Effects of surface water interactions with karst groundwater on microbial biomass, metabolism, and production

Adrian Barry-Sosa[1], Madison K. Flint[2], Justin C. Ellena[1], Jonathan B. Martin[2], Brent C. Christner[1]

[1]Department of Microbiology and Cell Science, University of Florida, Gainesville, 32611, USA
5 [2]Department of Geological Sciences, University of Florida, Gainesville, 32611, USA

Correspondence to: Brent C. Christner (xner@ufl.edu)

Abstract. Unearthing the effects of surface water and groundwater interactions on subsurface biogeochemical reactions is crucial for developing a more mechanistic understanding of carbon and energy flow in aquifer ecosystems. To examine physiological characteristics across groundwater microbial communities that experience varying degrees of interaction with
10 surface waters, we investigated ten springs and a river sink and rise system in North Central Florida that discharge from and/or mix with the karstic Upper Floridan Aquifer (UFA). Groundwater with longer residence times in the aquifer had lower concentrations of dissolved oxygen, dissolved and particulate organic carbon, and microbial biomass, as well as the lowest rates of respiration (0.102 to 0.189 mg O_2 L^{-1} d^{-1}) and heterotrophic production (198 to 576 µg C L^{-1} d^{-1}). Despite these features, oligotrophic UFA groundwater (< 0.5 mg C L^{-1}) contained bioavailable organic matter that supported
15 doubling times (14 to 62 h) and cell specific production rates (0.0485 to 0.261 pmol C $cell^{-1}$ h^{-1}) comparable to those observed for surface waters (17 to 20 h; 0.105 to 0.124 pmol C $cell^{-1}$ h^{-1}). The relatively high specific rates of dissimilatory and assimilatory metabolism indicate a subsurface source of labile carbon to the groundwater (e.g., secondary production and/or chemoautotrophy). Our results link variations in UFA hydrobiogeochemistry to the physiology of its groundwater communities, providing a basis to develop new hypotheses related to microbial carbon cycling, trophic hierarchy, and
20 processes generating bioavailable organic matter in karstic aquifer ecosystems.

1 Introduction

Groundwater is a vital natural resource that sustains aquatic ecosystems and provides approximately half of the water used globally for agriculture and human consumption (Jasechko and Perrone, 2021)(Jasechko and Perrone, 2021)(Jasechko and Perrone, 2021). Information on microbial biogeochemical reactions that affect organic matter degradation, nutrient cycling,
25 and the transformation of contaminants in aquifers is therefore highly relevant for understanding the processes contributing to groundwater quality. Moreover, knowledge of groundwater food webs is necessary for enabling meaningful assessments of their resilience to human impacts and environmental change. Aquifers vary in lithology and characteristics such as permeability, depth, and water storage capacity. In the case of kKKarst terrain, which covers ~15 % of Earth's ice-free land surface (Goldscheider et al., 2020)(Goldscheider et al., 2020)(Goldscheider et al., 2020) and represents the largest reservoir
30 of carbon on the Earth with potential impacts on the carbon and climate cycle (Martin, 2017). In the case of karst aquifers, , and the dissolution of the underlying carbonate rock, which represents the largest reservoir of carbon on the Earth with

fractures; and conduits from a few cm to tens of m in diameter ~~-~~; Worthington et al., 2000)~~(Worthington et al., 2000)))(Worthington et al., 2000)~~. Because the hydrogeological features of karst aquifers provide opportunities for rapid and direct exchange between surface waters and groundwater, they are extremely vulnerable to surface contaminants (e.g., Kalhor et al., 2019)~~(Kalhor et al., 2019)(Kalhor et al., 2019)~~.

Studies of karstic aquifers from around the world have shown their groundwaters to be generally oligotrophic (< 0.5 mg C L^{-1}) and contain low standing stocks of microbial cells and biomass (Farnleitner et al., 2005; Wilhartitz et al., 2009, 2013; Hershey et al., 2018; Hershey and Barton, 2018; Malki et al., 2020, 2021)~~(Farnleitner et al., 2005; Hershey et al., 2018; Hershey and Barton, 2018; Malki et al., 2020, 2021; Wilhartitz et al., 2009, 2013)(Farnleitner et al., 2005; Wilhartitz et al., 2009, 2013; Hershey et al., 2018; Hershey and Barton, 2018; Malki et al., 2020, 2021)~~. While these properties imply energy- and growth-limited conditions, new observations from karst aquifers are needed to decipher the biogeochemical contributions and rates of carbon metabolism by microbes in their groundwaters. Supplies of organic matter for subsurface microbial activity include photosynthetically derived material originating from the surface (e.g., Jin et al., 2014)~~(Jin et al., 2014)(Jin et al., 2014)~~ and ~~that produced~~ in situ production via chemosynthesis. The current paradigm for karstic aquifers is that biogeochemical reactions are chiefly driven by the oxidation of surface-derived organic matter (Hershey and Barton, 2018)~~(Hershey and Barton, 2018)(Hershey and Barton, 2018)~~. Based on this assumption, rates of carbon cycling and microbial growth should depend on the nature of the organic matter pool, presence of suitable electron acceptors, and residence time of groundwater. Though secondary production (i.e., heterotrophic production of biomass) may be the dominant component of subsurface carbon and energy flow, the data available to assess rates of organic carbon recycling and heterotrophic growth in karst aquifer ecosystems are sparse. Remarkably, studies that have compared the chemistry of organic matter in surface water to groundwater have shown higher hydrogen to carbon ratios that indicate the groundwater contains more labile forms of organic carbon (McDonough et al., 2022)~~(McDonough et al., 2022)(McDonough et al., 2022)~~. Sources of labile carbon to karstic groundwater remain poorly characterized; however, recent ~~global~~ estimates of global dark primary production in carbonate aquifer ecosystems (0.11 Pg C y^{-1}; Overholt et al., 2022)~~Overholt et al., 2022a))Overholt et al., 2022a~~ suggest chemoautotrophy may have a larger contribution than previously thought.

The Upper Floridan Aquifer (UFA) is one of the largest and most hydrologically productive karstic aquifers in the world, with an area of ~260,000 km^2 and depth of up to 500 m below the surface (Miller, 1986, 1997; Williams and Kuniansky, 2016)~~(Miller, 1986, 1997; Williams and Kuniansky, 2016) (Miller, 1986, 1997; Williams and Kuniansky, 2016)~~. Most of the UFA is confined by the Hawthorn Group, which consists of siliciclastic sands and clays interbedded with thin carbonate units (Scott, 1988)~~(Scott, 1988)(Scott, 1988)~~. At the erosional edge of the Hawthorn Group in northwest Florida, the

confining unit has been ~~removed by~~ eroded~~sion~~, promoting extensive surface water–groundwater exchange. The presence of numerous hydrological springs throughout this region provides direct access to groundwater that has had varying degrees of interaction with surface water and an experimentally tractable system to investigate the effects of hydrogeochemistry on microbial biomass and ~~community~~ metabolism. Previous work has shown that groundwater ~~(Katz et al., 2001; Duarte et al., 2010; Flint et al., 2021)~~Rresidence time is inversely proportional to concentrations of dissolved oxygen (~~a key factor, with~~ DO) and dissolved organic carbon (DOC; ~~concentrations decreasing at locations with longer residence times~~ Martin and Gordon, 2000; Martin et al., 2016; Oberhelman et al., 2024)~~(Martin et al., 2016; Martin and Gordon, 2000; Oberhelman et al., 2024)I~~ ~~;~~ however, the effects of hydrogeochemistry on microbial physiology and production in the groundwater are not known. Importantly, interactions with surface waters ~~can~~that ~~dramatically~~ increase ~~the~~ groundwater DO and DOC ~~and DO~~ concentrations can shift groundwater ~~, altering the~~ redox ~~balance in the system~~state and pH, which in turn ~~has~~alter ~~significant effects on the aquifer's~~ biogeochemical reactions in the aquifer~~stry~~ (Brown et al. 2014). For instance, microbial production of the greenhouse gas~~es production, particularly~~ nitrous oxide (Flint et al., 2021, 2023)~~(Flint et al., 2021, 2023)(Flint et al., 2021, 2023)~~ and methane (Oberhelman et al., 2023)~~(Oberhelman et al., 2023)(Oberhelman et al., 2023) has been~~are known to be enhanced by surface water mixing into phreatic cave systems of the UFA. ~~observed in previous studies. In both cases, concentrations increase at locations of frequent mixing, suggesting changes in the system biogeochemical dynamics (Flint et al., 2021, 2023; Oberhelman et al., 2023).~~

In this study, we tested the hypothesis that groundwater residence time and the availability and quality (i.e., composition, chemical structure, and nutrient content) of organic matter correlate with microbial biomass, metabolism, and production. This concept was examined by studying ten locations (Fig. 1) that discharge groundwater with differing organic matter characteristics and estimated residence times ranging from ~18 h to ~40 years (Martin and Dean, 1999; Martin et al., 2016)~~(Martin et al., 2016; Martin and Dean, 1999)(Martin and Dean, 1999)~~. The effects of mixing with surface water bodies were studied by sampling water from springs that reverse flow under flood stage conditions (Gulley et al., 2011)~~(Gulley et al., 2011)(Gulley et al., 2011)~~ as well as the Santa Fe River before and after it emerges from the subsurface after ~~~6 km~~ conduit flow and interaction with groundwater (Moore et al., 2009)~~conduit flow and interaction with groundwater (Moore et al., 2009)conduit flow and interaction with groundwater (Moore et al., 2009) of conduit flow and interaction with groundwater~~. Our results ~~from the UFA microbial communities~~ furnish new insight on microbial carbon flow and partitioning over an

energy gradient ranging from nutrient-rich surface waters to oligotrophic groundwaters that had been stored in the ~~aquifer~~ UFA for decades.

95 **2 Materials and methods**

2.1 Site description

Water from 10 springs and a river sink-rise system (O'Leno State Park) in North Central Florida (Fig. 1) were sampled between December 2018 and December 2022 (Table S1). Based on the geochemical properties of the groundwater discharged from the springs and extent of interaction with surface waters, the sites investigated can be classified into three
100 groups (Flint et al., 2021)~~(Flint et al., 2021)(Flint et al., 2021)~~: (1) the Ichetucknee springs group, which includes Blue Hole Spring ~~(BH)~~, Head Spring ~~(HS)~~, Mission Spring ~~(MS)~~, Coffee Spring ~~(CS)~~ and Devil´s Eye Spring ~~(DE)~~; (2) the reversing springs group, which includes Madison Blue Spring ~~(MB)~~, Peacock Springs ~~(PKS)~~, Little River Spring ~~(LRS)~~, and Gilchrist Blue Springs ~~(GBS)~~; and (3) the Santa Fe River sink-rise system, where the River Sink ~~(RS)~~ and the River Rise ~~(RR)~~ are located (Fig. 1). ~~We define t~~The rRiver sssink-rise system ~~a~~is a ~~a~~ swallet or opening where the Santa Fe~~a~~ rRiver directly
105 recharges water-filled caves and that is dischargesd to the surface ~~at a point~~ ~6 km downstream~~resurgence where river water disappears underground through a sinkhole, circulates underground through a series of karts conduits and reappears back into the surface through a resurgence~~. Blue Hole Spring, Madison Blue Spring, and River Rise are first magnitude springs (discharge > 2.8 m^3 s^{-1}); Gilchrist Blue Springs (vents 1 and 2), Little River Spring, Head Spring, and Devil's Eye Spring are second magnitude (discharge 2.8 – 0.28 m^3 s^{-1}); and Peacock Springs, Mission Spring, and Coffee Spring are third magnitude
110 (discharge < 0.28 m^3 s^{-1}).

Springs of the Ichetucknee springs group (blue symbols in Fig. 1) show low temporal geochemical variability, are located far from point recharge of sinking streams ~~point recharge of sinking streamsthe main recharge area~~, and have a low degree of mixing with surface waters (Martin et al., 2016; Henson et al., 2017)~~(Henson et al., 2017; Martin et al., 2016)(Martin et al.,~~
115 ~~2016; Henson et al., 2017)~~. The Ichetucknee springs group is comprised of two subgroups, with groundwater from springs in subgroup I (Head Spring, Blue Hole Spring, and Coffee Spring) having shorter apparent ages (~30 years) and higher average dissolved oxygen (DO; ~3 mg L^{-1}) in comparison to springs in subgroup II (Mission Spring and Devil´s Eye Spring), which have apparent ages of ~40 years and average DO concentrations of ~0.5 mg L^{-1} ~~(Martin and Gordon, 2000)(Martin and Gordon, 2000)~~(Martin and Gordon, 2000; Martin et al., 2016). The Ichetucknee springs group discharge water draining a 960
120 km^2 springshed (Katz et al., 2009)~~(Katz et al., 2009)(Katz et al., 2009);~~; approximately half of this area is located within the unconfined portion of the UFA, and the watershed is dominated by diffusive recharge with minimal point recharge. Approximately half of the landscape in the Ichetucknee springshed is forested and about one quarter is used for agriculture (Katz et al., 2009)~~(Katz et al., 2009)(Katz et al., 2009)~~.

125 When water levels of the spring vents in the reversing springs group (Peacock Springs, Madison Blue Spring, Little River Spring, and Gilchrist Blue Springs; green symbols in Fig. 1) are higher than that of the receiving water, these springs discharge clear, fresh groundwater to spring runs. However, increases in the river stage during periods of high discharge may raise surface water elevations above groundwater heads, causing a reversal of spring flow direction that injects DO- and ~~dissolved organic carbon~~ (DOC~-~)-rich surficial waters into subsurface conduits hydraulically connected to the spring vent

130 (Gulley et al., 2011)~~(Gulley et al., 2011)(Gulley et al., 2011)~~. At Madison Blue Spring, the river water intrusions have been documented at distances of at least 1 km from the vent (Brown et al., 2014)~~(Brown et al., 2014)(Brown et al., 2014)~~. Depending on rainfall and the spring's physiographic characteristics, the frequency of reversals for a given spring can vary from a few times a year to once every few years, and the effects of reversals on groundwater chemistry have been shown to persist for a period of at least 1 month (Brown et al., 2014, 2019; Oberhelman et al., 2023, 2024)~~(Brown et al., 2014, 2019;~~

135 ~~Oberhelman et al., 2023, 2024)(Brown et al., 2014, 2019)~~.

In the Santa Fe Sink and Rise system (orange symbols in Fig. 1), the Santa Fe River flows over a confined portion of the UFA to a region that is unconfined; this boundary is a topographic feature known as the Cody Scarp (Puri and Vernon, 1964)~~(Puri and Vernon, 1964)(Puri and Vernon, 1964)~~. At ~~the~~ River Sink, river water enters a sinkhole, flows underground

140 for approximately 6 km through anastomosing water filled caves (herein called conduits) that have been ~~extensively~~ partially mapped by cave divers, and reemerges at ~~the~~ River Rise, a first magnitude spring (Fig. 1) (Moore et al., 2009)~~(Moore et al., 2009)(Moore et al., 2009)~~. Water transit times through the conduit system from the River Sink to the River Rise are estimated to be from 18 h to six days, depending on river stage (Martin and Dean, 1999)~~(Martin and Dean, 1999)(Martin and Dean, 1999)~~. At lower rates of river discharge (i.e., < ~15 m^3 s^{-1} at River Rise), there is a net gain of water in the conduits

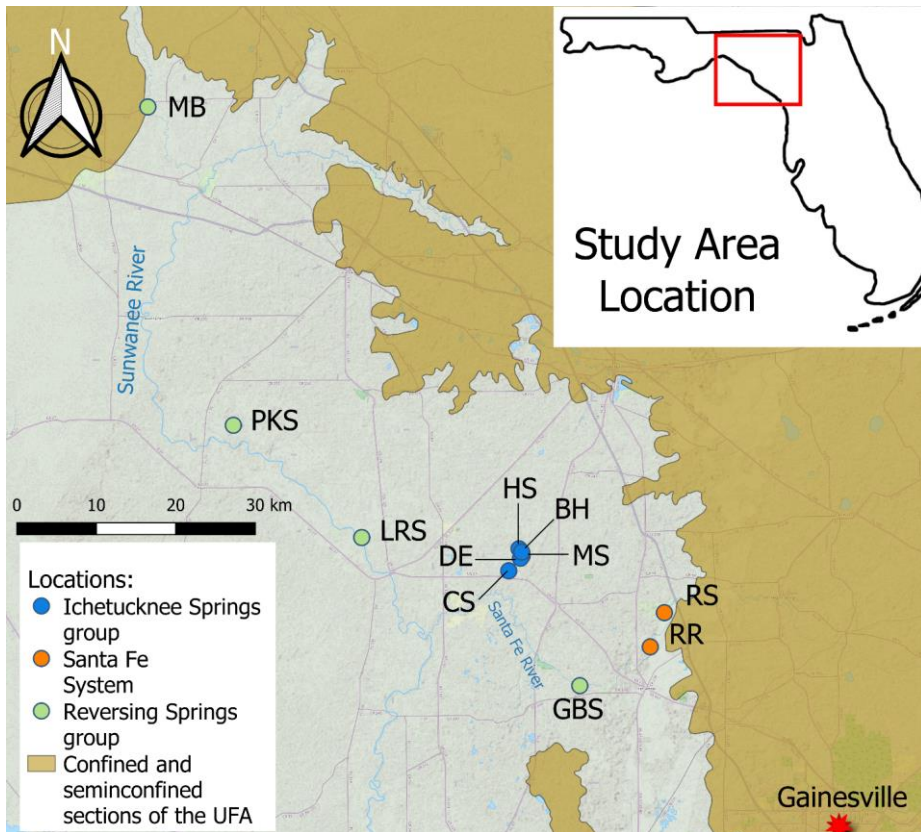
145 from groundwater that flows from the rock matrix porosity (Martin and Dean, 2001; Flint et al., 2023)~~(Flint et al., 2023; Martin and Dean, 2001)(Martin and Dean, 2001; Flint et al., 2023)~~. When discharge is > ~15 m^3 s^{-1} at River Rise, river water in the conduits flows into the matrix to recharge the aquifer. For simplicity, we subsequently use the descriptors 'low' and 'high' flow to ~~distinguish between~~separate samples collected ~~during these contrasting hydrological conditions~~when discharge is < or > 15 m^3 s^{-1} at River Rise, respectively.

150

Figure 1. Locator map of the sites sampled in this study. MB: Madison Blue Spring; PKS: Peacock Spring; LRS: Little River Spring; DE: Devil's Eye Spring; HS: Head Spring; BH: Blue Hole Spring; MS: Mission Spring; CS: Coffee Spring. GBS: Gilchrist Blue Springs; RS: Santa Fe River Sink; RR: Santa Fe River Rise. The figure was generated using QGIS with base map data from © OpenStreetMap contributors 2021. Distributed under the Open Data Commons Open Database License (ODbL) v1.0.

155 **2.2 Groundwater sampling**

Spring discharge was sampled by performing hydrocasts from a canoe with a 5 L Niskin bottle (General Oceanics Inc., Miami, FL) or by pumping with a peristaltic pump (GeoTech) through PVC tubing led from the spring vent to the shore. Prior to deployment, the Niskin bottle was thoroughly cleaned with 10 % (v/v) bleach followed by thorough rinsing with autoclaved deionized (DI) water. The PVC tubing was decontaminated by circulating a solution of 5 % (v/v) hydrogen

160 peroxide for at least 1 minute and then rinsed with DI. Water samples collected in the Niskin bottle were carefully
transferred to sterile 100 ml BOD bottles (radioisotope incorporation analyses) or 40 ml serum bottles (DIC production and
oxygen consumption analyses) via clean silicon tubing that was inserted into the bottles. Each bottle was filled with sample
until no headspace remained and sealed with a glass or butyl stopper. To determine the initial DIC concentration, the water
sample was filtered using a 0.1 µm PVDF syringe filter (Merck Milllipore, Cork, Ireland) and collected in 20 ml glass DIC

165 bottles with no headspace. T~~For~~ transport to the laboratory was done within 4 h of collection, with~~the~~ samples ~~were~~ stored in
an insulated cooler that contained water from each location to minimize temperature changes prior to analysis.~~,~~ ~~which was~~
~~always within 4 h of collection.~~

The POC and δ^{13}C isotopic composition of POC ($\delta^{13}C_{POC}$) were analyzed using samples collected on 25 mm glass fiber

170 filters (GF/F; Whatman). All sampling equipment was thoroughly cleaned with detergent and soaked overnight in 15 % HCl,
followed by rinsing six times with Milli-Q water. Residual organic carbon on the tweezers and glass fiber filters was
removed by combustion at 450 ° C for 4 h in a muffle furnace. Particles were purged from the tubing by pumping spring
water through it for several minutes before attaching the filter housing and collecting the particulate samples. After filtration,
the samples were kept chilled upon return to the laboratory and stored at -20°C until processed.

175 **2.3 Water properties and organic carbon content**

Water temperature, pH, ORP, specific conductivity, dissolved oxygen, turbidity, and depth data were obtained with a
ProDSS multiparameter meter (YSI Inc.) that was calibrated prior to deployment. The data presented were collected at
depths that were in the immediate vicinity and as close as possible to the vent discharge point.

