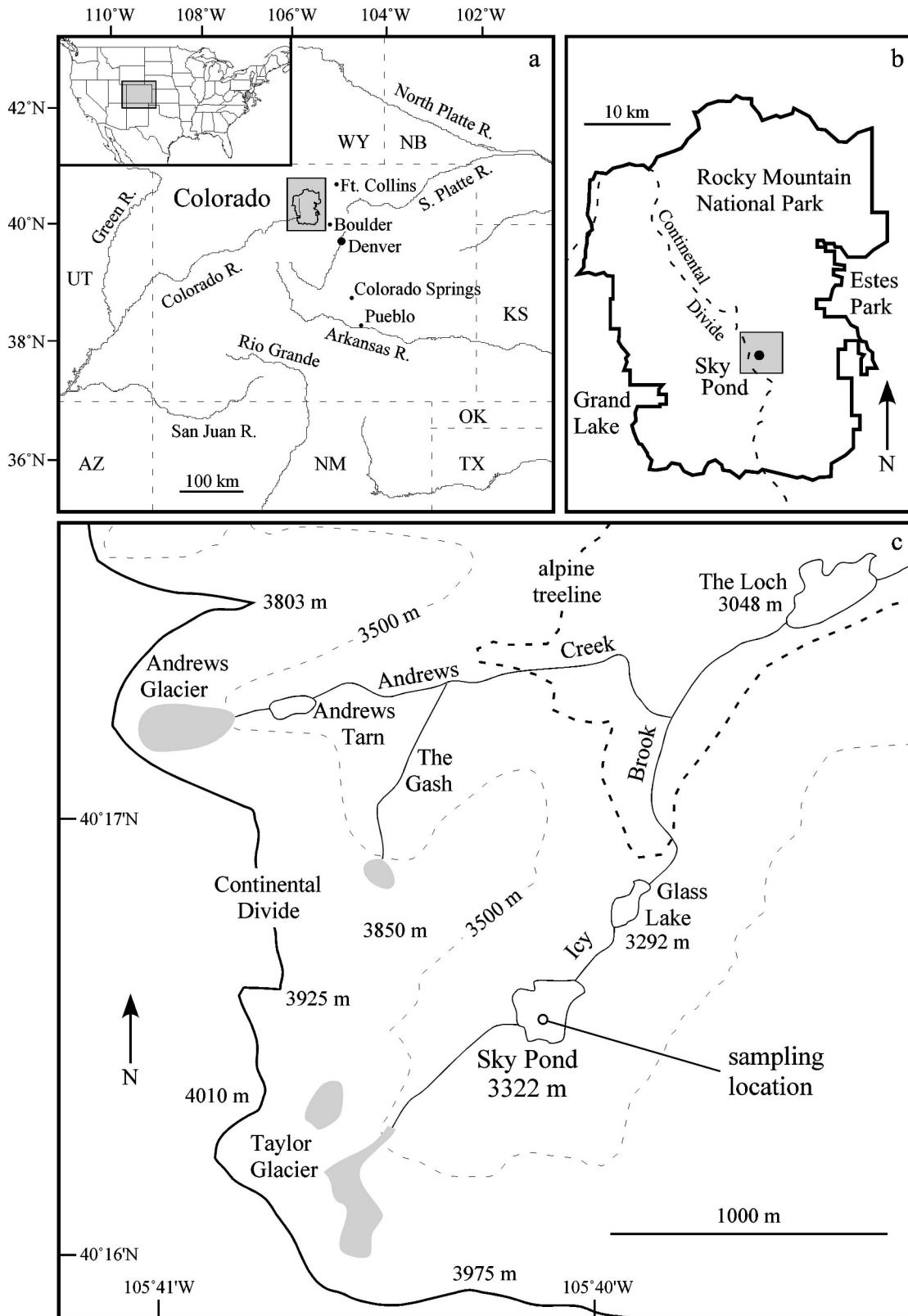


Fig. 1. Location of (a) Rocky Mountain National Park, (b) Sky Pond, and (c) the catchment of Sky Pond and coring location.

Table 1. Location and summary water chemistry for Sky Pond, showing mean values from long-term measurements ( $n = 142\text{--}210$ ).

Parameter	
Latitude (N)	40°16'42"
Longitude (W)	105°40'06"
Elevation (m above sea level)	3,322
Area (m <sup>2</sup> )	0.031 km <sup>2</sup>
Maximum depth (m)	7.2
Mean pH	6.7±0.2
Conductivity	13.3±1.2
Mean [NO <sub>3</sub> <sup>-</sup> ] (μmol L <sup>-1</sup> )	17.35±2.35
Mean [NH <sub>4</sub> <sup>+</sup> ] (μmol L <sup>-1</sup> )	2.66±0.42
Mean [TP] (μmol L <sup>-1</sup> )	0.09±0.02

all cores and corresponds to ~1950 according to the robust prior <sup>210</sup>Pb chronology (Fig. 2).

**Chlorin extraction for isotopic analysis**—Wet sediments (4 to 9 g dry weight) were sequentially extracted with 30-mL solutions of 100% MeOH, 50% MeOH:MeCl<sub>2</sub>, 25% MeOH:MeCl<sub>2</sub>, and 100% MeCl<sub>2</sub> by ultrasonication while immersed in an ice water bath. Total lipid extracts were applied to a flash column of deactivated SiO<sub>2</sub> with 600 mL of 7% MeOH:MeCl<sub>2</sub>. The first 100 mL that eluted, an orange fraction containing β-carotene and other carotenoids, was discarded. The following 200 mL, a darker green fraction containing sedimentary chlorins, was collected and dried. Purification of sedimentary chlorins was performed according to the protocols of Sachs et al. (1999). Purity was checked by analytical high-performance liquid chromatography (HPLC) monitored at 440 and 666 nm. Throughout handling, sediments were protected from exposure to light and heat. Chlorin concentrations were quantified between purification steps by ultraviolet–visible spectrometry to monitor losses. The final extract was redissolved in 200 μL

7% MeOH:MeCl<sub>2</sub> and passed through a Kromasil Kr100-5-C-18 preparative column (10 × 250 mm), with a 10 × 50 mm guard column on a Shimadzu HPLC. A 35-min program gradient of methanol and acetone at a variable flow rate of 6 to 7 mL min<sup>-1</sup> was used to separate Chl *a*, pheophytin *a*, and pheophorbide *a*. These compounds were detected using a photodiode array detector set at 440 and 666 nm and manually collected as they eluted from the column. Chlorins were further purified on a 4.6 × 150 mm SiO<sub>2</sub> analytical column (Supelco LC-Si, 3 μm), with acetone:hexane (10:90) in an isocratic mode at 2 mL min<sup>-1</sup>. Purified compounds were recombined for isotopic analysis.

