



Methane cycling in the carbonate critical zone

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ABSTRACT

The carbonate critical zone (CZ) is characterized by extensive groundwater-surface water exchange that leads to highly variable redox states of groundwater. Changes in redox condition may cause either production or consumption of methane (CH₄), thereby providing an atmospheric source or sink of this important greenhouse gas. To assess how groundwater-surface water exchange affects redox state and CH₄ cycling in the carbonate CZ, we measured CH₄ concentrations and ¹³C isotopes in water from streams, spring systems, and wells in north-central Florida. Sampled groundwater has subsurface residence times ranging from hours at a stream sink-rise system, to months following a flood recharge event into a spring vent, to decades at springs with limited point recharge. Concentrations of CH₄ ranged from 0.002 to 89 μM, with an inverse relationship in springs between subsurface residence time and CH₄ concentration. Where residence time is short, low CH₄ concentrations result from methanotrophy linked to elevated dissolved oxygen (DO) concentrations. Following flooding, methanotrophy occurs soon after recharge and is followed by methanogenesis as groundwater becomes increasingly reducing. Groundwater extracted from wells had CH₄ concentrations greater than spring water indicating CH₄ is lost during flow to spring vents. CH₄ concentrations covary with δ¹³C-CH₄ values, which supports both methanogenesis and methanotrophy with changing residence times. Mean fluxes of CH₄ ranged from −0.05 to 1.0 mg m^{−2} d^{−1} at spring vents, with negative values caused by CH₄ uptake in water undersaturated with respect to atmospheric concentration. Most springs are dominated by methanotrophy, limiting atmospheric evasion of CH₄ produced in the carbonate CZ. We estimate CH₄ emissions to be 12.6 × 10^{−6} Tg a^{−1} across all Florida springs or about two orders of magnitude less than emissions from Floridan aquifer groundwater abstraction (3041 × 10^{−6} Tg a^{−1}). Although CH₄ is produced in the carbonate CZ, natural attenuation limits its effects on the global carbon cycle.

1. Introduction

Earth's critical zone (CZ), which extends from the base of groundwater to the top of the canopy at Earth's surface (Brantley et al., 2007), is important to the global carbon cycle, including local methane (CH₄) dynamics. Because CH₄ is a powerful greenhouse gas, its processing, including production (methanogenesis) and consumption (methanotrophy) in the CZ may be a factor in global climate (Saunio et al., 2016; Myhre et al., 2013; Bastviken et al., 2011). Most CZ CH₄ is generated in wetlands, although CH₄ forms in other anoxic regions, including aquifers, lakes, reservoirs, and river sediments where reducing conditions favor methanogenesis (Saunio et al., 2016). The produced CH₄ can be oxidized as it migrates from reducing to oxidizing conditions in the subsurface because it is an important energy source for heterotrophic microbes (Brankovits et al., 2017; Shelley et al., 2014; Matthey et al., 2013; Opsahl and Chanton, 2006; Hutchens et al., 2004), thereby limiting its loss to the atmosphere. Despite oxidation and oxic

conditions, many streams and rivers have CH₄ concentrations in excess of saturation with atmospheric CH₄, and thus provide an atmospheric source. Some of the excess CH₄ in streams is thought to originate from groundwater inflow (Stanley et al., 2016).

Although CH₄ can form through thermal decomposition of organic matter, most CZ CH₄ is produced during low temperature anaerobic microbial processes (Conrad, 2007). This biogenic CH₄ forms by two major metabolic pathways (acetoclastic or hydrogenotrophic) of Archaea belonging to the phylum *Euryarchaeota* (methanogens) (Ferry, 2011; Conrad, 2007). The hydrogenotrophic pathway uses CO₂ and H₂ as substrates while the acetoclastic pathway uses acetate generated by microbial decomposition of larger organic molecules (Fenchel et al., 2012; Ferry, 2011; Conrad, 2007). In the acetoclastic pathway, methanogens dismutate acetate to CO₂ and CH₄ in a fermentative process. In the hydrogenotrophic pathway, which dominates in SO₄^{2−} rich (>0.2 mM) environments, methanogens reduce CO₂ with H₂ to form CH₄ and H₂O. These two pathways and CH₄ oxidation create distinct δ¹³C values

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of CH₄ because the magnitude of isotope fractionation between precursor substrate and produced CH₄ for the acetoclastic pathway (25–35 ‰ lighter) is smaller than for hydrogenotrophic pathway (>55 ‰ lighter) while oxidation enriches residual CH₄ in ¹³C (Whiticar, 1999).

Concern about fugitive CH₄ from unconventional shale gas development has produced much of the current knowledge of the origin, transport, and fate of CH₄ in groundwater, and delivery of CH₄ to the atmosphere from aquifers (Molofsky et al., 2021; Cahill et al., 2017). Groundwater CH₄ concentrations range from ~1 to 9000 μM. These values are in excess of concentrations expected in freshwater equilibrated with atmospheric CH₄ (~0.01 μM) (Kulongoski and McMahon, 2019; Darling and Goody, 2006; Goody and Darling, 2005) and thus can provide a source of atmospheric CH₄ (Zhang et al., 2022; Brankovits et al., 2017; Cahill et al., 2017; Stanley et al., 2016; Matthey et al., 2013). Groundwater CH₄ evades to the atmosphere through diffusion across static air-water interfaces of surface water bodies and the water table, by ebullition from springs, and during well pumping (Kulongoski and McMahon, 2019; Cahill et al., 2017). Evasion from pumped well water constitutes ~0.2 % (~0.53 Tg CH₄) of annual global emissions (Kulongoski and McMahon, 2019). Diffuse groundwater CH₄ emissions are difficult to measure but do not likely exceed estimated emissions from streams, which represent ~4.8 % (26.8 Tg CH₄) of annual global CH₄ emissions (Stanley et al., 2016; Crawford et al., 2013; Jones and Mulholland, 1998). Most diffuse groundwater CH₄ inputs originate from shallow sources in river-adjacent riparian soils or wetlands with smaller contributions from CH₄ in deep upwelling groundwater.

Most work on natural CH₄ emissions centers on groundwater in siliciclastic aquifers (Molofsky et al., 2021). For example, in the siliciclastic Po River Valley in northern Italy 14 springs have CH₄ concentrations ranging from 0.002 to 0.1 μM, with an estimated average diffusive atmospheric flux of $9 \pm 9 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ (Laini et al., 2011). This value is minor compared to the average diffusive flux from rivers of $130 \pm 410 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ (Stanley et al., 2016). In contrast, little work exists on CH₄ distribution in the carbonate CZ although it has geomorphic characteristics suggesting it could impact CH₄ dynamics, including congruent dissolution of carbonate minerals and subsurface drainage networks connected with air- and water-filled caves, sinkholes, springs, and sinking streams (Covington et al., 2023; Martin et al., 2021). These features permit rapid and extensive exchange of water, nutrients, and dissolved gases between the surface and subsurface that create heterogeneous subsurface redox conditions and may allow both methanogenesis and methanotrophy, depending on subsurface residence times of recharged water.

Infiltration of surface water with dissolved oxygen (DO) concentrations at equilibrium with atmospheric oxygen creates local oxic conditions in karst aquifers over short residence times (Flint et al., 2021; Brown et al., 2014; Moore et al., 2009). CH₄ oxidation is commonly mediated by microbes in both vadose and phreatic zones of karst systems. Consumption of atmospheric and vadose zone CH₄ has been observed in air-filled caves around the world (Ojeda et al., 2019; Webster et al., 2016; Brankovits et al., 2017; Nguyễn-Thụy et al., 2017; Matthey et al., 2013; Hutchens et al., 2004). Conversely, karst aquifer matrix porosity may store water for sufficiently long periods of time to favor methanogenesis, particularly if recharged surface water has elevated dissolved organic carbon concentrations. These conditions can cause CH₄ concentrations >1000 μM in well water from carbonate aquifers (Kulongoski and McMahon, 2019). Reducing conditions may also occur in shallow portions of the aquifer matrix porosity even if water in solution features remains oxic (Einsiedl et al., 2007). Redox related controls in the carbonate CZ are also linked to production of N₂O, another important greenhouse gas, to excess with respect to atmospheric equilibrium (Flint et al., 2021). All of these characteristics indicate complex CH₄ cycling in the carbonate CZ and a poor understanding of its role in global CH₄ cycling (e.g., Molofsky et al., 2021; Kulongoski and McMahon, 2019; Cahill et al., 2017; Harkness et al., 2017; McMahon et al., 2017; Laini et al., 2011; Goody and Darling,

2005).

In this work, we evaluate the role of the carbonate CZ in CH₄ cycling and whether it is a net source or sink of atmospheric CH₄. To address these questions, we assess the occurrence and fate of CH₄ in 14 springs, wells, and a sinking stream in the carbonate CZ of north-central Florida, USA. These systems have variable levels of interaction with surface water and a range of groundwater residence times, which make them ideal locations to evaluate possible biogeochemical and hydrologic processes that may lead to CH₄ consumption, production, and transport in the carbonate CZ. The Upper Floridan aquifer (UFA) is an eogenetic karst system and therefore does not represent all types of karst or carbonate CZs. Nonetheless, information about biogeochemical processes in this setting will provide useful insights for future assessments of the potential role of the carbonate CZ in global CH₄ budgets and OC cycling. Assessments of CH₄ cycling in phreatic karst features will also complement similar assessments made in human accessible caves that sample mostly the vadose zone and at the water table (e.g. Ojeda et al., 2019).

2. Methods

2.1. Study location

The field area for this study is the Suwannee River watershed, which is underlain by the karstic Floridan aquifer (Fig. 1A). This aquifer consists of pre-Miocene eogenetic carbonate rocks and is partially confined by Miocene Hawthorn Group siliciclastic rocks. The Floridan aquifer is divided by a middle semi-confining unit into the UFA, composed of the Oligocene Suwannee and Eocene Ocala limestones, and the Lower Floridan aquifer. Ocala Limestone has a porosity and average matrix permeability of ~30 % and 10^{-13} m^2 , respectively (Florea and Vacher, 2006; Budd and Vacher, 2004). Within the Suwannee River watershed, the Hawthorn Group reaches a maximum thickness of 95 m and has been completely removed by erosion in the southwestern region (Scott, 1988). The erosional edge of the Hawthorn Group, between thicknesses of 0 to 30 m, forms a geomorphic feature called the Cody Scarp and represents a region of semi-confinement of the UFA (Fig. 1B). Surface water is abundant northeast of the Cody Scarp where the Hawthorn Group is confining, but is limited southwest of the scarp where the UFA is unconfined and carbonate rocks of the Ocala Limestone crop out. Southwest of the Cody Scarp is a karst landscape characterized by numerous sinkholes and springs with surface water limited to the Suwannee River and its tributary, the Santa Fe River.

