

# Underwater sinkhole sediments sequester Lake Huron's carbon

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**Abstract** Lake Huron's submerged sinkhole habitats are impacted by high-conductivity groundwater that allows photosynthetic cyanobacterial mats to form over thick, carbon-rich sediments. To better understand nutrient cycling in these habitats, we measured the stable isotopic content of carbon and nitrogen in organic and inorganic carbon pools in Middle Island sinkhole, a ~23 m deep feature influenced by both groundwater and overlying lake water. Two distinct sources of dissolved CO<sub>2</sub> (DIC)

were available to primary producers. Lake water DIC ( $\delta^{13}\text{C} = -0.1\text{‰}$ ) differed by +5.9‰ from groundwater DIC ( $\delta^{13}\text{C} = -6.0\text{‰}$ ). Organic carbon fixed by primary producers reflected the two DIC sources. Phytoplankton utilizing lake water DIC were more enriched in <sup>13</sup>C ( $\delta^{13}\text{C} = -22.2$  to  $-23.2\text{‰}$ ) than mat cyanobacteria utilizing groundwater DIC ( $\delta^{13}\text{C} = -26.3$  to  $-30.0\text{‰}$ ). Sinkhole sediments displayed an isotopic signature ( $\delta^{13}\text{C} = -23.1\text{‰}$ ) more similar to sedimenting phytoplankton than the cyanobacterial mat. Corroborated by sediment C/N ratios, these data suggest that the carbon deposited in sinkhole sediments originates primarily from planktonic rather than benthic sources. <sup>210</sup>Pb/<sup>137</sup>Cs radiodating suggests rapid sediment accumulation and sub-bottom imaging indicated a massive deposit of organic carbon beneath the sediment surface. We conclude that submerged sinkholes may therefore act as nutrient sinks within the larger lake ecosystem.

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## Abbreviations

SGD Subsurface groundwater discharge  
DIC Dissolved inorganic carbon  
DOC Dissolved organic carbon  
IRMS Isotope ratio mass spectrometry  
POC Particulate organic carbon  
SOC Sedimentary organic carbon  
SPOC Sedimenting particulate organic carbon

## Introduction

Subsurface groundwater discharge (SGD) into aquatic habitats can play a significant role in shaping ecosystem properties, especially in areas of karst geology (Church 1996). Groundwater can transport nutrients, trace elements, metals, and pollutants to surface waters (Beck et al. 2007; Gonzalez et al. 2008; Knee et al. 2010; Montlucon and Sanudo-Wilhelmy 2001; Swarzenski et al. 2006), modify biogeochemical cycles (Basterretxea et al. 2010; Garcia-Solsona et al. 2010; Hebbing et al. 2006), and alter an ecosystem's trophic status (Camacho et al. 2001; Capone and Bautista 1985). We have been studying the effects of SGD on the submerged sinkholes of Lake Huron (Fig. 1). Submerged sinkholes are potentially numerous formations in the Michigan Basin created when underwater caves collapse (Coleman 2002; Gardner 1974). Modified by the limestone, shale, and evaporite layers through which it flows, groundwater is deposited through sinkholes onto the lake floor (Biddanda et al. 2006, 2009; Ruberg et al. 2005, 2008). Since the surrounding Lake Huron water has significantly different physicochemical properties, steep environmental gradients are formed, creating hotspots of microbial diversity and biogeochemical activity (Nold et al. 2010a).

Middle Island sinkhole is a geologic feature where cold, dense groundwater pours through vents in a rocky bowl-shaped basin (the “alcove”, Fig. 2) and flows over a ledge into a flat expanse of sediment (the “arena”, Fig. 2) that extends ~0.5 km northward into Lake Huron (Ruberg et al. 2008). The groundwater is chemically distinct from the overlying lake water, exhibiting a relatively low pH (7.3 vs. 8.4), high conductivity due to high levels of dissolved sulfate and chloride, and low oxygen (3–4 mg l<sup>-1</sup>) and nitrate (<0.01 mg l<sup>-1</sup>) concentrations (Ruberg et al. 2008). Light penetration to the lake bottom (4–10 % of surface irradiance) supports growth of purple-pigmented, filamentous cyanobacterial mats (*Phormidium* spp.) that cover the lake floor (Nold et al. 2010a, b; Voorhies et al. 2012). These mats represent modern analogs of Precambrian communities that played a critical role in atmospheric evolution on early earth (Bekker et al. 2004; Johnston et al. 2009). Underlying the mats lay thick organic-rich sediments that represent an enormous accumulation of nutrients whose origin is unknown.

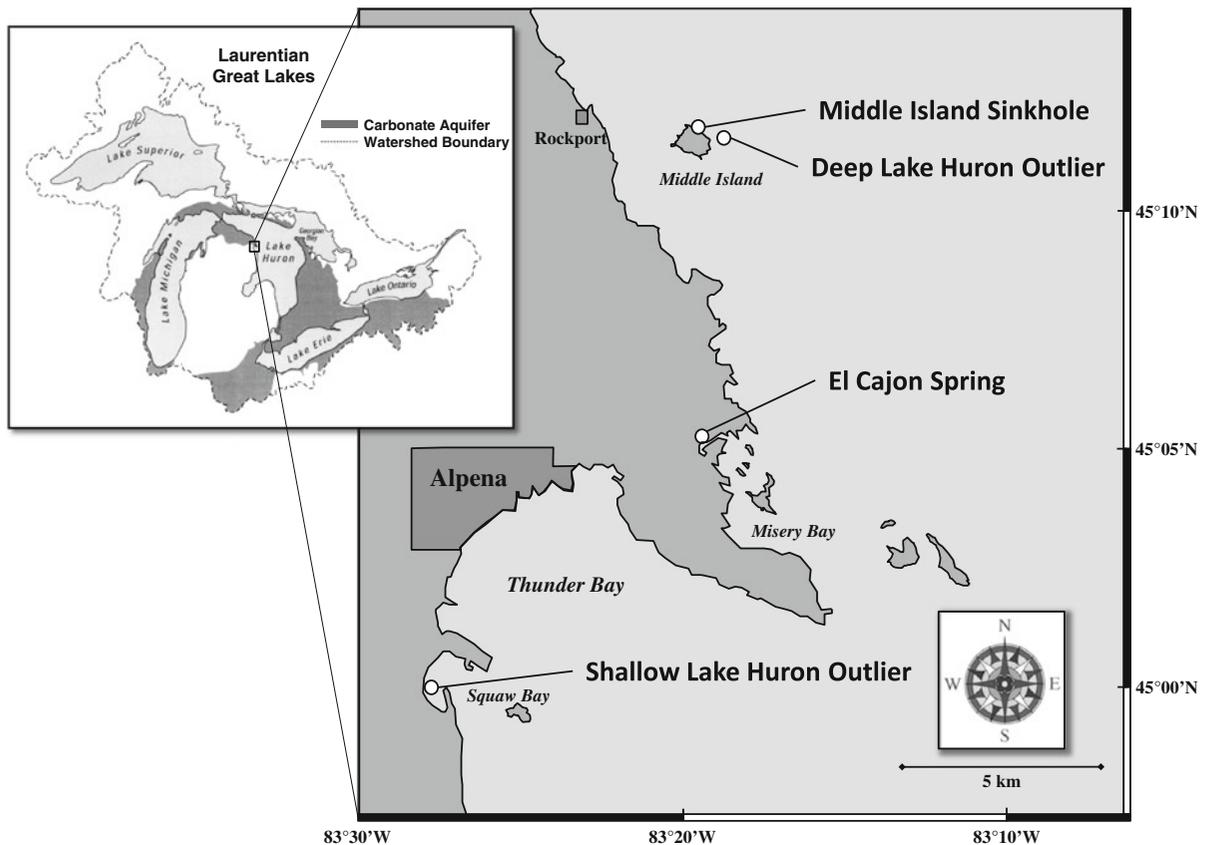
Two competing hypotheses can explain carbon deposition in these sediments. Cyanobacterial mats may dominate primary production, fixing the organic carbon found in sinkhole sediments. Cyanobacterial primary production has resulted in substantial nutrient accumulation in hypersaline lagoons (DesMarais et al. 1992; Wieland et al. 2008) and may be responsible for sinkhole sediment accretion. Alternatively, suspended phytoplankton may dominate primary production, fixing the carbon found in sinkhole sediments. Phytoplankton production varies by location in Lake Huron (Fahnenstiel and Carrick 1992, 1988; Karim et al. 2008), but deposition over time could result in significant sediment accumulation.