180 DOC concentrations were measured on a Shimadzu TOC-V CSN total carbon analyzer. Three-dimensional fluorescence
spectra were obtained using a Hitachi F-7000 Fluorescence Spectrophotometer across an excitation and emission range from
240–450 nm (5 nm intervals) and 250–550 nm (2 nm intervals), respectively. Three variables were parameterized from the
spectral matrix data to describe organic matter quality: HIX (Humification index), BIX (Biological index) and FI
(Fluorescence index). When A HIX values are above ~~differentiates between organic matter with humic characteristics (>~~ 16,

185 ~~indicates~~ the presence of complex mixtures of organic molecules with low H/C ratios and higher molecular weights are
inferred, whereas a ~~HIX~~values below 4 indicates ~~)~~ ~~compared with~~ less humic characteristics that ~~approximat~~eing
autochthonous aquatic bacterial ~~origin~~ (< 4). ~~;~~ BIX differentiates dissolved organic matter of allochthonous (≤ 0.6) and
autochthonous (~0.8–1.0) origin, while ~~;~~ ~~and~~ FI distinguishes fulvics of terrestrial origin (≤ 1.4) from those produced by
microbes (≥ 1.9) (Flint et al., 2023)~~(Flint et al., 2023)(Flint et al., 2023)~~.

190

Filters for POC, $\delta^{13}C_{POC}$, and the procedural blanks were decarbonated for 3 h under HCl fumes, dried at 40 °C, and stored at
-20 °C until analyzed. Measurements for POC and the $\delta^{13}C_{POC}$ were made using a Thermo Electron DeltaV Advantage

isotope ratio mass spectrometer coupled with a ConFlo II interface linked to a Carlo Erba NA 1500 CNHS Elemental Analyzer. All carbon isotopic data are expressed in standard delta notation relative to Vienna Peedee Belemnite (VPDB).

2.4 Cell and biomass concentration

Estimates of cell concentration were performed with 15 ml water samples that were preserved with formalin (final concentration 4 % v/v) immediately after collection. The fixed samples were subsequently vacuum filtered (< 23.7 kPa) through black 25 mm 0.2 µm pore size Isopore polycarbonate filter (Millipore) and a 0.45 µm pore size nitrocellulose backing filter (Whatman). DNA-containing cells on the filters were stained for 15 minutes with a $25\times$ SYBR Gold stain (Invitrogen) solution that was diluted in 0.2 µm-filtered TBE (Tris Borate EDTA). After staining, the filter towers were washed with clean TBE, vacuum was applied to filter the excess material, and the filters were mounted on glass microscope slides with a drop of a 1:1 antifade solution (0.1 % w/v phenylenediamine:glycerol). The stained cells were visualized and digitized using a Nikon Eclipse Ni-E epifluorescence microscope equipped with a C-FL GFP filter set (excitation 450–490 nm; dichroic mirror 495 nm; emission 500–550 nm) and 4.2 megapixel Zyla 4.2 PLUS camera.

For each sample, digital images were obtained for 40 random fields of view. To maximize depth of field for the analysis, a z-stack of 40 images was captured in 0.4 µm intervals (z-range of 16 µm) and compiled into a single image file for each field of view. The number, size, and shape of epifluorescent cells in the acquired images were determined by software-assisted tracing and individual observations were measured using NIS-Elements AR v4.51.00 (Nikon Inc.). The number of cells were determined based on the "brightest pixel detection" algorithm. For shape and size, a uniform "threshold cut-off" value was not applied to all samples due to differences in background fluorescence among the samples. Instead, light threshold and contrast were manually adjusted for each sample in tandem with histogram analysis of binned intensity data. This allowed visual verification that the threshold parameters selected were processing data collected from individually stained cells. Contiguous pixels with intensity values above the threshold cut-off were used to define a particle boundary, from which the area, circularity, equivalent diameter, major axis length, and minor axis length were calculated by the software. Spherical volume based on the major axis length was used to estimate biovolume following the assumption of equivalent diameter. Estimates of cell carbon using biovolume data were calculated according to Verity et al., 1992(Verity et al., 1992)(Verity et al., 1992) and their conversion of 0.36 pg C µm^{-3} for 10 µm^3 cells.

The 50 ml water samples for measurement of cellular adenosine triphosphate (ATP (Adenosine triphosphate) were collected in triplicate and sequentially filtered through 0.2 µm pore size Millex MF Millipore MCE membranes and 0.1 µm pore size Millex Durapore PVDF membrane filters using 60 ml syringes. Immediately upon return to the laboratory, the filters were stored at -20 ºC and processed less than 48 h after collection. ATP was extracted from cells on the filters using the BioTherma ATP Biomass Kit HS and following the manufacturer´s instructions except for the following modification: 500 µl of Extractant B/S was added to and used to extract ATP from each filter by purging the material into a test tube with an

air-filled sterile syringe. Subsequently, 100 µl of the extracted material was mixed with 400 µl of reconstituted ATP reagent HS and immediately measured using a Turner BioSystems E5331 luminometer. The data presented for each filtered sample are the average values from three technical replications. The amount of ATP in each sample was calculated as follows: $ATP_{smpl} = I_{smp} / (I_{smp} + std - I_{smpl})$, where ATP_{smpl} is the amount of ATP in the sample (in pmol), I_{smp} is the sample tube

230 intensity in relative luminosity units (RLUs), and $I_{smp} + std$ is the intensity (in RLUs) in the sample tube after adding 10 µl of the 10^{-7} mol L^{-1} internal ATP standard. ATP concentration was converted into carbon biomass based on a molar ratio 250:1 for C:ATP (Karl, 1980)(Karl, 1980)(Karl, 1980).

2.5 Microbial respiration

Rates of oxygen consumption and production of dissolved inorganic carbon (DIC) were determined at in situ temperatures
235 and calculated from the slope of linear regression models for concentration data obtained during time course experiments.

Oxygen concentration was measured in triplicate using stoppered 40 ml serum bottles containing OXSP5 oxygen sensor spots (Pyroscience) and no headspace. Killed controls were prepared in triplicate by amending samples with benzalkonium chloride to a final concentration of 0.01 % (w/v). Bottles were incubated in an Innova 44 incubator (New Brunswick) at 21º
240 C, except for samples collected at River Sink during low flow, which were incubated at the in situ temperature of 15º C. At daily intervals, oxygen concentration was measured using a calibrated FireSting®-O2 (1 channel) fiber-optical oxygen meter (Pyroscience). A serum bottle containing deionized water was incubated with the samples to serve as the temperature reference for the oxygen measurements.

245 Upon return to the laboratory, the sample bottles for measurement of DIC production were incubated a 21 º C (except River Sink during low flow, which was incubated at 15 º C). After 24, 48, and 96 h of incubation, triplicate bottles of the water were filtered through a 0.1 µm pore size syringe filter (Merck Milllipore, Cork, IRL) into clean 20 ml DIC vials. Vials were stored at 4 º C and measured within 2 weeks of terminating the experiment. Total DIC was measured using a UIC (Coulometrics) 5017 CO_2 coulometer coupled with an AutoMate automated carbonate preparation device (AutoMateFX,
250 Inc). Approximately 5 ml of sample was weighed into septum top tubes and placed into the AutoMate carousel. Acid and CO_2-free nitrogen carrier gas was then injected into the sample vial through a double needle assembly and evolved CO_2 is carried through a silver nitrate scrubber to the coulometer where total carbon is measured.

The respiratory quotient (RQ) was determined by dividing the molar rate of DIC production by the rate of O_2 consumption.
255 The average oxygen utilization rate (OUR) was calculated for paired samples at the Sink Rise system by subtracting the oxygen concentration at River Sink from the oxygen concentration at River Rise and dividing by the estimated residence time based on the hydrological stage (ranging fromassumed to be 18 h for high flow and tpand 6 days for low flow; Martin and Dean, 1999)Martin and Dean, 1999))Martin and Dean, 1999. For Head Spring and Devil's Eye Spring, OUR was

calculated by assuming the initial oxygen concentration when recharge occurred to be atmospherically equilibrated water at

260   21° C and sea level (8.5 mg L^{-1}).

2.6 ^{3}H-leucine and -thymidine incorporation

Radioassays consisted of 0.8 ml water samples that were placed into 1.5 ml microcentrifuge tubes and amended with 0.2 ml of a solution containing ^{3}H-thymidine (thymidine [Methyl-^{3}H], 50.8 Ci mmol^{-1} in sterile water) or ^{3}H-leucine (L-leucine [4, 5-^{3}H], 160 Ci mmol^{-1} in ethanol water 2:98; Perkin-Elmer). The final concentration for each was 20 nM, which corresponded

265   to 1 μCi per sample for the ^{3}H-thymidine assays and 3.2 μCi per sample for ^{3}H-leucine. Killed controls were prepared by adding 200 μl of 50 % (v/v) formalin to designated water samples. Six replicates per time point were established for the experimental and control groups. All samples were incubated at 21° C in the dark.

At designated time intervals during the experiment (48 h for Devil's Eye, Madison Blue and ~~the~~ River Rise at high flow; 24

270   h for all other samples)~~,~~ subsets of the samples were killed by adding 200 μl of 50 % (v/v) formalin and ~~storing~~ stored at 4° C. Acid insoluble macromolecules were precipitated by adding 200 μl of an ice-cold solution of 100 % (w/v) TCA followed by centrifugation at 15000 *x g* for 15 min. Samples were then sequentially washed with 5 % (w/v) TCA and 70 % (v/v) ethanol, with a 5 min centrifugation at 15000 *x g* after each wash. The washed pellets were suspended in 1 ml of scintillation cocktail (CytoScint, MP Biomedicals), the tubes were placed into scintillation vials, and radioactivity was measured on the

275   ^{3}H channel of a Beckman LS6500 scintillation counter for 10 min. To determine disintegrations per minute from the count per minute data, the counting efficiency was calculated using a quench curve and ^{3}H-Toluene standard (Perkin-Elmer) with a specific activity of 2.552 dpm g^{-1} (20 μl). The curve was based on data generated by mixing 20 ml of scintillation cocktail (CytoScint) with 42,335 dpm of ^{3}H-Toluene (MP Biomedicals)~~,~~ and 0 %, 0.25 %, 0.5 %, 1 %, 1.5 %, 2 %, 2.5 %, 4 % and 5 % acetone (v/v).

280

To convert ^{3}H-thymidine and ^{3}H-leucine incorporation rates to cell carbon and estimate heterotrophic production, standard conversion factors of 2.0 x 10^{18} cells mol^{-1} were used for ^{3}H-thymidine (Fuhrman and Azam, 1980)~~(Fuhrman and Azam, 1980)(Fuhrman and Azam, 1980)~~ and 1.42 x 10^{17} cells mol^{-1} for ^{3}H-leucine (Chin-Leo and Kirchman, 1988)~~(Chin-Leo and Kirchman, 1988)(Chin-Leo and Kirchman, 1988)~~. Cellular carbon content was based on values estimated from biovolume.

285   Bacterial growth efficiency (BGE) was calculated as the quotient between carbon incorporated into biomass and the sum of carbon incorporated into biomass plus that respired as DIC.

3 Results

3.1 Hydrogeochemistry

The groundwater discharged from sites in the Ichetucknee springs group (Head Spring, Blue Hole Spring, Coffee Spring, Devil's Eye Spring, and Mission Spring) had little interaction with surface waters during its subsurface residence time and these springs discharge continuously at rates that vary by less than a factor of three (Martin and Gordon, 2000; Katz, 2004)~~(Katz, 2004; Martin and Gordon, 2000)~~~~(Martin and Gordon, 2000; Katz, 2004)~~. In comparison, members of the reversing springs group (Madison Blue Spring, Gilchrist Blue Spring, Peacock Springs, and Little River Spring) experience reversal of flow when river water levels exceed groundwater heads, which is indicated by negative discharge. For instance, there were eight occasions from 2018 to 2022 when the stage of the Withlacoochee River resulted in flow reversal of Madison Blue Spring. We had opportunities to sample Madison Blue Spring and Peacock Springs during periods when they were reversing (Table 1 and Table S1) ~~as shown by the USGS gauging station (02319302), which is located on the Madison Blue Spring run, which~~ ~~where~~ ~~show reversals~~ are ~~indicated as negative discharge values and discharging flow as positive values~~. Observed DO concentrations during reversals (7.21 and 3.86 mg L^{-1}~~,~~ for Madison Blue and Peacock Springs, respectively) were at least twice as high as the values when discharge was positive. The samples we analysed from Madison Blue Spring for POC, DIC, oxygen consumption, and heterotrophic production were all collected in 2022 and at timeframes of 1 to 104 d after it had transitioned from negative to positive discharge.

The physical and chemical properties of groundwater discharged from the Ichetucknee and reversing springs groups showed minimal variation over 48 months of observation, and their clear (turbidity values near 0 FNU), circumneutral waters had an average temperature of 21.83 ± 0.07° C (n=65; ± the standard error; Table 1). There are statistically significant differences in specific conductance (SpC; ANOVA, $p < 0.05$), oxidation-reduction potential (ORP; $p < 0.001$), and DO concentration ($p < 0.001$) among the Ichetucknee and reversing springs groups. Discharge from Peacock Springs and Little River Spring had the highest SpC values, and Devil's Eye Spring and Mission Spring had the lowest DO concentration (average of 0.21 and 0.55 mg L^{-1}, respectively; Table 1). Water quality parameters from the Santa Fe Sink and Rise system ranged widely depending on hydrological conditions (Table 1). When groundwater was transported into the conduit during periods of low flow, water at River Rise had characteristics similar to ~~regional~~ matrix ~~ground~~water (i.e. water ~~stored and circulating through the aquifer rock porosity~~contained in matrix porosity; Moore et al. 2009). For example, temperature that tended to approach 21° C and SpC higher than Santa Fe River source waters. Variation in these physical water properties during periods of high flow coincided with increasing DOC concentration and HIX (Table 2), indicating that the distinct hydrological regimes in the ~~river~~ sink-rise system were associated with appreciable changes in the quantity and quality of organic matter.

Parameter	Ichetucknee Subgroup I			Ichetucknee Subgroup II		Reversing Springs Group							Santa Fe ~~ink-~~ Sink-Rise System	
Spring designation	HS (n=11)	BH (n=4)	CS (n=4)	DE (n=14)	MS (n=6)	GBS1 (n=5)	GBS2 (n=3)	PKS (n=6)	PKS (During reversal) (n=1)	LRS (n=4)	MB (n=8)	MB (During reversal) (n=1)	RS (n=9)	RR (n=13)
Water temperature (ºC)	21.71 ± 0.01	21.60 ± 0	21.85 ± 0.04	21.78 ± 0.01	21.70 ± 0	22.56 ± 0.03	22.50 ± 0.00	21.63 ± 0.03	25.7	21.93 ± 0.09	20.90 ± 0.02	15.70	14.7–27.10	15.7–26.30
pH	7.26 ± 0.04	7.42 ± 0.07	7.40 ± 0.07	7.28 ± 0.04	7.25 ± 0.07	7.24 ± 0.04	7.37 (n = 1)	7.35 ± 0.10	6.48	7.14 ± 0.06	7.46 ± 0.08	6.26	6.09–7.79	6.14–8.25
ORP (mV)	161 ± 6	169 ± 14	178 ± 7	143 ± 6	133 ± 8	189.95 ± 9.55	182.5 (n = 2)	116 ± 10	206	133 ± 7	210 ± 3	190	103–207	30.4–205
DO (mg L^{-1})	3.70 ± 0.01	1.46 ± 0.04	2.75 ± 0.14	0.21 ± 0.02	0.55 ± 0.04	4.60 ± 0.04	4.55 ± 0.02	1.84 ± 0.3	3.86	1.42 ± 0.06	1.62 ± 0.11	7.21	4.02–6.93	0.01–6.44
Sp. Cond. (µS cm^{-1})	346.0 ± 1.5	309.8 ± 1.9	301.0 ± 1.8	347.4 ± 1.2	323.5 ± 2.1	385.4 ± 2.6	390.6 ± 8.48	430.4 ± 2.8	64.3	417.1 ± 6.2	299.0 ± 2.7	76.9	71.8–319	78.2–532.3
Salinity (PSU)	0.166 ± 0.001	0.148 ± 0.001	0.142 ± 0.001	0.166 ± 0.001	0.153 ± 0.001	0.184 ± 0.001	0.187 ± 0.004	0.207 ± 0.002	0.03	0.200 ± 0.011	0.145 ± 0.001	0.040	0.030–0.150	0.040–0.260
TDS (mg L^{-1})	224 ± 1	201 ± 1	196 ± 1	226 ± 1	210 ± 1	250 ± 2	254 ± 5	280 ± 2	42	271 ± 4	195 ± 2	50	47–208	51–346
Turbidity (FNU)	0	0.25 ± 0.12	0.05 ± 0.03	0.31 ± 0.22	0.16 ± 0.10	0.07 ± 0.04	0	0	3.15	0.29 ± 0.10	0.28 ± 0.12	10.90	0.77–26.37	0.32–9.47
DIC (µg C g^{-1})	40.76 ± 0.05	N.d.	N.d.	39.3 ± 0.1	N.d.	N.d.	N.d.	N.d.	N.d.	N.d.	30.51 ± 0.03	N.d.	28.39 – 2.27	33.64 – 10.21
Depth to vent from surface (m)	4.90	11.42	0.71	3.56	1.29	5.47	5.22	6.01	8.53	3.92	7.2	13.26	1.30	11.13
Location (Lat, Long, WSG 84)	29.984º N 82.762º W	29.981º N 82.759º W	29.959º N 82.775º W	29.974º N 82.760º W	29.976º N 82.758º W	29.830º N 82.683º W	29.830º N 82.681º W	30.122º N 83.133º W	30.122º N 83.133º W	29.997º N 82.966º W	30.481º N 83.244º W	30.481º N 83.244º W	29.912º N 82.573º W	28.874º N 82.591º W

Table 1. Summary of basic physical and geochemical data collected from each location sampled from 2018 to 2022.
ORP: Redox potential; DO: Dissolved oxygen; Sp. Cond.: Specific conductivity; TDS: Total dissolved solids; and N.d.: No data.

Results of a one-way ANOVA and post-hoc Tukey HSD analysis showed POC concentration was significantly higher at locations with the highest surface water influence ~~the~~ (River Sink and River Rise; ~~) in comparison to the other sites sampled~~ ~~(~~ANOVA; p < 0.001, F = 22.007; Fig. 2 a). Head Spring, Devil's Eye Spring, and Madison Blue Spring had the lowest

concentrations of POC (< 0.03 mg C L^{-1}; ANOVA, $p < 0.001$, F = 22.007) and DOC (0.2 to 0.4 mg C L^{-1} (Flint et al., 2021)(Flint et al., 2021)(Flint et al., 2021). The heaviest δ^{13}C value for POC was observed at Madison Blue Springs (average of -28.7 ± 0.3 ‰), which was enriched by approximately 2 ‰ and 4 ‰ relative to locations with less surface water influence [(that for Devil's Eye Spring (; (-31.6 ± 0.3 ‰) and) and Head Spring (; (-32.5 ± 1.4 ‰), respectively;), respectively (Fig. 2 b).]. Though average values for δ^{13}C$_{POC}$ were isotopically lighter in samples from the Santa Fe Sink and Risesink-rise system during low flow, the differences are not statistically significantly different from those at high flow (Fig. 2 b).

Dissolved organic matter with HIX values below 10, BIX above 0.8, and FI above 1.9 is generally assumed viewed to be of high quality (e.g., Flint et al., 2023)(Flint et al., 2023)(Flint et al., 2023). HIX values indicate thatt the springs with the largest influence ofoldest groundwater (the Ichetucknee springs group) had the lowest humic content, and higher values for FI and BIX imply the organic matter was of higher quality relative to the other springs sampled (Table 2). Though much higher concentrations of DOC were observed in water from the river

sink-rise system (Flint et al., 2023)(Flint et al., 2023)(Flint et al., 2023), the HIX, FI, and BIX values are indicative of lower quality organic matter. Higher concentrations of DOC and POC were observed during periods of high flow at River Sink, but there iwas not a statistical difference in POC concentration with flow stage at River Rise (Fig. 2 a).