Chlorin recovery averaged 30% (range: 13% to 45%). Isolated chlorins (~55 to 874 nmol) were analyzed for δ<sup>15</sup>N on a Finnigan MAT DeltaPlus isotope ratio mass spectrometer, after combustion to N<sub>2</sub> in a Carlo Erba/Fisons elemental analyzer at Woods Hole Oceanographic Institute (Houghton et al. 2000). All δ<sup>15</sup>N values were blank corrected. An analytic uncertainty of ±0.34‰ was determined by glycine and ammonium sulfate standards with N concentrations of 50 to 900 nmol. Cellular δ<sup>15</sup>N values (δ<sup>15</sup>N<sub>alg</sub>) were calculated by adding 5.1‰ to chlorin δ<sup>15</sup>N (Sachs et al. 1999).

**Compound-specific δD and δ<sup>13</sup>C measurements**—Sediment samples (4 to 9 g dry weight) were freeze-dried and extracted using an accelerated solvent extractor (ASE200, Dionex) with a 9:1 azeotrope of dichloromethane and methanol at 90°C and 1.03 × 10<sup>4</sup> kPa. Neutral and acid fractions were separated via saponification with potassium hydroxide. Alkanes were isolated from the neutral fraction by solid-phase extraction with hexane. The *n*-acids fraction was methylated by refluxing with 3% boron trifluoride (BF<sub>3</sub>) in methanol. Methylated *n*-acid δD values were corrected for the isotopic contribution of hydrogen added during methylation (Sessions et al. 2002); the δD composition of added methyl groups from the BF<sub>3</sub> solution was

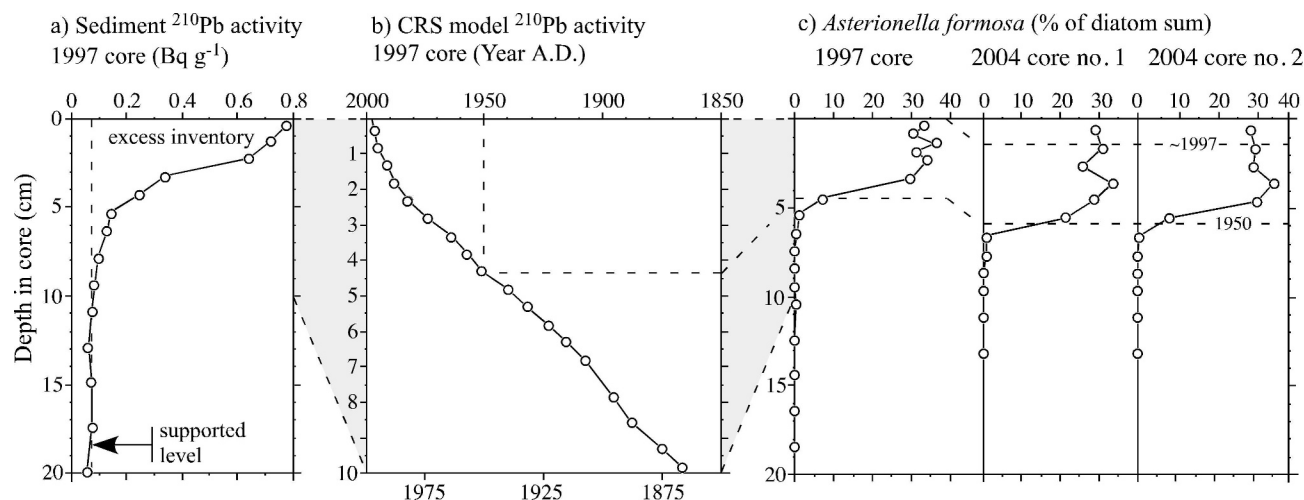


Fig. 2. Chronological scheme based on (a) the sediment <sup>210</sup>Pb inventory of a core taken in 1997, which produces a chronology (b) that places the rapid rise of (c) the diatom *Asterionella formosa* at ~1950 (Wolfe et al. 2001). The same diatom stratigraphic change is clearly recognized in both 2004 cores (panel c), enabling a correlation of the year ~1950 that suggests a realistic 1.2 cm of accumulation between 1997 and 2004.

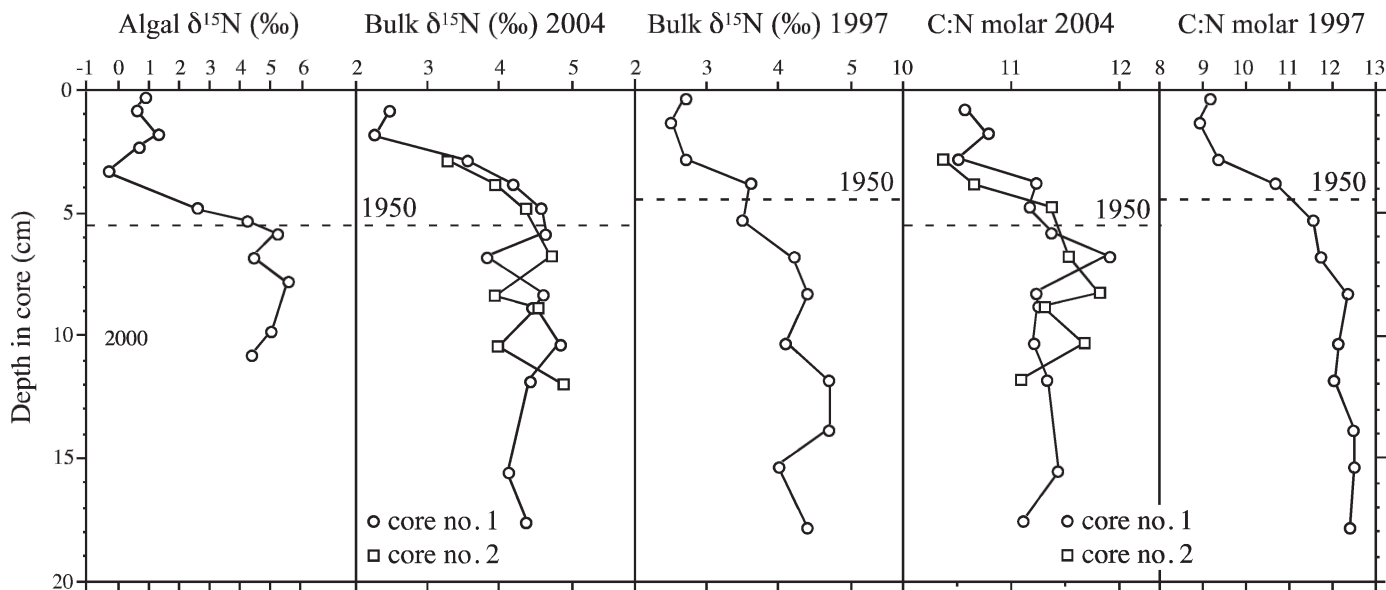


Fig. 3. Sediment profiles of algal  $\delta^{15}\text{N}$  alongside bulk  $\delta^{15}\text{N}$  from the 2004 Sky Pond cores. Bulk  $\delta^{15}\text{N}$  is also shown for the 1997 core (Wolfe et al. 2001), and molar C:N trends from all cores.

determined by methylating phthalic acid of a known isotopic composition (Schimmelmänn pers. comm.).  $\delta^{13}\text{C}$  values of methylated fatty acids were not corrected.