We used stable isotopes to test our hypotheses. Stable isotopes have been used to track nutrient cycling and energy flow in aquatic ecosystems (Fry and Sherr 1984, 1987) and were recently employed to investigate food web interactions in Lake Huron sinkholes (Sanders et al. 2011). Nitrogen isotopic ratios ( $\delta^{15}\text{N}$ ) can be used to dissect trophic relationships in food webs (Hebert et al. 2006; Keough et al. 1996), while carbon isotopic ratios ( $\delta^{13}\text{C}$ ) can be used to determine the source of carbon input into benthic communities (Asche et al. 2003; Peterson 1999; Raymond and Bauer 2001). We also used radioisotope dating and sub-bottom imaging to investigate the nutrient fluxes, sediment age, and depth of accumulated sediments in Middle Island sinkhole. Our data suggest that cyanobacterial carbon minimally contributes to nutrient deposition. Instead, sinkholes act to focus and capture phytoplankton-derived carbon into poorly degraded sediments.

## Materials and methods

### Sample sites

Samples were collected (summer 2007) from sinkhole sites near Alpena, MI (Fig. 1), including the deep (23 m) Middle Island sinkhole (N 45.19838°, W 83.32756°) and the shallower (<1 m) El Cajon spring (N 45.08540°, W 83.32437°), a shoreline groundwater seep exhibiting similar groundwater chemistry. Middle Island sinkhole has 22 m of overlying lake water to sustain phytoplankton growth while El Cajon spring is exclusively supplied by groundwater with limited phytoplankton growth. Like Middle Island sinkhole, El Cajon spring contains carbon-rich sediments, but is



**Fig. 1** Map depicting sampling locations within Lake Huron

dominated by extensive growth of eukaryotic algae (primarily *Oedogonium* spp.) with limited growth of benthic cyanobacteria. Control sites included locations with no evidence of high-conductivity groundwater including the shallow (<1 m) Lake Huron outlier (Squaw Bay, N 45.00508°, W 83.45822°) characterized by sandy sediments and a deep (20 m) Lake Huron outlier (N 45.19739°, W 83.31625°) characterized by sand and cobble sediments. Depth profiles of water quality parameters were collected using a YSI 6600 sonde (YSI Incorporated, Yellow Springs, OH) equipped with sensors for depth, conductivity, temperature, pH, oxidation–reduction potential, turbidity, and chlorophyll. Limestone reference samples were gathered from an abandoned quarry near Rockport, MI (N 45.20218°, W 083.38180°).

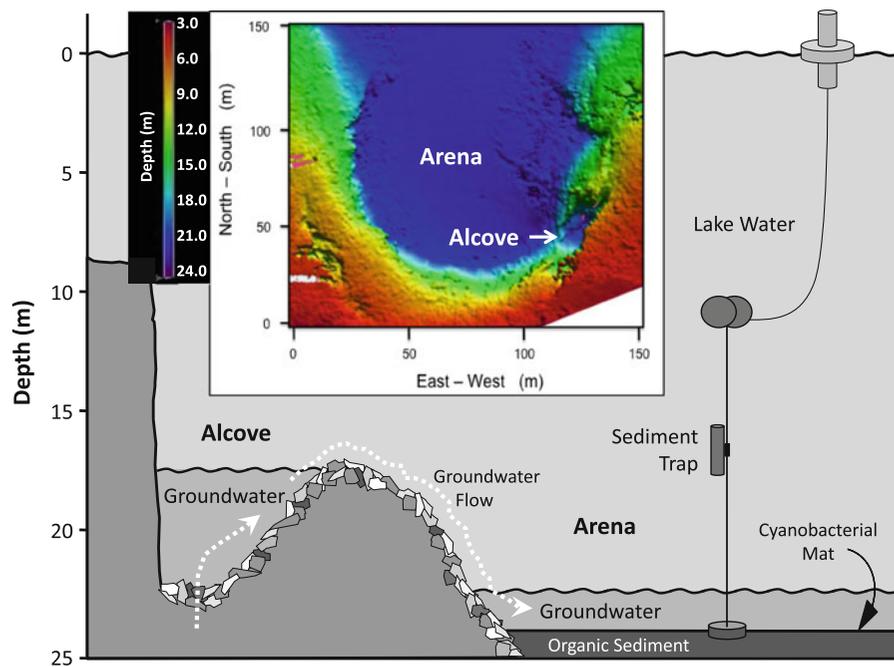
#### Water samples

Replicate water samples ( $n \geq 4$ ) were collected from shallow and deep sites using Niskin bottles. Surface

water was hand-collected directly overboard, while bottom and groundwater samples were collected by divers. Water samples (250 ml) were transferred to screw-capped plastic bottles containing no headspace for DIC analysis. A subset of DIC samples was later collected in glass BOD bottles with ground-glass stoppers for comparison of  $\delta^{13}\text{C}$  values. DIC samples were stored at 4 °C until analysis within 4 days of collection.

#### Phytoplankton POC/SPOC samples

Water column phytoplankton POC and sedimenting particulate organic carbon (SPOC) samples were collected from shallow and deep sampling sites using a Nutex<sup>®</sup> plankton tow net (20  $\mu\text{m}$  screen size, Wildco, Buffalo, NY). At deep sites, the net was retrieved from 2 m above the lake floor to minimize contamination by sediment carbon or particles in the groundwater. At shallow sites, surface water was



**Fig. 2** Profile view of Middle Island sinkhole along a NW transect from Middle Island; *inset* bathymetric contour map of alcove and arena, *pink areas* indicate uncertainty of measurements at the described location; the alcove appears in the lower right

poured through the phytoplankton screen. Samples were later filtered through a 112- $\mu\text{m}$  Nytex<sup>®</sup> screen. The 20–112  $\mu\text{m}$  size fraction includes phytoplankton POC and SPOC, but excludes larger zooplankton and smaller bacteria (APHA 1992). Phytoplankton POC/SPOC samples were stored at  $-20\text{ }^{\circ}\text{C}$  until analysis.