Sink-Rise System	Ichetucknee Subgroup I	Ichetucknee Subgroup II	Reversing Springs Group	Sink-Rise System			
	HS	DE	MB*	RS	RS-H	RR	RR-H
Subsurface residence time	~30 y[f]	~40 y[f]	N.d.	N.a.	N.a.	~6 d[e]	~18 h[e]
DOC (mg C L⁻¹)[a]	0.2–0.4	<0.1–1.0	0.3–14.4	3.9—30.1	24.6—58.8	2.4—23.5	21.5—50.2
HIX[b]	1.90–4.40	2.52–13.61	5.07–20.21	8.28—24.46	16.06—30.45	13.42—23.77	17.40—30.40
BIX[b]	0.77–0.94	0.55–0.75	0.47–0.76	0.42—0.53	0.40—0.45	0.46—0.54	0.40—0.47
FI[b]	1.84–2.04	1.54–1.81	1.44–1.71	1.37—1.51	1.34—1.43	1.44—1.51	1.35—1.43
Biovolume cell carbon (fg C cell⁻¹)	64.9 ± 0.7	52.2 ± 0.9	105 ± 4.9	36.3 ± 0.1	N.d.	50.1 ± 0.2	N.d.
OUR (mg L⁻¹ d⁻¹)	0.0004	0.0006	N.d.	N.a.	N.a.	0.5517	6.4133
O₂ consumption (mg L⁻¹ d⁻¹)[c]	0.19 ± 0.01	0.10 ± 0.01	0.17 ± 0.02 0.10 ± 0.01 N.d. 0.19 ± 0.03	0.18 ± 0.02	0.32 ± 0.01 0.319± 0.006	0.137 ± 0.008	0.38 ± 0.02
DIC production (mg C L⁻¹ d⁻¹)[c]	BLD	BLD	N.d. 0.09 ± 0.01 N.d. 0.19 ± 0.03	0.36 ± 0.07	0.096 ± 0.009 0.08 ± 0.01	0.49 ± 0.05	0.16 ± 0.03
Respiratory Quotient	N.d.	N.d.	N.d. 1.23 N.d. 0.62	2.66	0.39 0.35	4.85	0.56
Heterotrophic production (µg C L⁻¹ d⁻¹)[d]	576 ± 89	198 ± 22	60 ± 6 N.d. 42 ± 2 90 ± 11	4653 ± 88	18328 ± 110	1284 ± 85	14225 ± 343
Cell-specific heterotrophic production (pmol C cell⁻¹ h⁻¹)[d]	0.2607	0.0485	0.0056 N.d. 0.0071 0.0262	0.1242	0.1046	0.0135	0.1135
Specific growth rate (d⁻¹)[d]	1.16	0.27	0.02 N.d. 0.02 0.07	0.99	0.83	0.08	0.65
Doubling time[d] (h)	14	62	1028 N.d. 856 232	17	20	215	25
BGE[d] (%)	87[g]	81[g]	N.d. N.d. 39.3 32.1	92.9	99.5	72	98.9

Leu:TdR			0.42 N.d. 0.29				
	6.24	2.72	0.44	3.10	3.56	3.86	4.06

Table 2. DOC concentration and quality, biogeochemical data, and microbial physiological properties derived from the rate data. RS-H and RR-H: River Sink and Rise under high flow conditions, respectively. [a] Data from Flint et al., 2021; [b] DOC quality data were collected between August 2018 through March 2022. [c] Error (±) is based on the slope uncertainty and not the standard error; [d] Based on ^{3}H-leucine incorporation and DIC production rates; [g] Based on the oxygen consumption data and a theoretical RQ = 1.2 (Berggren et al. 2012); Residence time data are from [e] Martin and Dean, 1999 and [f] Martin et al., 2016. Multiple values for a given parameter represent independent measurements from different dates in chronological order (see Table S1), and for Madison Blue Spring, these samples correspond to 51, 1, 104, and 91 d, respectively, after a reversal. N.d.: no data. N.a.: Not applicable; BLD: Below the level of detection of this analytical procedure.

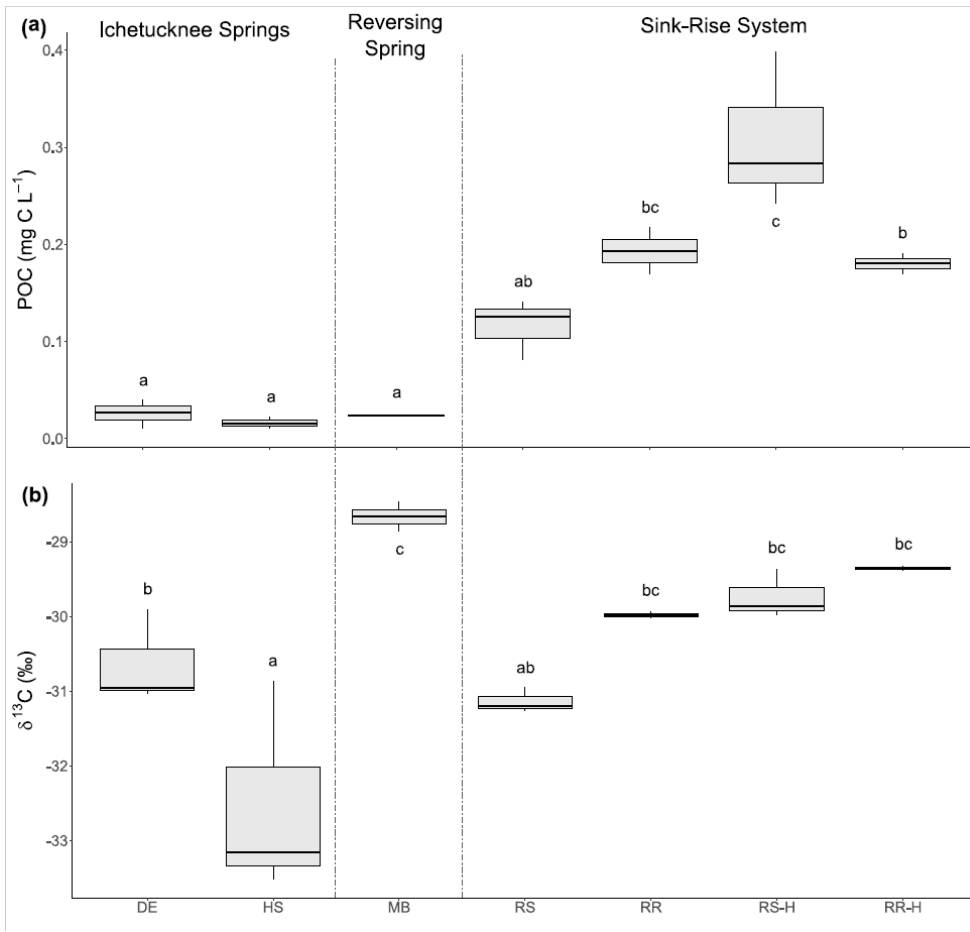
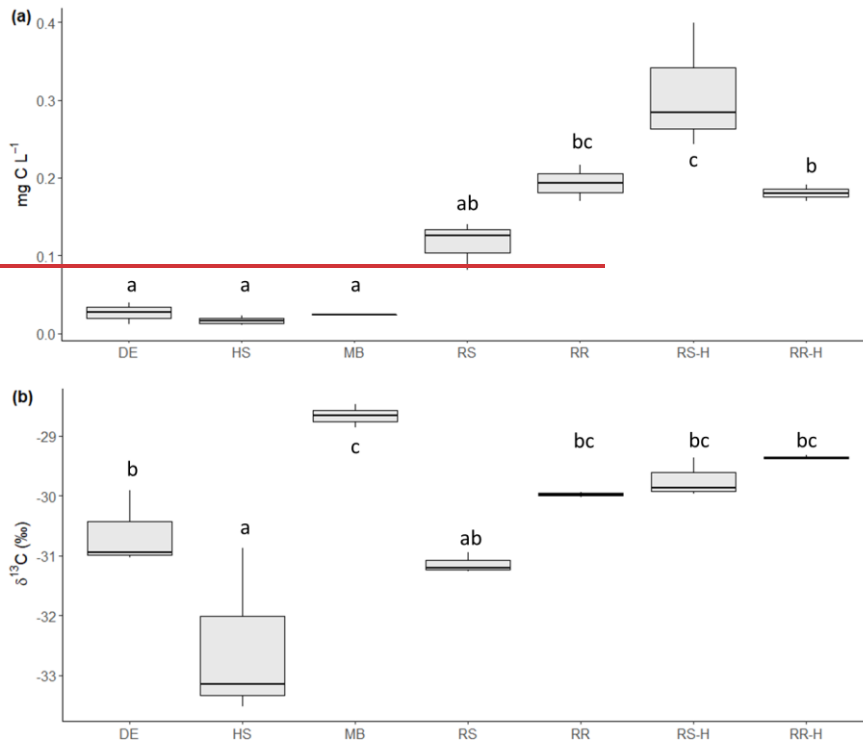



Figure 2. POC concentration (a) and the $\delta^{13}C$ of POC (b) in samples from the springs and sink-rise system. DE: Devil's Eye Spring; HS: Head Spring; MB: Madison Blue Spring; RS: Santa Fe River Sink; RR: Santa Fe River Rise. Lowercase letters indicate the significance groups based on a Tukey HSD test. RR-H and RS-H are samples taken during high flow at the sink-rise system.

3.2 Microbial Cell and Biomass Concentrations

The results of a one-way ANOVA ($p < 0.001$, $F = 28.35$) indicate significant differences in cell concentration means among analyzed samples from the ten springs and ~~river~~ the sink-rise system ~~analyzed~~ (Fig. 3 a). The lowest cell concentrations were observed in samples from the Ichetucknee springs group ~~(largest influence of groundwater)~~, which ranged from $4.83 \pm 0.50 \times 10^6$ cells L^{-1} at Blue Hole Spring to $1.29 \pm 0.05 \times 10^7$ cells L^{-1} at Devil's Eye Spring. ~~Springs that had periodically experienced flow reversal~~Reversing springs (i.e., Madison Blue Spring, Peacock Springs, and Little River Spring) generally had higher average cell concentrations at baseflow than those in the Ichetucknee springs group, but only samples from Little River Spring were significantly higher (Tukey HSD test, $p < 0.001$). Cell concentration means at locations with the largest

360 surface weaterwater contributions (i.e. for the River Sink and River Rise: (6.08 ± 0.8 and 4.35 ± 0.4 × 10^8 cells L^{-1},

respectively) are significantly higher (p < 0.001) than all other samples except those from Little River Spring and Madison

Blue Spring when high river stage had during reversed spring flow (Fig. 3 a).

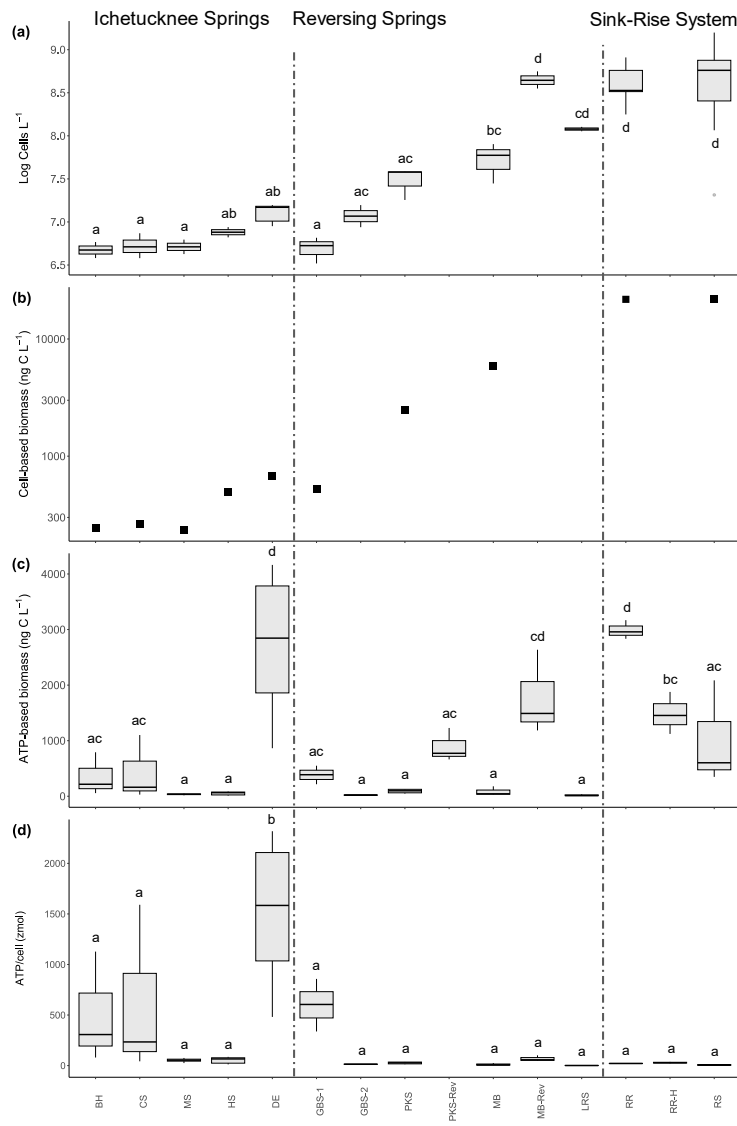
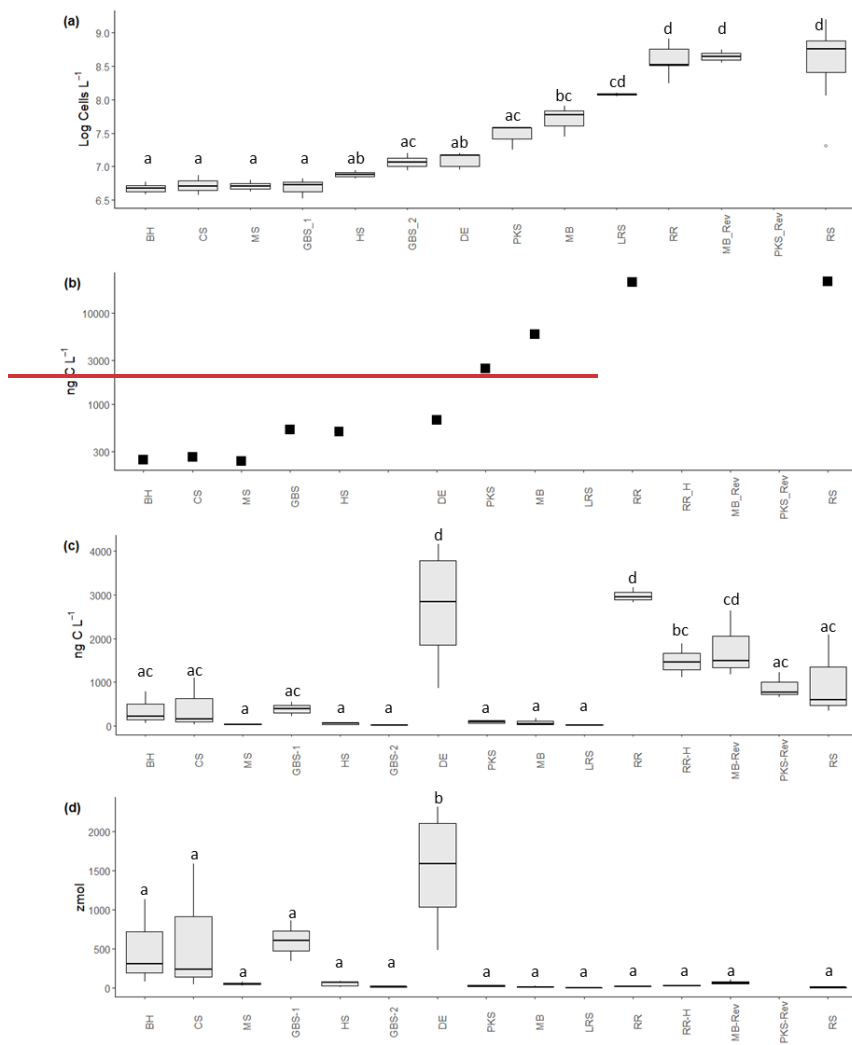



Figure 3. Cell abundance and biomass estimates in samples collected from various springs and sink-rise system. (a) Log10-transformed data for cell abundance. ~~Sample order in panels (a)–(d) is based on sites with increasing mean cell concentrations based on direct epifluorescent microscopic counts.~~ (b) Microbial biomass based on cell volumetric data derived from microscopic observations. (c)

Microbial biomass based on the concentration of cellular ATP retained in the 0.2 µm fraction. (d) ATP concentration per cell (in zeptomol). BH: Blue Hole Spring; CS: Coffee Spring. MS: Mission Spring; HS: Head Spring; DE: Devil's Eye Spring; GBS-1: Gilchrist Blue Springs vent 1; GBS-2: Gilchrist Blue Springs vent 2; PKS: Peacock Spring; MB: Madison Blue Spring; LRS: Little River Spring; RR: Santa Fe River Rise; RS: Santa Fe River Sink. Samples labeled with the suffix -Rev indicate those taken during a spring reversal and the suffix -H indicates those taken during high flow at River Rise. Lower case letters indicate the significance groups based on a Tukey HSD test.

From a total of 198,700 individual biovolume measurements, 1,029 extreme outliers [i.e., values of Q3 + (3 × IQR) or Q1 - (3 × IQR)], Q3, 3rd Quartile; IQR, Interquartile range; Q1, 1st Quartile, were identified among the samples and removed. ~~S, and s~~ubsequent analyses were conducted on an average of 19,762 ± 6,160 observations per sample. Average biovolume in the groundwaters and surface waters sampled was 0.1259 ± 0.0003 µm^{-3}, ranging from a high of 0.36 ± 0.02 µm^{-3} (Madison Blue Spring) to a low of 0.1010 ± 0.0003 µm^{-3} (River Sink). A Kruskal–Wallis test indicated there were significant differences in biovolume among the samples ($p < 0.001$). Therefore, cell carbon estimates were calculated separately for each spring. A sample from River Sink at low flow had the lowest average cellular carbon content (36.3 ± 0.1 fg C cell^{-1}; Fig. S1), which is significantly lower than values at other sites (post -Hoc Dunn test; $p < 0.001$). The highest cellular carbon content of 105 ± 5 fg C cell^{-1} was observed at Madison Blue Spring (Table 2) from a sample collected 83 d after the spring had reversed from negative to positive discharge. Multiplying cell carbon by cell concentration provides a total estimate of microbial biomass (Fig. 3 b), which is strongly correlated with cell abundance (Spearman r = 0.988;)(Fig. 3 a). Microbial biomass in samples from the sink-rise system, ~~highly influenced by surface water,~~ (River Sink, 22,065 ng C L^{-1}; River Rise, 21,799 ng C L^{-1}; Fig. 3 b) was ~100-times higher than that of the oldest groundwater from the Ichetucknee springs group, (236 to 673 ng C L^{-1})~~, where groundwater influence predominates~~. The quantity of carbon biomass in samples from Devil's Eye Spring and Head Spring corresponded to 3 % of the carbon in POC (Fig. 2 a), whereas much higher fractions of the POC were inferred to be microbial biomass in samples from Madison Blue Spring (24 %) and the sink-rise system (24 % and 11 % for River Sink and River Rise, respectively).

~~Experiments that sequentially passed water samples through 0.2 and 0.1 µm pore size filters showed m~~Most extractable ATP (79 to 99 %) was associated with cells retained on the 0.2 µm pore sized filters. ~~One exception was vent two of Gilchrist Blue Spring, where total extractable ATP in the 0.1 to 0.2 µm fraction (0.38 pM) exceed that in the > 0.2 µm fraction by ~2-fold (Fig. S2).~~ Unexpectedly, cells in the > 0.2 µm fraction from Devil's Eye Spring contained ATP concentrations ~15 times higher than those observed in samples from other springs in the Ichetucknee springs group as well as reversing spring group, with values similar to those for the sink-rise system (Fig. S2). The inferred ~~To exclude a technical error or contamination as explanations for the high ATP concentration measured at Devil's Eye Spring, repeat sampling conducted within one month of the initial observation confirmed that the cells contained higher cellular ATP concentrations than other springs. Dividing ATP by cell concentration provides an average estimate of ATP content per cell (Fig. 3 d), and these D~~data ~~show that cellular~~ ATP concentration per cell (Fig. 3 d) in samples from ~~the groundwater predominant~~ Devil's Eye Spring are significantly higher ($p < 0.001$; 1516 ± 300 zmol ATP cell^{-1}) than all other observations (range of 1.25 to 622.32 zmol ATP cell^{-1}). The trend in carbon biomass estimated from the cellular ATP concentration (Fig. 3 c) generally agrees with that

based on biovolume (Fig. 3 b) but ~~there are~~with exceptions. For instance, low biomass is inferred for most Ichetucknee and reversing springs with positive rates of discharge, the latter of which significantly increased during periods of reversal when contributions from ~~, where~~ surface water predominate~~s~~ (Fig. 3 c). ~~However, a~~Although ~~all~~the Ichetucknee springs ~~are highly influenced~~have ~~by~~ groundwater with the longest residence time, biomass carbon for Devil's Eye Spring based on ATP data

410 was significantly higher than for the other Ichetucknee springs (Fig. 3 c) and not statistically different from River Rise samples that contained ~15-fold more cells (Fig. 3 a). ATP-based biomass concentrations in samples from River Rise at low flow are significantly higher than values at high flow and ~3-fold lower than those observed for River Sink at low flow (Fig. 3 c). As ATP is considered a proxy for viable biomass (e.g., Oulahal-Lagsir et al., 2000; Köster and Meyer-Reil, 2001)~~(Köster and Meyer-Reil, 2001; Oulahal-Lagsir et al., 2000)(Oulahal-Lagsir et al., 2000; Köster and Meyer-Reil, 2001)~~,

415 we used the ratio between biomass estimates derived from biovolume (Fig. 3 b) and ATP concentration (Fig. 3 c) to assess trends that may be related to viability of the groundwater communities. ATP-based biomass in the reversing springs group ranged from 1.4 % (Madison Blue Spring) to 38.5 % (Gilchrist Blue Spring) of the biovolume-based biomass estimates, whereas values for the River Rise and River Sink were 4.6 % and 13.7 %, respectively. In contrast, ATP-based biomass was up to 4-fold higher than estimates inferred from biovolume for the oldest groundwaters in the Ichetucknee springs group

420 (Blue Hole Spring, Coffee Spring, and Devil´s Eye Spring; 145 %, 163 %, and 404 %, respectively)~~; (Fig. 2 c)~~.