*N*-alkanes and *n*-acids were analyzed for  $\delta^{13}\text{C}$  and  $\delta\text{D}$  on a Thermo Finnigan MAT 253 isotope ratio monitoring (irm) mass spectrometer (MS) coupled to Thermo Finnigan trace gas chromatograph (GC) combustion III (for  $\delta^{13}\text{C}$ ) and high temperature conversion (for  $\delta\text{D}$ ) systems (irm-GCMS). Individual *n*-alkanes and *n*-acids were separated using a J&W Scientific DB-1 capillary column (60 cm  $\times$  0.25 mm  $\times$  0.25  $\mu\text{m}$ ). The temperature program was from 60°C (held for 1 min) at 6°C min<sup>-1</sup> to 320°C (isothermal for 25 min). Helium was used as a carrier gas with a column flow rate of 2.0 mL min<sup>-1</sup>. The injected volume was adjusted between 0.5  $\mu\text{L}$  and 1.2  $\mu\text{L}$ , depending on the sample, to obtain sufficient response on the irm-GCMS system for individual peaks. Programmed-temperature variation injection was executed at 40°C, solvent evaporated at 180°C, and transfer to the reactors occurred at 260°C.

Pyrolytic conversion of organic hydrogen to H<sub>2</sub> was conducted at 1,400°C. The H<sup>3</sup>-factor was determined daily using H<sub>2</sub> reference gas with a  $\delta\text{D} = -230\text{‰}$  standard mean ocean water (SMOW). H<sub>2</sub> peak heights varied over an eightfold range, which was within the range of most H<sub>2</sub> peaks from analyzed compounds. Precision of isotopic measurements of H<sub>2</sub> reference gas after H<sub>3</sub><sup>+</sup> correction was  $\leq 5\text{‰}$ . Analytical accuracy and precision of the system were analyzed at least once per day and were calibrated using a standard mixture of alkanes *n*-C<sub>16</sub> through *n*-C<sub>30</sub> ( $\delta\text{D}$  values for individual *n*-alkanes are available upon request) and 5 $\alpha$ -androstane ( $\delta\text{D} = -256.2\text{‰}$  SMOW), measured off-line at Indiana University by Arndt Schimmelmänn.  $\delta^{13}\text{C}$  and  $\delta\text{D}$  of both *n*-alkanes and *n*-acids are reported as the average of duplicate analyses. Analytical uncertainty, based on daily standard measurements, was  $\pm 0.15\text{‰}$  for  $\delta^{13}\text{C}$  ( $n = 154$ ) and  $\pm 3.3\text{‰}$  for  $\delta\text{D}$  ( $n = 105$ ).

*$\delta\text{D}$  of Sky Pond waters*—Archived samples of Sky Pond epilimnetic waters were obtained from the Loch Vale Watershed Research Project for  $\delta\text{D}$  measurements. The analyzed samples were collected during winter and spring between December 1991 and January 1996 and stored in opaque plastic bottles at 4°C. Hydrogen isotopic compositions were determined using a hydrogen device connected to a Finnigan MAT 253 mass spectrometer. Analytical uncertainty was less than  $\pm 1\text{‰}$ .

For all reported stable isotopic measurements, standard delta notation is used:

$$\delta X = \left[ \frac{R_{\text{Sample}}}{R_{\text{Standard}}} - 1 \right] \times 1,000 \quad (1)$$

where  $\delta X$  represents  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$ , or  $\delta\text{D}$ , and  $R$  is the abundance ratio  $^{15}\text{N}:^{14}\text{N}$ ,  $^{13}\text{C}:^{12}\text{C}$ , or  $\text{D}:\text{H}$ , respectively. All  $\delta$  values are calculated relative to international standards of atmospheric N<sub>2</sub> for N, VPBD for C, and VSMOW for H.

## Results

*Bulk measurements*—Sediment  $\delta^{15}\text{N}_{\text{bulk}}$  averages 4.3‰ ( $\pm 0.41\text{‰}$ ) between 18 cm and 5.5 cm before trending to more negative values within the upper 5 cm. The most  $^{15}\text{N}$ -depleted compositions (2.4‰) occur at the top of the core (Fig. 3). In contrast, variability in  $\delta^{13}\text{C}_{\text{bulk}}$  is small, ranging between  $-23.9\text{‰}$  and  $-24.6\text{‰}$ , with no consistent relationship to  $\delta^{15}\text{N}_{\text{bulk}}$  trends. Sediment C:N molar ratios decrease over the same interval as the negative  $\delta^{15}\text{N}_{\text{bulk}}$  shift (Fig. 3).

Both the abundances of *A. formosa* (Fig. 2) and the C:N ratios (Fig. 3) are sufficiently similar between the 1997 and 2004 cores to enable the reliable transfer of the <sup>210</sup>Pb-dated initial rise of *A. formosa* (circa the year 1950) to a depth of 5.5 cm in the 2004 cores.