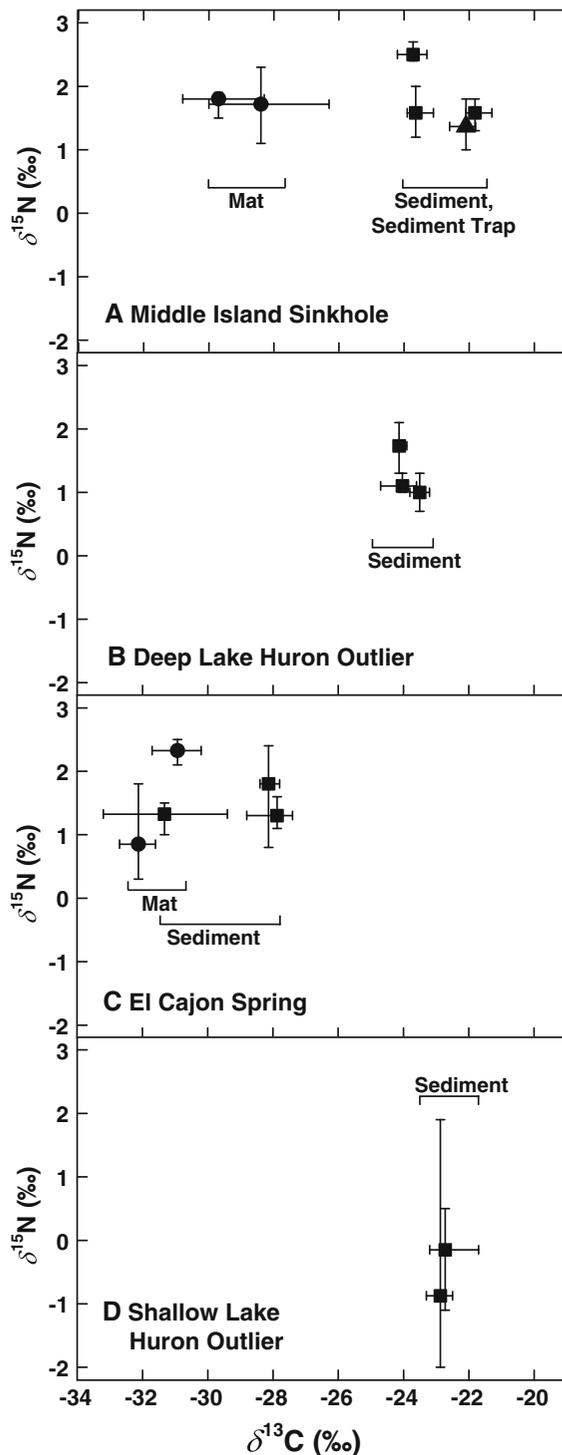
#### SPOC samples

SPOC was collected using three integrating sediment traps deployed in Middle Island sinkhole from May through September 2007 as previously described (Eadie et al. 1984; Eadie 1997). Sediment traps consisted of a 10  $\times$  50 cm Plexiglas<sup>®</sup> cylinder above a funnel opening into a 500-ml polyethylene bottle containing chloroform (20 ml) as a preservative. Each trap was deployed on separate moorings distributed  $\sim$ 30 m apart and 7 m above the sediment surface (16 m water depth) (Fig. 2). Traps collected SPOC throughout the summer phytoplankton bloom. To determine any residual carbon signal from the chloroform or collection bottles, glass beads (0.1-mm diameter) were placed in a muffle furnace (400  $^{\circ}\text{C}$  for 4 h) to remove organic carbon, then placed in an

identical bottle containing chloroform and distilled water and incubated for the same time period. Water and chloroform were evaporated to deposit residual carbon onto the glass beads, and beads were later analyzed. Residual carbon signal was below detection limits.

#### Sediment sampling

Sediment cores were collected using 30 cm  $\times$  7.5 cm Plexiglas<sup>®</sup> tubes either by hand from shallow sites or by divers from deep-water sites. Middle Island sinkhole sediment cores ( $n \geq 4$ ) were extruded and sectioned as previously described (Brandes and Devol 1995) according to visual cues in the sediment profile; surface cyanobacterial mat (0–0.25 cm) and three samples from the thick, organic-rich sediment were collected from depths that appear in Table 2. One core was sectioned every 1 cm to a depth of 7.5 cm with larger intervals thereafter. El Cajon spring sediment cores were also organic-rich but lacked a surface cyanobacterial mat. Cores from the shallow and deep Lake Huron outliers were sandy. At the shallow outlier site, the sediment surface displayed minimal detritus



**Fig. 3** Stable isotope characterization of Lake Huron sinkhole and open lake ecosystems; error bars represent minimum and maximum of collected data and the symbol is placed at the mean ( $n = 4$ ); (square), sediment samples; (circle), cyanobacterial mat samples; (triangle), SPOC collected from sediment traps ( $n = 3$ )

but at the deep outlier site, a thin layer of loose detritus was collected for analysis and included in the uppermost sample. Outlier sediments were sectioned from depths appearing in Table 2.

#### Photosynthetic mat samples

Mat samples were gathered into polypropylene tubes and frozen at  $-20\text{ }^{\circ}\text{C}$  until analysis. El Cajon spring contained two distinct photosynthetic communities. A small ( $100\text{ cm}^2$ ) cyanobacterial mat (*Phormidium* spp.) was attached to rocks at the spring's source. The eukaryotic green algae (*Oedogonium* sp.) was also sampled. In Middle Island sinkhole, curious cyanobacterial “fingers” (protrusions 5–15 cm long, 1–3 cm in diameter) were buoyed by an enclosed gas bubble. Bathed in groundwater, these structures were primarily filamentous cyanobacteria with minimal additional organic matter (Biddanda et al. 2006).

#### Sample preparation

Since drying methods can affect  $\delta$ -value determinations, all organic samples were dehydrated by freeze-drying to minimize analytic error (Carabel et al. 2006). All organic material was further acidified to remove inorganic carbonates. Sediment, mat, and phytoplankton samples were lyophilized, acidified by adding 1 N HCl drop wise until cessation of gaseous evolution, and incubated at room temperature for 2 h. Acidified samples were again dehydrated and homogenized using a mortar and pestle. Samples were transferred into tin cups after which their masses were determined using an analytic balance and stored at  $-80\text{ }^{\circ}\text{C}$  until analysis. These standard procedures were followed to minimize dilution of the  $\delta^{13}\text{C}$  signal in the organic carbon fraction due to contamination by inorganic carbon (Carabel et al. 2006; Ogrinc et al. 2005).

#### DIC analysis

Stable isotope analysis of DIC and limestone samples was performed by the Stable Isotope Facility for Environmental Research at the University of Utah (SIRFER, Salt Lake City, UT) according to methods described by Fessenden et al. (2002). Briefly, water samples were transferred to a vial with a sealed headspace and acidified. The evolved gas was separated in an elemental analyzer and  $\delta^{13}\text{C}$  values were

obtained by IRMS using appropriate standards. A difference of  $-2.6\text{‰}$  ( $n = 8$ ) was observed between identical water samples collected in plastic versus glass biological oxygen demand bottles, so the reported DIC values for samples collected in plastic bottles was corrected by adding  $2.6\text{‰}$ . The limestone samples were treated with  $100\%$  phosphoric acid at  $90\text{ °C}$  and the evolved  $\text{CO}_2$  was cryogenically separated through a CarboFlo device and run through the dual inlet of the IRMS.