3.3 Microbial respiration

The rate of oxygen consumption was highest in samples from River Rise and River Sink, with rates at high flow (0.382 and 0.322 mg L^{-1} d^{-1}, respectively) being approximately twice those observed during low flow (0.137 and 0.178 mg L^{-1} d^{-1}, respectively; Fig. 4 a; Table 2; Fig. S3). In contrast, rates of DIC production during low flow were at least three times higher

425 than those observed during high flow conditions (Fig. 4; Table 2). The lowest oxygen consumption rates were in groundwaters from Devil's Eye Spring (0.102 mg L^{-1} d^{-1}) and samples taken at Madison Blue Spring 1 d after a period of flow reversal (31 March 2022; 0.101 mg L^{-1} d^{-1}). Significantly higher rates of oxygen consumption (ANOVA F = 44.912, p < 0.001) were measured in discharge from Head Spring (0.189 mg L^{-1} d^{-1}), as well as in samples from Madison Blue Spring (0.168 mg L^{-1} d^{-1}, and 0.199 mg L^{-1} d^{-1}; Fig. 4 a) that occurred 51~~,~~ and 91 d, respectively, after it had transitioned from

430 negative to positive rates of discharge. A higher rate of oxygen consumption in the 91 d sample from Madison Blue Spring matched a DIC production rate that was ~2-fold higher than values observed immediately after the transition from reversing conditions (Fig. 4 b). Unfortunately, poor fit of the DIC data from Devil's Eye Spring and Head Spring to the regression models ($r^2 \leq 0.2$; Fig. S4) coupled with no statistically significant change in concentration over time prevented an estimate of DIC production by this method. The observed molar ratio of DIC produced to oxygen consumed (i.e., respiratory quotient,

435 RQ) for Madison Blue Spring 91 d after a reversal event (0.62) was approximately half that measured after one day of positive discharge (1.23). At the sink-rise system, RQ was much higher during low flow~~.~~ (4.85 and 2.66 for River Rise and River Sink, respectively)~~,~~ when there wa~~s~~ more influence from groundwater ~~influence,~~ than at high flow when surface water contributions to the conduits were the largest (0.56 and 0.37, respectively; Table 2).

440 At low flow in the sink-rise system, the OUR (0.55 mg L^{-1} d^{-1}) was higher but similar to measured rates of oxygen consumption (0.14 mg L^{-1} d^{-1}); however, OUR at higher flow (6.41 mg L^{-1} d^{-1}) was ~16-fold higher than measured oxygen consumption rates (Table 2). The largest discrepancy documented was for the oldest groundwaters ~~members~~ of the Ichetucknee springs group~~, where groundwater influence is predominant,~~ which had measured rates of oxygen consumption (Fig. 4 a) three orders of magnitude higher than OUR (1.26 to 5.68 x 10^{-4} mg L^{-1} d^{-1}; Table 2).

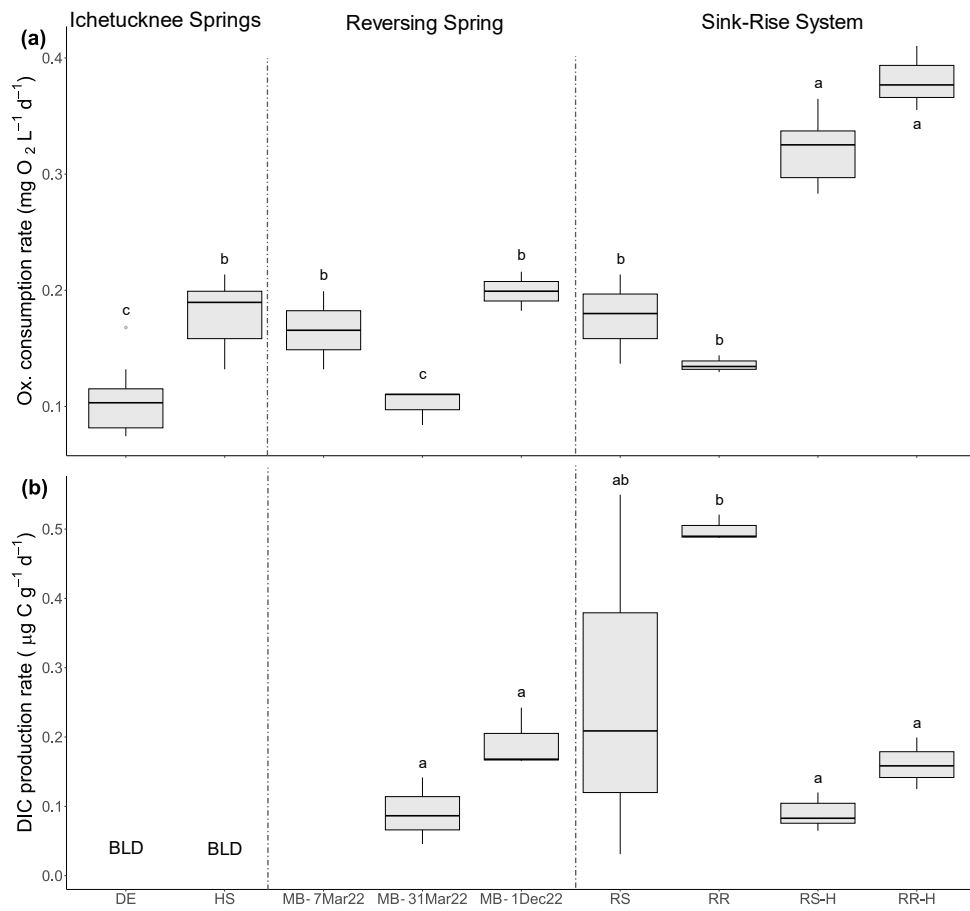
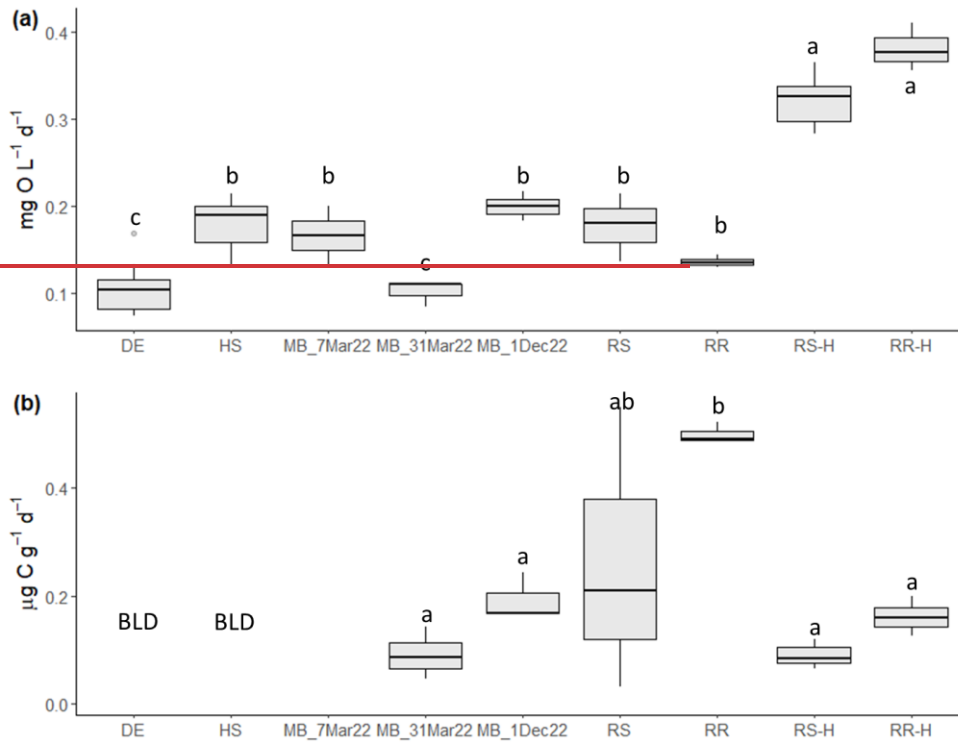



Figure 4. Rates of oxygen consumption (a) and DIC production (b) in select springs and sink-rise system. Letters indicate significance groups based on a Tukey HSD test. DE: Devil's Eye Spring; HS: Head Spring; MB: Madison Blue Spring; RS: Santa Fe River Sink; RR: Santa Fe River Rise. DIC production data are not available for the Madison Blue Spring sample collected on 7 March 2022. Samples labeled with the suffix -H indicate those taken during high flow at River Sink and River Rise. BLD: Below detection limit.

3.4 Heterotrophic Carbon Assimilation

~~Time series experiments that were performed to quantify the amount of ^{3}H-leucine and ^{3}H-thymidine incorporated into acid insoluble macromolecules provided data to estimate assimilatory metabolic and growth rates for heterotrophic members of the communities.~~ Observed rates of ^{3}H-leucine incorporation exceeded those for ^{3}H-thymidine, except for the samples from Madison Blue Spring, where ^{3}H-thymidine incorporation rates were up to 3-fold higher than those for ^{3}H-leucine incorporation (Fig. S5). The highest rates of incorporation were observed in samples from the River Sink and River Rise that also contained the highest cell and biomass concentrations (Fig. 3). The molar ratio of ^{3}H-leucine to ^{3}H-thymidine

incorporation (Leu:TdR) was very low (< 0.45) for observations from Madison Blue Spring and much lower than the longest residence time groundwater (Devil's Eye, 2.72; Table 2). The largest Leu:TdR ratios were observed in samples from the River Sink (3.10), River Rise (4.06), and Head Springs (6.24).

To estimate doubling times from the ^{3}H-leucine and ^{3}H-thymidine incorporation data, we assumed that all cells in the samples (Fig. 3 a) were viable and capable of incorporating the radiotracers into newly synthesized protein and DNA, respectively. Therefore, the values derived correspond to maximum reproduction estimates for the cell populations. Doubling times calculated from cell specific rates of ^{3}H-leucine (Table 2) and ^{3}H-thymidine incorporation negatively correlate with dissolved oxygen (r = -0.79 to -0.86, p < 0.05). However, the separate radiotracers did not provide matching reproduction rates, with values based on ^{3}H-thymidine incorporation 2 to 50-times shorter than those based on ^{3}H-leucine incorporation. Given that freshwater bacteria have shown preferential uptake of leucine over thymidine (e.g., Pérez et al., 2010) we used the ^{3}H-leucine incorporation rates to further evaluate rates of growth and cell carbon production in the groundwaters and surface waters studied. Doubling times of 20 h at the River Sink were shorter than those at the River Rise, and under low flow conditions, with doubling time increasing to nearly 9 days at River Rise, when groundwater had been flowing into the conduits (Table 2). Much longer doubling times of 10 to 42 d were inferred at Madison Blue Spring (i.e., when discharge rates were positive) in comparison to the older groundwaters analyzed from the Ichetucknee springs group (14 h for Head Spring and 62 h for Devil's Eye Spring).

A standard leucine-to-carbon conversion factor was used to estimate heterotrophic productivity based on the rate of ^{3}H-leucine incorporation (Fig. 5). The values obtained for heterotrophic productivity on three dates at Madison Blue Spring were the lowest observed (42.9 to 90.6 µg C L^{-1} d^{-1}). Based on an ANOVA (p < 0.001, F = 144.183) and Tukey HSD post-hoc analysis, heterotrophic productivity for Devil's Eye Spring (198 µg C L^{-1} d^{-1}) and Head Spring (576 µg C L^{-1} d^{-1}) were significantly higher than at least two of the three observations from Madison Blue Spring (Fig. 5). The highest rates of heterotrophic production were measured during high flow and surface water inputs in the sink-rise system (River Sink and Rise; 18,328 µg C L^{-1} d^{-1} and 14,225 µg C L^{-1} d^{-1}, respectively), which were significantly different from (ANOVA p < 0.001, F = 188.553) and 4- and 11-fold higher, respectively, than values at low flow and, when groundwater enters the conduits (Fig. 5). Low flow conditions coincided with significantly higher rates of heterotrophic production at the sink (ANOVA p < 0.001, F = 205.016; 4,653 µg C L^{-1} d^{-1}) versus the rise (1,285 µg C L^{-1} d^{-1}). High correlation of heterotrophic production rates with DOC concentration (r = 0.86, p < 0.05; Fig. S7 a) and quality (HIX, r = 0.86; Fig. S7 d BIX and FI, r = -0.85; p < 0.05; Fig. S7 b and c) support that both the availability and characteristics of DOC influenced growth of the surface water and groundwater communities we investigated. Cell carbon incorporation rates derived from ^{3}H-thymidine follow a similar pattern among samples as those derived from ^{3}H-leucine, but in general, implied higher rates of heterotrophic production at most sites (Fig. S8). The trend

for cell specific rates of heterotrophic productivity based on the ^{3}H-leucine data (Table 2) is quite different from that for bulk values (Fig. 5), and with the highest cell specific rates were observed at Head Spring , which exceedinged those for surface waters at the River Sink by ~2-fold.

495

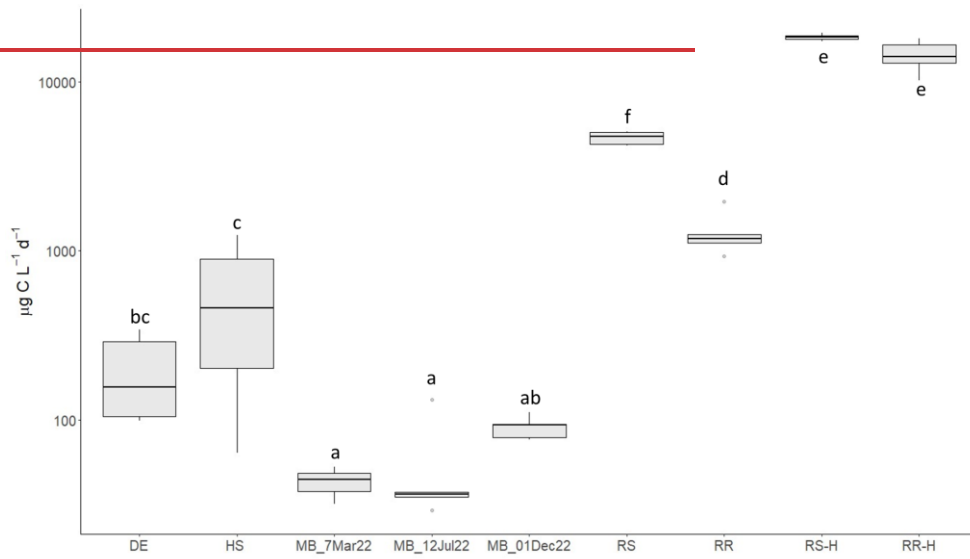
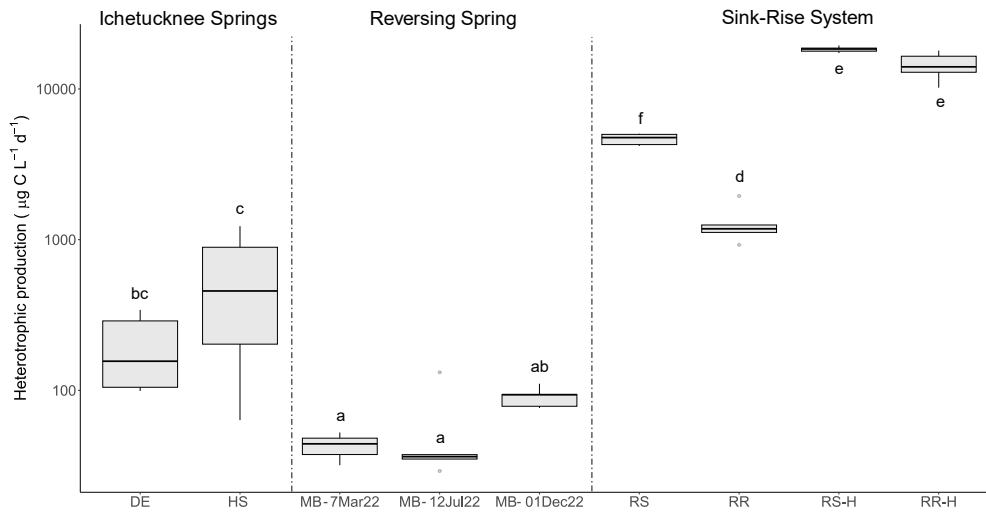
Very high growth efficiencies were inferred from estimates of heterotrophic production (^{3}H-leucine data) and respiration (DIC production data) for samples from thee sink-rise system (93 to 99%), with a decreased BGE of 72% at River Rise under low flow conditions and when groundwater contributions were highesthas more influence under (low flow conditions).
500   The lowest BGEs (39% and 32%) were found in samples collected from Madison Blue Spring at least three months after a period of flow reversal (Table 2). Although we were unable to empirically determine respiration rates using the DIC concentration data collected from Devil's Eye Spring and Head Spring, BGE values calculated usingbased on the oxygen consumption rate and a theoretical RQ of 1.2 (Berggren et al., 2012)(Berggren et al., 2012)(Berggren et al., 2012) were high (81% and 87%, respectively) and implied that a lower proportion of the DOC utilized was lost as CO_2 in comparison to the
505   groundwater sampled from Madison Blue Spring.

510

Figure 5. Rates of heterotrophic carbon production based on ^{3}H-leucine incorporation data. Letters indicate significance groups based on a Tukey post -hoc analysis. DE: Devil's Eye Spring; HS: Head Spring; MB: Madison Blue Spring; RS: Santa Fe River Sink; RR: Santa Fe River Rise. Samples labeled with the suffix -H indicate those taken during high flow at River Sink and River Rise.

4 Discussion

4.1 Groundwater and surface water mixing in karst landscapes

Studies that have examined microbial production in aquifer ecosystems have deepened understanding of subsurface carbon cycling and the biogeochemical processes contributing to groundwater quality (e.g., Wilhartitz et al., 2009; Hofmann and Griebler, 2018; Karwautz et al., 2022)~~(Hofmann and Griebler, 2018; Karwautz et al., 2022; Wilhartitz et al., 2009)(Wilhartitz et al., 2009; Hofmann and Griebler, 2018; Karwautz et al., 2022)~~. Research over recent decades in silicate-dominated aquifer systems has contributed greatly to these discussions, but there has been less emphasis on landscapes composed predominately of carbonate minerals (Covington et al., 2023)~~(Covington et al., 2023)(Covington et al., 2023)~~. A key aspect of karstic aquifers are the fractures and conduits that enhance permeability and water flow within their geological formations, facilitating relatively rapid exchange of dissolved gases, nutrients, organic matter, and microbes,~~ and~~ between surface water bodies and groundwater. The groundwater discharged from springs in North Central Florida tends to be organic carbon poor and suboxic in contrast to surface waters that are organic carbon-rich and with DO near equilibration with atmospheric oxygen (Moore et al., 2009; Martin et al., 2016; Flint et al., 2021; Oberhelman et al., 2023)~~(Flint et al., 2021; Martin et al., 2016; Moore et al., 2009; Oberhelman et al., 2023)(Moore et al., 2009; Martin et al., 2016; Flint et al., 2021; Oberhelman et al., 2023)~~. In recent decades~~addition~~, there have been ~~Over recent decades,~~ alarming trends in groundwater quality ~~trends~~that have raised concerns about the health of Florida's springs ~~over recent decades~~, including decreased rates of discharge (Florida Springs Institute, 2018)~~(Florida Springs Institute, 2018)(Florida Springs Institute, 2018)~~, increased abundance of reactive nitrogen species (Katz, 2004; Katz et al., 2001, 2009; Denizman, 2018)~~(Denizman, 2018; Katz, 2004; Katz et al., 2001, 2009)(Katz et al., 2001; Katz, 2004; Katz et al., 2009; Denizman, 2018)~~, and lower DO concentration in the groundwater and spring runs (Heffernan et al., 2010)~~(Heffernan et al., 2010)(Heffernan et al., 2010)~~. Consequently, this region provides a model experimental setting to evaluate the effects of surface-derived substrate delivery on microbial consumption of organic matter and biomass production in karstic groundwater.