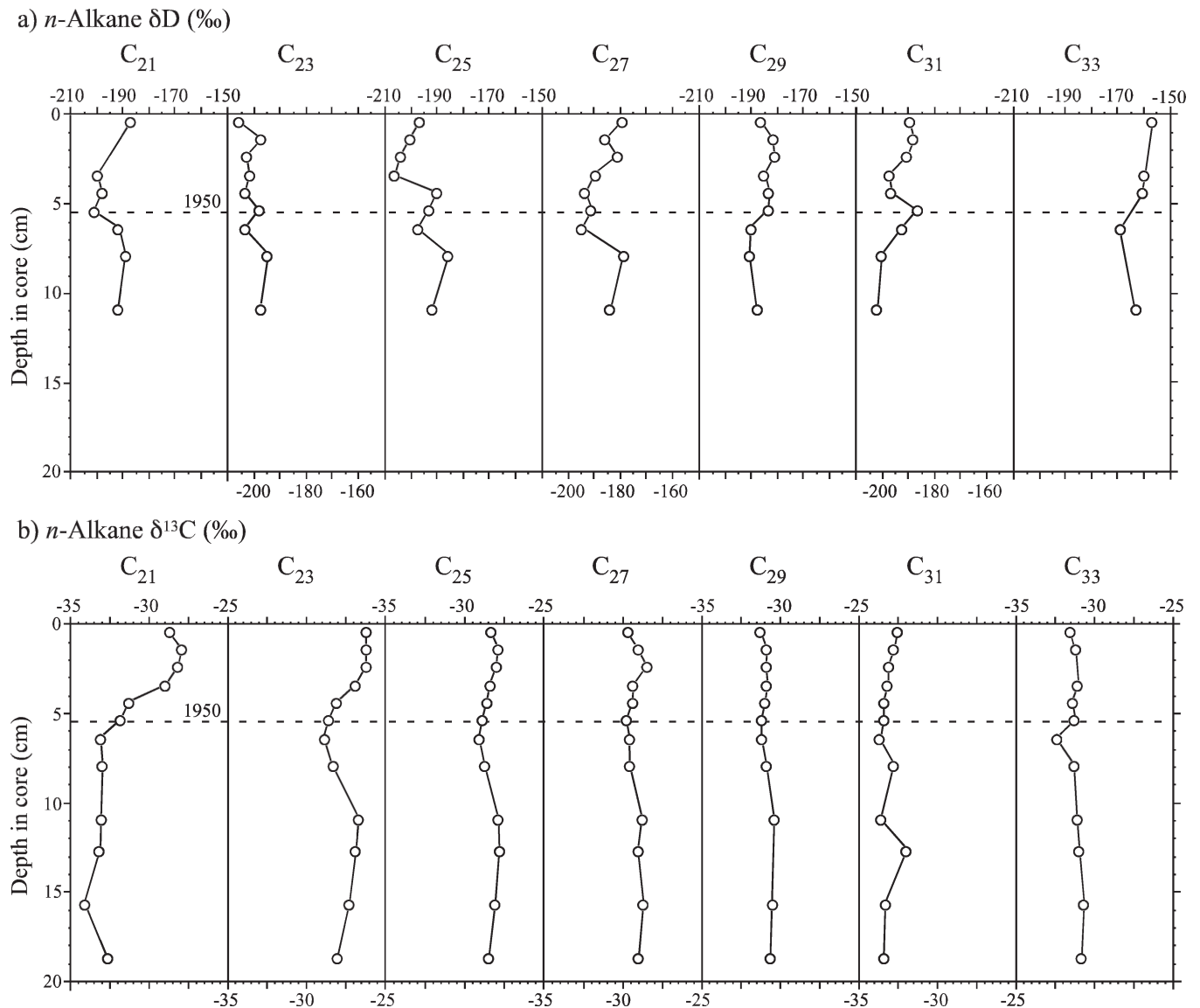


Fig. 4. Sediment profiles of (a) *n*-alkane  $\delta D$  and (b)  $\delta^{13}C$ , from carbon-chain lengths of C<sub>21</sub> to C<sub>33</sub>.

Sediment  $\delta^{15}N_{alg} - \delta^{15}N_{alg}$  values average 4.8‰ ( $\pm 0.54$ ‰) below 6 cm and become increasingly negative between 5.25 cm and the top of the core. The total  $\delta^{15}N_{alg}$  shift ( $-4.5$ ‰) over the upper 5.25 cm is approximately 2.5‰ greater than that expressed by  $\delta^{15}N_{bulk}$  (Fig. 3). However, values of  $\delta^{15}N_{alg}$  and  $\delta^{15}N_{bulk}$  are similar within analytical error below 6 cm. At the mud–water interface,  $\delta^{15}N_{alg}$  is 2‰ depleted relative to  $\delta^{15}N_{bulk}$ .

*n*-Alkanes—*n*-C<sub>25</sub> through *n*-C<sub>33</sub> show a strong odd-over-even predominance throughout the sediment core with a maximum at *n*-C<sub>29</sub> (data not shown).  $\delta D$  values of sediment *n*-alkanes with chain lengths *n*-C<sub>21</sub> to *n*-C<sub>31</sub> range between  $-179$ ‰ and  $-215$ ‰ and show no compelling temporal trends (Fig. 4a). *n*-C<sub>33</sub> is consistently D enriched relative to this range ( $> -170$ ‰), implicating a different source for this compound. Variability of  $\delta^{13}C$  values below 6 cm is generally low ( $< 3$ ‰) for all *n*-alkanes (Fig. 4b).

Above 6.25 cm,  $\delta^{13}C$  values remain relatively constant, with the exception of *n*-C<sub>21</sub>, which shows marked enrichment toward the top of the core. In general,  $\delta^{13}C$  values decrease with longer carbon-chain length, but *n*-C<sub>21</sub> and *n*-C<sub>33</sub> are exceptions to this pattern.

*Fatty acids*—Saturated *n*-acids are dominated by *n*-C<sub>16:0</sub> (palmitic acid), with a predominance of even-over-odd-number homologues (data not shown). Fatty acid  $\delta D$  values have greatest variability in the *n*-C<sub>16:0</sub> fraction, including a striking 63‰ depletion between 8 cm and the top of the core (Fig. 5a) preceding major changes in N cycling (Fig. 3). *n*-C<sub>20:0</sub> and *n*-C<sub>22:0</sub> show somewhat similar  $\delta D$  trends, but of considerably lower magnitude. *n*-C<sub>16:0</sub> exhibits a negative  $\delta D$  shift from 4 cm to the top of the core that is not observed for any other fatty acid.  $\delta^{13}C$  values of *n*-C<sub>20:0</sub> through C<sub>28:0</sub> vary  $\leq 2$ ‰, and temporal trends are similar. However,  $\delta^{13}C$  values for *n*-C<sub>16:0</sub> ( $\delta^{13}C_{PA}$ , where