### $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis of organic samples

Stable carbon and nitrogen isotopes from organic samples were analyzed on a ThermoFinnigan Delta-PlusXP IRMS coupled to a Costech ECS 4010 elemental analyzer at the Large Lakes Observatory of the University of Minnesota-Duluth. Phytoplankton POC fractions were analyzed on a GV Instruments Isoprime Mass Spectrometer interfaced with a Euro-Vector 3000 Series Elemental Analyzer at the Biogeochemistry and Paleoproteomics Laboratory at Michigan State University. For all data, stable isotope results were corrected after comparison to appropriate standards, weight % (wt%) of C and N were calculated, and isotope signatures were reported using delta notation, a parts per thousand deviation from standard materials according to the following equation, (Fry and Sherr 1984):

$$\delta X = [R_{(\text{SAMPLE})}/R_{(\text{STANDARD})} - 1] \times 1000 \quad (1)$$

where  $X = \text{C}$  (carbon),  $\text{N}$  (nitrogen), or  $\text{O}$  (oxygen) and  $R$  is the ratio of the heavy isotope over the light isotope ( $^{13}\text{C}/^{12}\text{C}$  for carbon,  $^{15}\text{N}/^{14}\text{N}$  for nitrogen, and  $^{18}\text{O}/^{16}\text{O}$  for oxygen). Data was reported relative to the standards VPDB (Pee Dee Belemnite) for carbon and oxygen and atmospheric nitrogen levels for nitrogen. C/N ratios were calculated by dividing the wt% C by the wt% N values and converting to molar ratios by multiplying by 1.166.

### Data analysis

A  $Q$  test was performed at  $90\%$  confidence to exclude any data points exhibiting high variation (none were removed). Carbon and nitrogen signals from all four sample sites were compared to the Middle Island sinkhole cyanobacterial mat and all samples from the

El Cajon spring using a two-tailed Student's  $t$  test. Confidence intervals ( $95\%$ ) were calculated using a 2-tailed T-distribution. Sediment  $\delta^{13}\text{C}$  values were subjected to mixing model calculations according to the following model, (Fry 2006):

$$f_1 = (\delta_{\text{sample}} - \delta_{\text{source2}})/(\delta_{\text{source1}} - \delta_{\text{source2}}) \quad (2)$$

where  $f_1$  is equal to the fraction of carbon originating from the Middle Island sediment trap (source 1), carbon from the cyanobacterial mat was assigned source 2, and sediment carbon was used as the sample. By pooling organic carbon into bulk categories, this model ignores processes that could result in depletion of  $^{13}\text{C}$  within sedimentary organic carbon pools.

### Radiodating

Lead and cesium radioisotopes released during above-ground nuclear testing are deposited in lake sediments via atmospheric deposition. Deposition rates have declined since the mid-1960s but these isotopes can still be used to effectively source deposited materials.  $^{210}\text{Pb}$  and  $^{137}\text{Cs}$  geochronologies were obtained from a 70 cm Middle Island sediment core collected by divers (September 2008). Sediment sections (1–2 cm) were harvested by extrusion and samples were dried to a constant weight at  $60\text{ °C}$ . Porosities were determined from water content assuming a density of  $2.4\text{ g cm}^{-3}$  dry sediment.  $^{210}\text{Pb}$  activities were determined via a modified procedure of Robbins and Edgington (1975). Briefly, an internal  $^{209}\text{Po}$  standard was added to determine recovery efficiency and sediments were acid-digested and oxidized. Resulting solutions were plated onto a polished copper disk and counted via alpha spectrometry.  $^{210}\text{Po}$  was assumed to be in secular equilibrium with its  $^{210}\text{Pb}$  parent. Supported  $^{210}\text{Pb}$  was estimated from  $^{214}\text{Bi}$  activities.  $^{214}\text{Bi}$  (609.3 keV) and  $^{137}\text{Cs}$  (661.6 keV) were determined by direct gamma counting using high resolution low background Ge detectors. The observed excess  $^{210}\text{Pb}$  activities were compared to a rate of supply model that assumes constant fallout of atmospheric  $^{210}\text{Pb}$  to the lake water (Robbins and Edgington 1975). The mass sedimentation rate ( $\omega$ ) was calculated by fitting a regression line through the excess  $^{210}\text{Pb}$  data. Sediment age estimates were compared to reference points in the cesium fallout record (Robbins and Edgington 1975) to confirm dates that correspond to sediment depths.

**Table 1** Water characteristics and stable isotopic characterization of dissolved organic carbon in Lake Huron sinkhole and open lake ecosystems

Sample type Sampling site	Water source	Sampling depth (m)	Depth above sediment surface (m)	[O <sub>2</sub> ] (mg ml <sup>-1</sup> )	Specific conductance (μS cm <sup>-1</sup> )	pH	Temperature (°C)	SO <sub>4</sub> <sup>2-</sup> (mg l <sup>-1</sup> )	Cl <sup>-</sup> (mg l <sup>-1</sup> )	[DIC] (mg ml <sup>-1</sup> ) <sup>a</sup>	DIC δ <sup>13</sup> C (‰) <sup>a</sup>
<b>Groundwater</b>											
El Cajon spring	Vent	<1	0.1	0	2,400	7.3	10	1,304	46	0.15 ± 0.01	-4.7 ± 0.3
Middle Island sinkhole	Alcove vent	23	0.1	0	2,300	7.2	9	1,678	40	0.14 ± 0.01	-7.1 ± 0.3
Middle Island sinkhole	Lake floor	23	0.2	3.5	1,600	7.4	9 <sup>b</sup>	907	19	0.13 ± 0.02	-6.0 ± 0.2
<b>Lake water</b>											
Middle Island	Surface	<1	23	11	200	8.3	13 <sup>c</sup>	19	7	0.06 ± 0.00	+0.6 ± 0.7
Deep Lake Huron outlier	Surface	<1	20	11	214	8.3	13 <sup>c</sup>	ND	ND	0.06 ± 0.01	+0.5 ± 0.7
Deep Lake Huron outlier	Bottom	20	1	12	217	8.4	9	ND	ND	0.06 ± 0.00	+0.6 ± 0.2
Shallow Lake Huron outlier	Surface	<1	0.1	11	250	8.4	23	13	8	0.09 ± 0.02	-2.1 ± 0.5

ND not determined

<sup>a</sup> Error reflects a 95 % confidence interval:  $s(t^*/\sqrt{n}, n = 4)$

<sup>b</sup> Reported groundwater temperatures are from June 2007; time series data demonstrated that lake floor temperatures fluctuated from 8 to 16 °C from May to September 2007 (Ruberg et al. 2008)

<sup>c</sup> Reported surface temperatures are from June 2007; time series data demonstrated that surface temperatures fluctuated from 4 to 21 °C from May to September 2007 (Ruberg et al. 2008)

## Sinkhole and sub-bottom profiling

A high-resolution depth profile of Middle Island Sinkhole was obtained using a Kongsberg Simrad EM3002 multibeam sonar operating at an acoustic frequency of 300 kHz collecting data from 254 separate channels. Measurements of Middle Island sinkhole sub-bottom topography were made using a Ross Labs 4900 acoustic sub-bottom profiler operating at a frequency of 7 kHz. Acoustic energy at this frequency passes through the soft sediments and echoes off the rocky bottom, resulting in a profile that displays both the soft sediment surface and the rocky substratum.

## Results

### Sample characteristics

Groundwater from Middle Island sinkhole and El Cajon spring displayed lower pH values and O<sub>2</sub> concentrations, and higher specific conductance, sulfate, and chloride concentrations than lake water (Table 1). Groundwater temperatures remained stable throughout the summer (~9–10 °C) while lake water temperatures were generally higher (Table 1) [For time-series hydrological data see Ruberg et al. (2008)]. Sediment cores were covered with purple-pigmented filamentous cyanobacterial mats. Beneath the mat lay thick, black, organic-rich sediment containing methane bubbles (Nold et al. 2010a). Methane ebullition is common at Middle Island sinkhole but was absent at the control sites.