4.2 Effects of groundwater residence time on microbes, biomass, and organic carbon~~Abundance of microbes, biomass, and organic carbon~~

Cell concentrations in groundwater from the Ichetucknee springs group (Fig. 3 a) are similar to those reported in previous studies of springs in this region (Malki et al., 2020, 2021)~~(Malki et al., 2020, 2021)(Malki et al., 2020, 2021)~~ and other oligotrophic karstic groundwaters (Farnleitner et al., 2005; Wilhartitz et al., 2007, 2009, 2013; Hershey et al., 2018)~~(Farnleitner et al., 2005; Hershey et al., 2018; Wilhartitz et al., 2007, 2009, 2013)(Farnleitner et al., 2005; Hershey et al., 2018; Wilhartitz et al., 2007, 2009, 2013)~~. In comparison, water samples from the reversing springs and sink-rise system

540 had higher DO (Table 1), DOC (Table 2), cell (~10^7 to 10^9 cells L^{-1}), and biomass (Fig. 3 b and c) concentrations. Biomass estimates based on biovolume (Fig. 3 b) are largely congruent with those derived using ATP data for most of the Ichetucknee springs and the reversing ~~springs~~ spring group when their discharge rates were positive (Fig. 3 c). The ATP-based biomass data reveal an effect of surface waters on the microbial communities, showing significantly higher values during periods of spring reversal and during hydrological conditions when groundwater mixing occurred at ~~the~~ River Rise. For the Ichetucknee

545 springs group, the ATP data provided higher biomass estimates in comparison to those derived from biovolume, with ~~and the largest discrepancy is for~~ Devil's Eye Spring having the largest discrepancy ~~, where the ATP-based biomass estimate is 4 times higher~~ (Fig. 3 b and c). Our data and related calculations imply that average cellular ATP contents in populations discharged with suboxic, oligotrophic groundwater at Devil's Eye Spring (Fig. 3 d) were at least ~~4~~four-fold higher than those at other sites and approach those reported in laboratory cultured bacteria (e.g., 2,000 zmol; Thore et al., 1975)~~Thore et al.,~~

550 ~~1975)(Thore et al., 1975)~~. High ATP contents are associated with cells ~~that have~~with large biovolumes and rapid metabolisms (e.g., Eydal and Pedersen, 2007)~~(Eydal and Pedersen, 2007)(Eydal and Pedersen, 2007)~~, but neither of these explanations are consistent with the ~~biovolume or metabolic rate~~ data ~~(Table 2)~~ observed at Devil's Eye Spring (Table 2). As such, we hypothesize that the lower anabolic rates and energy consumption allows ATP to accumulate within cells. ~~While future work will be necessary to provide a clear understanding about why Devil's Eye has an unusually large ATP~~

555 ~~concentration compared with other springs , we believe that the most likely hypothesis is that low anabolic rates that slowly consume ATP allow its accumulation within the cells.~~

The quantity and quality ~~(i.e., composition, chemical structure, and nutrient content)~~ of organic matter is a crucial determinant of heterotrophic metabolism and biomass production rates (Hosen et al., 2014; Wu et al., 2018)~~(Hosen et al.,~~

560 ~~2014; Wu et al., 2018) (Hosen et al., 2014; Wu et al., 2018)~~. Surface water from the sink-rise system had the highest concentrations of DOC (Table 2) and POC (Fig. 2 a), but according to high HIX and low FI and BIX values (Table 2), ~~suggest~~ the DOC origin was terrigenous and ~~had~~ ~~of~~ lower quality. Although groundwater discharged from Head Spring and Devil´s Eye Spring had DOC and POC concentrations similar to those at Madison Blue Spring, DOC quality in the latter reversing spring was intermediate to values for River Rise and River Sink (Table 2). Bioavailability of the microbially

565 derived, protein-like DOC in the Ichetucknee springs (Flint et al., 2023)~~(Flint et al., 2023) (Flint et al., 2023)~~ was confirmed by the relatively high rates of cell-specific production observed in these samples (Table 2). The $\delta^{13}C_{POC}$ data also provided evidence for differences in the carbon sources available (Fig. 2 b), with the isotopically lightest $\delta^{13}C_{POC}$ from Head Spring coinciding with the shortest community doubl~~ing~~e time observed (14 h) among the groundwaters and surface waters sampled (Table 2).

570 **4.3 Hydrology and DOC influences ~~on~~ microbial respiration~~Microbial respiration~~**

The rates of oxygen consumption in oligotrophic groundwaters discharged from the Ichetucknee and reversing springs (0.1 to 0.2 mg L^{-1} d^{-1}) were within the range observed in the DOC-rich waters of the sink-rise system during low flow (Table 2).

Significantly lower values occurred only in suboxic groundwater discharging from Devil's Eye Spring and Madison Blue Spring 1 d after it transitioned from negative to positive discharge (Fig. 4 a). The low rates of oxygen consumption and DIC production observed in samples from Madison Blue Spring immediately following a reversal event may represent a temporal biogeochemical response of reversing spring systems. In the organic-rich waters at the sink-rise system, the highest rates of oxygen consumption were detected under high flow conditions, whereas the highest rates of DIC production were observed during low flow (Fig. 4; Table 2) and when there was a substantial contribution of groundwater to the conduits (Flint et al., 2023). Transitions in hydrological stage in the sink-rise system during river flooding led to increases in the DO (from an average of 1.6 and 3.2 mg L^{-1} at River Rise to 4.18 and 5.29 at the River Sink during low and high flow, respectively; Table 1) and DOC concentration, but higher values of HIX suggest the DOC was of poorer quality (Table 2). While incomplete oxidation of lower quality DOC at high flow is possible, reduced oxygen concentrations during low flow should enable additional microbial sources of DIC from fermentative and anaerobic respiratory metabolisms. In particular, water discharged at River Rise contain supersaturated concentrations of dissolved N_2O that has been generated via denitrification, and N_2O concentrations are consistently higher under low flow conditions (Flint et al., 2023). Hence, a component of DIC produced at River Rise, as well as in the N_2O-saturated discharge of Madison Blue Spring (Flint et al., 2021) has likely originated from heterotrophic denitrification in the groundwater.

RQ values based on DIC production and oxygen consumption rates (0.35 to 4.85; Table 2) deviate from the generally assumed range of 0.8 to 1.2 that is based on the stoichiometry of oxidation for specific organic compounds. However, they are commensurate to the wide range of values that have been reported for natural bacterial assemblages in freshwater ecosystems (0.25 to 4.6; Berggren et al., 2012). At the sink-rise system, the increase of RQ from < 0.6 at high flow to 2.66 and 4.85 at low flow implies a carbon source transition to highly oxidized, low molecular weight organic acids (Berggren et al., 2012; Allesson et al., 2016; Hilman et al., 2022) and/or an effect of supplementary DIC sources from anaerobic metabolisms (Flint et al., 2021, 2023). Decreasing methane concentrations and enrichment of $\delta^{13}CH_4$ from River Sink to River Rise indicates a contribution from methanotrophy (Oberhelman et al., 2023), which has a theoretical RQ of 0.5 (Bastviken et al., 2008) and may partially explain the low RQ values observed during high flow (Table 2). RQ values for Madison Blue Spring (1.23 and 0.62) were based on data collected 1 and 94 d, respectively, after a period of reversal, supporting the contention that relatively short residence time in the aquifer affects physiology of and carbon consumption by the groundwater community.

To compare the empirical oxygen consumption rates with estimates based on mixing of groundwater with atmospheric gases (i.e., OUR), we assumed that water enters the aquifer saturated with atmospheric oxygen, constant rates of oxygen consumption during the subsurface residence time, and aerobic respiration was the only oxygen sink. For springs discharging the oldest groundwater (i.e., Head Spring and Devil's Eye Spring), OUR grossly underestimates the observed oxygen consumption rate by 433- and 180-fold, respectively (Table 2). OUR values for River Rise are more congruent with measured rates and overestimate oxygen consumption by 4- and 16-fold during low and high flow, respectively (Table 2). A "dramatic" decrease in the DO concentrations of groundwater discharged by springs in this region has been documented since the 1970s (Heffernan et al., 2010)(Heffernan et al., 2010)(Heffernan et al., 2010). Although the biogeochemical basis for the decrease in DO is not well understood, the temporal trend coincides with increasing NO$_3^-$ concentrations that have also been observed in spring discharge over the last ~70 years (Hornsby et al., 2004; Munch et al., 2006)(Hornsby et al., 2004; Munch et al., 2006)(Hornsby et al., 2004; Munch et al., 2006). Elevated levels of reactive nitrogen species have been implicated in enhancing N$_2$O production in the UFA (Flint et al., 2021)(Flint et al., 2021)(Flint et al., 2021), but it is currently not knowknown if nitrogen eutrophication of the groundwater may also be enhancing microbial metabolic rates in the subsurface.

4.4 Effect of surface water-groundwater interactions on heterotrophic growth Heterotrophic production and growth do not scale with groundwater ageHeterotrophic production and growth

High correlation of heterotrophic production rates with DOC concentration (r = 0.86, p < 0.05) and quality (HIX, r = 0.86; BIX and FI, r = -0.85; p < 0.05) support that both the availability and characteristics of DOC influenced growth of the surface water and groundwater communities we investigated. In the organic matter- and DO-rich surface waters of River Sink, biomass was produced at rates exceeding those reported for the Amazon River (28 µg C L^{-1} d^{-1}; Benner et al., 1995Benner et al., 1995Benner et al., 1995) and were comparable to values for high organic matter river systems such as the Columbia River estuary (3,120 to 114,240 µg C L^{-1}d^{-1}; Herfort et al., 2017)Herfort et al., 2017)Herfort et al., 2017). Groundwater contributions to the sink-rise system are minimized when discharge rates are high (> 15 m^3 s^{-1}), and while these hydrological conditions tend to increase DO and DOC concentration, the terrigenous-derived DOC was generally of lower quality than that observed when groundwater is entering the conduits (Flint et al., 2023)(Flint et al., 2023)(Flint et al., 2023). Nevertheless, rates of heterotrophic production, cell reproduction, and oxygen consumption in the sink-rise system decreased during low flow conditions (Tables 1 and 2). One possibility is that metabolism becomes limited by electron acceptor availability, similar to biogeochemical scenarios described for the conduits of Madison Blue Spring during periods of reversal (Brown et al., 2014, 2019)(Brown et al., 2014, 2019)(Brown et al., 2014, 2019). Though BGEs as high as 80% have been reported in aquatic systems (Del Giorgio and Cole, 1998; Eiler et al., 2003)(Eiler et al., 2003; Del Giorgio and Cole, 1998)(Del Giorgio and Cole, 1998; Eiler et al., 2003), the values inferred from the ^{3}H-leucine and DIC data for the River Sink and River Rise (> 92 %) are so high they suggest our experimental approach underestimated microbial respiration or overestimated heterotrophic production in these samples. The former possibility is less likely given that we expect

additional CO_2 formed via anaerobic metabolisms in these waters, which should result in an overestimate of aerobic
640 respiration based on ΔDIC. Very similar and high BGEs values (> 90 %) are also derived from the ^{3}H-thymidine
incorporation data, leading us to conclude that both of these methods overestimated heterotrophic production (e.g., see
Giering and Evans, 2022)(e.g., Giering and Evans, 2022)e.g., (Giering and Evans, 2022) in samples from the sink-rise
system.

645 Heterotrophic production was lower in groundwater discharging from the reversing and Ichetucknee springs than surface
waters from the Santa Fe River, and four to eight orders of magnitude higher than that reported for a karstic aquifer in the
European Alps (Wilhartitz et al., 2009)(Wilhartitz et al., 2009)(Wilhartitz et al., 2009) and oligotrophic groundwater from
sand and gravel aquifers (Hofmann and Griebler, 2018; Karwautz et al., 2022).(Hofmann and Griebler, 2018b; Karwautz et
al., 2022b). (Hofmann and Griebler, 2018b; Karwautz et al., 2022b). Temperature-dependent reaction kinetics alone do not
650 fully explain these differences, aAssuming standard responses for reaction kinetics (i.e., Q_{10} values of 2 to 3; Gillooly et al.,
2001), tGillooly et al., 2001), t, tGillooly et al., 2001 emperature-dependent reaction kinetics cannot fully explain the
differences between when comparing the warmer groundwater temperaturesrates in of ~22-°C in the UFA (~22-°C) with
temperatures ofand 4 and 12-°C, in the Aalpine and sand and-gravel aquifers (, 4 and 12-° C, respectively; Hofmann and
Griebler, 2018; Karwautz et al., 2022)Hofmann and Griebler, 2018; Karwautz et al., 2022)Hofmann and Griebler, 2018;
655 Karwautz et al., 2022 . There are mMultiple mechanisms may explainpotential reasons for the observed higher rates of
microbial productivity in UFA groundwater, and several observations make a strong case for higher bioavailability of
organic matter. Higher availability of metabolizable organic matter in the UFA groundwaters samples was substantiated by
the the much shorter inferred doubling times (from 0.58 d for Head Spring to 43 d for Madison Blue Spring; Table 2) when
compared to those for alpine karst aquifer (712 d, groundwater residence time of ~22 years; Wilhartitz et al.,
660 2009)(Wilhartitz et al., 2009)(Wilhartitz et al., 2009) and oligotrophic groundwater (533 d; Karwautz et al., 2022)Karwautz
et al., 2022)Karwautz et al., 2022) systems. In addition,We also observed that microbes discharged from Head Spring had
the highest rate of leucine relative to thymidine incorporation, potentially representing a response to growth limiting
conditions (e.g., Church 2008). Nevertheless, these populations had rates of specific heterotrophic production (0.2607 pmol
C cell^{-1} h^{-1}) that were twice values for surface waters (Table 2), and 2 to 5 orders of magnitude higher than those for
665 groundwater of comparable residence time (Wilhartitz et al., 2009)(Wilhartitz et al., 2009)(Wilhartitz et al., 2009). Lastly,
the DOC examined in telogenetic alpine aquifers was generally of lower quality (i.e., average FI of 1.6; Harjung et al.,
2023Harjung et al., 2023Harjung et al., 2023) relative to that we have documented in the Ichetucknee springs (average FI of
1.78).

670 When Withlacoochee River level is low, groundwater discharging to Madison Blue Sspring run has a similar composition
and DOC concentration to that in the nutrient-limited Ichetucknee springs (Table 1), yet a large disparity was observed in
reproduction rates (Table 2). During the period of study, Madison Blue Spring reversed flow eight times; events that

transported electron donor- and acceptor-rich river water directly into the subsurface. Large volumes of water can recharge during reversals and . ~~D~~during a 7.5 d reversal in 2009, an estimated ~5.8×10^4 m^3 of river water recharged the conduits of
675 Madison Blue Spring (Gulley et al., 2011)~~(Gulley et al., 2011)(Gulley et al., 2011)~~. Increases in bioreactive solute concentration during reversals coincide with transient microbial blooms that have been observed by cave divers in the typically nutrient limited environment of the conduits (Gulley et al., 2011, 2013; Brown et al., 2014)~~(Brown et al., 2014; Gulley et al., 2011, 2013)(Gulley et al., 2011, 2013; Brown et al., 2014)~~. Based on these observations, we ~~initially~~ hypothesized that metabolic rates of subsurface communities in reversing springs would be intermediate to those of the
680 Ichetucknee springs and sink-rise system. Though respiration rates (Fig. 4) and DO, DOC, and POC concentrations (Table 2; Fig. 2 a) at Madison Blue Spring were similar to other UFA groundwaters, the cell carbon production rates were at least 2-fold lower than those for the Ichetucknee springs (Fig. 5; Table 2) and do not ~~provide~~ fully support ~~for~~ our initial hypothesis. The heterotrophic production and respiration data from Madison Blue Spring produced BGEs (32 % and 39 %) that are lower than other sites (Table 2) and comparable to values from oligotrophic portions of the ocean (Del Giorgio and Cole,
685 1998)~~(Del Giorgio and Cole, 1998)(Del Giorgio and Cole, 1998)~~ and subsurface aquatic systems in Antarctica (Vick-Majors et al., 2016)~~(Vick-Majors et al., 2016)(Vick-Majors et al., 2016)~~. Low BGE at Madison Blue Spring could indicate uncoupling between catabolism and anabolism, which is consistent with low leucine to thymidine incorporation ratios (Table 2) that imply its groundwater community was investing most of their energy flow in maintenance metabolism rather than growth (e.g., Chin-Leo and Kirchman, 1990; Wos and Pollard, 2012)~~(Chin-Leo and Kirchman, 1990; Wos and Pollard,~~
690 ~~2012)(Wos and Pollard, 2012; Chin-Leo and Kirchman, 1990)~~. Previous investigations at Madison Blue Spring have shown altered groundwater chemistry for a period of ~1 month after each reversal (Brown et al., 2014)~~(Brown et al., 2014)(Brown et al., 2014)~~. Considering ~~this,~~ our sampling ~~the~~ intervals after reversal ~~we sampled~~ (i~~.~~e.~~,~~ Figure 5 data were collected 52 to 107 d after a transition from negative to positive discharge), there may be a protracted ~~might have been deemed sufficient to avoid the transient geochemical effects of flow reversal. However, our data make clear that further studies that include~~
695 ~~high temporal resolution sampling are needed to constrain the legacy~~ effect~~s~~ of spring flow reversal on~~on~~ the availability of ~~nutrients and redox couples for~~ microbial metabolism in ~~the~~ groundwater.

5 Conclusion

Groundwater discharged from unconfined portions of the UFA contained low standing stocks of microbes that divided and produced cell carbon at rates greatly exceeding those previously documented for oligotrophic aquifers. Biogeochemical
700 activity in the aquifer is enhanced due to extensive exchange of organic matter- and DO-rich surface water with groundwater across the karst landscape. Comparably high rates of cell specific metabolism and reproduction in the groundwater —some of which exceeded values observed for surface waters— indicate the presence of readily oxidizable and assimilable organic carbon sources. Since labile pools of organic carbon in surface waters would not be expected to persist for the timeframes necessary to be transported with the oldest groundwaters, ~~the~~ our results indicate a subsurface source of organic matter that

705 may be supplied via a combination of chemoautotrophy, secondary production, and degradation of necromass (e.g., Geesink et al., 2022)(e.g., Geesink et al., 2022)e.g., (Geesink et al., 2022). ~~Over recent decades, alarming groundwater quality trends have raised concerns about the health of Florida's springs, including decreased rates of discharge (Florida Springs Institute, 2018), increased abundance of reactive nitrogen species (Katz et al., 2001; Katz, 2004; Katz et al., 2009; Denizman, 2018), and lower DO concentration in the groundwater and spring runs (Heffernan et al., 2010).~~ Similar environmental

710 ~~changes~~Environmental challenges threatening aquifer health is a global trend that is also affecting the UFA. ~~are occurring globally, and T~~thus, evaluations of mechanisms controlling microbial processes in the UFA may be useful to provide insights in other settings ~~experiencing similar menaces.~~ An improved understanding of the microbial ~~biogeochemical processes~~ activities affecting UFA groundwater quality is essential for developing strategies to mitigate anthropic ~~these challenging environmental issues~~disturbance and manage this vital natural resource under changing climate and land-use

715 regimes. ~~In addition to providing a basis for f~~Future studies that decipher the sources of organic matter driving biogeochemical processes in the UFA are necessary to fully explain ~~,~~the relationships we have documented among microbial biomass, physiology, and hydrogeochemistry. ~~provide an important case study about groundwater microbial communities~~ Given that anthropogenic alteration of groundwater biogeochemistry and quality is an issue that is not unique to the region of study, our work from the UFA provides an important case study on groundwater microbial communities that

720 may be compared with conditions in different geological and environmental contexts of global karst landscapes.

Data availability

The DOC concentration data were previously published by Flint et al., (2021). DOC quality data are available at hydroshare: http://www.hydroshare.org/resource/a876020b85d6413f8486c57dc0b0e3bf. All other data collected and analyzed in this study are available at: http://www.hydroshare.org/resource/e8e4994bb1a740d8bb3fd65acf342cb6.

725 **Author contributions**

ABS: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing – original draft, and visualization. MKF: methodology, formal analysis, investigation, data curation, and writing – review and editing. JCE: methodology, formal analysis, investigation, and writing – review and editing. JBM: conceptualization, resources, writing – review and editing, supervision, and funding acquisition. BCC: conceptualization, methodology, formal analysis, resources,

730 writing – original draft, supervision, project administration, and funding acquisition.

Competing interests

The authors declare that they have no conflict of interest.

Acknowledgements

This study was supported by funding from the University of Florida's Biodiversity Institute (Seed grant #020518 to BCC and JBM; graduate fellowship to ABS) and a Fulbright scholarship (to ABS). Partial support was also provided by the Institute of Food and Agricultural Sciences at the University of Florida. Research at springs in North Central Florida was conducted under the Florida Department of Environmental Protection permits 06281812, 03211912, 07092012, 07162112A, and 08122212, and we are indebted to Christine Housel for her assistance with permit acquisition. We thank Patricia Spellman, Robert Sharping, and Jason Gulley for discussions; Quincy Faber for assisting with collecting the ATP data; and Kelenna O. Irving, Madison Tharp, Victoria Cassady, Arianna Insenga, Rachel Pinsky, and Katelyn Palmer for their assistance with field work.