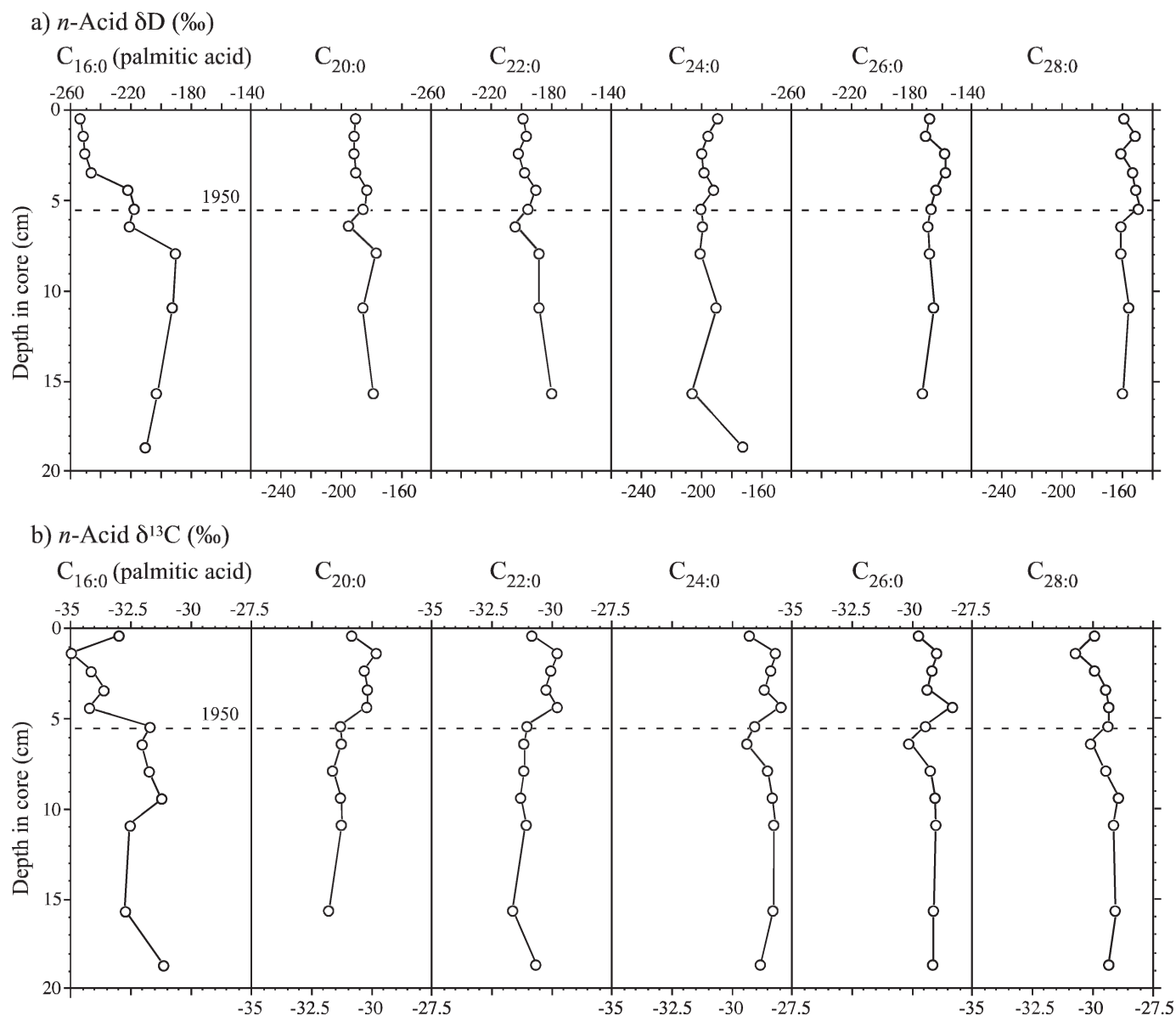


Fig. 5. Sediment profiles of (a)  $n$ -acid  $\delta D$  and (b)  $\delta^{13}C$  from the saturated  $n$ -acids:  $n$ - $C_{16:0}$ ,  $n$ - $C_{20:0}$ ,  $n$ - $C_{22:0}$ ,  $n$ - $C_{24:0}$ ,  $n$ - $C_{26:0}$ , and  $n$ - $C_{28:0}$ .

PA is palmitic acid) are distinctly different:  $\delta^{13}C_{PA}$  shifts opposite in direction to the other  $n$ -acids with a range of  $\sim 4\%$  and a trend toward more negative values (2‰ to 3‰) since  $\sim 1950$  (Fig. 5b).

$\delta D$  of Sky Pond surface water— $\delta D$  values of surface water in Sky Pond ( $\delta D_{LW}$ , where LW is lake water) reveal considerable intraannual and interannual variability and a consistent negative trend over a 5-yr period (Fig. 6). Using the  $\delta D$  value of modern Sky Pond surface water ( $-119\%$ ; Fig. 6) and the  $\delta D$  value of palmitic acid ( $\delta D_{PA}$ ) over the sediment depth interval of 0 to 0.5 cm ( $-254\%$ ), we estimate an isotopic fractionation between palmitic acid and lake water ( $\epsilon_{PA-water}$ ) of  $-153\%$ , consistent with values reported in the literature ( $-145\%$  to  $-213\%$ ) (Huang et al. 2002, 2004).

## Discussion

$\delta^{15}N$  and the Sky Pond N reservoir—Our results confirm that the negative  $\delta^{15}N$  shift from  $\sim 1950$  to the present reflects a primary feature in Sky Pond. The  $\delta^{15}N_{alg}$  record indicates that the excursion is 2.5‰ larger in photosynthate relative to bulk sediment (Fig. 3). The departure of  $\delta^{15}N_{alg}$  from  $\delta^{15}N_{bulk}$  at  $\sim 5$  cm indicates that the decompositional environment changed circa 1950. We attribute the +2‰ up-core offset in  $\delta^{15}N_{bulk}$  relative to  $\delta^{15}N_{alg}$  to increased microbial activity and loss of  $^{14}N$  stimulated by greater primary production and deposition of nitrogen-rich organic matter, as evidenced by decreasing C:N ratios. Our results confirm that the use of bulk N isotopes to interpret changes in primary production and nitrogen availability is problematic, since the processes exert opposing influences