### DIC and limestone characterization

Inorganic carbon pools differed between groundwater and lake water (Table 1). Lake water DIC concentrations (mean = 0.07 mg ml<sup>-1</sup>, *n* = 16) were significantly lower (*p* < 0.001) than groundwater (mean = 0.14 mg ml<sup>-1</sup>, *n* = 12). In addition, the venting groundwater displayed δ<sup>13</sup>C values significantly more depleted than lake water (*p* < 0.001). The mean DIC δ<sup>13</sup>C value for all groundwater samples was -6.0 ‰ (*n* = 12), while the mean lake water value was -0.1 ‰ (*n* = 16), nearly 6 ‰ more enriched in <sup>13</sup>C than the groundwater. The lowest δ<sup>13</sup>C value was detected in samples collected from a vent at the

alcove's bottom (-7.1 ‰). Collected by divers, this sample represents pure, undiluted groundwater. The δ<sup>13</sup>C value of limestone collected from a quarry near Rockport, MI (Fig. 1) was similar to the PeeDee Belemnite standard and lake water DIC (+0.9 ± 0.3 ‰, *n* = 4) while the δ<sup>18</sup>O of the limestone was -6.7 ± 1.1 ‰, (*n* = 4).

### C and N content

Sediment nitrogen and organic carbon were generally higher in Middle Island sinkhole and El Cajon spring than the Lake Huron outliers (Table 2). Algal and cyanobacterial mats displayed high C and N contents compared to the underlying sediments while the cyanobacterial fingers displayed the highest wt% C and wt% N values (34.8 and 7.8 %, respectively). Middle Island sediment C/N molar ratios (Table 2) were generally lower in the mat (6.9) and cyanobacterial fingers (5.3) compared to the sediments (8.6–10.0). C/N ratios of the sediment trap (11.1), deep Lake Huron outlier (9.1–11.1), and phytoplankton POC and SPOC samples collected from the water column (9.2–9.5) were similar to Middle Island sinkhole sediments. El Cajon spring generally displayed higher C/N ratios than Middle Island sinkhole (Table 2).

### Stable isotope variation

Data appearing in Tables 1 and 2 are reported as means ±95 % confidence intervals (*n* ≥ 4). Stable isotope analysis segregated samples from Middle Island sinkhole into two distinct clusters (Fig. 3a; Table 2). Cyanobacterial mat samples were significantly more depleted in <sup>13</sup>C than sediments and SPOC from the sediment trap (*p* < 0.001, *n* = 30). Sinkhole sediments exhibited δ<sup>13</sup>C values similar to the deep (Fig. 3b) and shallow (Fig. 3d) Lake Huron outliers. In El Cajon spring, C and N stable isotopes were similar in sediments, algal mats, and cyanobacterial mats (Fig. 3c). The cyanobacterial mat in El Cajon spring and the fingers in Middle Island sinkhole displayed the most depleted δ<sup>13</sup>C values (Table 2). Differences in δ<sup>15</sup>N between any of the samples we measured were minimal (Fig. 3; Table 2).

Phytoplankton POC/SPOC collected via phytoplankton tow net displayed consistent δ<sup>13</sup>C values (-22.2 to -23.2 ‰, *n* = 3) regardless of sampling

**Table 2** Stable isotope characterization of Lake Huron sinkhole and open lake ecosystems

Sampling site type	Sample	Collection depth (m)	Core depth (cm)	wt% C <sup>a</sup>	$\delta^{13}\text{C}$ (‰) <sup>a</sup>	wt% N <sup>a</sup>	$\delta^{15}\text{N}$ (‰) <sup>a</sup>	C/N molar ratio
Middle Island sinkhole								
Cyanobacterial mat	23		0–0.25	17.9 ± 4.2	−28.1 ± 1.1	3.1 ± 0.9	1.6 ± 0.3	6.8 ± 0.4
Cyanobacterial “fingers”	23		n/a	34.8 ± 8.0	−29.7 ± 1.6	7.8 ± 2.1	1.8 ± 0.3	5.2 ± 0.3
Sediment core	23		0.25–2.0	6.2 ± 0.3	−23.7 ± 0.4	0.8 ± 0.1	1.6 ± 0.4	8.6 ± 0.3
Sediment core	23		7.5–9.5	6.1 ± 1.2	−21.8 ± 0.4	0.8 ± 0.2	1.5 ± 0.2	9.4 ± 0.3
Sediment trap	16		n/a	12.9 ± 1.1	−22.8 ± 0.5	1.3 ± 0.2	1.6 ± 0.2	11.1 ± 1.1
Deep Lake Huron outlier								
Sediment core	23		0–0.5	2.4 ± 4.7	−23.5 ± 3.5	0.3 ± 0.7	1.0 ± 3.5	9.1 ± 3.5
Sediment core	23		0.5–1.0	2.6 ± 2.4	−24.1 ± 0.5	0.3 ± 0.4	1.7 ± 1.0	10.1 ± 3.2
Sediment core	23		2.0–4.0	1.6 ± 1.9	−24.0 ± 1.4	0.2 ± 0.2	1.1 ± 0.4	10.6 ± 1.7
El Cajon spring								
Green algae	0		Water surface	27.3 ± 5.6	−30.9 ± 1.2	2.4 ± 0.4	2.3 ± 0.3	13.4 ± 2.9
Cyanobacterial mat	<1		Rock surface	10.5 ± 15.3	−32.1 ± 0.8	1.6 ± 2.2	0.9 ± 1.3	7.2 ± 4.0
Sediment core	<1		0–1.0	15.9 ± 7.2	−31.3 ± 2.7	1.1 ± 1.2	1.3 ± 0.4	13.8 ± 1.7
Sediment core	<1		7.0–11.0	6.8 ± 4.7	−27.9 ± 1.0	0.5 ± 0.3	1.3 ± 0.4	17.5 ± 3.9
Sediment core	<1		14.5–20.5	8.3 ± 2.0	−28.1 ± 0.8	0.7 ± 0.6	1.8 ± 2.2	14.0 ± 8.1
Shallow Lake Huron outlier								
Sediment core	<1		0.0–0.5	1.3 ± 0.8	−22.7 ± 1.4	0.2 ± 0.1	−0.1 ± 1.4	8.9 ± 3.1
Sediment core	<1		2.5–3.5	1.9 ± 5.3	−22.9 ± 0.8	0.1 ± 0.0	−0.9 ± 3.7	6.9 ± 3.3
Sediment core	<1		6.0–8.0	0.2 ± 0.1	−23.0 ± 0.9	BDL	BDL	n/a
Sediment core	<1		16.0–18.0	0.2 ± 0.1	−23.6 ± 2.1	BDL	BDL	n/a

n/a not applicable, BDL below detection limit

<sup>a</sup> Error reflects a 95 % confidence interval:  $s \cdot (t^*/\sqrt{n})$ ,  $n \geq 4$

site. These values were similar to SPOC collected in sediment traps (Table 2). In contrast, POC/SPOC collected near El Cajon Spring displayed a more depleted  $\delta^{13}\text{C}$  value (−29.6 ‰) relative to the shallow control (−23.3 ‰), but was similar to green algal and cyanobacterial primary producers in this groundwater-dominated habitat (Table 2) (Sanders et al. 2011).