Water discharging from springs in the region may have recharged the aquifer from days to decades prior to its discharge (Martin et al., 2016; Gulley et al., 2011; Katz et al., 2001; Martin and Dean, 1999). The variation in the subsurface residence times affects biogeochemical reactions and chemical compositions of the springs (Gulley et al., 2011; Martin and Gordon, 2000) and thus both spatial and temporal variations in spring water compositions can be used to assess links between hydrologic processes, residence time, and biogeochemical reactions (Flint et al., 2021; Brown et al., 2019; Gulley et al., 2011).

2.2. Sampling locations

The sampling sites include Madison Blue Spring, Peacock Springs, Little River Spring, Otter Spring, eight vents in the Ichetucknee Springs group, and the Santa Fe River Sink-Rise system (Fig. 1B-D). These sites have a range of residence times, flow paths, and OC delivery within the UFA. Additional water and dissolved gas samples were collected from wells near some of the sampled springs. Selected sites were sampled twice along the ~110 km longitudinal transect of the Santa Fe River. Because of their geographic proximity, all sampled locations have similar aquifer characteristics and climatic conditions.

Madison Blue Spring, Otter Spring, Little River Spring, and Peacock Spring discharge water to short spring runs (less than a few hundred meters) that flow to the Withlacoochee or Suwannee rivers (Fig. 1B).

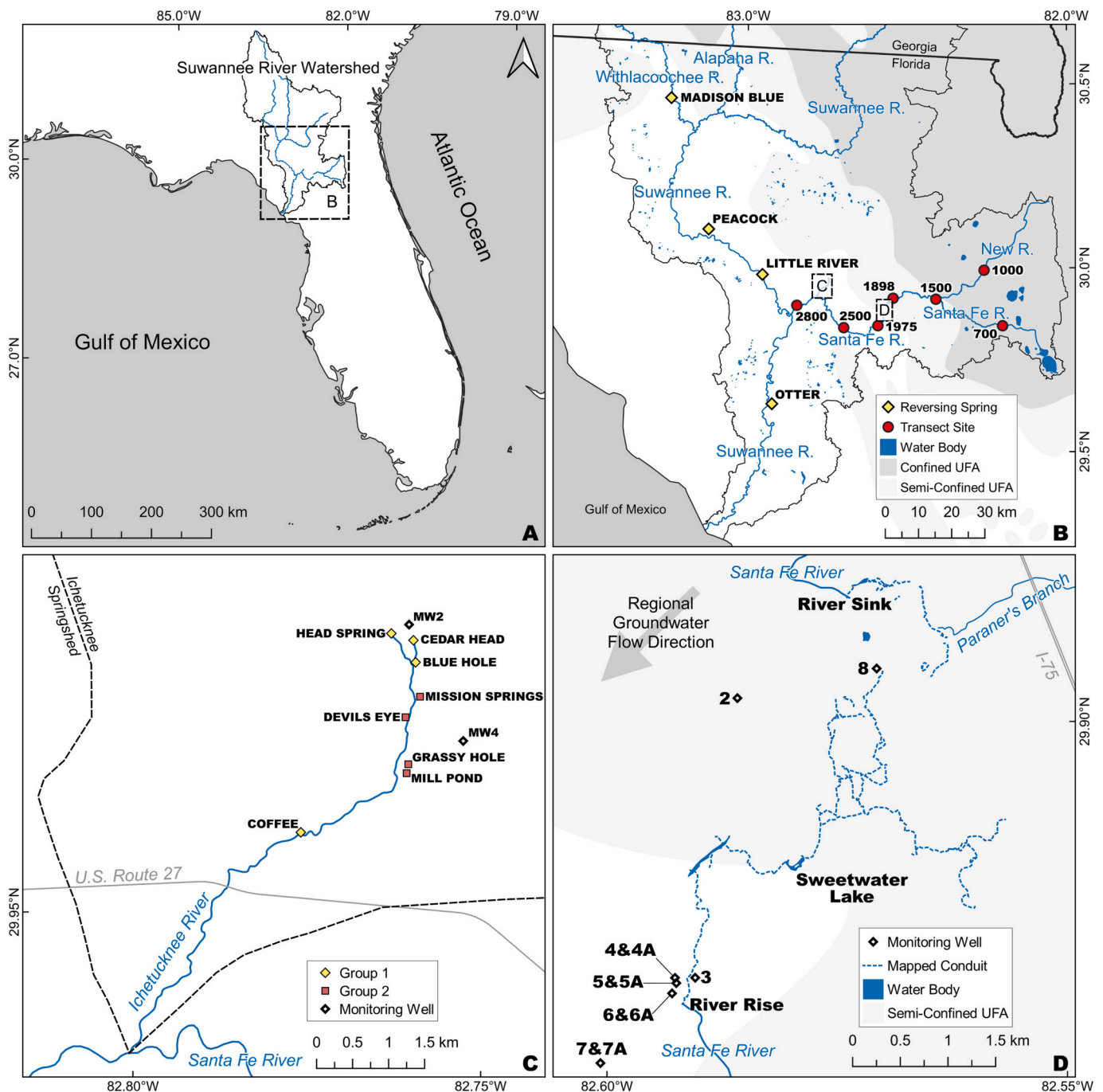


Fig. 1. Map of the study area and sample sites. (A) Suwannee River watershed with the main study area outline by the dashed box. (B) Map of the main study area in north-central Florida showing the major drainages, sampled reversing springs, Santa Fe River transect sample sites, and the Cody Scarp, which is approximated by where the UFA is semi-confined. Location of the Ichetucknee River (C) and the Santa Fe River Sink-Rise system (D) are outline by dashed boxes. (C) Map of the Ichetucknee River and springshed showing sampled springs sorted by group and two sampled monitoring wells. (D) Map of the Santa Fe River Sink-Rise system showing the three flow path sample points, mapped conduit, and sampled monitoring well locations. Wells noted with an 'A' are screened at the water table.

These springs are classified as 1st ($\geq 2.8 \text{ m}^3/\text{s}$; Madison Blue), 2nd ($0.28\text{--}2.8 \text{ m}^3/\text{s}$; Otter and Little River), or 3rd ($0.03\text{--}0.28 \text{ m}^3/\text{s}$; Peacock) magnitude (Meinzer, 1927). When the rivers flood, their stage can exceed the hydraulic head at these spring vents and cause flow direction to reverse allowing river water to flow into the conduits (referred to here as a spring reversal; Gulley et al., 2011). Water recharged during spring reversals has elevated oxygen, OC, and trace metal concentrations compared with the groundwater and may have residence times of days to weeks in the aquifer. These events alter redox state and pH of the conduit and surrounding aquifer matrix and drive OC remineralization,

metal oxide dissolution and precipitation, and carbonate mineral dissolution (Brown et al., 2019; Brown et al., 2014; Gulley et al., 2011), which suggest they may be important to methanogenesis and methanotrophy. Water discharged as the river stage decreases during the flood recession has an initial composition similar to the recharged stream water with a gradual shift to groundwater compositions. The change in composition reflects mixtures of recharged surface water and groundwater with long subsurface residence time, which can be up to decades (Katz et al., 2001).

The Ichetucknee springs group contains eight named springs and

numerous minor springs and seeps (Kurzt et al., 2015), which source the Ichetucknee River in its upper reaches before it flows ~8 km to the Santa Fe River (Fig. 1C). The long spring run prevents reversals of these springs and they have limited point recharge in the upland area. Consequently, Ichetucknee spring water has average ages on the order of decades (Martin et al., 2016), which represents the length of residence time in the subsurface. Sampled springs range from 1st to 3rd magnitude and include Head (2nd), Cedar Head (3rd), Blue Hole (1st), Coffee (3rd), Mission (2nd), Devil's Eye (2nd), Grassy Hole (3rd), and Mill Pond (2nd) springs. These springs fall into two groups (Martin et al., 2016; Martin and Gordon, 2000). Group 1 springs (Head, Cedar Head, Blue Hole, Coffee) have higher DO concentrations, more variable temperatures, and younger apparent age (by ~5–8 years) than Group 2 springs (Mission, Devil's Eye, Grassy, Mill Pond), indicating shorter subsurface residence times and shallower flow paths. Water chemistry varies little through time, including low OC concentrations within both groups. Group 1 water has mean apparent ages of 30 to 35 years and Group 2 has mean apparent ages of 38 to 42 years, although apparent age of both groups has increased by 10–20 years over the past couple of decades, reflecting regional scale shifts in climate and increased groundwater extraction (Martin et al., 2016). Groundwater was sampled near the Ichetucknee springs from two water table monitoring wells.

The Santa Fe River Sink-Rise system (herein the Sink-Rise system) occurs at the Cody Scarp (Fig. 1D). It is located between a ~36 m deep sinkhole (River Sink) that captures all of Santa Fe River flow, except during extreme floods when overland flow occurs, and extends to a first magnitude spring (River Rise) that represent the headwaters of the lower Santa Fe River. Between River Sink and River Rise, cave divers have mapped ~8 km of conduits with additional conduits unexplored. Several sinkholes along the flow path have collapsed into the conduits and provide a surface connection with the conduits. The closest collapse feature to River Rise is Sweetwater Lake, which occurs mid-way between River Sink and River Rise. Along the flow path between River Sink and River Rise water can exchange between the conduits and aquifer matrix porosity, depending on conduit hydraulic head. Gaining and losing conditions can be identified by differences between flow into River Sink and discharge from River Rise, with losing conditions common during high flow events when flow captured by River Sink exceeds River Rise discharge (Martin et al., 2006; Bailly-Comte et al., 2010). Recharge at River Sink provides OC, oxygen, nutrients, and trace metals to the conduit system and near-conduit aquifer matrix porosity when water is lost from the conduits (Moore et al., 2010). Subsurface residence times of the Sink-Rise system average 1.8 days and range between 1 and 15 days, based on the lag in temperature anomalies of recharged water, with an inverse exponential relationship with River Sink discharge (Bailly-Comte et al., 2010; Martin and Dean, 2001; Martin and Dean, 1999). However, water lost from the conduits to the matrix porosity will have residence times longer than expected based on the temperature tracing. The Sink-Rise system has multiple groundwater monitoring wells that are distributed near the mapped location of conduits. The wells are cased with 5.1 cm diameter PVC casing and extend either to the water table (~1–3 m below land surface) or to the depth of conduits (~30 m below land surface) (Ritorto et al., 2009). The shallow wells have a 3 m screened interval and the deep wells have a 6 m screened interval.