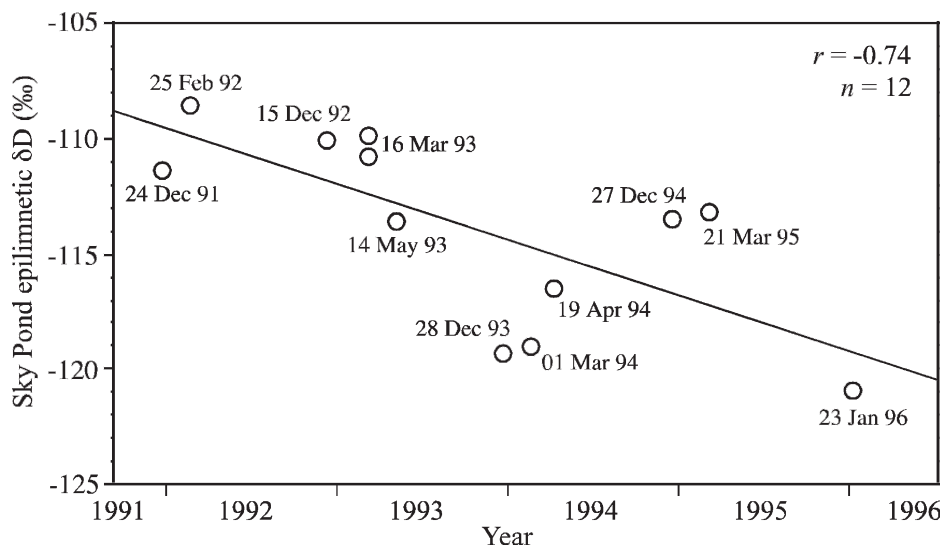


Fig. 6. Sky Pond lake-water  $\delta D$  values over the 24 December 1991 to 23 January 1996 interval showing progressive D depletion.

on  $\delta^{15}N_{\text{bulk}}$ . However in this region at least, the amplitude of diagenetic effects appears to be less than the fingerprint of regional changes in N biogeochemistry, so that bulk sediments retain the signature of decreasing lake-sediment  $\delta^{15}N$ .

The large  $-4.5\%$  decrease in  $\delta^{15}N_{\text{alg}}$  values could result from three dominant processes: (1) a decrease in the  $\delta^{15}N$  of atmospheric N, (2) an increase in high altitude N delivery leading to an increase in the usable N pool, and (3) changes in the biological composition of Sky Pond.

A decrease in the  $\delta^{15}N$  of atmospheric N could result from increased regional production of anthropogenic N from plant fertilizer, fossil fuel combustion, and ammonium volatilized from confined feeding operations—activities which all increased dramatically c. 1950 in the Colorado Front Range (Baron et al. 2000). Anthropogenic N exhibits a wide range of  $\delta^{15}N$  values, from  $-15\%$  to  $+25\%$  (Macko and Ostrom 1994, and references therein), which implies that a minimum (i.e., applying the most  $^{15}N$ -depleted anthropogenic value) of 30% of the available N pool at Sky Pond derives from anthropogenic sources. However, more reasonable estimates of anthropogenic  $\delta^{15}N$  values from the Front Range region ( $\sim 0\%$  to more positive values) negate the possibility that a decrease in the  $\delta^{15}N$  of atmospheric N is the primary cause for the observed shift in  $\delta^{15}N_{\text{alg}}$ , and this suggests that changes in N availability at Sky Pond contributed to the  $\delta^{15}N_{\text{alg}}$  shift.

Increased N availability would relieve N limitation in Sky Pond, enhance algal production, and lead to a decrease in  $\delta^{15}N_{\text{alg}}$  values as kinetic isotope effects related to N uptake are more fully expressed (Goerick et al. 1994). Changes in hydrology have the potential to alter catchment nitrogen cycling and lake ecology, with or without alterations in the concentration of N compounds in atmospheric deposition. A scenario of enhanced algal production due to a decrease in N limitation over the past 60 yr is supported by both  $\delta^{15}N_{\text{alg}}$  values and C:N molar ratio trends (Meyers and Ishiwatari 1993; Fig. 3).

Increases in the contribution of fall blooming cyanophytes could also have influenced the observed decrease in  $\delta^{15}N_{\text{alg}}$  given that the  $\delta^{15}N$  of N fixers is  $\sim 0\%$ , or about 3% to 4% more negative than autotrophic algal  $\delta^{15}N$  (France et al. 1998). However, enrichment experiments in Sky Pond suggest that the resident cyanophytes are not obligate N fixers (McKnight et al. 1990). Similarly, isotopic effects due to changes in species composition are difficult to assess. For example, while the 5.1% isotopic depletion of chlorins relative to total algal nitrogen is considered a robust average in diverse marine environments, differences of  $\pm 2.5\%$  have been observed between individual species (Sachs et al. 1999). Thus, it is possible that changes in chlorin contributions from changing algal species also influenced  $\delta^{15}N_{\text{alg}}$  trends. However, this possibility remains speculative until more information regarding the  $\delta^{15}N$  of chlorophyll from the dominant primary producers in Sky Pond is obtained. Therefore, we argue that historical changes in  $\delta^{15}N_{\text{alg}}$  were primarily driven by changes in N delivery to Sky Pond.

$\delta^{13}C_{n-C_{21}}$  and catchment ecology—The  $\delta^{13}C$  values of most *n*-alkanes appear relatively invariant except for *n*-C<sub>21</sub>, which becomes substantially  $^{13}C$ -enriched by 4% to 5%, synchronous with changes in  $\delta^{15}N_{\text{alg}}$  (Fig. 4b). *N*-alkanes with carbon-chain lengths in the range of C<sub>25</sub> to C<sub>35</sub> and with odd-over-even predominance derive from the waxy cuticles of higher plants (Eglinton and Hamilton 1967). The isotopic deviation of *n*-C<sub>21</sub> indicates that this compound derives from sources distinct from terrestrial plants. *n*-C<sub>21</sub> is found in bacteria, some phytoplankton (Parrish 1988), and macrophytes (Ficken et al. 2000), and also represents an important hydrocarbon of some lichen, where it is found in concentrations of 7% to 15% (Zygodlo et al. 1993). Our analysis of lichens with chlorophyte and cyanobacterial symbionts collected on a bedrock outcrop near the Sky Pond outflow contained relatively high concentrations of *n*-C<sub>21</sub> (not shown), consistent with published records.