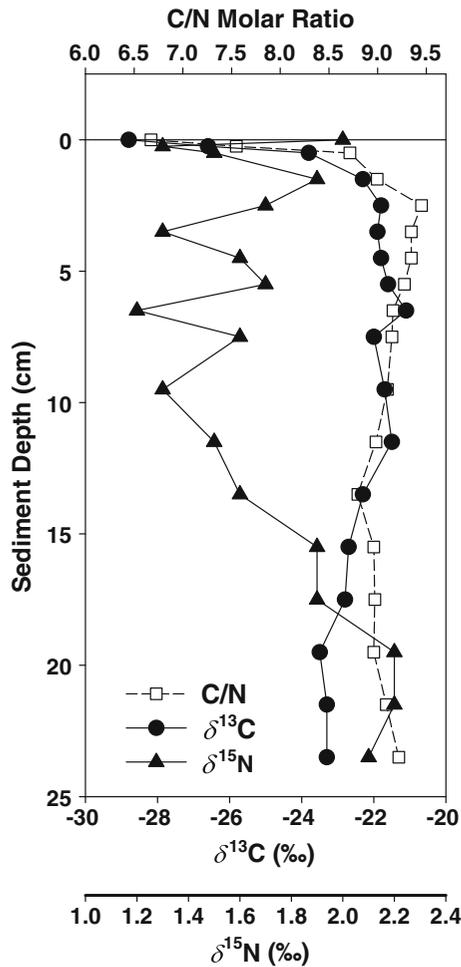
Carbon isotopes varied with depth in Middle Island sediments (Fig. 4). SOC was most depleted at the surface ( $\delta^{13}\text{C} = -28.8$  ‰,  $n = 5$ ) but became more enriched in  $^{13}\text{C}$  with depth, stabilizing at values between −21 and −24 ‰. The nitrogen stable isotope varied little with depth ( $\delta^{15}\text{N} = +1.2$  to +2.7). However, C/N ratios increased with depth, creating a profile very similar to differences in  $\delta^{13}\text{C}$  (Fig. 4).

Mixing model results (Eq. 2) suggested that the majority of carbon deposited in Middle Island sinkhole sediments originated from water column SPOC.

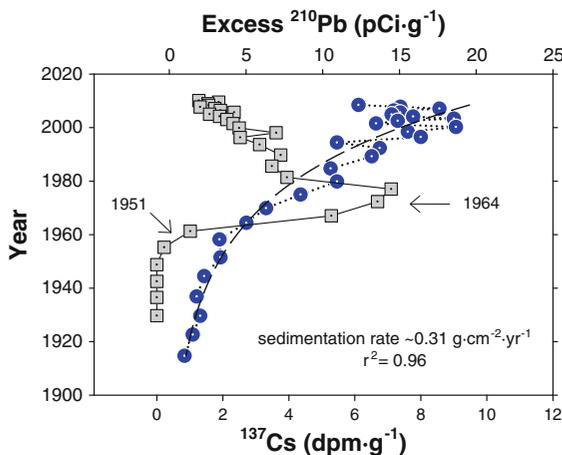
Depending on which values were used as the sample carbon, the model estimated that between 83.1 and 118.8 % of the sediment carbon originated from sedimenting phytoplankton. The lowest value (83.1 %) came from calculations based on the deepest sediment samples (−27.5 to −28.5 cm). Using average data from all observations, ca. 96 % of the sedimenting carbon likely comes from planktonic sources.

#### Sediment geochronology

Both  $^{210}\text{Pb}$  and  $^{137}\text{Cs}$  geochronologies yielded an estimated mass sediment accumulation rate of  $0.31 \text{ g cm}^{-2} \text{ year}^{-1}$  (Fig. 5).  $^{210}\text{Pb}$  and  $^{137}\text{Cs}$  inventories both exceed atmospheric inputs, with  $^{210}\text{Pb}$  levels ca. 13 times more abundant than values expected from atmospheric deposition alone.  $^{137}\text{Cs}$



**Fig. 4** Differences in  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and C/N ratios with depth in Middle Island sinkhole sediment (data were not replicated)



**Fig. 5** Plot of  $^{210}\text{Pb}$  (circles) and  $^{137}\text{Cs}$  (squares) activities vs. cumulative mass depth for Middle Island sediment

levels were ca. 6 times atmospheric inputs (Robbins and Edgington 1975).

#### Organic carbon and total nitrogen sedimentary mass balance

Models of organic matter diagenesis assume that fractions are mineralized at different rates depending on the abilities of the resident microbial decomposers (Boudreau and Ruddick, 1991; Westrich and Berner 1984). In practice, we are interested in only two of these fractions: one that is remineralized and returned to the overlying water ( $G_M$ ), and one that is permanently buried ( $G_{BUR}$ ). The organic matter deposited onto the sediment surface represents the sum of these two fractions ( $G_O$ ). Based on this theory, we can calculate the depositional flux ( $J_{IN}$ ), the burial flux ( $J_{BURIED}$ ), and the recycled flux ( $J_{RECYCLED}$ ) for organic carbon and total nitrogen by multiplying the mass sediment accumulation rate ( $\omega$ ) with the difference in concentration between the two end members (the concentration at the surface and the concentration at depth in  $\text{mol g}^{-1}$  sediment) according to the following formulae (Klump et al. 2009):

$$\begin{aligned} J_{RECYCLE} &= J_{IN} - J_{BURIED} = \omega(G_M) \\ &= \omega(G_O - G_{BUR}) \end{aligned} \quad (3)$$

$$\begin{aligned} \text{Fraction regenerated} &= f_r = J_{RECYCLE}/J_{IN} \\ &= (G_O - G_{BUR})/G_O \end{aligned} \quad (4)$$

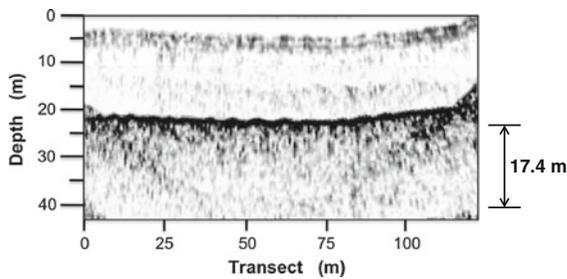
Depending on the source of sedimenting organic matter ( $G_O$ ) used in the formulations, between 23 and 73 % of the organic carbon and 25–81 % of the total nitrogen deposited in Middle Island sediments is remineralized and released back into the overlying water (Table 3). Overall, the highest carbon and nitrogen remineralization rates were found in the sandy sediments of the shallow Lake Huron outlier.

#### Sub-bottom profiling

Sub-bottom imaging revealed a large sinkhole filled with sediment (Fig. 6). The image displays the sediment surface at 22 m water depth overlying a crescent-shaped bowl ca. 17.4 m deep at its nadir 60 m from the transect start. By modeling the sinkhole shape as sphere, we can calculate sediment volume. Given the sediment depth of 17.4 m and sinkhole

**Table 3** Sediment organic carbon and total nitrogen fluxes

Sampling site source of incoming sediment ( $G_O$ )	Organic carbon				Total nitrogen			
	Sediment fluxes ( $\text{mol m}^{-2} \text{ year}^{-1}$ )				Sediment fluxes ( $\text{mol m}^{-2} \text{ yr}^{-1}$ )			
	$J_{IN}$	$J_{BURIED}$	$J_{RECYCLED}$	$f_r$ (%)	$J_{IN}$	$J_{BURIED}$	$J_{RECYCLED}$	$f_r$ (%)
Middle Island Sinkhole								
Cyanobacterial mat as $G_O$	46.2	12.4	33.8	73	6.9	1.3	5.5	81
Sediment trap as $G_O$	33.3	12.4	20.9	63	2.9	1.3	1.6	54
0.25–2.0 cm sediment as $G_O$	16.0	12.4	3.6	23	1.8	1.3	0.4	25
Deep Lake Huron outlier	6.2	4.1	2.1	33	0.7	0.4	0.2	33
El Cajon Spring	41.1	21.4	19.6	48	2.4	1.6	0.9	36
Shallow Lake Huron outlier	3.4	0.5	2.8	85	0.4	0	0.4	100

**Fig. 6** Acoustic sub-bottom profile of Middle Island sinkhole showing sediment surface (thick line at 22 m water depth), rocky substratum (fainter hemispherical line beneath sediment surface) and extent of sediment accumulation (ca. 17 m) within sinkhole

radius of  $\sim 60$  m, the volume of sediment contained within the sinkhole boundaries is ca.  $1 \times 10^5 \text{ m}^3$ . Using a sediment porosity of 0.5, a wt% carbon value of 5 %, and a density of  $2 \text{ g cm}^{-3}$ , we estimate that  $5 \times 10^6 \text{ kg}$  of carbon is sequestered within Middle Island sinkhole.