A total of 217 samples were collected for this study. During 2018–2020 and 2022, samples were collected at an irregular interval. During 2021, the Sink-Rise system was sampled biweekly, the Ichetucknee Group springs every four months, and the reversing springs every three months. Madison Blue Spring experiences spring reversal on average once or twice per year, one of which was sampled at an average rate of 1 sample every 4 days between mid-February and mid-April 2021, with greater frequency during the recession following the reversal. Wells were sampled at least twice during 2020–2021. Exact sample dates and times are listed in the accompanying data set (Oberhelman et al., 2023).

The Santa Fe River was sampled from its headwaters to its confluence with the Suwannee River twice (August 7–9, 2020 and March 6, 2022) to complement sampling across the Cody Scarp at the Sink-Rise system (Fig. 1B and D). The headwaters of the upper Santa Fe River consist of tannic surface run off and shallow interflow from wetlands and lakes perched on the Hawthorn Group. Along with water from River Rise, the lower Santa Fe River is sourced by clear groundwater flow from discrete springs, diffuse seepage, and the Ichetucknee River. Transect sample sites correspond to USGS monitoring stations on the Santa Fe River. Sites upstream of River Sink include stations 02320700, 02321000, 02321500, and 02321898 referred to here as Sites 700, 1000, 1500, and 1898, respectively. Sites downstream of River Rise include stations 02321975, 02322500, and 02322800 referred to here as Sites 1975, 2500, and 2800, respectively. Mean discharge at Site 700 is 1.7 m³/s while at Site 2800 mean discharge is 55 m³/s (<https://dashboard.waterdata.usgs.gov> – retrieved August 2022).

2.3. Field, laboratory, and modeling methods

Water was pumped from spring vents, River Sink, Sweetwater Lake, River Rise, and transect sites using a Geotech peristaltic pump and weighted PVC tubing inserted into the water body. The pump outlet was connected to an overflow cup where a YSI ProQuatro Multiparameter Meter was used to monitor temperature (°C), DO (% saturation and mg/l), specific conductivity (µS/cm), and pH until values stabilized and were recorded after which water and gas samples were collected. Monitoring wells were sampled using a Proactive Environmental Products 12-Volt Submersible Tornado Pump with PVC tubing connected to an overflow cup that contained the YSI electrodes. Samples were collected after purging at least three well-volumes and YSI parameters stabilized.

CH₄ and CO₂ samples were collected by head space extraction. Extractions used HDPE media bottles that hold 600 ml of water. The bottles are closed with rubber stoppers fitted with two 1-way valves that allow the transfer of gas and water. Bottles were filled from the bottom and allowed to overflow with unfiltered sample water and immediately stoppered with no head space. A syringe was used to replace 60 ml of water with 60 ml of ultra-high purity grade N₂ without contact with the atmosphere. The bottle was shaken for ~3 min to equilibrate the head space N₂ with the remaining 540 ml of sample water. Once equilibrated, the headspace gas was transferred using a syringe and hypodermic needle to pre-evacuated 75 ml glass vials stoppered with butyl-rubber septa and aluminum crimp caps. Gas samples were analyzed for CH₄ concentration and δ¹³C-CH₄ and δ¹³C-CO₂ (reference to Vienna Pee Dee Belemnite) values within two weeks of collection by cavity ring-down spectroscopy on a Picarro G2201-i. Dissolved gas concentrations were calculated from headspace gas concentration using Henry's Law and the ideal gas law (Pain et al., 2019; Sander et al., 2011). Atmospheric gas samples were collected at each site by filling a syringe with air ~2 m above the land surface at each site and injecting 60 ml into a pre-evacuated 75 ml glass vials.

Water samples were collected to measure major cations (Ca²⁺, Mg²⁺, Na⁺, K⁺) and major anions (Cl⁻, SO₄²⁻, F⁻, NO₃⁻) concentrations. Samples were filtered with in-line 0.45 µm GeoTech medium-capacity capsule filters and collected in two 20 ml HDPE bottles. Cation samples were acidified to pH < 2 in the field with trace-metal grade nitric acid while no preservative was used for anion samples. All samples were stored on ice in the field and refrigerated at 4 °C upon return to the lab. Major ions were measured by ion chromatography on Dionex ICS-2100 (anions) and ICS-1100 (cations) instruments.

The quality of CH₄ and major ion data was assessed with independent check standards, sample blanks, and sample duplicates, which indicate no contamination of samples and good instrument calibration and analytical reproducibility. Mann-Whitney *U* tests were performed on CH₄ concentration, δ¹³C isotope, and major ion data to investigate the significance of changes in water chemistry due to surface water-groundwater interaction. These statistical analyses were performed in

the R software environment. Significance of the test results are reported as p values using a 0.05 significance level.

Mixing models developed in two previous studies (Brown et al., 2014; Moore et al., 2009) using conservative major ion concentrations at Madison Blue Spring and at the Sink-Rise system were used to assess the influence of mixing and dilution on CH_4 concentrations (supplemental information). The Madison Blue Spring mixing model is a binary model based on average Cl^- concentrations in spring water (0.17 mM) and intruding river water (0.21 mM) during spring reversal (Brown et al., 2014). A three end-member model was developed for the Sink-Rise system using concentrations of SO_4^{2-} and Mg^{2+} to estimate proportion of River Rise discharge that is (1) surface water recharging at River Sink, (2) shallow groundwater represented by water collected at Well 4, and (3) deep upwelling mineralized groundwater represented by water collected at Well 2 (Moore et al., 2009). These ions were chosen because they behave conservatively at the Sink-Rise system and because they have the greatest differences in concentrations between the three sources (Moore et al., 2009).

The atmospheric flux of CH_4 was estimated for Sweetwater Lake, River Rise, Madison Blue Spring, Little River Springs, Head Spring, Blue Hole Spring, Cedar Head Spring, Devil's Eye Spring, and Mill Pond Spring. These springs were chosen to represent the varied residence times of the discharging groundwater. Gas exchange between air and water was estimated by.

$$F = k(c_m - c_{eq}) \quad (1)$$

where c_m is the gas concentration measured in spring discharge (μM), c_{eq} is the gas concentration expected in water at equilibrium with the atmosphere ($\sim 0.01 \mu\text{M}$), and k is the gas transfer velocity (cm/h). k was obtained by averaging values estimated from windspeed relationships published in Crusius and Wanninkhof (2003) and Cole and Caraco (1998) (supplemental information). Windspeeds used in calculating k were the average for the day of gas sample collection from the two Weather Underground weather stations nearest to the sample site. The stations include KFLHIGHS13 and KFLHIGHS17 for the Sink-Rise system, KFLJENNI2 and KFLJENNI5 for Madison Blue Spring, KFLBRANF8 and KFLOBRIE20 for Little River Springs, and KFLFORTW112 and KFLFORTW138 for Ichetucknee Springs.

3. Results

3.1. Concentrations and loading of CH_4

Measured CH_4 concentrations ranged from 0.002 to 89 μM for all samples (Fig. 2A). The highest median concentrations occur in deep wells at the Sink-Rise system and previously reported water supply wells in the Floridan aquifer (McMahon et al., 2017), with values of 1.6 and 0.74 μM , respectively. Lower CH_4 concentrations occur in water table wells at the Sink-Rise system and near Ichetucknee springs, with median concentrations of 0.12 and 0.004 μM , respectively. Most of the groundwater concentrations are $>0.01 \mu\text{M}$, which is the concentration expected for water at equilibrium with the local average atmospheric CH_4 concentration of 137 μM (2.2 ppm).

Surface water and springs show a trend of increasing median CH_4 concentration as subsurface residence times decrease in regions where surface water-groundwater interactions are increasingly common (Fig. 2A). CH_4 concentrations were lowest in Group 2 Ichetucknee springs (~ 40 yr residence time) followed by Group 1 springs (~ 30 yr residence time), with the highest concentrations occurring at Cedar Head Spring for all Ichetucknee springs. CH_4 concentration in the Ichetucknee River ~ 1.4 km downstream of the sampled springs was 0.32 μM , which is 1–2 order of magnitude higher than any of the Ichetucknee Springs and more similar to River Sink concentrations. The highest CH_4 concentrations occur at the Sink-Rise system where subsurface residence times are hours to days (Martin and Dean, 1999).

Concentrations of CH_4 at the reversing springs, where residence times are on the order of weeks to months, are intermediate between those at Ichetucknee springs and the Sink-Rise system.

Along the longitudinal transect of the Santa Fe River, CH_4 concentrations decrease downstream from $\sim 1.5 \mu\text{M}$ at Site 700 to 0.05 μM at River Rise and then increase to 0.34 μM at Site 2800 (Fig. 3A). A consistent trend of decreasing CH_4 concentration was present across the Sink-Rise system (Fig. 4A). Median CH_4 concentrations decreased significantly ($p < 0.05$) from River Sink (0.34 μM) to Sweetwater Lake (0.15 μM) and River Rise (0.10 μM). The CH_4 load (discharge times concentration) increased from 1000 to 3000 $\mu\text{g-CH}_4 \text{ s}^{-1}$ upstream at Site 700 to approximately 250,000 $\mu\text{g-CH}_4 \text{ s}^{-1}$ downstream at Site 2800 although the Sink-Rise system shows a reversal in this trend with a local minimum of 6000 $\mu\text{g-CH}_4 \text{ s}^{-1}$ at River Rise (Fig. 3B). This minimum represents a significant ($p < 0.05$) decrease in load for most Sink-Rise samples with the median CH_4 load of 77,000 and 29,000 $\mu\text{g-CH}_4 \text{ s}^{-1}$ at River Sink and River Rise, respectively (Fig. 4B).

All sample pairs that were collected from River Sink and River Rise when discharge was less than average ($n = 20$) show a decrease in CH_4 loading from River Sink to River Rise (Fig. 5). Most of the sample pairs collected at higher than average discharge ($n = 16$) also show a decrease in loading from River Sink to River Rise. Only five Sink-Rise sample pairs had higher CH_4 loading at River Sink than at River Rise. All five pairs occurred at flows greater than the annual average of 14.8 m^3/s at River Sink. Three of the five pairs exhibited both lower CH_4 concentration and mass loading at River Sink than River Rise.