References

Allesson, L., Ström, L., and Berggren, M.: Impact of photochemical processing of DOC on the bacterioplankton respiratory quotient in aquatic ecosystems, Geophys Res Lett, 43, 7538–7545, https://doi.org/10.1002/2016GL069621, 2016.

Bastviken, D., Cole, J. J., Pace, M. L., and Van de-Bogert, M. C.: Fates of methane from different lake habitats: Connecting whole-lake budgets and CH4 emissions, J Geophys Res Biogeosci, 113, 1–13, https://doi.org/10.1029/2007JG000608, 2008.

Benner, R., Opsahl, S., Chin-Leo, G., Richey, J. E., and Forsberg, B. R.: Bacterial carbon metabolism in the Amazon River system, Limnol Oceanogr, 40, 1262–1270, https://doi.org/10.4319/lo.1995.40.7.1262, 1995.

Berggren, M., Lapierre, J. F., and Del Giorgio, P. A.: Magnitude and regulation of bacterioplankton respiratory quotient across freshwater environmental gradients, ISME Journal, 6, 984–993, https://doi.org/10.1038/ismej.2011.157, 2012.

Brown, A. L., Martin, J. B., Screaton, E. J., Ezell, J. E., Spellman, P., and Gulley, J.: Bank storage in karst aquifers: The impact of temporary intrusion of river water on carbonate dissolution and trace metal mobility, Chem Geol, 385, 56–69, https://doi.org/10.1016/j.chemgeo.2014.06.015, 2014.

Brown, A. L., Martin, J. B., Kamenov, G. D., Ezell, J. E., Screaton, E. J., Gulley, J., and Spellman, P.: Trace metal cycling in karst aquifers subject to periodic river water intrusion, Chem Geol, 527, 0–1, https://doi.org/10.1016/j.chemgeo.2018.05.020, 2019.

Chin-Leo, G. and Kirchman, D.: Unbalanced growth in natural assemblages of marine bacterioplankton, Mar Ecol Prog Ser, 63, 1–8, https://doi.org/10.3354/meps063001, 1990.

765 Chin-Leo, G. and Kirchman, D. L.: Estimating bacterial production in marine waters from the simultaneous incorporation of thymidine and leucine, Appl Environ Microbiol, 54, 1934–1939, https://doi.org/10.1128/aem.54.8.1934-1939.1988, 1988.

Covington, M. D., Martin, J. B., Toran, L. E., Macalady, J. L., Sekhon, N., Sullivan, P. L., García Jr., Á. A., Heffernan, J. B., and Graham, W. D.: Carbonates in the Critical Zone Earth ' s Future, Earths Future, 11,
770 https://doi.org/10.1029/2022EF002765, 2023.

Denizman, C.: Land use changes and groundwater quality in Florida, Appl Water Sci, 8, 1–17, https://doi.org/10.1007/s13201-018-0776-9, 2018.

775 Eiler, A., Langenheder, S., Bertilsson, S., and Tranvik, L. J.: Heterotrophic bacterial growth efficiency and community structure at different natural organic carbon concentrations, Appl Environ Microbiol, 69, 3701–3709, https://doi.org/10.1128/AEM.69.7.3701-3709.2003, 2003.

Eydal, H. S. C. and Pedersen, K.: Use of an ATP assay to determine viable microbial biomass in Fennoscandian Shield
780 groundwater from depths of 3-1000 m, J Microbiol Methods, 70, 363–373, https://doi.org/10.1016/j.mimet.2007.05.012, 2007.
Farnleitner, A. H., Wilhartitz, I., Ryzinska, G., Kirschner, A. K. T., Stadler, H., Burtscher, M. M., Hornek, R., Szewzyk, U., Herndl, G., and Mach, R. L.: Bacterial dynamics in spring water of alpine karst aquifers indicates the presence of stable autochthonous microbial endokarst communities, Environ Microbiol, 7, 1248–1259, https://doi.org/10.1111/j.1462-2920.2005.00810.x, 2005.

785

Flint, M. K., Martin, J. B., Summerall, T. I., Barry-Sosa, A., and Christner, B. C.: Nitrous oxide processing in carbonate karst aquifers, J Hydrol (Amst), 594, 125936, https://doi.org/10.1016/j.jhydrol.2020.125936, 2021
.
Flint, M. K., Martin, J. B., Oberhelman, A., Janelle, A. J., Black, M., Barry-Sosa, A., and Christner, B.: Hydrologic and Organic
790 Carbon Quality Controls on Nitrous Oxide Dynamics Across a Variably Confined Karst Aquifer, J Geophys Res Biogeosci, 128, 1–20, https://doi.org/10.1029/2023JG007493, 2023.

Florida Springs Institute: Florida Springs Conservation Plan, High Springs, Florida, 2018.

795 Fuhrman, J. A. and Azam, F.: Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California, Appl Environ Microbiol, 39, 1085–1095, https://doi.org/10.1128/aem.39.6.1085-1095.1980, 1980.

Geesink, P., Taubert, M., Jehmlich, N., von Bergen, M., and Küsel, K.: Bacterial Necromass Is Rapidly Metabolized by Heterotrophic Bacteria and Supports Multiple Trophic Levels of the Groundwater Microbiome, Microbiol Spectr, 10, 1–14, https://doi.org/10.1128/spectrum.00437-22, 2022.

Giering, S. L. C. and Evans, C.: Overestimation of prokaryotic production by leucine incorporation—and how to avoid it, Limnol Oceanogr, 67, 726–738, https://doi.org/10.1002/lno.12032, 2022.

Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M., and Charnov, E. L.: Effects of size and temperature on metabolic rate, Science (1979), 293, 2248–2251, https://doi.org/10.1126/science.1061967, 2001.

Del Giorgio, P. A. and Cole, J. J.: Bacterial growth efficiency in natural aquatic systems, Annu Rev Ecol Syst, 29, 503–541, https://doi.org/10.1146/annurev.ecolsys.29.1.503, 1998.

Goldscheider, N., Chen, Z., Auler, A. S., Bakalowicz, M., Broda, S., Drew, D., Hartmann, J., Jiang, G., Moosdorf, N., Stevanovic, Z., and Veni, G.: Global distribution of carbonate rocks and karst water resources, Hydrogeol J, 28, 1661–1677, https://doi.org/10.1007/s10040-020-02139-5, 2020.

Gulley, J., Martin, J. B., Screaton, E., and Moore, P. J.: River reversals into karst springs: A model for cave enlargement in eogenetic karst aquifers, Bulletin of the Geological Society of America, 123, 457–467, https://doi.org/10.1130/B30254.1, 2011.

Gulley, J., Martin, J., Spellman, P., Moore, P., and Screaton, E.: Dissolution in a variably confined carbonate platform: Effects of allogenic runoff, hydraulic damming of groundwater inputs, and surface-groundwater exchange at the basin scale, Earth Surf Process Landf, 38, 1700–1713, https://doi.org/10.1002/esp.3411, 2013.

Harjung, A., Schweichhart, J., Rasch, G., and Griebler, C.: Large-scale study on groundwater dissolved organic matter reveals a strong heterogeneity and a complex microbial footprint, Science of the Total Environment, 854, 158542, https://doi.org/10.1016/j.scitotenv.2022.158542, 2023.

Heffernan, J. B., Liebowitz, D. M., Frazer, T. K., Evans, J. M., and Cohen, M. J.: Algal blooms and the nitrogen-enrichment hypothesis in Florida springs: Evidence, alternatives, and adaptive management, Ecological Applications, 20, 816–829, https://doi.org/10.1890/08-1362.1, 2010.

Henson, W. R., Huang, L., Graham, W. D., and Ogram, A.: Nitrate reduction mechanisms and rates in an unconfined eogenetic karst aquifer in two sites with different redox potential, J Geophys Res Biogeosci, 122, 1062–1077, https://doi.org/10.1002/2016JG003463, 2017.

835 Herfort, L., Crump, B. C., Fortunato, C. S., McCue, L. A., Campbell, V., Simon, H. M., Baptista, A. M., and Zuber, P.: Factors affecting the bacterial community composition and heterotrophic production of Columbia River estuarine turbidity maxima, Microbiologyopen, 6, 1–15, https://doi.org/10.1002/mbo3.522, 2017.

Hershey, O. S. and Barton, H. A.: The Microbial Diversity of Caves, 69–90, https://doi.org/10.1007/978-3-319-98852-8_5,
840 2018.

Hershey, O. S., Kallmeyer, J., Wallace, A., Barton, M. D., and Barton, H. A.: High microbial diversity despite extremely low biomass in a deep karst aquifer, Front Microbiol, 9, 1–13, https://doi.org/10.3389/fmicb.2018.02823, 2018.

845 Hilman, B., Weiner, T., Haran, T., Masiello, C. A., Gao, X., and Angert, A.: The Apparent Respiratory Quotient of Soils and Tree Stems and the Processes That Control It, J Geophys Res Biogeosci, 127, https://doi.org/10.1029/2021JG006676, 2022.
Hofmann, R. and Griebler, C.: DOM and bacterial growth efficiency in oligotrophic groundwater: Absence of priming and co-limitation by organic carbon and phosphorus, Molecular Plant-Microbe Interactions, 31, 311–322, https://doi.org/10.3354/ame01862, 2018.
850
Hornsby, D., Mattson, R., and Mirti, T.: Surfacewater Quality and Biological Annual Report, Live Oak, Florida, 2004.

Hosen, J. D., McDonough, O. T., Febria, C. M., and Palmer, M. A.: Dissolved organic matter quality and bioavailability changes across an urbanization gradient in headwater streams, Environ Sci Technol, 48, 7817–7824,
855 https://doi.org/10.1021/es501422z, 2014.

Jasechko, S. and Perrone, D.: Global groundwater wells at risk of running dry, Science (1979), 372, 418–421, https://doi.org/10.1126/science.abc2755, 2021.

860 Jin, J., Zimmerman, Andrew R., Moore, Paul J., and Martin, J. B.: Organic and inorganic carbon dynamics in a karst aquifer: Santa Fe River Sink-Rise system, north Florida, USA, J Geophys Res Biogeosci, 340–357, https://doi.org/10.1002/2013JG002350, 2014.

Kalhor, K., Ghasemizadeh, R., Rajic, L., and Alshawabkeh, A.: Assessment of groundwater quality and remediation in karst aquifers: A review, Groundw Sustain Dev, 8, 104–121, https://doi.org/10.1016/j.gsd.2018.10.004, 2019.

Karl, D. M.: Cellular nucleotide measurements and applications in microbial ecology, Microbiol Rev, 44, 739–796, https://doi.org/10.1128/mmbr.44.4.739-796.1980, 1980.

Karwautz, C., Zhou, Y., Kerros, M. E., Weinbauer, M. G., and Griebler, C.: Bottom-Up Control of the Groundwater Microbial Food-Web in an Alpine Aquifer, Front Ecol Evol, 10, 1–14, https://doi.org/10.3389/fevo.2022.854228, 2022.

Katz, B. G.: Sources of nitrate contamination and age of water in large karstic springs of Florida, Environmental Geology, 46, 689–706, https://doi.org/10.1007/s00254-004-1061-9, 2004.

Katz, B. G., Böhlke, J. K., and Hornsby, H. D.: Timescales for nitrate contamination of spring waters, northern Florida, USA, Chem Geol, 179, 167–186, https://doi.org/10.1016/S0009-2541(01)00321-7, 2001.

Katz, B. G., Sepulveda, A. A., and Verdi, R. J.: Estimating nitrogen loading to ground water and assessing vulnerability to nitrate contamination in a large karstic springs Basin, Florida, J Am Water Resour Assoc, 45, 607–627, https://doi.org/10.1111/j.1752-1688.2009.00309.x, 2009.

Köster, M. and Meyer-Reil, L. A.: Characterization of carbon and microbial biomass pools in shallow water coastal sediments of the southern Baltic Sea (Nordrügensche Bodden), Mar Ecol Prog Ser, 214, 25–41, https://doi.org/10.3354/meps214025, 2001.

Kurtz, Z. D., Müller, C. L., Miraldi, E. R., Littman, D. R., Blaser, M. J., and Bonneau, R. A.: Sparse and Compositionally Robust Inference of Microbial Ecological Networks, PLoS Comput Biol, 11, 1–25, https://doi.org/10.1371/journal.pcbi.1004226, 2015.

Malki, K., Rosario, K., Sawaya, N. A., Székely, A. J., Tisza, M. J., and Breitbart, M.: Prokaryotic and Viral Community Composition of FreshwaterSprings in Florida, USA, Applied and Environmental Science, 11, 1–18, 2020.

Malki, K., Sawaya, N. A., Tisza, M. J., Coutinho, F. H., Rosario, K., Székely, A. J., and Breitbart, M.: Spatial and Temporal Dynamics of Prokaryotic and Viral Community Assemblages in a Lotic System (Manatee Springs, Florida), Appl Environ Microbiol, 87, 1–18, https://doi.org/10.1128/AEM.00646-21, 2021.

Martin, J. and Gordon, S. L.: Surface and ground water mixing, flow paths, and temporal variations in chemical compositions of karst springs, Groundwater Flow and Contaminant Transport in Carbonate Aquifers, 65–92, 2000.

900

Martin, J. B.: Carbonate minerals in the global carbon cycle, Chem Geol, 449, 58–72, https://doi.org/10.1016/j.chemgeo.2016.11.029, 2017.

Martin, J. B. and Dean, R. W.: Temperature as a natural tracer of short residence times for groundwater in karst aquifers, Karst
905   Modeling. Karst Waters Institute Special Publication, 5, 236–242, 1999.

Martin, J. B. and Dean, R. W.: Exchange of water between conduits and matrix in the Floridan aquifer, Chem Geol, 179, 145–165, https://doi.org/10.1016/S0009-2541(01)00320-5, 2001.

910   Martin, J. B., Kurz, M. J., and Khadka, M. B.: Climate control of decadal-scale increases in apparent ages of eogenetic karst spring water, J Hydrol (Amst), 540, 988–1001, https://doi.org/10.1016/j.jhydrol.2016.07.010, 2016.
McDonough, L. K., Andersen, M. S., Behnke, M. I., Rutlidge, H., Oudone, P., Meredith, K., O'Carroll, D. M., Santos, I. R., Marjo, C. E., Spencer, R. G. M., McKenna, A. M., and Baker, A.: A new conceptual framework for the transformation of groundwater dissolved organic matter, Nat Commun, 13, 1–11, https://doi.org/10.1038/s41467-022-29711-9, 2022.

915

Miller, J. A.: Hydrogeologic framework of the Floridan Aquifer System in Florida and in parts of Georgia, Alabama, and South Carolina—Regional Aquifer-system Analysis: U.S. Geological Survey Professional Paper 1403–B, U.S. Geological Survey Professional Paper, 1403-B, 91, 1986.

920   Miller, J. A.: Hydrogeology of Florida, in: The Geology of Florida, edited by: Randazzo, A. and Douglas, J., University Press of Florida, Gainesville, 69–88, 1997.

Moore, P. J., Martin, J. B., and Screaton, E. J.: Geochemical and statistical evidence of recharge, mixing, and controls on spring discharge in an eogenetic karst aquifer, J Hydrol (Amst), 376, 443–455, https://doi.org/10.1016/j.jhydrol.2009.07.052,
925   2009.

Munch, D. A., Toth, D. J., Huang, C.-T., Davis, J. B., Fortich, C. M., Osburn, W. L., Phlips, E. J., Quinlan, E. L., Allen, M. S., Woods, M. J., Cooney, P., Knight, R. L., Clarke, R. A., and Knight, S. L.: Fifty-Year Retrospective Study of the Ecology of Silver Springs, 2006.

930

Oberhelman, A., Martin, J. B., and Flint, M. K.: Methane cycling in the carbonate critical zone, Science of the Total Environment, 899, 165645, https://doi.org/10.1016/j.scitotenv.2023.165645, 2023.

Oberhelman, A., Martin, J. B., and Flint, M. K.: Groundwater-surface water interaction, dissolved organic carbon oxidation and dissolution in carbonate aquifers, Earth Surf Process Landf, https://doi.org/10.1002/esp.5830, 2024.

Oulahal-Lagsir, N., Martial-Gros, A., Bonneau, M., and Blum, L. J.: Ultrasonic methodology coupled to ATP bioluminescence for the non-invasive detection of fouling in food processing equipment - Validation and application to a dairy factory, J Appl Microbiol, 89, 433–441, https://doi.org/10.1046/j.1365-2672.2000.01132.x, 2000.

Overholt, W. A., Trumbore, S., Xu, X., Bornemann, T. L. V., Probst, A. J., Krüger, M., Herrmann, M., Thamdrup, B., Bristow, L. A., Taubert, M., Schwab, V. F., Hölzer, M., Marz, M., and Küsel, K.: Carbon fixation rates in groundwater similar to those in oligotrophic marine systems, Nat Geosci, 15, 561–567, https://doi.org/10.1038/s41561-022-00968-5, 2022.

Pérez, M. T., Hörtnagl, P., and Sommaruga, R.: Contrasting ability to take up leucine and thymidine among freshwater bacterial groups: Implications for bacterial production measurements, Environ Microbiol, 12, 74–82, https://doi.org/10.1111/j.1462-2920.2009.02043.x, 2010.

Puri, H. S. and Vernon, R. O.: Summary of the Geology of Florida and a Guidebook to the Classic Exposures, Gainesville, FL, 312 pp., 1964.

Scott, T. M.: The lithostratigraphy of the Hawthorn Group (Miocene) of Florida, Florida Geological Survey, 59, 1–148, 1988.
Thore, A., Ansehn, S., Lundin, A., and Bergman, S.: Detection of bacteriuria by luciferase assay of adenosine triphosphate, J Clin Microbiol, 1, 1–8, https://doi.org/10.1128/jcm.1.1.1-8.1975, 1975.

Verity, P. G., Robertson, C. Y., Tronzo, C. R., Andrews, M. G., Nelson, J. R., and Sieracki, M. E.: Relationships between cell volume and the carbon and nitrogen content of marine photosynthetic nanoplankton, Limnol Oceanogr, 37, 1434–1446, https://doi.org/10.4319/lo.1992.37.7.1434, 1992.

Vick-Majors, T. J., Mitchell, A. C., Achberger, A. M., Christner, B. C., Dore, J. E., Michaud, A. B., Mikucki, J. A., Purcell, A. M., Skidmore, M. L., Priscu, J. C., Adkins, W. P., Anandakrishnan, S., Barbante, C., Barcheck, G., Beem, L., Behar, A., Beitch, M., Bolsey, R., Branecky, C., Edwards, R., Fisher, A., Fricker, H. A., Foley, N., Guthrie, B., Hodson, T., Horgan, H., Jacobel, R., Kelley, S., Mankoff, K. D., McBryan, E., Powell, R., Sampson, D., Scherer, R., Siegfried, M., and Tulaczyk, S.:

Physiological ecology of microorganisms in subglacial lake whillans, Front Microbiol, 7, 1–16, https://doi.org/10.3389/fmicb.2016.01705, 2016.

Wilhartitz, I., Mach, R. L., Teira, E., Reinthaler, T., Herndl, G. J., and Farnleitner, A. H.: Prokaryotic community analysis with CARD-FISH in comparison with FISH in ultra-oligotrophic ground- and drinking water, J Appl Microbiol, 103, 871–881, https://doi.org/10.1111/j.1365-2672.2007.03319.x, 2007.

Wilhartitz, I. C., Kirschner, A. K. T., Stadler, H., Herndl, G. J., Dietzel, M., Latal, C., MacH, R. L., and Farnleitner, A. H.: Heterotrophic prokaryotic production in ultraoligotrophic alpine karst aquifers and ecological implications, FEMS Microbiol Ecol, 68, 287–299, https://doi.org/10.1111/j.1574-6941.2009.00679.x, 2009.

Wilhartitz, I. C., Kirschner, A. K. T., Brussaard, C. P. D., Fischer, U. R., Wieltschnig, C., Stadler, H., and Farnleitner, A. H.: Dynamics of natural prokaryotes, viruses, and heterotrophic nanoflagellates in alpine karstic groundwater, Microbiologyopen, 2, 633–643, https://doi.org/10.1002/mbo3.98, 2013.

Williams, L. J. and Kuniansky, E. L.: Revised Hydrogeologic Framework of the Floridan Aquifer System in Florida and Parts of Georgia, Alabama, and South Carolina, U.S. Geological Survey Professional Paper 1807, https://doi.org/http://dx.doi.org/10.3133/pp1807., 2016.

Worthington, S., Davies, G., and Clifford, F.: Matrix, fracture, and channel components of storage and flow in a Paleozoic limestone aquifer, 113–128, 2000.

Wos, M. and Pollard, P.: Bacterial physiological state in wastewater: Monitoring maintenance and production with Leu/TdR ratio for less pollution, Water Air Soil Pollut, 223, 4507–4513, https://doi.org/10.1007/s11270-012-1213-6, 2012.