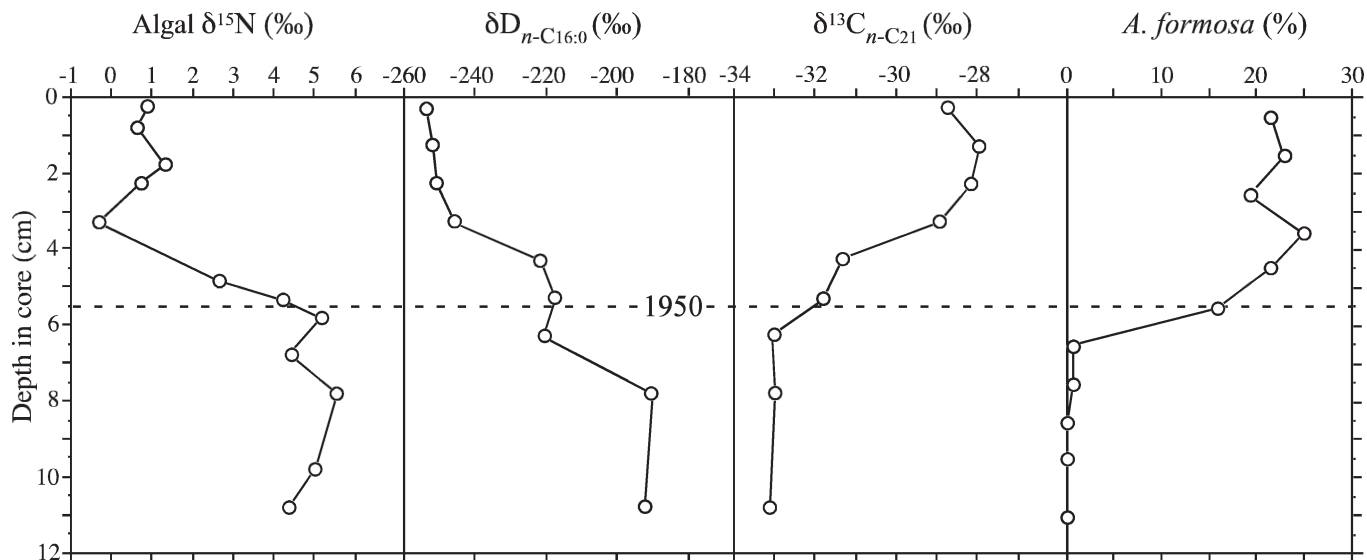


Fig. 7. Summary of proxies recording post-1950 biogeochemical and ecological change at Sky Pond: algal  $\delta^{15}\text{N}$  ( $\delta^{15}\text{N}_{\text{alg}}$ ),  $\delta\text{D}_{n\text{-C}_{16:0}}$  ( $\delta\text{D}_{\text{PA}}$ ),  $\delta^{13}\text{C}_{n\text{-C}_{21}}$ , and relative frequencies of *Asterionella formosa*.

Submerged macrophytes are ruled out as a possible source of  $n\text{-C}_{21}$  because they are absent in Sky Pond.

The source of  $n\text{-C}_{21}$  is inferred to be algal, with an origin either in the lake or in upslope talus communities, in free-living or symbiotic (with lichen) form. The highly negative  $\delta^{13}\text{C}_{n\text{-C}_{21}}$  values seen downcore ( $-32.5\text{‰}$  to  $-34\text{‰}$ ) are associated with carbon fixation via Rubisco, while the positive trend in  $\delta^{13}\text{C}$  values is associated with increased growth rates, increased carbon limitation, and with fixation via phosphoenolpyruvate carboxylase, all scenarios consistent with increased primary production accompanying N enrichment. In contrast, methanogenesis produces highly negative  $\delta^{13}\text{C}$  values, but a decline in methanogenesis necessary to produce the up-core  $^{13}\text{C}$ -enrichment is inconsistent with the scenario of decreased oxygen availability indicated by the offset in  $\delta^{14}\text{N}_{\text{alg}}$  from  $\delta^{14}\text{N}_{\text{bulk}}$ , which leads us to reject bacteria as the source of the  $\delta^{13}\text{C}_{n\text{-C}_{21}}$  signature.

A probable source of  $n\text{-C}_{21}$  derives from green lichen. Very low  $\delta^{13}\text{C}$  values ( $-31\text{‰}$  to  $-35\text{‰}$ ) occur in green lichen under dry conditions because photobionts are able to photosynthesize in equilibrium with water vapor (Lange et al. 1988). This scenario results in high intercellular  $\text{CO}_2$  concentrations and more negative  $\delta^{13}\text{C}_{\text{CO}_2}$  values due to the kinetic isotope effects associated with carbon fixation and diffusion through air ( $-4.4\text{‰}$ ). In contrast, higher diffusion resistances accompany liquid phase diffusion of  $\text{CO}_2$  and result in lower ambient and intercellular  $\text{CO}_2$  concentrations, less  $^{13}\text{C}$  discrimination, and more positive  $\delta^{13}\text{C}$  values comparable to those typical of blue-green lichens ( $-14\text{‰}$  to  $-23\text{‰}$ ) (Lange et al. 1988). On this basis,  $\delta^{13}\text{C}$  trends of  $n\text{-C}_{21}$  support a temporal increase in moisture delivery to the rock surfaces and talus soils in the catchment surrounding Sky Pond in the form of rain, snow, and/or ice melt—a supposition supported by  $\delta\text{D}$  trends.

*$\delta\text{D}_{\text{PA}}$  and catchment hydrology*—Hydrogen isotopic compositions of sedimentary lipids record the  $\delta\text{D}$  signature

of source water, as well as effects of biosynthetic fractionation and exchange of hydrogen. Lake sediment and epilimnion water  $\delta\text{D}_{\text{PA}}$  records have been shown to track the  $\delta\text{D}$  of source water with a fractionation between  $-145\text{‰}$  and  $-213\text{‰}$  (Huang et al 2002, 2004), making  $\delta\text{D}_{\text{PA}}$  a tracer of the local hydrologic cycle.

Evidence for historical changes in the hydrological cycle at Sky Pond is potentially expressed by a  $\sim 60\text{‰}$  negative shift in  $\delta\text{D}_{\text{PA}}$  within the upper 8 cm of the core (Fig. 5a). Similar isotopic patterns with substantially smaller magnitudes are evident in  $n\text{-C}_{20:0}$  and  $n\text{-C}_{22:0}$ , which contrast with the  $\delta\text{D}$  trends of  $n\text{-C}_{24:0}$  to  $n\text{-C}_{28:0}$ . These relationships imply distinct sources for  $n\text{-C}_{16:0}$  to  $n\text{-C}_{22:0}$  and  $n\text{-C}_{24:0}$  to  $n\text{-C}_{28:0}$ , which is consistent with common interpretations of a higher plant source for longer chain compounds ( $>\text{C}_{22}$ ) and a microorganism source of shorter chain fatty acids.

Interpreting changes in hydrological conditions is not necessarily straightforward in light of substantial differences in D:H fractionation among different species of green algae and among different families identified in a recent study (Zhang and Sachs 2007). Therefore, the pattern observed in  $\delta\text{D}_{\text{PA}}$  could be driven by changes in the source of palmitic acid as a result of changes in lake ecology (Baron et al. 2000; Wolfe et al. 2001, 2003; Zhang and Sachs 2007) and/or changes in  $\delta\text{D}$  of the Sky Pond lake-water source.