CH_4 concentrations in recharging water at Madison Blue Spring during the sampled spring reversal are significantly greater ($p < 0.05$) than concentrations following the flood recession (Fig. 6). The spring reversed flow twice during the event, with the first period lasting 11 days and the second lasting 6 days (Fig. 6A). The Cl^- mixing model (e.g., Brown et al., 2014) shows a steady decline in the proportion of intruded river water during the 30 days following the end of the spring reversal. Four samples taken during the spring reversal indicate the average CH_4 concentration of the recharging water (0.16 μM) is greater than the average concentration at baseflow ($\sim 0.03 \mu\text{M}$). Samples collected between the two intrusion events have values between ~ 0.04 and $\sim 0.09 \mu\text{M}$. Although the spring discharged mixed river water and groundwater for approximately 30 days following the second intrusion event, CH_4 returned to and remained near baseflow concentrations around 10 to 30 h after spring discharge resumed except for a single large spike to 0.53 μM about 20 days after the second reversal period.

3.2. Atmospheric flux of CH_4

Mean CH_4 flux between dissolved and atmospheric gases ranges from -0.05 to $1.0 \text{ mg m}^{-2} \text{ d}^{-1}$, with negative numbers indicating dissolution of atmospheric CH_4 (Table 1). Positive CH_4 fluxes range from 0.01 to 7.4 $\text{mg m}^{-2} \text{ d}^{-1}$. Negative CH_4 fluxes range from -0.06 to $-0.004 \text{ mg m}^{-2} \text{ d}^{-1}$. Except Devil's Eye and Mill Pond springs, all springs show a positive mean flux of CH_4 . All Ichetucknee springs had periods of negative CH_4 flux but only Mill Pond Spring has a constant negative CH_4 flux. The greatest positive mean CH_4 flux occurred at Sweetwater Lake.

3.3. Isotopic signature of CH_4 and CO_2

The median dissolved $\delta^{13}\text{C-CH}_4$ value was -43 ‰ and ranged from -81 to -5.0 ‰ while the median dissolved $\delta^{13}\text{C-CO}_2$ value was -17 ‰ and ranged from -22 to $+1.9 \text{ ‰}$. Surface water exhibits a wider range of $\delta^{13}\text{C-CH}_4$ values than groundwater. Although groundwater and surface water $\delta^{13}\text{C-CH}_4$ values overlap at the Sink-Rise system, the groundwater $\delta^{13}\text{C-CH}_4$ values are mostly lower than River Sink and River Rise water. The reversing spring and both groups of Ichetucknee springs water also had $\delta^{13}\text{C-CH}_4$ values that overlapped with River Sink and River Rise water but are mostly lower.

The $\delta^{13}\text{C-CH}_4$ values show a maximum at the Sink-Rise system

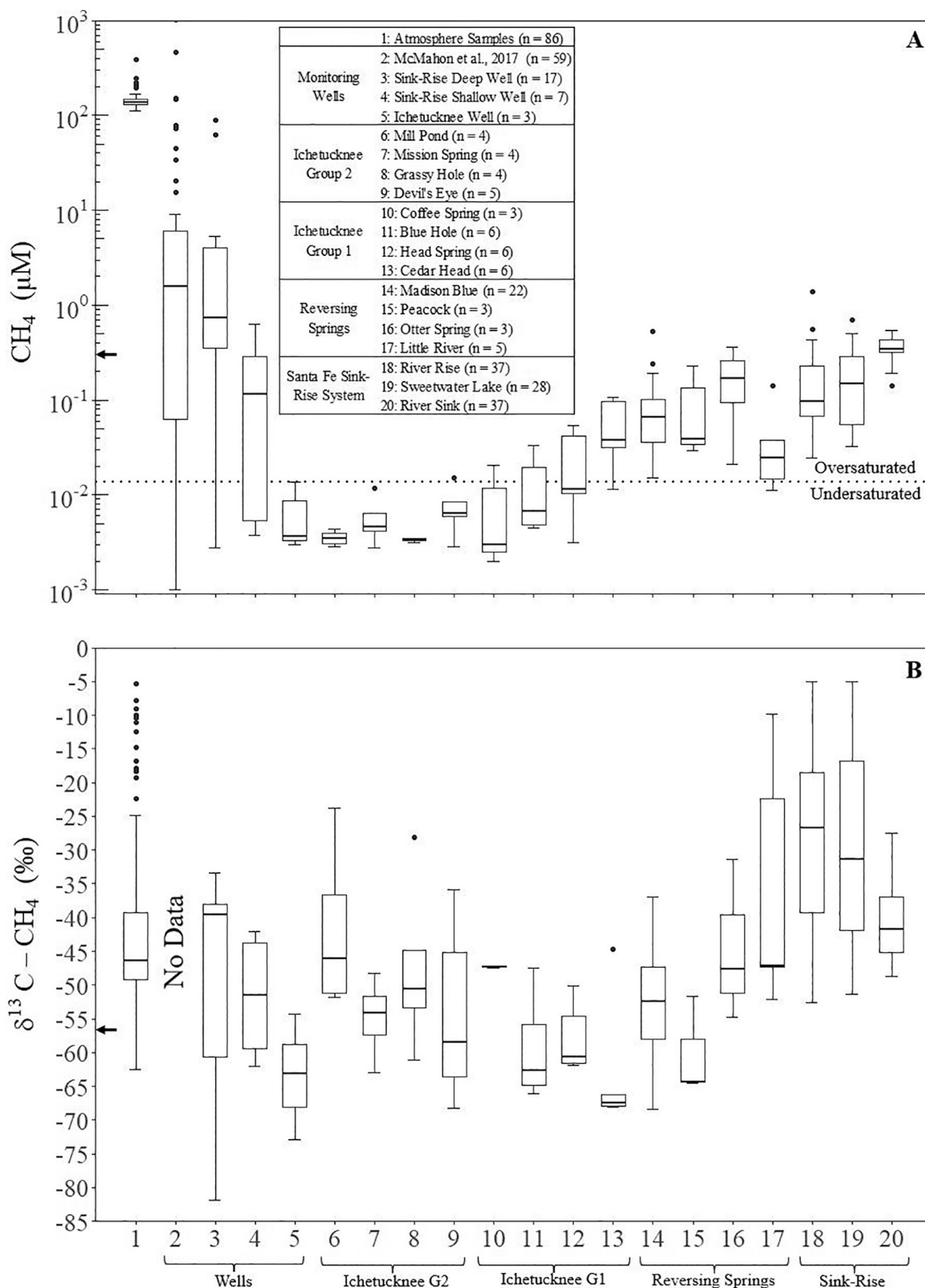


Fig. 2. Box plots showing the distributions of CH_4 concentration (A) on a log scale and $\delta^{13}\text{C}-\text{CH}_4$ (B) across atmosphere, spring, and well samples. The dotted line on A represents the CH_4 concentration of water in equilibrium with atmospheric CH_4 ($0.01 \mu\text{M}$) concentrations in 1A. The arrow on A at $0.32 \mu\text{M}$ and on B at -56.2 ‰ represent the average CH_4 concentration and $\delta^{13}\text{C}-\text{CH}_4$ value in the Ichetucknee River at USGS monitoring station 02322700, respectively. Boxplots follow the standard Tukey definition.

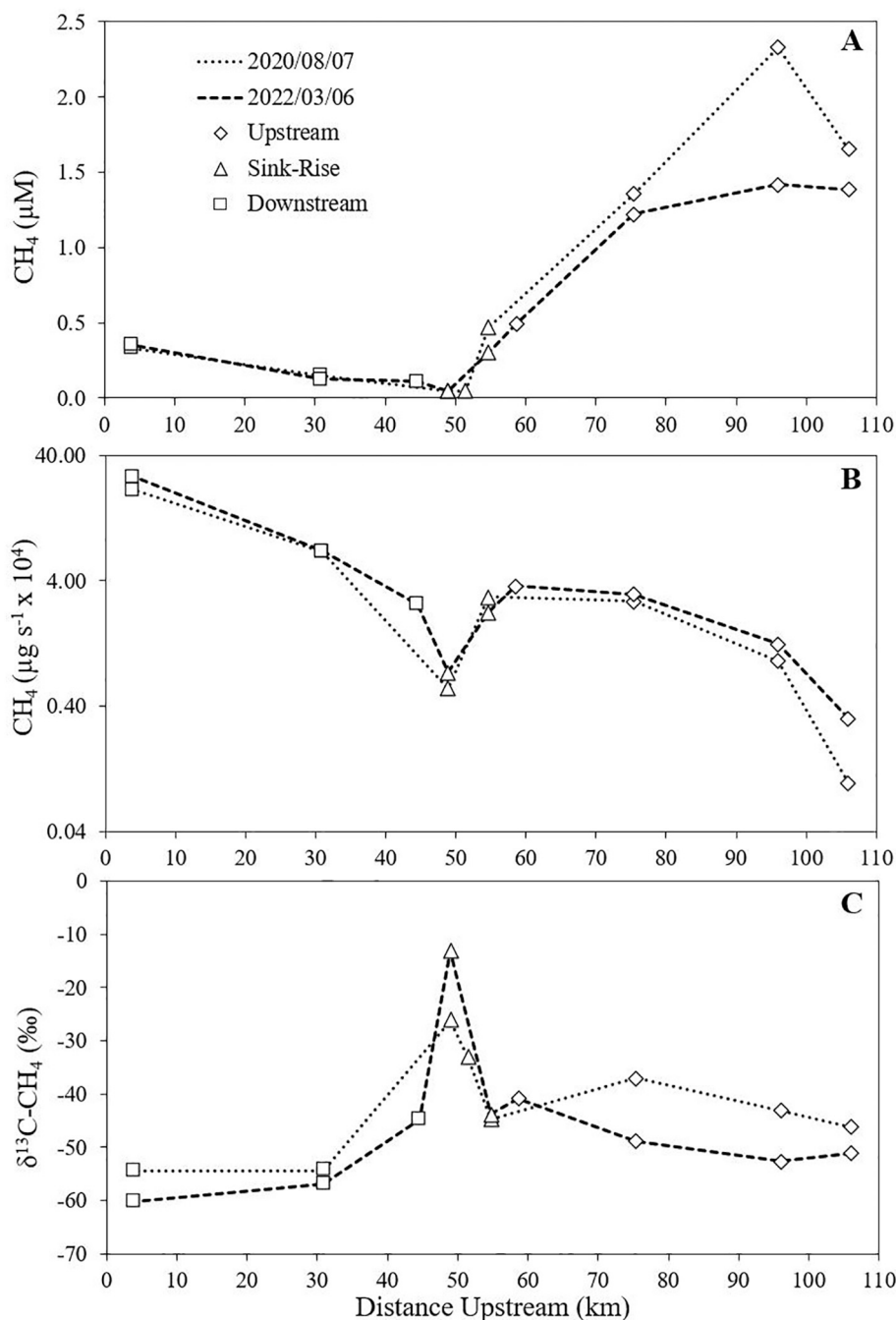


Fig. 3. Profiles of (A) CH₄ concentration, (B) CH₄ loading, and (C) δ¹³C-CH₄ along the Santa Fe River transect. Sites sampled during the 2020 transect from upstream to downstream are 700, 1000, 1500, River Sink, Sweetwater Lake, River Rise, 2500, and 2800. As no discharge data is available at Sweetwater Lake it is not included on B. Sites sampled during the 2022 transect from upstream to downstream are 700, 1000, 1500, 1898, River Sink, River Rise, 1975, 2500, and 2800.

compared with approximately constant, but distinct, values at the upstream and downstream sites (Fig. 3C). The downstream δ¹³C-CH₄ values are lower by about 5 to 15 ‰ than the upstream values. Median δ¹³C-CH₄ values increase significantly ($p < 0.05$) from River Sink to River Rise and range from -42 ‰ at River Sink, -31 ‰ at Sweetwater Lake, and -27 ‰ at River Rise (Fig. 4C).