Wu, X., Wu, L., Liu, Y., Zhang, P., Li, Q., Zhou, J., Hess, N. J., Hazen, T. C., Yang, W., and Chakraborty, R.: Microbial interactions with dissolved organic matter drive carbon dynamics and community succession, Front Microbiol, 9, 1–12, https://doi.org/10.3389/fmicb.2018.01234, 2018.

Allesson, L., Ström, L., and Berggren, M.: Impact of photochemical processing of DOC on the bacterioplankton respiratory quotient in aquatic ecosystems, Geophys Res Lett, 43, 7538–7545, https://doi.org/10.1002/2016GL069621, 2016.

Bastviken, D., Cole, J. J., Pace, M. L., and Van de Bogert, M. C.: Fates of methane from different lake habitats: Connecting whole-lake budgets and CH4 emissions, J Geophys Res Biogeosci, 113, 1–13, https://doi.org/10.1029/2007JG000608, 2008.

Benner, R., Opsahl, S., Chin-Leo, G., Richey, J. E., and Forsberg, B. R.: Bacterial carbon metabolism in the Amazon River system, Limnol Oceanogr, 40, 1262–1270, https://doi.org/10.4319/lo.1995.40.7.1262, 1995.

Berggren, M., Lapierre, J. F., and Del Giorgio, P. A.: Magnitude and regulation of bacterioplankton respiratory quotient across freshwater environmental gradients, ISME Journal, 6, 984–993, https://doi.org/10.1038/ismej.2011.157, 2012.

1000 Brown, A. L., Martin, J. B., Screaton, E. J., Ezell, J. E., Spellman, P., and Gulley, J.: Bank storage in karst aquifers: The impact of temporary intrusion of river water on carbonate dissolution and trace metal mobility, Chem Geol, 385, 56–69, https://doi.org/10.1016/j.chemgeo.2014.06.015, 2014.

Brown, A. L., Martin, J. B., Kamenov, G. D., Ezell, J. E., Screaton, E. J., Gulley, J., and Spellman, P.: Trace metal cycling in karst aquifers subject to periodic river water intrusion, Chem Geol, 527, 0–1, https://doi.org/10.1016/j.chemgeo.2018.05.020,

1005 2019.

Chin-Leo, G. and Kirchman, D.: Unbalanced growth in natural assemblages of marine bacterioplankton, Mar Ecol Prog Ser, 63, 1–8, https://doi.org/10.3354/meps063001, 1990.

Chin-Leo, G. and Kirchman, D. L.: Estimating bacterial production in marine waters from the simultaneous incorporation of thymidine and leucine, Appl Environ Microbiol, 54, 1934–1939, https://doi.org/10.1128/aem.54.8.1934-1939.1988, 1988.

1010 Covington, M. D., Martin, J. B., Toran, L. E., Macalady, J. L., Sekhon, N., Sullivan, P. L., García Jr., Á. A., Heffernan, J. B., and Graham, W. D.: Carbonates in the Critical Zone Earth's Future, Earths Future, 11, https://doi.org/10.1029/2022EF002765, 2023.

Denizman, C.: Land use changes and groundwater quality in Florida, Appl Water Sci, 8, 1–17, https://doi.org/10.1007/s13201-018-0776-9, 2018.

1015 Eiler, A., Langenheder, S., Bertilsson, S., and Tranvik, L. J.: Heterotrophic bacterial growth efficiency and community structure at different natural organic carbon concentrations, Appl Environ Microbiol, 69, 3701–3709, https://doi.org/10.1128/AEM.69.7.3701-3709.2003, 2003.

Eydal, H. S. C. and Pedersen, K.: Use of an ATP assay to determine viable microbial biomass in Fennoscandian Shield groundwater from depths of 3–1000 m, J Microbiol Methods, 70, 363–373, https://doi.org/10.1016/j.mimet.2007.05.012, 2007.

1020 Farnleitner, A. H., Wilhartitz, I., Ryzinska, G., Kirschner, A. K. T., Stadler, H., Burtscher, M. M., Hornek, R., Szewzyk, U., Herndl, G., and Mach, R. L.: Bacterial dynamics in spring water of alpine karst aquifers indicates the presence of stable autochthonous microbial endokarst communities, Environ Microbiol, 7, 1248–1259, https://doi.org/10.1111/j.1462-2920.2005.00810.x, 2005.

Flint, M. K., Martin, J. B., Summerall, T. I., Barry-Sosa, A., and Christner, B. C.: Nitrous oxide processing in carbonate karst

1025 aquifers, J Hydrol (Amst), 594, 125936, https://doi.org/10.1016/j.jhydrol.2020.125936, 2021.

Flint, M. K., Martin, J. B., Oberhelman, A., Janelle, A. J., Black, M., Barry-Sosa, A., and Christner, B.: Hydrologic and Organic Carbon Quality Controls on Nitrous Oxide Dynamics Across a Variably Confined Karst Aquifer, J Geophys Res Biogeosci, 128, 1–20, https://doi.org/10.1029/2023JG007493, 2023.

Florida Springs Institute: Florida Springs Conservation Plan, High Springs, Florida, 2018.

1030 Fuhrman, J. A. and Azam, F.: Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California, Appl Environ Microbiol, 39, 1085–1095, https://doi.org/10.1128/aem.39.6.1085-1095.1980, 1980.

Geesink, P., Taubert, M., Jehmlich, N., von Bergen, M., and Küsel, K.: Bacterial Necromass Is Rapidly Metabolized by Heterotrophic Bacteria and Supports Multiple Trophic Levels of the Groundwater Microbiome, Microbiol Spectr, 10, 1–14, https://doi.org/10.1128/spectrum.00437-22, 2022.

1035 Giering, S. L. C. and Evans, C.: Overestimation of prokaryotic production by leucine incorporation — and how to avoid it, Limnol Oceanogr, 67, 726–738, https://doi.org/10.1002/lno.12032, 2022.

Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M., and Charnov, E. L.: Effects of size and temperature on metabolic rate, Science (1979), 293, 2248–2251, https://doi.org/10.1126/science.1061967, 2001.

Del Giorgio, P. A. and Cole, J. J.: Bacterial growth efficiency in natural aquatic systems, Annu Rev Ecol Syst, 29, 503–541,
1040 https://doi.org/10.1146/annurev.ecolsys.29.1.503, 1998.

Goldscheider, N., Chen, Z., Auler, A. S., Bakalowicz, M., Broda, S., Drew, D., Hartmann, J., Jiang, G., Moosdorf, N., Stevanovic, Z., and Veni, G.: Global distribution of carbonate rocks and karst water resources, Hydrogeol J, 28, 1661–1677, https://doi.org/10.1007/s10040-020-02139-5, 2020.

Gulley, J., Martin, J. B., Screaton, E., and Moore, P. J.: River reversals into karst springs: A model for cave enlargement in
1045 eogenetic karst aquifers, Bulletin of the Geological Society of America, 123, 457–467, https://doi.org/10.1130/B30254.1, 2011.

Gulley, J., Martin, J., Spellman, P., Moore, P., and Screaton, E.: Dissolution in a variably confined carbonate platform: Effects of allogenic runoff, hydraulic damming of groundwater inputs, and surface-groundwater exchange at the basin scale, Earth Surf Process Landf, 38, 1700–1713, https://doi.org/10.1002/esp.3411, 2013.

1050 Harjung, A., Schweichhart, J., Rasch, G., and Griebler, C.: Large-scale study on groundwater dissolved organic matter reveals a strong heterogeneity and a complex microbial footprint, Science of the Total Environment, 854, 158542, https://doi.org/10.1016/j.scitotenv.2022.158542, 2023.

Heffernan, J. B., Liebowitz, D. M., Frazer, T. K., Evans, J. M., and Cohen, M. J.: Algal blooms and the nitrogen-enrichment hypothesis in Florida springs: Evidence, alternatives, and adaptive management, Ecological Applications, 20, 816–829,
1055 https://doi.org/10.1890/08-1362.1, 2010.

Henson, W. R., Huang, L., Graham, W. D., and Ogram, A.: Nitrate reduction mechanisms and rates in an unconfined eogenetic karst aquifer in two sites with different redox potential, J Geophys Res Biogeosci, 122, 1062–1077, https://doi.org/10.1002/2016JG003463, 2017.

Herfort, L., Crump, B. C., Fortunato, C. S., McCue, L. A., Campbell, V., Simon, H. M., Baptista, A. M., and Zuber, P.: Factors
1060 affecting the bacterial community composition and heterotrophic production of Columbia River estuarine turbidity maxima, Microbiologyopen, 6, 1–15, https://doi.org/10.1002/mbo3.522, 2017.

Hershey, O. S. and Barton, H. A.: The Microbial Diversity of Caves, 69–90, https://doi.org/10.1007/978-3-319-98852-8_5, 2018.

Hershey, O. S., Kallmeyer, J., Wallace, A., Barton, M. D., and Barton, H. A.: High microbial diversity despite extremely low
1065 biomass in a deep karst aquifer, Front Microbiol, 9, 1–13, https://doi.org/10.3389/fmicb.2018.02823, 2018.

Hilman, B., Weiner, T., Haran, T., Masiello, C. A., Gao, X., and Angert, A.: The Apparent Respiratory Quotient of Soils and Tree Stems and the Processes That Control It, J Geophys Res Biogeosci, 127, https://doi.org/10.1029/2021JG006676, 2022.

Hofmann, R. and Griebler, C.: DOM and bacterial growth efficiency in oligotrophic groundwater: Absence of priming and co-limitation by organic carbon and phosphorus, Molecular Plant Microbe Interactions, 31, 311–322, https://doi.org/10.3354/ame01862, 2018.

Hornsby, D., Mattson, R., and Mirti, T.: Surfacewater Quality and Biological Annual Report, Live Oak, Florida, 2004.

Hosen, J. D., McDonough, O. T., Febria, C. M., and Palmer, M. A.: Dissolved organic matter quality and bioavailability changes across an urbanization gradient in headwater streams, Environ Sci Technol, 48, 7817–7824, https://doi.org/10.1021/es501422z, 2014.

Jasechko, S. and Perrone, D.: Global groundwater wells at risk of running dry, Science (1979), 372, 418–421, https://doi.org/10.1126/science.abc2755, 2021.

Jin, J., Zimmerman, Andrew R., Moore, Paul J., and Martin, J. B.: Organic and inorganic carbon dynamics in a karst aquifer: Santa Fe River Sink-Rise system, north Florida, USA, J Geophys Res Biogeosci, 340–357, https://doi.org/10.1002/2013JG002350, 2014.

Kalhor, K., Ghasemizadeh, R., Rajic, L., and Alshawabkeh, A.: Assessment of groundwater quality and remediation in karst aquifers: A review, Groundw Sustain Dev, 8, 104–121, https://doi.org/10.1016/j.gsd.2018.10.004, 2019.

Karl, D. M.: Cellular nucleotide measurements and applications in microbial ecology, Microbiol Rev, 44, 739–796, https://doi.org/10.1128/mmbr.44.4.739-796.1980, 1980.

Karwautz, C., Zhou, Y., Kerros, M. E., Weinbauer, M. G., and Griebler, C.: Bottom-Up Control of the Groundwater Microbial Food-Web in an Alpine Aquifer, Front Ecol Evol, 10, 1–14, https://doi.org/10.3389/fevo.2022.854228, 2022.

Katz, B. G.: Sources of nitrate contamination and age of water in large karstic springs of Florida, Environmental Geology, 46, 689–706, https://doi.org/10.1007/s00254-004-1061-9, 2004.

Katz, B. G., Böhlke, J. K., and Hornsby, H. D.: Timescales for nitrate contamination of spring waters, northern Florida, USA, Chem Geol, 179, 167–186, https://doi.org/10.1016/S0009-2541(01)00321-7, 2001.

Katz, B. G., Sepulveda, A. A., and Verdi, R. J.: Estimating nitrogen loading to ground water and assessing vulnerability to nitrate contamination in a large karstic springs Basin, Florida, J Am Water Resour Assoc, 45, 607–627, https://doi.org/10.1111/j.1752-1688.2009.00309.x, 2009.

Köster, M. and Meyer-Reil, L. A.: Characterization of carbon and microbial biomass pools in shallow water coastal sediments of the southern Baltic Sea (Nordrügensche Bodden), Mar Ecol Prog Ser, 214, 25–41, https://doi.org/10.3354/meps214025, 2001.

Malki, K., Rosario, K., Sawaya, N. A., Székely, A. J., Tisza, M. J., and Breitbart, M.: Prokaryotic and Viral Community Composition of FreshwaterSprings in Florida, USA, Applied and Environmental Science, 11, 1–18, 2020.

Malki, K., Sawaya, N. A., Tisza, M. J., Coutinho, F. H., Rosario, K., Székely, A. J., and Breitbart, M.: Spatial and Temporal Dynamics of Prokaryotic and Viral Community Assemblages in a Lotic System (Manatee Springs, Florida), Appl Environ Microbiol, 87, 1–18, https://doi.org/10.1128/AEM.00646-21, 2021.

Martin, J. and Gordon, S. L.: Surface and ground water mixing, flow paths, and temporal variations in chemical compositions of karst springs, Groundwater Flow and Contaminant Transport in Carbonate Aquifers, 65–92, 2000.

Martin, J. B.: Carbonate minerals in the global carbon cycle, Chem Geol, 449, 58–72, https://doi.org/10.1016/j.chemgeo.2016.11.029, 2017.

Martin, J. B. and Dean, R. W.: Temperature as a natural tracer of short residence times for groundwater in karst aquifers, Karst Modeling. Karst Waters Institute Special Publication, 5, 236–242, 1999.

Martin, J. B. and Dean, R. W.: Exchange of water between conduits and matrix in the Floridan aquifer, Chem Geol, 179, 145–165, https://doi.org/10.1016/S0009-2541(01)00320-5, 2001.

Martin, J. B., Kurz, M. J., and Khadka, M. B.: Climate control of decadal-scale increases in apparent ages of eogenetic karst spring water, J Hydrol (Amst), 540, 988–1001, https://doi.org/10.1016/j.jhydrol.2016.07.010, 2016.

McDonough, L. K., Andersen, M. S., Behnke, M. I., Rutlidge, H., Oudone, P., Meredith, K., O'Carroll, D. M., Santos, I. R., Marjo, C. E., Spencer, R. G. M., McKenna, A. M., and Baker, A.: A new conceptual framework for the transformation of groundwater dissolved organic matter, Nat Commun, 13, 1–11, https://doi.org/10.1038/s41467-022-29711-9, 2022.

Miller, J. A.: Hydrogeologic framework of the Floridan Aquifer System in Florida and in parts of Georgia, Alabama, and South Carolina—Regional Aquifer-system Analysis: U.S. Geological Survey Professional Paper 1403-B, U.S. Geological Survey Professional Paper, 1403-B, 91, 1986.

Miller, J. A.: Hydrogeology of Florida, in: The Geology of Florida, edited by: Randazzo, A. and Douglas, J., University Press of Florida, Gainesville, 69–88, 1997.

Moore, P. J., Martin, J. B., and Screaton, E. J.: Geochemical and statistical evidence of recharge, mixing, and controls on spring discharge in an eogenetic karst aquifer, J Hydrol (Amst), 376, 443–455, https://doi.org/10.1016/j.jhydrol.2009.07.052, 2009.

Munch, D. A., Toth, D. J., Huang, C. T., Davis, J. B., Fortich, C. M., Osburn, W. L., Phlips, E. J., Quinlan, E. L., Allen, M. S., Woods, M. J., Cooney, P., Knight, R. L., Clarke, R. A., and Knight, S. L.: Fifty-Year Retrospective Study of the Ecology of Silver Springs, 2006.

Oberhelman, A., Martin, J. B., and Flint, M. K.: Methane cycling in the carbonate critical zone, Science of the Total Environment, 899, 165645, https://doi.org/10.1016/j.scitotenv.2023.165645, 2023.

Oberhelman, A., Martin, J. B., and Flint, M. K.: Groundwater-surface water interaction, dissolved organic carbon oxidation and dissolution in carbonate aquifers, Earth Surf Process Landf, https://doi.org/10.1002/esp.5830, 2024.

Oulahal-Lagsir, N., Martial-Gros, A., Bonneau, M., and Blum, L. J.: Ultrasonic methodology coupled to ATP bioluminescence for the non-invasive detection of fouling in food processing equipment—Validation and application to a dairy factory, J Appl Microbiol, 89, 433–441, https://doi.org/10.1046/j.1365-2672.2000.01132.x, 2000.

Overholt, W. A., Trumbore, S., Xu, X., Bornemann, T. L. V., Probst, A. J., Krüger, M., Herrmann, M., Thamdrup, B., Bristow, L. A., Taubert, M., Schwab, V. F., Hölzer, M., Marz, M., and Küsel, K.: Carbon fixation rates in groundwater similar to those in oligotrophic marine systems, Nat Geosci, 15, 561–567, https://doi.org/10.1038/s41561-022-00968-5, 2022.

1135 Pérez, M. T., Hörtnagl, P., and Sommaruga, R.: Contrasting ability to take up leucine and thymidine among freshwater bacterial groups: Implications for bacterial production measurements, Environ Microbiol, 12, 74–82, https://doi.org/10.1111/j.1462-2920.2009.02043.x, 2010.

Puri, H. S. and Vernon, R. O.: Summary of the Geology of Florida and a Guidebook to the Classic Exposures, Gainesville, FL, 312 pp., 1964.

1140 Scott, T. M.: The lithostratigraphy of the Hawthorn Group (Miocene) of Florida, Florida Geological Survey, 59, 1–148, 1988.

Thore, A., Ansehn, S., Lundin, A., and Bergman, S.: Detection of bacteriuria by luciferase assay of adenosine triphosphate, J Clin Microbiol, 1, 1–8, https://doi.org/10.1128/jcm.1.1.1-8.1975, 1975.

Verity, P. G., Robertson, C. Y., Tronzo, C. R., Andrews, M. G., Nelson, J. R., and Sieracki, M. E.: Relationships between cell volume and the carbon and nitrogen content of marine photosynthetic nanoplankton, Limnol Oceanogr, 37, 1434–1446, 1145 https://doi.org/10.4319/lo.1992.37.7.1434, 1992.

Vick-Majors, T. J., Mitchell, A. C., Achberger, A. M., Christner, B. C., Dore, J. E., Michaud, A. B., Mikucki, J. A., Purcell, A. M., Skidmore, M. L., Priscu, J. C., Adkins, W. P., Anandakrishnan, S., Barbante, C., Barcheck, G., Beem, L., Behar, A., Beitch, M., Bolsey, R., Branecky, C., Edwards, R., Fisher, A., Fricker, H. A., Foley, N., Guthrie, B., Hodson, T., Horgan, H., Jacobel, R., Kelley, S., Mankoff, K. D., McBryan, E., Powell, R., Sampson, D., Scherer, R., Siegfried, M., and Tulaczyk, S.: 1150 Physiological ecology of microorganisms in subglacial lake whillans, Front Microbiol, 7, 1–16, https://doi.org/10.3389/fmicb.2016.01705, 2016.

Wilhartitz, I., Mach, R. L., Teira, E., Reinthaler, T., Herndl, G. J., and Farnleitner, A. H.: Prokaryotic community analysis with CARD-FISH in comparison with FISH in ultra-oligotrophic ground- and drinking-water, J Appl Microbiol, 103, 871–881, https://doi.org/10.1111/j.1365-2672.2007.03319.x, 2007.

1155 Wilhartitz, I. C., Kirschner, A. K. T., Stadler, H., Herndl, G. J., Dietzel, M., Latal, C., MacH, R. L., and Farnleitner, A. H.: Heterotrophic prokaryotic production in ultraoligotrophic alpine karst aquifers and ecological implications, FEMS Microbiol Ecol, 68, 287–299, https://doi.org/10.1111/j.1574-6941.2009.00679.x, 2009.

Wilhartitz, I. C., Kirschner, A. K. T., Brussaard, C. P. D., Fischer, U. R., Wieltschnig, C., Stadler, H., and Farnleitner, A. H.: Dynamics of natural prokaryotes, viruses, and heterotrophic nanoflagellates in alpine karstic groundwater, Microbiologyopen, 1160 2, 633–643, https://doi.org/10.1002/mbo3.98, 2013.

Williams, L. J. and Kuniansky, E. L.: Revised Hydrogeologic Framework of the Floridan Aquifer System in Florida and Parts of Georgia, Alabama, and South Carolina, U.S. Geological Survey Professional Paper 1807, https://doi.org/http://dx.doi.org/10.3133/pp1807., 2016.

Worthington, S., Davies, G., and Clifford, F.: Matrix, fracture, and channel components of storage and flow in a Paleozoic 1165 limestone aquifer, 113–128, 2000.

Wos, M. and Pollard, P.: Bacterial physiological state in wastewater: Monitoring maintenance and production with Leu/TdR ratio for less pollution, Water Air Soil Pollut, 223, 4507–4513, https://doi.org/10.1007/s11270-012-1213-6, 2012.