Culture work has identified differences in D and H fractionation between different species of green algae of  $\sim 10\text{‰}$  to  $15\text{‰}$  and between different families of  $\sim 90\text{‰}$  to  $100\text{‰}$  (Zhang and Sachs 2007). Given observed changes in diatom assemblages, the influence of changing lake ecology on  $\delta\text{D}_{\text{PA}}$  at Sky Pond could be important. A relative increase in autochthonous productivity could also decrease  $\delta\text{D}_{\text{PA}}$ , because higher plant lipids are D enriched by up to  $60\text{‰}$  relative to aquatic lipids (Sachse et al. 2004). However, it is notable that the onset of substantial changes in  $\delta\text{D}_{\text{PA}}$  occurs  $\sim 2$  cm (more than a decade) before the shifts in  $\delta^{15}\text{N}_{\text{alg}}$ ,  $\delta^{13}\text{C}_{n\text{-C}_{21}}$ , and percentage *A. formosa*

(Fig. 7). And the isotopic value for higher plant lipids as inferred from  $\delta D_{n-C_{28:0}}$  ( $-160\%$ , Fig. 5) suggests that only an unrealistic shift in the balance between terrestrial and aquatic production (from 70% terrestrial production to 5% terrestrial production) could account for the  $\delta D_{PA}$  shift. Moreover, while  $\delta^{13}C_{PA}$  exhibits variability, which can reflect changes in the source of palmitic acid, periods of rapid change in  $\delta D_{PA}$  are not accompanied by significant shifts in  $\delta^{13}C_{PA}$  (Fig. 5). Therefore, while changes in the source of palmitic acid production c. 1950 potentially affected  $\delta D_{PA}$ , the available evidence suggests that the negative shift recorded in the sedimentary  $\delta D_{PA}$  of Sky Pond is tracking changes in the  $\delta D$  values of lake water.

Direct evidence for a negative isotope trend in the  $\delta D$  of Sky Pond ( $\delta D_{LW}$ ) is found in recent waters sampled from under-ice epilimnetic conditions between 1992 and 1996 (Fig. 6). Intraannual and interannual variability in  $\delta D$  values of surface water in Sky Pond (Fig. 6) can relate to seasonal differences in the lake's precipitation: evaporation balance, water residence time, and fraction derived directly from melt. The modern data support a sustained negative  $\delta D$  trend, consistent with the pattern observed in  $\delta D_{PA}$ , suggesting modern trends are a continuation of hydrological changes initiated more than 60 yr ago.

Historical changes in the  $\delta D$  of water in Sky Pond ultimately derive from changes in either the  $\delta D$  of precipitation or meltwater and/or changes in the ratio of evaporation and precipitation. The  $\delta D$  of *n*-alkanes and *n*-acids associated with higher plants show little variability (Figs. 4a and 5a), suggesting that the  $\delta D$  of summer precipitation and soil evaporation and transpiration effects (Yapp and Epstein 1982) are unlikely to be important controls on  $\delta D_{PA}$ . Snowmelt is the principal source of water to Sky Pond and the sole source for the spring phytoplankton bloom. Therefore, changing meltwater dynamics and evaporative D enrichment during late spring and early summer (Back 1992) are likely the primary factors forcing  $\delta D_{LW}$  with relevance for  $\delta D_{PA}$ . Seasonal evaporative enrichment at Sky Pond is relatively small ( $\sim +10\%$ ) (Back 1992), although it could have been larger in the past if the supply of meltwater during the summer was lower. A larger decrease in  $\delta D_{LW}$  could result from increased contributions of snow and ice melt ( $-110\%$  to  $-131\%$ ) relative to precipitation ( $-35.5\%$  to  $-86.5\%$ ) (Back 1992) to Sky Pond. Relatively negative  $\delta D$  values ( $-120\%$  to  $-150\%$ ) are exhibited in permafrost in the American Rocky Mountains (Steig et al. 1998), and an increased contribution of waters from permafrost due to rising summer temperatures (Clow et al. 2003) would deplete  $\delta D_{LW}$  and  $\delta D_{PA}$ . As a corollary, this supposition implies that higher plant *n*-acids derive from distal sources or have D:H compositions dominated by processes associated with summertime precipitation and evapotranspiration.

Cryosphere melting—including snowpacks, glaciers, and permafrost—has increased at high elevation sites in the Front Range over recent decades (Mote et al. 2005). At the elevation of Sky Pond, which roughly coincides with the altitudinal limit of permafrost, summer temperatures have risen at a rate of  $0.15^\circ\text{C yr}^{-1}$  since the early 1990s,

superimposed on a more gradual 20th-century warming trend observed in alpine regions globally (Clow et al. 2003). Importantly, snowmelt carries the DIN that would relieve N limitation, stimulate major blooms of *Asterionella* (McKnight et al. 1990), and account for the observed isotopic signals. The consequences of increased melt for talus communities are poorly understood (Campbell et al. 2000), but may have important ecological implications given that talus is home to transient but active pools of microbially cycled organic and inorganic N (Brooks et al. 1999; Ley et al. 2004). Shifts in  $\delta D_{PA}$  ( $\sim 7$  cm) predate changes in diatom assemblages and  $\delta^{13}C_{n-C_{21}}$ , which suggests a delay in organism response to environmental change. C:N ratios arguably shift to more negative values beginning at  $\sim 7$  cm, supporting the interpretation that changes in hydrology and associated increases in N delivery implied by decreasing  $\delta D_{PA}$  stimulated an increase in primary production prior to triggering algal community reorganization.

Our results coupled with independent microfossil evidence (Baron et al. 2000; Wolfe et al. 2001, 2003) indicate that increased DIN concentrations in Sky Pond since  $\sim 1950$  have driven a reorganization of the lake's algal community. Changes in meltwater dynamics, as evidenced in  $\delta D$  values of palmitic acid, commenced prior to observed changes in algal assemblages and  $\delta^{15}N_{alg}$  and have acted to alleviate N limitation and moisture availability, enhancing production by algae within the lake and in talus communities. Changes in N delivery to Sky Pond are thus attributed both to increased anthropogenic N inputs and to hydrologic changes in the region.

Collectively, our data demonstrate that the biogeochemical cycles of N, C, and H have all entered new states at Sky Pond, resulting from the complex interplay among atmospheric deposition, climate change, and lake biology. Despite the current levels of environmental protection administered in protected areas such as RMNP, it is clear that changes proceeding apace are sufficiently potent to reshape the base of food chains in this relatively undisturbed alpine wilderness. Further, given that bulk sediments from lakes throughout the Rocky Mountains show similar  $\delta^{15}N$  and phytoplankton trends (Wolfe et al. 2003; Saros et al. 2005; Wolfe and Saros unpubl. data), it is likely that the environmental changes discussed here represent a regional phenomenon.

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