At Madison Blue Spring, the median δ¹³C-CH₄ value of intruding river water (-46 ‰) is significantly higher ($p < 0.05$) from baseflow spring discharge (-57 ‰). As intruded water discharged during flood recession, the δ¹³C-CH₄ value decreased to a minimum value of -65 ‰ on March 31 when discharging water was ~42 % intruded water and then increases to -50 ‰ on April 16 when discharging water was ~11 %

intruded water (Fig. 6). The exception to this overall trend occurred on March 26 when δ¹³C-CH₄ showed a maximum value of -41 ‰, which occurred with little change in CH₄ concentration. This isotope maximum occurred immediately prior to an increase in CH₄ concentration, which is associated with the minimum δ¹³C-CH₄ value of -65 ‰.

Differences are significant between the median δ¹³C-CH₄ value for surface and well waters ($p < 0.05$) and surface and spring waters ($p < 0.05$). Surface water was defined as water composition at sites with minimal input of groundwater and include the upstream transect sites, River Sink, and Madison Blue Spring during spring reversal. Samples from Sweetwater Lake and River Rise are excluded from spring waters because they are heavily influenced by surface water recharging to River

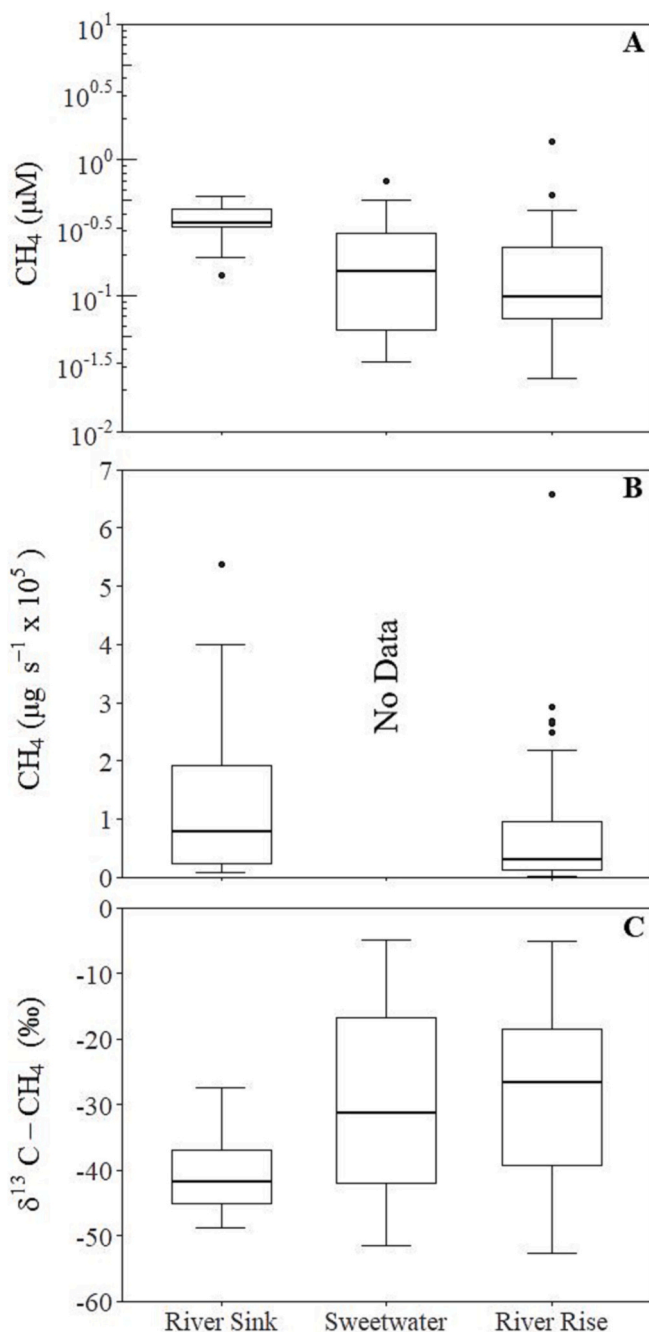


Fig. 4. Box plots of (A) CH_4 concentration, (B) CH_4 loading, and (C) $\delta^{13}\text{C}-\text{CH}_4$ values for 36 sample times from River Sink and River Rise, for which only 28 times included Sweetwater Lake. Discharge is unavailable at Sweetwater Lake, preventing calculations of loading there. Boxplots follow the standard Tukey definition.

Sink and thus are not representative of baseflow at most UFA springs.

3.4. Mixing models and water chemistry

Mixing models (supplemental information) predict concentrations of Cl^- at River Rise (Fig. 8A) and Na^+ at Madison Blue Spring (Fig. 8b). These two solutes are conservative but were not used in mixing models at River Rise and Madison Blue Spring. Good correlations exist between modeled and observed Cl^- concentrations at River Rise ($R^2 = 0.97$) and modeled and observed Na^+ concentrations at Madison Blue Spring ($R^2 = 0.97$). However, mixing does not predict the variations in CH_4

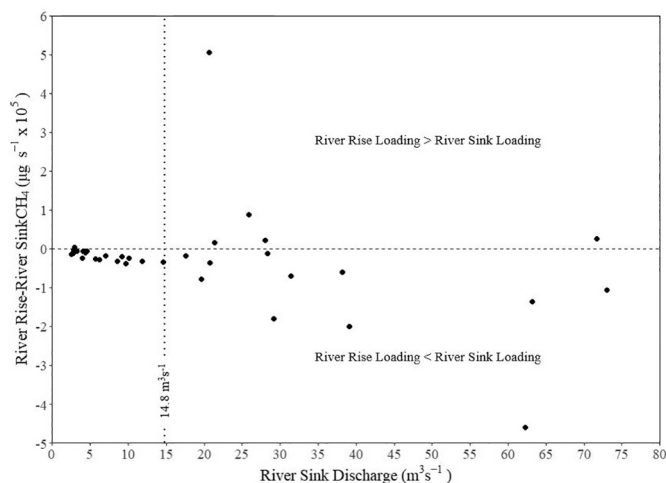


Fig. 5. Difference in CH_4 mass loading between River Rise and River Sink versus the discharge at River Sink. The horizontal dashed line represents no change in CH_4 loading between River Sink and River Rise while the vertical dotted line represents the average discharge at the River Sink ($14.8 \text{ m}^3/\text{s}$). The horizontal dashed line separates fields where CH_4 loading at River Rise is greater (above the line) or less than (below the line) loading at River Sink.

concentration at River Rise ($R^2 = 0.09$) or Madison Blue Spring ($R^2 = 0.001$) reflecting controls by processes in addition to mixing.

4. Discussion

Surface water-groundwater exchange and its control on redox conditions should affect both CH_4 consumption and production and thus CH_4 concentrations within and fluxes from the carbonate CZ. Distributions of CH_4 in springs, surface water, and groundwater of north-central Florida suggest surface water-groundwater interaction may produce CH_4 and generate an atmospheric source. However, the CH_4 distributions also suggest at certain locations CH_4 may be sufficiently low to be an atmospheric sink. Depending on magnitudes of and processes controlling CH_4 gain or loss, which are poorly known, CH_4 in the carbonate CZ may positively or negatively impact global climate through its strong greenhouse warming potential. We explore below relationships between exchange of surface water and groundwater and magnitudes, timing, and controls of CH_4 gain and loss in the carbonate CZ. We compare CH_4 fluxes from north-central Florida to global fluxes, recognizing the uncertainty of this comparison caused by the range of carbonate CZ characteristics (Covington et al., 2023; Martin et al., 2021), which also likely alters CH_4 fluxes from individual sites.

4.1. Possible sources of CH_4

Distributions of CH_4 concentrations from streams, springs, and wells across north-central Florida reflect differences in CH_4 production and consumption within the UFA. In the upper Santa Fe River, downstream increases in CH_4 loading could reflect shallow interflow out of river sediments or wetlands (Fig. 3B). A source from interflow is supported by CH_4 concentrations in the Ichetucknee River that increase by 1 to 2 orders of magnitude $\sim 5 \text{ km}$ downstream of the headwaters compared to its headwater springs (Fig. 2A). The elevated concentrations correspond with an $\sim 13\%$ increase in river flow that originates from small ungauged springs and seeps (Kurz et al., 2015) and implies a CH_4 source from interflow. Additional support for a source of CH_4 from interflow is elevated CH_4 concentrations at Cedar Head Spring (Fig. 2A), which has low discharge from a vent choked with sediment with no visible boil and is surrounded by wetlands.