Wu, X., Wu, L., Liu, Y., Zhang, P., Li, Q., Zhou, J., Hess, N. J., Hazen, T. C., Yang, W., and Chakraborty, R.: Microbial interactions with dissolved organic matter drive carbon dynamics and community succession, Front Microbiol, 9, 1–12, https://doi.org/10.3389/fmicb.2018.01234, 2018.

Allesson, L., Ström, L., and Berggren, M.: Impact of photochemical processing of DOC on the bacterioplankton respiratory quotient in aquatic ecosystems, Geophys Res Lett, 43, 7538–7545, https://doi.org/10.1002/2016GL069621, 2016.

Bastviken, D., Cole, J. J., Pace, M. L., and Van de Bogert, M. C.: Fates of methane from different lake habitats: Connecting whole-lake budgets and CH4 emissions, J Geophys Res Biogeosci, 113, 1–13, https://doi.org/10.1029/2007JG000608, 2008.

Benner, R., Opsahl, S., Chin-Leo, G., Richey, J. E., and Forsberg, B. R.: Bacterial carbon metabolism in the Amazon River system, Limnol Oceanogr, 40, 1262–1270, https://doi.org/10.4319/lo.1995.40.7.1262, 1995.

Berggren, M., Lapierre, J. F., and Del Giorgio, P. A.: Magnitude and regulation of bacterioplankton respiratory quotient across freshwater environmental gradients, ISME Journal, 6, 984–993, https://doi.org/10.1038/ismej.2011.157, 2012.

Brown, A. L., Martin, J. B., Screaton, E. J., Ezell, J. E., Spellman, P., and Gulley, J.: Bank storage in karst aquifers: The impact of temporary intrusion of river water on carbonate dissolution and trace metal mobility, Chem Geol, 385, 56–69, https://doi.org/10.1016/j.chemgeo.2014.06.015, 2014.

Brown, A. L., Martin, J. B., Kamenov, G. D., Ezell, J. E., Screaton, E. J., Gulley, J., and Spellman, P.: Trace metal cycling in karst aquifers subject to periodic river water intrusion, Chem Geol, 527, 0–1, https://doi.org/10.1016/j.chemgeo.2018.05.020, 2019.

Chin-Leo, G. and Kirchman, D.: Unbalanced growth in natural assemblages of marine bacterioplankton, Mar Ecol Prog Ser, 63, 1–8, https://doi.org/10.3354/meps063001, 1990.

Chin-Leo, G. and Kirchman, D. L.: Estimating bacterial production in marine waters from the simultaneous incorporation of thymidine and leucine, Appl Environ Microbiol, 54, 1934–1939, https://doi.org/10.1128/aem.54.8.1934-1939.1988, 1988.

Covington, M. D., Martin, J. B., Toran, L. E., Macalady, J. L., Sekhon, N., Sullivan, P. L., García Jr., Á. A., Heffernan, J. B., and Graham, W. D.: Carbonates in the Critical Zone Earth's Future, Earths Future, 11, https://doi.org/10.1029/2022EF002765, 2023.

Denizman, C.: Land use changes and groundwater quality in Florida, Appl Water Sci, 8, 1–17, https://doi.org/10.1007/s13201-018-0776-9, 2018.

Duarte, C. M., Prairie, Y. T., Frazer, T. K., Hoyer, M. V., Notestein, S. K., Martínez, R., Dorsett, A., and Canfield, D. E.: Rapid accretion of dissolved organic carbon in the springs of Florida: The most organic-poor natural waters, Biogeosciences, 7, 4051–4057, https://doi.org/10.5194/bg-7-4051-2010, 2010.

Eiler, A., Langenheder, S., Bertilsson, S., and Tranvik, L. J.: Heterotrophic bacterial growth efficiency and community structure at different natural organic carbon concentrations, Appl Environ Microbiol, 69, 3701–3709, https://doi.org/10.1128/AEM.69.7.3701-3709.2003, 2003.

Eydal, H. S. C. and Pedersen, K.: Use of an ATP assay to determine viable microbial biomass in Fennoscandian Shield groundwater from depths of 3–1000 m, J Microbiol Methods, 70, 363–373, https://doi.org/10.1016/j.mimet.2007.05.012, 2007.

Farnleitner, A. H., Wilhartitz, I., Ryzinska, G., Kirschner, A. K. T., Stadler, H., Burtscher, M. M., Hornek, R., Szewzyk, U., Herndl, G., and Mach, R. L.: Bacterial dynamics in spring water of alpine karst aquifers indicates the presence of stable autochthonous microbial endokarst communities, Environ Microbiol, 7, 1248–1259, https://doi.org/10.1111/j.1462-2920.2005.00810.x, 2005.

Flint, M. K., Martin, J. B., Summerall, T. I., Barry-Sosa, A., and Christner, B. C.: Nitrous oxide processing in carbonate karst aquifers, J Hydrol (Amst), 594, 125936, https://doi.org/10.1016/j.jhydrol.2020.125936, 2021.

Flint, M. K., Martin, J. B., Oberhelman, A., Janelle, A. J., Black, M., Barry-Sosa, A., and Christner, B.: Hydrologic and Organic Carbon Quality Controls on Nitrous Oxide Dynamics Across a Variably Confined Karst Aquifer, J Geophys Res Biogeosci, 128, 1–20, https://doi.org/10.1029/2023JG007493, 2023.

Florida Springs Institute: Florida Springs Conservation Plan, High Springs, Florida, 2018.

Fuhrman, J. A. and Azam, F.: Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California, Appl Environ Microbiol, 39, 1085–1095, https://doi.org/10.1128/aem.39.6.1085-1095.1980, 1980.

Geesink, P., Taubert, M., Jehmlich, N., von Bergen, M., and Küsel, K.: Bacterial Necromass Is Rapidly Metabolized by Heterotrophic Bacteria and Supports Multiple Trophic Levels of the Groundwater Microbiome, Microbiol Spectr, 10, 1–14, https://doi.org/10.1128/spectrum.00437-22, 2022.

Giering, S. L. C. and Evans, C.: Overestimation of prokaryotic production by leucine incorporation — and how to avoid it, Limnol Oceanogr, 67, 726–738, https://doi.org/10.1002/lno.12032, 2022.

Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M., and Charnov, E. L.: Effects of size and temperature on metabolic rate, Science (1979), 293, 2248–2251, https://doi.org/10.1126/science.1061967, 2001.

Del Giorgio, P. A. and Cole, J. J.: Bacterial growth efficiency in natural aquatic systems, Annu Rev Ecol Syst, 29, 503–541, https://doi.org/10.1146/annurev.ecolsys.29.1.503, 1998.

Goldscheider, N., Chen, Z., Auler, A. S., Bakalowicz, M., Broda, S., Drew, D., Hartmann, J., Jiang, G., Moosdorf, N., Stevanovic, Z., and Veni, G.: Global distribution of carbonate rocks and karst water resources, Hydrogeol J, 28, 1661–1677, https://doi.org/10.1007/s10040-020-02139-5, 2020.

Gulley, J., Martin, J. B., Screaton, E., and Moore, P. J.: River reversals into karst springs: A model for cave enlargement in eogenetic karst aquifers, Bulletin of the Geological Society of America, 123, 457–467, https://doi.org/10.1130/B30254.1, 2011.

Gulley, J., Martin, J., Spellman, P., Moore, P., and Screaton, E.: Dissolution in a variably confined carbonate platform: Effects of allogenic runoff, hydraulic damming of groundwater inputs, and surface-groundwater exchange at the basin scale, Earth Surf Process Landf, 38, 1700–1713, https://doi.org/10.1002/esp.3411, 2013.

Harjung, A., Schweichhart, J., Rasch, G., and Griebler, C.: Large-scale study on groundwater dissolved organic matter reveals a strong heterogeneity and a complex microbial footprint, Science of the Total Environment, 854, 158542, https://doi.org/10.1016/j.scitotenv.2022.158542, 2023.

Heffernan, J. B., Liebowitz, D. M., Frazer, T. K., Evans, J. M., and Cohen, M. J.: Algal blooms and the nitrogen-enrichment hypothesis in Florida springs: Evidence, alternatives, and adaptive management, Ecological Applications, 20, 816–829, https://doi.org/10.1890/08-1362.1, 2010.

Henson, W. R., Huang, L., Graham, W. D., and Ogram, A.: Nitrate reduction mechanisms and rates in an unconfined eogenetic karst aquifer in two sites with different redox potential, J Geophys Res Biogeosci, 122, 1062–1077, https://doi.org/10.1002/2016JG003463, 2017.

Herfort, L., Crump, B. C., Fortunato, C. S., McCue, L. A., Campbell, V., Simon, H. M., Baptista, A. M., and Zuber, P.: Factors affecting the bacterial community composition and heterotrophic production of Columbia River estuarine turbidity maxima, Microbiologyopen, 6, 1–15, https://doi.org/10.1002/mbo3.522, 2017.

Hershey, O. S. and Barton, H. A.: The Microbial Diversity of Caves, 69–90, https://doi.org/10.1007/978-3-319-98852-8_5, 2018.

Hershey, O. S., Kallmeyer, J., Wallace, A., Barton, M. D., and Barton, H. A.: High microbial diversity despite extremely low biomass in a deep karst aquifer, Front Microbiol, 9, 1–13, https://doi.org/10.3389/fmicb.2018.02823, 2018.

Hilman, B., Weiner, T., Haran, T., Masiello, C. A., Gao, X., and Angert, A.: The Apparent Respiratory Quotient of Soils and Tree Stems and the Processes That Control It, J Geophys Res Biogeosci, 127, https://doi.org/10.1029/2021JG006676, 2022.

Hofmann, R. and Griebler, C.: DOM and bacterial growth efficiency in oligotrophic groundwater: Absence of priming and co-limitation by organic carbon and phosphorus, Molecular Plant-Microbe Interactions, 31, 311–322, https://doi.org/10.3354/ame01862, 2018.

Hornsby, D., Mattson, R., and Mirti, T.: Surfacewater Quality and Biological Annual Report, Live Oak, Florida, 2004.

Hosen, J. D., McDonough, O. T., Febria, C. M., and Palmer, M. A.: Dissolved organic matter quality and bioavailability changes across an urbanization gradient in headwater streams, Environ Sci Technol, 48, 7817–7824, https://doi.org/10.1021/es501422z, 2014.

Jasechko, S. and Perrone, D.: Global groundwater wells at risk of running dry, Science (1979), 372, 418–421, https://doi.org/10.1126/science.abc2755, 2021.

Jin, J., Zimmerman, Andrew R., Moore, Paul J., and Martin, J. B.: Organic and inorganic carbon dynamics in a karst aquifer: Santa Fe River Sink-Rise system, north Florida, USA, J Geophys Res Biogeosci, 340–357, https://doi.org/10.1002/2013JG002350, 2014.

Kalhor, K., Ghasemizadeh, R., Rajic, L., and Alshawabkeh, A.: Assessment of groundwater quality and remediation in karst aquifers: A review, Groundw Sustain Dev, 8, 104–121, https://doi.org/10.1016/j.gsd.2018.10.004, 2019.

Karl, D. M.: Cellular nucleotide measurements and applications in microbial ecology, Microbiol Rev, 44, 739–796, https://doi.org/10.1128/mmbr.44.4.739-796.1980, 1980.

Karwautz, C., Zhou, Y., Kerros, M. E., Weinbauer, M. G., and Griebler, C.: Bottom-Up Control of the Groundwater Microbial Food-Web in an Alpine Aquifer, Front Ecol Evol, 10, 1–14, https://doi.org/10.3389/fevo.2022.854228, 2022.

Katz, B. G.: Sources of nitrate contamination and age of water in large karstic springs of Florida, Environmental Geology, 46, 689–706, https://doi.org/10.1007/s00254-004-1061-9, 2004.

Katz, B. G., Böhlke, J. K., and Hornsby, H. D.: Timescales for nitrate contamination of spring waters, northern Florida, USA, Chem Geol, 179, 167–186, https://doi.org/10.1016/S0009-2541(01)00321-7, 2001.

Katz, B. G., Sepulveda, A. A., and Verdi, R. J.: Estimating nitrogen loading to ground water and assessing vulnerability to nitrate contamination in a large karstic springs Basin, Florida, J Am Water Resour Assoc, 45, 607–627, https://doi.org/10.1111/j.1752-1688.2009.00309.x, 2009.

Köster, M. and Meyer-Reil, L. A.: Characterization of carbon and microbial biomass pools in shallow water coastal sediments of the southern Baltic Sea (Nordrügensche Bodden), Mar Ecol Prog Ser, 214, 25–41, https://doi.org/10.3354/meps214025, 2001.

Malki, K., Rosario, K., Sawaya, N. A., Székely, A. J., Tisza, M. J., and Breitbart, M.: Prokaryotic and Viral Community Composition of FreshwaterSprings in Florida, USA, Applied and Environmental Science, 11, 1–18, 2020.

Malki, K., Sawaya, N. A., Tisza, M. J., Coutinho, F. H., Rosario, K., Székely, A. J., and Breitbart, M.: Spatial and Temporal Dynamics of Prokaryotic and Viral Community Assemblages in a Lotic System (Manatee Springs, Florida), Appl Environ Microbiol, 87, 1–18, https://doi.org/10.1128/AEM.00646-21, 2021.

Martin, J. and Gordon, S. L.: Surface and ground water mixing, flow paths, and temporal variations in chemical compositions of karst springs, Groundwater Flow and Contaminant Transport in Carbonate Aquifers, 65–92, 2000.

Martin, J. B. and Dean, R. W.: Temperature as a natural tracer of short residence times for groundwater in karst aquifers, Karst Modeling. Karst Waters Institute Special Publication, 5, 236–242, 1999.

Martin, J. B. and Dean, R. W.: Exchange of water between conduits and matrix in the Floridan aquifer, Chem Geol, 179, 145–165, https://doi.org/10.1016/S0009-2541(01)00320-5, 2001.

Martin, J. B., Kurz, M. J., and Khadka, M. B.: Climate control of decadal-scale increases in apparent ages of eogenetic karst spring water, J Hydrol (Amst), 540, 988–1001, https://doi.org/10.1016/j.jhydrol.2016.07.010, 2016.

McDonough, L. K., Andersen, M. S., Behnke, M. I., Rutlidge, H., Oudone, P., Meredith, K., O'Carroll, D. M., Santos, I. R., Marjo, C. E., Spencer, R. G. M., McKenna, A. M., and Baker, A.: A new conceptual framework for the transformation of groundwater dissolved organic matter, Nat Commun, 13, 1–11, https://doi.org/10.1038/s41467-022-29711-9, 2022.

Miller, J. A.: Hydrogeologic framework of the Floridan Aquifer System in Florida and in parts of Georgia, Alabama, and South Carolina—Regional Aquifer-system Analysis: U.S. Geological Survey Professional Paper 1403-B, U.S. Geological Survey Professional Paper, 1403-B, 91, 1986.

Miller, J. A.: Hydrogeology of Florida, in: The Geology of Florida, edited by: Randazzo, A. and Douglas, J., University Press of Florida, Gainesville, 69–88, 1997.

Moore, P. J., Martin, J. B., and Screaton, E. J.: Geochemical and statistical evidence of recharge, mixing, and controls on spring discharge in an eogenetic karst aquifer, J Hydrol (Amst), 376, 443–455, https://doi.org/10.1016/j.jhydrol.2009.07.052, 2009.

Munch, D. A., Toth, D. J., Huang, C. T., Davis, J. B., Fortich, C. M., Osburn, W. L., Phlips, E. J., Quinlan, E. L., Allen, M. S., Woods, M. J., Cooney, P., Knight, R. L., Clarke, R. A., and Knight, S. L.: Fifty-Year Retrospective Study of the Ecology of Silver Springs, 2006.

Oberhelman, A., Martin, J. B., and Flint, M. K.: Methane cycling in the carbonate critical zone, Science of the Total Environment, 899, 165645, https://doi.org/10.1016/j.scitotenv.2023.165645, 2023.

Oulahal-Lagsir, N., Martial-Gros, A., Bonneau, M., and Blum, L. J.: Ultrasonic methodology coupled to ATP bioluminescence for the non-invasive detection of fouling in food processing equipment—Validation and application to a dairy factory, J Appl Microbiol, 89, 433–441, https://doi.org/10.1046/j.1365-2672.2000.01132.x, 2000.

Overholt, W. A., Trumbore, S., Xu, X., Bornemann, T. L. V., Probst, A. J., Krüger, M., Herrmann, M., Thamdrup, B., Bristow, L. A., Taubert, M., Schwab, V. F., Hölzer, M., Marz, M., and Küsel, K.: Carbon fixation rates in groundwater similar to those in oligotrophic marine systems, Nat Geosci, 15, 561–567, https://doi.org/10.1038/s41561-022-00968-5, 2022.

Pérez, M. T., Hörtnagl, P., and Sommaruga, R.: Contrasting ability to take up leucine and thymidine among freshwater bacterial groups: Implications for bacterial production measurements, Environ Microbiol, 12, 74–82, https://doi.org/10.1111/j.1462-2920.2009.02043.x, 2010.

Puri, H. S. and Vernon, R. O.: Summary of the Geology of Florida and a Guidebook to the Classic Exposures, Gainesville, FL, 312 pp., 1964.

Scott, T. M.: The lithostratigraphy of the Hawthorn Group (Miocene) of Florida, Florida Geological Survey, 59, 1–148, 1988.

Thore, A., Ansehn, S., Lundin, A., and Bergman, S.: Detection of bacteriuria by luciferase assay of adenosine triphosphate, J Clin Microbiol, 1, 1–8, https://doi.org/10.1128/jcm.1.1.1-8.1975, 1975.

Verity, P. G., Robertson, C. Y., Tronzo, C. R., Andrews, M. G., Nelson, J. R., and Sieracki, M. E.: Relationships between cell volume and the carbon and nitrogen content of marine photosynthetic nanoplankton, Limnol Oceanogr, 37, 1434–1446, https://doi.org/10.4319/lo.1992.37.7.1434, 1992.

Vick-Majors, T. J., Mitchell, A. C., Achberger, A. M., Christner, B. C., Dore, J. E., Michaud, A. B., Mikucki, J. A., Purcell, A. M., Skidmore, M. L., Priscu, J. C., Adkins, W. P., Anandakrishnan, S., Barbante, C., Barcheck, G., Beem, L., Behar, A., Beitch, M., Bolsey, R., Branecky, C., Edwards, R., Fisher, A., Fricker, H. A., Foley, N., Guthrie, B., Hodson, T., Horgan, H., Jacobel, R., Kelley, S., Mankoff, K. D., McBryan, E., Powell, R., Sampson, D., Scherer, R., Siegfried, M., and Tulaczyk, S.: Physiological ecology of microorganisms in subglacial lake whillans, Front Microbiol, 7, 1–16, https://doi.org/10.3389/fmicb.2016.01705, 2016.

Wilhartitz, I., Mach, R. L., Teira, E., Reinthaler, T., Herndl, G. J., and Farnleitner, A. H.: Prokaryotic community analysis with CARD-FISH in comparison with FISH in ultra-oligotrophic ground- and drinking-water, J Appl Microbiol, 103, 871–881, https://doi.org/10.1111/j.1365-2672.2007.03319.x, 2007.

Wilhartitz, I. C., Kirschner, A. K. T., Stadler, H., Herndl, G. J., Dietzel, M., Latal, C., MacH, R. L., and Farnleitner, A. H.: Heterotrophic prokaryotic production in ultraoligotrophic alpine karst aquifers and ecological implications, FEMS Microbiol Ecol, 68, 287–299, https://doi.org/10.1111/j.1574-6941.2009.00679.x, 2009.

Wilhartitz, I. C., Kirschner, A. K. T., Brussaard, C. P. D., Fischer, U. R., Wieltschnig, C., Stadler, H., and Farnleitner, A. H.: Dynamics of natural prokaryotes, viruses, and heterotrophic nanoflagellates in alpine karstic groundwater, Microbiologyopen, 2, 633–643, https://doi.org/10.1002/mbo3.98, 2013.

Williams, L. J. and Kuniansky, E. L.: Revised Hydrogeologic Framework of the Floridan Aquifer System in Florida and Parts of Georgia, Alabama, and South Carolina, U.S. Geological Survey Professional Paper 1807, https://doi.org/http://dx.doi.org/10.3133/pp1807., 2016.

Worthington, S., Davies, G., and Clifford, F.: Matrix, fracture, and channel components of storage and flow in a Paleozoic limestone aquifer, 113–128, 2000.

Wos, M. and Pollard, P.: Bacterial physiological state in wastewater: Monitoring maintenance and production with Leu/TdR ratio for less pollution, Water Air Soil Pollut, 223, 4507–4513, https://doi.org/10.1007/s11270-012-1213-6, 2012.

Wu, X., Wu, L., Liu, Y., Zhang, P., Li, Q., Zhou, J., Hess, N. J., Hazen, T. C., Yang, W., and Chakraborty, R.: Microbial interactions with dissolved organic matter drive carbon dynamics and community succession, Front Microbiol, 9, 1–12, https://doi.org/10.3389/fmicb.2018.01234, 2018.