## Discussion

### DIC sources

Two distinct sources supply DIC to Middle Island sinkhole and their  $\delta^{13}\text{C}$  values vary by nearly 6 ‰. Our value for Lake Huron water ( $-0.1$  ‰) is similar to reports from other Laurentian Great Lakes (Hodell et al. 1998) and displays a fractionation associated with freshwater at equilibrium with atmospheric  $\text{CO}_2$

(Mook et al. 1974; Vogel et al. 1970). In contrast, groundwater displays a more negative value ( $-6.0$  ‰) than would be expected if carbonate-bearing limestone ( $+0.9$  ‰) was simply dissolved. Fractionation of DIC is common throughout Michigan basin aquifers (McIntosh and Walter 2006). Bacterial and Archaeal metabolic pathways leading to the accumulation of DIC may explain why groundwater is  $^{13}\text{C}$ -depleted. For example, denitrifying bacteria can significantly lower the  $^{13}\text{C}$  content in groundwater (Nascimento et al. 1997) and lake water DIC (Atekwana and Krishnamurthy 1998; Bade et al. 2004; Keough et al. 1998). In Lake Huron sinkholes, emerging groundwater is depleted in both oxygen and nitrate (Biddanda et al. 2012), suggesting prior reduction of these electron acceptors and active subsurface denitrification. Methanogenic *Archaea* can also deplete the  $^{13}\text{C}$  in subsurface methane (Barker and Fritz 1981). The bacterial oxidation of  $^{13}\text{C}$ -depleted methane to  $\text{CO}_2$  can thereby result in  $^{13}\text{C}$ -depleted groundwater DIC (Gu et al. 2004). Both processes likely occur in Lake Huron sinkholes.

### Reflection of DIC in primary producers

Differences in  $\delta^{13}\text{C}$ -DIC values were retained as carbon entered the primary producers. Phytoplankton captured in sediment traps and plankton nets displayed a  $\delta^{13}\text{C}$  of  $-22.5$  ‰, similar to prior reports from the Great Lakes and freshwater phytoplankton in general (Fry 2006; Keeley and Sandquist 1992; Keough et al. 1996). In contrast, cyanobacterial mats incorporating groundwater DIC displayed a  $\delta^{13}\text{C}$  of  $-28.1$  ‰, roughly 5 ‰ more depleted than the phytoplankton.

These data suggest two distinct pools of organic carbon in sinkhole environments: phytoplankton POC and cyanobacterial mat POC, each with its characteristic signature carbon isotope ratio.

#### Source of sedimenting carbon

Two lines of evidence suggest that carbon in sinkhole sediment originates from phytoplankton rather than cyanobacteria. First, the  $^{13}\text{C}$  content of organic carbon in Middle Island sediments was similar to the carbon in sediment traps, POC, and groundwater-free lake sediments (Fig. 3). The cyanobacterial  $\delta^{13}\text{C}$  signature does not appear in sediments deeper than 2 cm (Table 2; Fig. 4), suggesting that the majority of sediment carbon is derived from phytoplankton biomass. Isotope mixing models further support this claim, as our calculations suggest that  $\sim 96\%$  of sediment POC originated from phytoplankton (Eq. 2). This model does not account for metabolic processes that can deplete  $^{13}\text{C}$  in sediment such as denitrification or the oxidation of isotopically depleted methane (Gu et al. 1996), so this value may overestimate the amount of sediment POC originating from phytoplankton. Second, the C/N ratios of sediment organic carbon are more similar to phytoplankton than cyanobacteria. C/N ratios can vary as organic carbon is modified, so this ratio is not strictly conserved (Sterner and Elser 2008). However, C/N ratios can serve as secondary proxies for drawing source-sink linkages in ecosystems (Cotner and Biddanda 2002). In Middle Island sinkhole, the low C/N signature of the cyanobacterial mat is not retained in the sediments. Rather, the higher C/N ratio representative of the phytoplankton is found in deeper sediments (Table 2; Fig. 4). Combined, these data suggest that cyanobacterial primary production does not significantly contribute to sinkhole sediment accumulation. Overall, Middle Island sinkhole appears to be a sink for phytoplankton-fixed carbon.

#### Evidence for carbon sequestration

Several lines of evidence suggest that Middle Island sinkhole focuses and traps SPOC from outside the basin. First, the  $^{137}\text{Cs}$  and  $^{210}\text{Pb}$  inventories greatly exceed atmospheric inputs (by ca.  $6\times$  and ca.  $13\times$ , respectively), implying collection of sedimenting material from a wider area than the water immediately

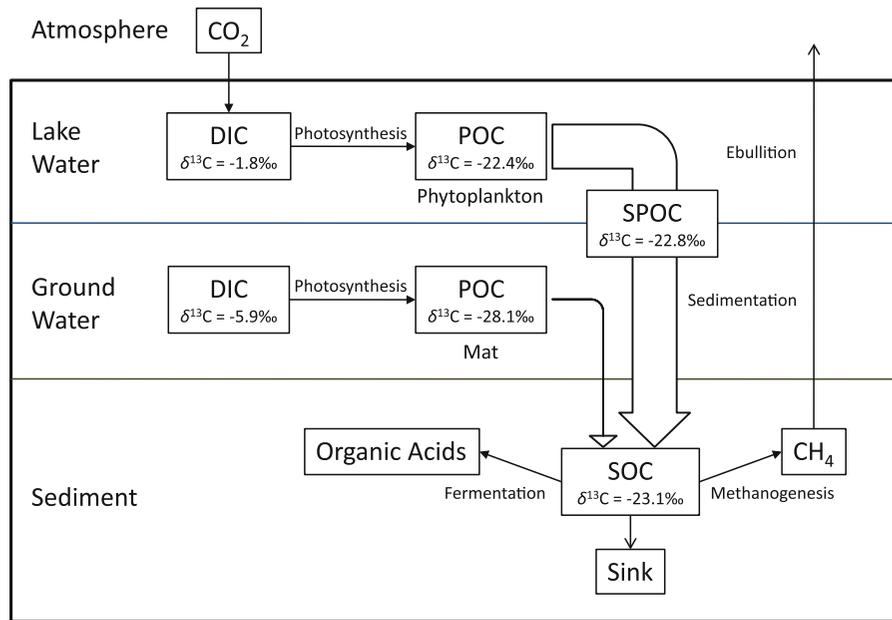
above the sinkhole. The difference between the  $^{137}\text{Cs}$  and  $^{210}\text{Pb}$  signals indicates a second, non-atmospheric source of  $^{210}\text{Pb}$  input, possibly from groundwater. Since Middle Island sinkhole groundwater is rich in  $^{226}\text{Ra}$  (pers. comm., Dr. Mark Baskaran, Wayne State University), radioactive decay of this element could result in additional inputs of  $^{210}\text{Pb}$ , a daughter isotope (Appleby 2001).