Most of the sampled CH_4 appears to originate from the acetoclastic pathway based on the $\delta^{13}\text{C}$ values of CO_2 and CH_4 , although five samples

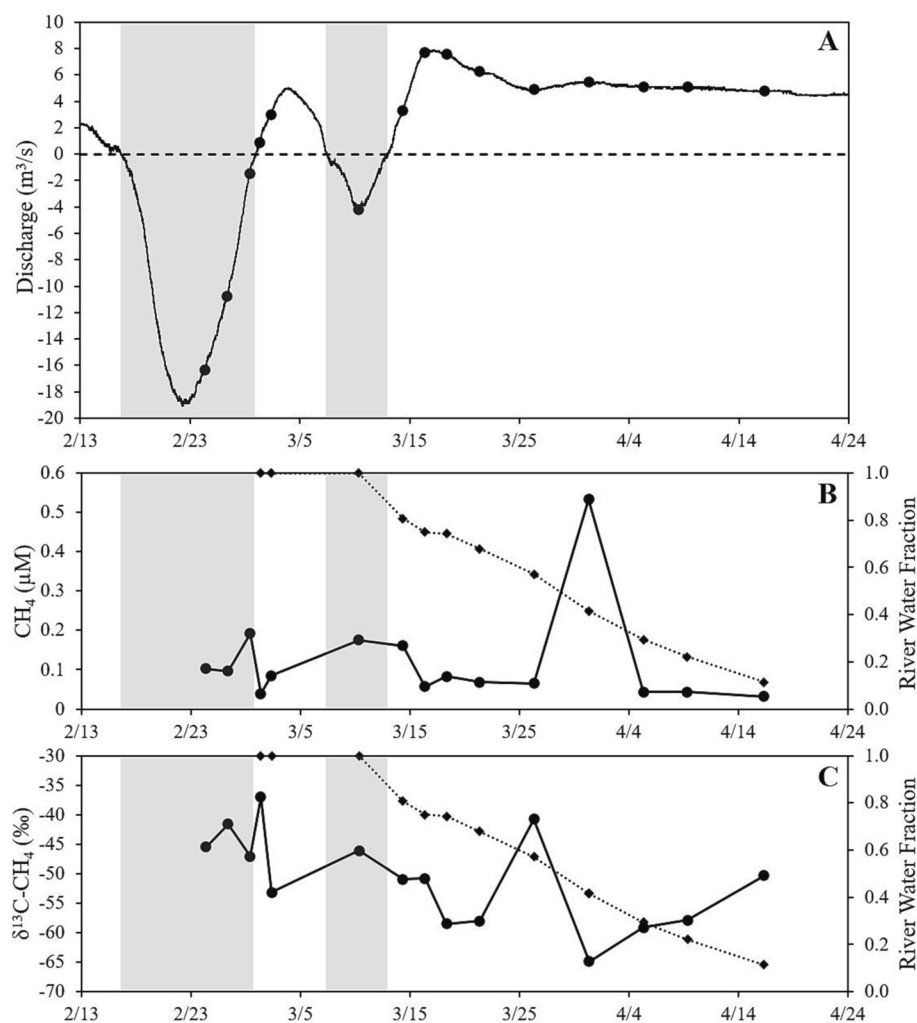


Fig. 6. Timeseries plots for the sampled reversal at Madison Blue Spring. (A) Plot of spring discharge during the reversal. The dashed line represents the shift between stream water intrusion and groundwater discharge. (B) Plot of CH_4 concentration. (C) Plot of $\delta^{13}\text{C}-\text{CH}_4$ values. The dotted line in plots A and B represents the fraction of river water in spring discharge as calculated by the mixing model. Shaded regions on each plot denote periods of stream water intrusion and the black circles represent sample points.

Table 1
 CH_4 flux at a subset of study springs.

n	Sweetwater Lake	River Rise	Madison Blue Spring	Little River Springs	Head Spring	Blue Hole Spring	Cedar Head Spring	Devil's Eye Spring	Mill Pond Spring
	28	34	22	5	4	5	5	5	4
CH_4 Flux ($\text{mg m}^{-2} \text{d}^{-1}$)									
Mean	1.0	0.94	0.37	0.18	0.05	0.01	0.22	-0.03	-0.05
Median	0.73	0.48	0.24	0.09	0.001	-0.03	0.17	-0.03	-0.04
σ	0.95	1.3	0.51	0.26	0.10	0.06	0.17	0.02	0.01
Min	0.11	0.05	0.01	-0.02	-0.02	-0.04	-0.004	-0.06	-0.06
Max	4.2	7.4	2.4	0.69	0.22	0.10	0.43	0.01	-0.04

have isotopic ratios indicative of the hydrogenotrophic pathway (Fig. 7). The ϵ_c value of 43 for the elevated CH_4 concentrations in the Ichetucknee River is within the expected range for the acetoclastic pathway. Production of CH_4 by acetoclastic methanogenesis can be linked to sufficiently high inputs of labile OC (e.g., Gruca-Rokosz and Koszelnik, 2018; Liu et al., 2017), which should occur where interflow water drains to streams or extensive surface water-groundwater interactions occur, such as along the Cody Scarp. Samples that reflect a hydrogenotrophic pathway include four deep wells at the Sink-Rise system and one Ichetucknee well (Fig. 7). Hydrogenotrophic methanogenesis in the UFA is supported by radiocarbon dead and ^{13}C depleted troglobitic fauna, which suggests a contribution to the food web from CH_4 partially produced with DIC from dissolution of the surrounding limestone aquifer (e.g., Opsahl and Chanton, 2006). Hydrogenotrophic methanogenesis is

the more dominant pathway where SO_4^{2-} concentrations are $>0.2 \text{ mM}$ (Whiticar, 1999) because SO_4^{2-} reducing bacteria out compete methanogens for substrates like acetate. SO_4^{2-} concentrations as great as 3.3 mM have been observed at Sink-Rise system wells from gypsum dissolution at the base of the Floridan aquifer coupled with upward flow through the aquifer (Moore et al., 2009). These elevated SO_4^{2-} concentrations favor hydrogenotrophic methanogenesis.

Regardless of the methanogenic pathway, a source for CH_4 in spring discharge is supported by elevated CH_4 concentrations in groundwater from most of the sampled wells as well as legacy well data (Fig. 2A). In contrast with these elevated CH_4 concentrations, median concentrations are below equilibrium with atmospheric CH_4 in wells near the Ichetucknee springs and all of the Ichetucknee springs except for Cedar Head Spring. The Ichetucknee springs have limited point surface water-

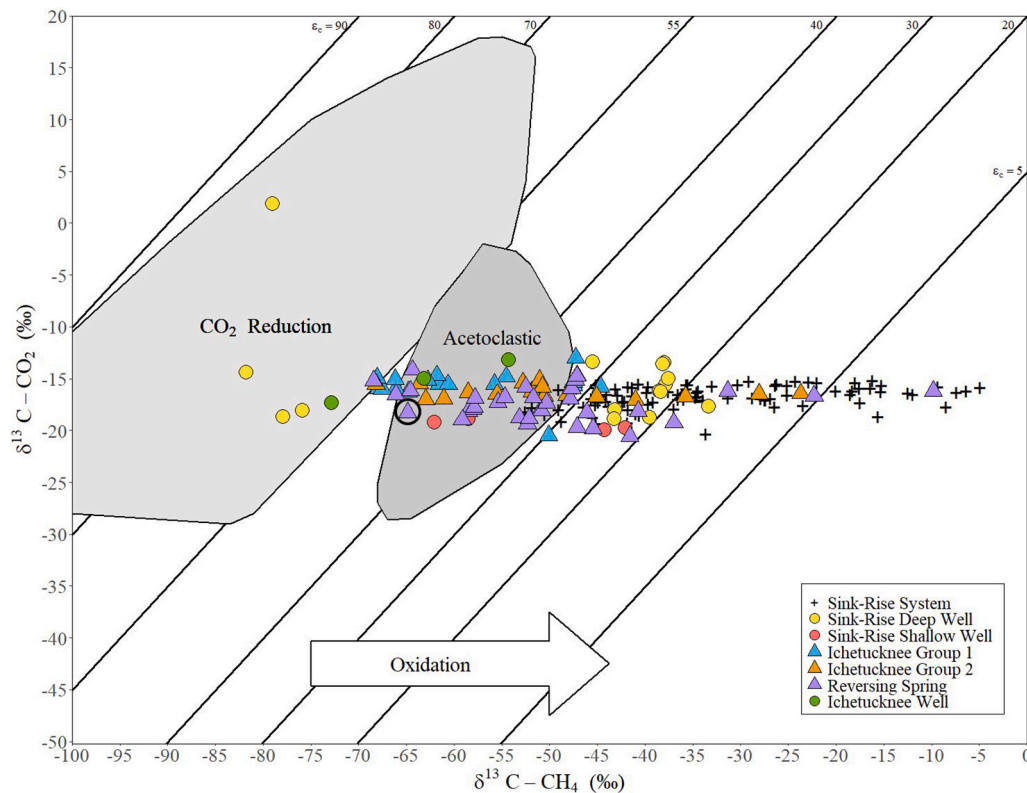


Fig. 7. Biplot after Whiticar (1999) showing the $\delta^{13}\text{C}$ of carbon in CH_4 and CO_2 . Lines represent trends of equal the isotope separation factor ϵ_c , which is approximately equal to the difference between $\delta^{13}\text{C}\text{-CO}_2$ and $\delta^{13}\text{C}\text{-CH}_4$. The arrow represents the direction indicative of a trend of CH_4 oxidation. Shaded regions represent isotopic signatures of the two major methanogenic pathways. The circled point is the ~ 8 -fold CH_4 spike during the Madison Blue Spring reversal.

groundwater exchange (e.g., sinking streams) and because of the long spring run (Fig. 1C) do not reverse flow. The limited amount of surface water recharge results in decades long subsurface residence times (Martin et al., 2016; Katz et al., 2001) compared to subsurface residence times of days to weeks at the Sink-Rise system and weeks to months following reversals at Madison Blue, Peacock, Little River and Otter springs. All sites where surface water-groundwater exchange is frequent, either from point recharge or spring reversals, have CH_4 concentrations greater than equilibrium with the atmosphere (Fig. 2A). This correspondence between surface water-groundwater exchange and variations in CH_4 concentrations at the sampled wells and springs implies that sources of DOC from surface systems are important to methanogenesis. However, most groundwater CH_4 concentrations are greater than sampled springs systems (Fig. 2a), which indicates that CH_4 concentrations decrease before groundwater discharges from springs.

4.2. Possible mechanisms for CH_4 loss

Processes that could decrease CH_4 concentrations following production in the aquifer or injection with point recharge could include physical degassing, methanotrophy, and/or mixing of water with low CH_4 concentrations. The potential for each of these mechanisms to decrease CH_4 concentrations is evaluated below relative to groundwater-surface water exchange and residence time in the subsurface. This evaluation is important to estimate the net effect on atmospheric fluxes from CH_4 cycling in the carbonate CZ.

4.2.1. Short subsurface residence times and CH_4 loss

The decrease in CH_4 concentration from the Santa Fe River headwaters to River Sink contrasts with increased CH_4 loading over this stretch of the river, while downstream of River Rise both concentration and loading increase (Fig. 3A and B). These differences in concentration

and loading upstream and downstream of the Sink-Rise system reflect variations in water sources. Upstream, CH_4 concentration and loading reflect runoff and interflow from surrounding wetlands that are perched on the confined aquifer, similar to downstream increases in CH_4 concentrations in the Ichetucknee River (Fig. 1B). This source contributes CH_4 and increases flow and CH_4 loading, but the source appears to have low CH_4 concentrations that dilute elevated CH_4 concentrations derived from the upstream runoff. In contrast, the simultaneous increase in CH_4 concentrations and loading downstream of River Rise reflects contributions of CH_4 enriched groundwater to the stream channel (e.g., Khadka et al., 2014; Flint et al., 2021). The downstream connection with the Floridan aquifer is shown by $\delta^{13}\text{C}\text{-CH}_4$ values that decrease to values similar to those in sampled wells (Figs. 2C & 3C). The isotopically heavier $\delta^{13}\text{C}\text{-CH}_4$ values upstream compared with downstream suggest that some of the CH_4 draining to the channel systems has been oxidized. These differences in CH_4 dynamics and $\delta^{13}\text{C}\text{-CH}_4$ values along the Santa Fe River highlight the importance of groundwater-surface water interactions to CH_4 dynamics in the Santa Fe River and these effects are amplified at the Sink-Rise system.

The decrease in median CH_4 concentrations and increase in median $\delta^{13}\text{C}\text{-CH}_4$ values at the Sink-Rise system disrupts the overall trend of changing CH_4 concentrations, loading, and isotope values along the stream channel (Figs. 3 & 4). Below we evaluate evasion, dilution by water with low CH_4 concentrations and distinct isotope ratios, and/or methanotrophy as causes of this disruption. The maximum potential loss of CH_4 from evasion was estimated based on the following assumptions. First, we assume water flows in a surface channel with a similar width to the ~ 8 km conduit (~ 18 m) connecting River Sink to River Rise. This assumption provides the maximum possible water surface area for evasion. Second, we assume travel time during which evasion could occur is 0.5 to 2.5 days, which represents the range of travel times through the conduits for flow conditions during sampling (Martin and

Dean, 1999). Third, we assume the average CH_4 evasion rate is the same as estimated at River Rise ($0.94 \text{ mg m}^{-2} \text{ d}^{-1}$). Even with these extreme conditions, the average decrease in CH_4 concentration at River Rise from evasion alone would be $0.01 \pm 0.01 \mu\text{M}$, which is an order of magnitude smaller than the average observed loss of $0.20 \pm 0.10 \mu\text{M}$. Considering a fractionation factor of 1.0008 for evasion of CH_4 to the atmosphere (Knox et al., 1992), and the estimated CH_4 loss, the $\delta^{13}\text{C}\text{-CH}_4$ values would be 5.0–25 ‰ more negative than values observed at River Rise, supporting limited loss of CH_4 from evasion.

Dilution is also unlikely to cause the observed decrease in CH_4 concentration seen in most of the paired River Sink and River Rise samples (Fig. 4A) considering the concentrations and isotopic compositions of the three sources of water to River Rise, including River Sink, shallow groundwater at Well 4, and deep groundwater at Well 2 (e.g., Moore et al., 2009). A decrease in CH_4 concentration from River Sink to River Rise was observed and predicted by modeled mixing and evasion for only 40 % of sample pairs. However, for these pairs, even with the combined effect of dilution and evasion, the observed River Rise CH_4 concentrations were on average $94 \pm 42 \%$ lower than expected based on the mixing modeled estimates. For sample pairs showing an increase in modeled CH_4 concentration, additional CH_4 originates from the deep groundwater source identified in Well 2, which has a CH_4 concentration ($0.77 \mu\text{M}$) greater than River Sink. Contributions from the deep source imply CH_4 losses are greater than expected from simple differences in concentration and loading between River Sink and River Rise. The average $\delta^{13}\text{C}\text{-CH}_4$ value for the three River Rise water sources is $\sim -40 \text{ ‰}$, which is more negative than the average $\delta^{13}\text{C}\text{-CH}_4$ at River Rise (-25 ‰) and cannot cause the observed increase in median $\delta^{13}\text{C}\text{-CH}_4$ values from River Sink to River Rise (Fig. 4C). This increase in $\delta^{13}\text{C}\text{-CH}_4$ value instead supports fractionation during methanotrophy (e.g., Whiticar, 1999), which likely dominates over evasion and dilution to the decrease in CH_4 concentrations and loading during flow from River Sink to River Rise (Fig. 5).

The loss of CH_4 through methanotrophy appears to have multiple controls considering the variability in CH_4 loading with changing discharge amounts (Fig. 5). Variability in loading increases as flow increases with loading at River Rise occasionally exceeding loading at River Sink. Causes of the increased variability in loading cannot be clearly determined with our current dataset although several possible explanations exist. First, allochthonous sources of CH_4 to the conduits, including sinkholes and wetlands near the channel, may become connected to the conduit during precipitation events that increase river discharge. These CH_4 -rich sources may contribute sufficient CH_4 to exceed loss by methanotrophy and thus more CH_4 may be lost than expected from the decline in concentrations from River Sink to River Rise (Fig. 4A). Second, during high flow events, the length of time for water to flow from River Sink to River Rise changes rapidly (Martin and Dean, 1999). Water sampled at River Sink and River Rise would have different lag times and may not be directly comparable. Regardless of the cause for increased loading, the scatter in loading between River Sink and River Rise and the occasional switch to greater loading at River Rise than River Sink indicates that flow variations impact CH_4 dynamics, transport, and fate in the carbonate CZ.

4.2.2. Production and consumption of CH_4 at intermediate residence times

During the initial stages of a spring reversal, the influx of redox sensitive solutes (DOC , O_2 , NO_3 , Fe , CH_4) fuels microbial oxidation of OC and reduction of electron acceptors (Brown et al., 2019; Carmichael et al., 2013). The initial oxic conditions could also support methanotrophy, which coupled with OC oxidation would increase CO_2 concentrations and contribute to carbonate mineral dissolution in the friable halo surrounding conduits (e.g., Moore et al., 2010; Gulley et al., 2011). Given sufficient time, oxidation of OC would lead to more reducing conditions, potentially to the point of methanogenesis.

Sequential methanotrophy and methanogenesis appear to occur at Madison Blue Spring during the sampled spring reversal and subsequent

recession. Low CH_4 concentrations during the initial recession reflect an initial loss of CH_4 (Fig. 6). The lack of a linear relationship between observed CH_4 concentrations and those derived from the mixing model indicates the loss is not caused by mixing (Fig. 8B). Evasion is also unlikely the cause of the loss because the observed changes in $\delta^{13}\text{C}\text{-CH}_4$ values are 5–10 ‰ greater than the $\sim 1 \text{ ‰}$ change expected if the CH_4 loss is from evasion. Like the Sink-Rise system, limited evidence for evasion and dilution implies that methanotrophy is the dominant process lowering CH_4 concentrations following the reversal. Methanotrophy is supported by an initial increase in $\delta^{13}\text{C}\text{-CH}_4$ values after the first intrusion. However, the decrease in CH_4 concentrations does not show a systematic correspondence with increasing in $\delta^{13}\text{C}\text{-CH}_4$ values during the 13 days following the intrusion, suggesting the two times of intrusion may lead to complex mixing of water with variable subsurface residence times with both methanotrophy and methanogenesis.

The anomalously high CH_4 concentration (~ 8 -fold increase above background values), which occurred ~ 21 days following the final period of intrusion when discharging water was $\sim 40 \%$ intruded river water, corresponds to the lowest $\delta^{13}\text{C}\text{-CH}_4$ value measured during the reversal. The simultaneous increase in concentration and decrease in $\delta^{13}\text{C}\text{-CH}_4$ value suggest redox conditions suitable for methanogenesis had developed by this time post-reversal. Assuming the precursor OC for methanogenesis has $\delta^{13}\text{C}$ values ranging from -22 to -34 ‰ , similar to typical terrestrial OC, the measured $\delta^{13}\text{C}\text{-CH}_4$ value (-65 ‰) falls within an expected 25 to 35 ‰ ^{13}C depletion relative to OC substrate associated with the acetoclastic pathway (Fig. 7). Low concentrations of SO_4^{2-} (0.12 mM) are also support acetoclastic methanogenesis.

The observed methanogenesis following the Madison Blue Spring reversal contrasts with observations of methanotrophy at the Sink-Rise system. Differences in CH_4 production and consumption at these two locations would depend on the two different mechanisms for recharge of

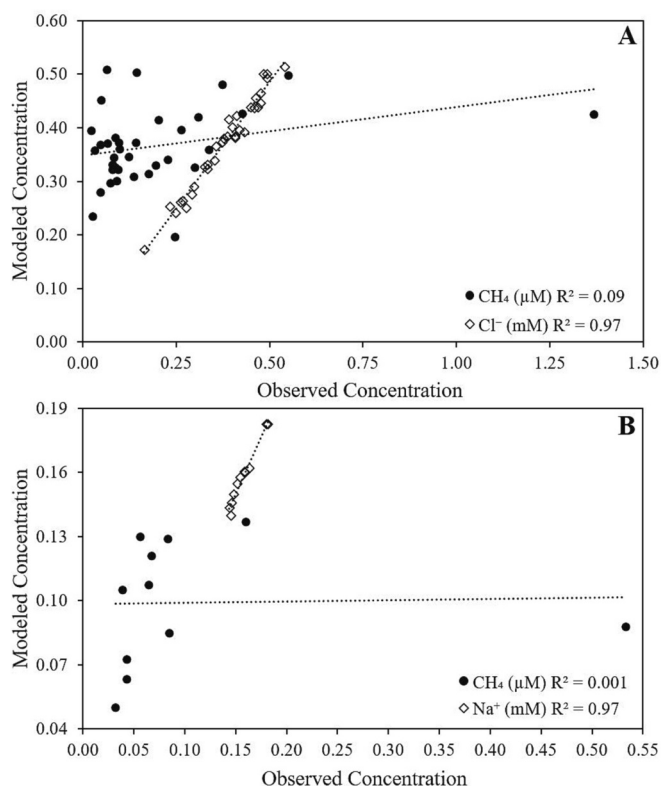


Fig. 8. Biplots of modeled concentration versus observed concentration for (A) CH_4 and Cl^- at River Rise and (B) CH_4 and Na^+ during the sampled Madison Blue Spring reversal. The Sink-Rise system model is based on Mg^{2+} and SO_4^{2-} while the Madison Blue Spring based Cl^- . Detailed descriptions of the two mixing models are in the supplemental material.

surface water and thus the relative magnitudes of DO supplied to the aquifer. During the spring reversal, the mass of recharged DO will be finite and with sufficiently long residence time energetically favorable electron acceptors such as DO would be consumed leading to methanogenesis. In contrast, the constant inflow of aerobic surface water at the Sink-Rise system would restrict methanogenesis and support the extensive methanotrophy observed there.