Second, the sedimentation rate we measured in Middle Island sinkhole is relatively high. Our reported sedimentation rate of  $0.31\text{ g cm}^{-2}\text{ year}^{-1}$  is higher than other oligotrophic Great Lakes habitats (Edgington et al. 1991) and roughly twice the highest value reported for the eutrophic Green Bay in Lake Michigan (Klump et al. 2009; Silliman et al. 2001). While we did not determine sedimentation rates for groundwater-free sampling sites, our high sedimentation rate is consistent with sinkhole sediment focusing.

Third, carbon and nitrogen input and burial fluxes are relatively high (Table 3), exceeding values reported for eutrophic Great Lakes habitats (Klump et al. 2009). Sedimentary carbon accumulation is accelerated when anaerobic decomposition such as fermentation and methanogenesis are dominant [e.g. (Fenchel et al. 1998; Hebbing et al. 2006)]. With an absence of favorable electron acceptors ( $\text{O}_2$ ,  $\text{NO}_3^-$ ), the unique sinkhole groundwater chemistry would favor slower anaerobic decomposition pathways.

Fourth, the fraction of carbon and nitrogen recycled back into the water column ( $f_r$ ) provide evidence for carbon sequestration. To accurately calculate ( $f_r$ ), the incoming sedimenting material ( $G_0$ ) must be appropriately chosen. The sediment just beneath the cyanobacterial mat (0.25–2.0 cm depth) likely best represents the SPOC contributing to organic carbon deposition since (1) the cyanobacterial mat offers little organic carbon to the sediment and (2) material in the sediment trap has not yet begun diagenesis. If we use the  $G_0$  from the 0.25–2.0 cm depth to calculate  $f_r$ , sinkhole habitats consistently display lower values of recycled carbon and nitrogen than the groundwater-free habitats we sampled (Table 3). Low levels of nutrient recycling would serve to sequester carbon and nitrogen in the sinkhole sediments.

Finally, in a study using stable isotopes to analyze food web interactions in Middle Island sinkhole (Sanders et al. 2011), benthic macroinvertebrates feeding on the mat and sediment were minimally linked to cyanobacterial primary production, and



**Fig. 7** Schematic of carbon isotopic signatures and potential carbon cycling pathways in Middle Island sinkhole: *DIC* dissolved inorganic carbon; *POC* particulate organic carbon; *SOC* sedimentary organic carbon; *SPOC* sedimenting particulate organic carbon

instead displayed isotopic signatures similar to phytoplankton. Higher trophic levels (piscivorous and planktivorous fish) utilizing macroinvertebrate carbon sources showed no signal from groundwater-derived nutrients, instead displaying isotopic signatures consistent with phytoplankton-derived nutrients. These data indicate that few of the sequestered sinkhole nutrients are used by higher trophic levels. Instead, nutrients appear to be captured and stored in the sinkhole sediments. Combined, these lines of evidence suggest that sinkhole sediments sequester and retain planktonic primary production.

#### Trophic considerations

Interestingly,  $\delta^{15}\text{N}$  did not vary significantly in the pools we sampled.  $\delta^{15}\text{N}$  values generally increase with level of trophic stature (Peterson and Fry 1987), and some have reported  $\delta^{15}\text{N}$  values as high as +12 ‰ for piscivorous fish in the Great Lakes (Keough et al. 1996; Sierszen et al. 2006). However,  $\delta^{15}\text{N}$  values reported for Great Lakes phytoplankton are generally near the atmospheric nitrogen standard, ranging from -0.6 to +2.1 ‰ (Keough et al. 1996). Since we find similar values throughout the Middle Island sediment core (+1.2 to +2.7 ‰), our data suggest that primary producers constitute the majority of deposited carbon

rather than higher trophic levels such as zooplankton or fish.

#### A model of sinkhole carbon flow and the role of cyanobacterial mats

Overall, these data allow us to construct a model of carbon flow through the Middle Island sinkhole ecosystem (Fig. 7). Anaerobic carbon remineralization rates are comparatively slow (Table 3) and significant amounts of carbon never leave the ecosystem resulting in deep accumulation of organic-rich sediment. However, cyanobacterial mats may play a large role in carbon sequestration. We have observed highly motile cyanobacterial behavior in laboratory mesocosms containing Middle Island sediments. Experimental tiles (2 × 2 cm) placed on the mat surface were completely covered by cyanobacterial filaments within two hours. This degree of motility leads us to speculate that cyanobacteria may facilitate burial of planktonic carbon by continually migrating toward light at the mat's surface during the day. At night when photosynthesis is no longer active, motile filamentous chemosynthetic and heterotrophic bacteria swarm over the cyanobacteria to optimally position themselves for feeding on oxygen diffusing from the overlying water and organic acids diffusing from the

**Table 4** Calculated age of Middle Island sinkhole sediments

Porosity	Linear sedimentation rate (cm year <sup>-1</sup> )	Sediment age (YBP)
0.7	0.41	4,300
0.6	0.31	5,700
0.5	0.24	7,150

Assuming mass sediment accumulation rate = 0.31 g cm<sup>-2</sup> year<sup>-1</sup>

sediment below (Biddanda et al. 2012). The following morning, cyanobacteria again strive toward the light, burying the white filamentous heterotrophs. Combined, these behaviors would quickly bury any SPOC deposited on the sediment surface, placing planktonic carbon in anoxic sediments where degradation proceeds more slowly.

#### Sinkhole sediment age

Since we have dated only the upper ~100 years of accumulated sediment, estimating the age of sediments that are 17.4 m deep (Fig. 7) and potentially thousands of years old requires significant extrapolation. Sediment age at the sinkhole bottom depends heavily on the porosity of the sediment and the sedimentation rate (Robbins and Edgington 1975). However, if we assume that there has been no change in the mass sedimentation rate (0.31 g cm<sup>-2</sup> year<sup>-1</sup>), we can calculate sediment age over a range of likely porosities (Table 4). Given these results, the age of sediment at the bottom of Middle Island sinkhole is likely between 5,000 and 6,000 YBP, reasonable given the history of glaciation in the region. Sediment accumulation over this period could result in the large amount of carbon (~5 × 10<sup>6</sup> kg C) sequestered within the sinkhole.

#### Cyanobacterial production

The fate of primary production by the cyanobacterial mat is unknown. Using metagenomic sequencing, Voorhies et al. (2012) demonstrated the potential of these communities to perform both oxygenic and anoxygenic photosynthesis. The resulting primary production is likely consumed within the mat community since production and respiration rates are tightly balanced in sinkhole sediments (Voorhies et al.

2012). Metabolic coupling between phototrophs and heterotrophs is common in cyanobacterial mat communities (Stal 1995; Ward et al. 1998). In some hot spring mats, nearly all of the carbon stored by phototrophs during the day is fermented by the cyanobacteria at night and released as small organic acids. This carbon and energy is transferred to heterotrophs and respired, leaving little for cell division and resulting in high rates of carbon remineralization (Nold and Ward 1996). Like hot spring phototrophs, sinkhole mat cyanobacteria likely transfer carbon and energy to their heterotrophic partners, fueling vertical motility that buries and preserves the sedimenting phytoplankton. Extensive transfer of cyanobacterial production to heterotrophs would explain how prior measurements of mat production and benthic respiration rates (Voorhies et al. 2012) are in tight metabolic balance—even as sedimentary carbon accumulates.

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