The time required to initiate methanogenesis at Madison Blue Spring is approximately similar to timing of changes in Fe and Mn concentrations following a smaller reversal at Madison Blue Spring in 2012. The 2012 reversal had an average water injection rate of $8200 \text{ m}^3 \text{ hr}^{-1}$ (injected river water volume/reversal length) over ~ 7.5 days (Brown et al., 2014) compared to $30,000 \text{ m}^3 \text{ hr}^{-1}$ over ~ 17.8 days for the reversal in 2021 (Fig. 6A). During the 2012 reversal, variations in dissolved ($0.45 \text{ }\mu\text{m}$ filter) Fe and Mn concentrations after the reversal indicate that the groundwater remained sufficiently oxic for ~ 20 days to precipitate Fe, Mn-oxides. Subsequently, development of reducing conditions caused dissolution of Mn-oxide for the final ~ 15 days of sampling although conditions were not sufficiently reducing for Fe-oxide dissolution. Conditions that were sufficiently reducing for methanogenesis in addition to Mn, Fe-oxide reduction may have occurred in the larger recharge event in 2021. Similar shifts in redox conditions following both reversals indicate that the duration of a spring reversal and subsurface residence times affect the extent of redox-sensitive reactions.

4.3. CH_4 fluxes from carbonate CZ springs

Previous work has shown springs discharging from the siliciclastic CZ to be supersaturated with CH_4 and thus an atmospheric source (Laini et al., 2011). Although the springs sampled here provide both sources and sinks of atmospheric CH_4 , most of the springs are supersaturated with respect to atmospheric CH_4 and thus a source (Table 1). Our measured range of CH_4 concentrations (0.002 – $1.4 \text{ }\mu\text{M}$) is similar to the range in siliciclastic springs (0.002 – $1.0 \text{ }\mu\text{M}$, Laini et al., 2011). Likewise, our range of flux estimates (-0.06 to $7.4 \text{ mg m}^{-2} \text{ d}^{-1}$) are similar to siliciclastic springs (0 – $18 \text{ mg m}^{-2} \text{ d}^{-1}$, Laini et al., 2011) and indicate little difference exists in the magnitude of CH_4 fluxes between springs discharging from the carbonate and siliciclastic CZ. These estimates fall at the low end of CH_4 fluxes from rivers, which range from <0.02 to $650 \text{ mg m}^{-2} \text{ d}^{-1}$ (Stanley et al., 2016) and are smaller than the average total flux (diffusive and ebullitive) of $70 \pm 135 \text{ mg m}^{-2} \text{ d}^{-1}$ from rivers.

Florida has the highest concentration of springs on earth, which our results suggest provides an atmospheric source of CH_4 . >1000 springs have been documented and 682 have had spring magnitude determined (Harrington, 2019). We estimate the springs' potential maximum CH_4 flux based on their assigned magnitude (e.g., 1st, 2nd, etc.; Meinzer, 1927) and the maximum CH_4 concentration, which was $1.37 \text{ }\mu\text{M}$ and occurred at River Rise. If a spring magnitude was unknown, it was assumed to be 3rd magnitude and 1st magnitude springs were assigned a discharge of $15 \text{ m}^3 \text{ s}^{-1}$, similar to average flow at Wakulla Spring the largest freshwater spring in Florida (Luzius et al., 2018). Given these assumptions, the total discharge was estimated to be $5.8 \times 10^8 \text{ m}^3 \text{ a}^{-1}$. This discharge was multiplied by the maximum CH_4 concentration minus the concentration expected from equilibrium with atmospheric CH_4 ($0.01 \text{ }\mu\text{M}$). This product indicates the maximum atmospheric flux of CH_4 from spring discharge in Florida of $12.6 \times 10^6 \text{ Tg a}^{-1}$.

For several reasons, our estimate is likely greater than the actual CH_4 emissions from Florida springs. The average CH_4 concentration in springs is $0.12 \text{ }\mu\text{M}$, which is about an order of magnitude less than the maximum value used in the estimate. All springs are assumed to have a positive CH_4 flux when six of the springs sampled in this study had negative fluxes on at least one sample day (Fig. 2). The calculation assumes the only sink for CH_4 at the surface is degassing while some CH_4 is likely lost to methanotrophy during surface flow. Springs do not always flow at their maximum discharge for their spring magnitude and lower

flow will reduce estimated fluxes.

For comparison with other CH_4 fluxes, abstraction from the Floridan aquifer wells is estimated to provide CH_4 emissions of $3041 \times 10^6 \text{ Tg a}^{-1}$, or more than two orders of magnitude greater than our estimates of spring discharge, while total global emissions from abstraction are estimated to be 0.53 Tg a^{-1} (Kulongsoski and McMahon, 2019). These estimates illustrate that CH_4 emissions from carbonate CZ springs are small relative to anthropogenically derived CH_4 emissions from groundwater abstraction on global and regional scales. Emissions from well water abstraction are also small compared to total global CH_4 emissions from freshwater bodies (122 Tg a^{-1}) and natural wetlands (185 Tg a^{-1}) (Saunio et al., 2016). However, like emissions from groundwater abstraction, emissions from springs may be significant at local scales. These estimates also highlight that abstraction represents a major modification to the natural loss of CH_4 from aquifers.

4.4. Implications for CH_4 cycling in the carbonate CZ

Both methanotrophic and methanogenic microbes have been identified in the vadose portion of the carbonate CZ (Waring et al., 2017; Brankovits et al., 2017; Hutchens et al., 2004). Methanotrophic bacteria are most abundant in air-filled cave sediments and soils while smaller populations exist on limestone surfaces (Waring et al., 2017). Methanotrophic depletion of CH_4 occurred in 87 of the 91 air-filled caves that were sampled across the United States, Spain, Vietnam, and Australia (Ojeda et al., 2019; Nguyễn-Thùy et al., 2017; Waring et al., 2017; Webster et al., 2016; Matthey et al., 2013). The CH_4 substrate that sustains methanotrophs originates from soil gas in the vadose zone, evasion from groundwater at the water table, and exchange with the atmosphere by convective ventilation. The magnitude of CH_4 consumption in air-filled caves is largely controlled by seasonal variations in air temperature and humidity, which control cave gas exchange (Waring et al., 2017; Nguyễn-Thùy et al., 2017). Regardless of seasonality, ventilation of the vadose zone and atmosphere indicates air-filled caves provide a net sink for atmospheric CH_4 .

Methanogenic and methanotrophic microbial communities have also been identified in phreatic portions of karst aquifers (e.g., Brankovits et al., 2017), although less is known about their occurrence. Possible sites where microbes may reside in phreatic portions of karst aquifers include microbial mats growing on conduit surfaces, in the aquifer matrix porosity where water exchanges with the conduit system providing nutrients for the microbial communities, or in sediment deposits within conduits. Nonetheless, activities of both methanotrophic and methanogenic microbes are shown by changes in CH_4 concentrations and $\delta^{13}\text{C-CH}_4$ at the Sink-Rise system and during the spring reversal sampled at Madison Blue Spring.

Unlike direct removal of CH_4 in karst vadose zones (Ojeda et al., 2019; Nguyễn-Thùy et al., 2017; Waring et al., 2017; Webster et al., 2016; Matthey et al., 2013), methanotrophy and methanogenesis in the carbonate CZ phreatic zone occur within groundwater, complicating exchange of dissolved and atmospheric CH_4 . Rather than loss to the atmosphere or vadose zone, methanotrophy in the phreatic zone only lowers the amount of CH_4 available to degas from supersaturated waters. Conversely, concentrations below equilibrium with the atmosphere (Fig. 2) will create a CH_4 sink through dissolution of atmospheric CH_4 . Groundwater that reaches the surface supersaturated with CH_4 by methanogenesis and degasses, such as across the water table at the base of the vadose zone or directly to the atmosphere at springs or during water abstraction, represents an atmospheric source but at rates dominated by flow through the aquifer and to the surface. The link between flow and magnitude of the atmospheric CH_4 sink or source from the phreatic portion of carbonate CZ complicates evaluation of the net flux to and from the atmosphere. Nonetheless, the magnitude of the flux is key to evaluate the role of the carbonate CZ to global CH_4 cycling and thus global climate.

5. Conclusions

This study presents a first evaluation of the occurrence and fate of CH₄ in the carbonate CZ, where extensive surface water-groundwater exchange impacts CH₄ cycling. This exchange alters redox conditions where point recharge of surface water introduces OC and DO to groundwater. Redox reactions include methanogenesis, which elevates CH₄ concentrations in settings with subsurface residence times on the scale of months and where groundwater has little point recharge and decadal long residence times. Methanotrophy occurs where sinking streams continuously recharge to the aquifer surface water with DO concentrations in equilibrium with atmospheric oxygen. Although groundwater may have elevated CH₄ concentrations, methanotrophy occurs as groundwater flows to springs, reducing CH₄ concentrations in some spring water to values undersaturated with respect to atmospheric CH₄ concentrations. Where springs are recharged by surface water during flooding, methanotrophy and methanogenesis occur sequentially because of the lag in generating reducing conditions favorable for methanogenesis. Atmospheric fluxes of CH₄ from springs that discharge water supersaturated with respect to atmospheric CH₄ concentrations are similar to those from springs discharging from siliciclastic settings. Emissions from Floridan aquifer springs are small compared to major atmospheric CH₄ sources from wetlands and lakes. However, the net impact of methanotrophy and methanogenesis on CH₄ cycling in the carbonate CZ will depend on variations in characteristics across the entire carbonate CZ. Particularly important characteristics are the differences in the magnitude and rates of surface water-groundwater exchange and residence time of water in the aquifer.

CRedit authorship contribution statement

Andrew Oberhelman: Conceptualization, Methodology, Formal analysis, Data curation, Investigation, Writing – original draft, Writing – review & editing, Visualization, Funding acquisition, Project administration, Resources, Supervision. **Jonathan B. Martin:** Conceptualization, Resources, Writing – review & editing, Visualization, Supervision, Funding acquisition. **Madison K. Flint:** Conceptualization, Writing – review & editing, Visualization, Project administration, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data is available on HydroShare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.165645>.

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