1. Introduction

Karst aquifers are formed from the chemical dissolution of soluble bedrock and serve as a drinking water resource for a quarter of the world’s population (Ford & Williams, 2007; Hartmann et al., 2014). Karst water resources are particularly susceptible to contamination as transport velocities in fractures and conduits are...
several orders of magnitude greater than in porous media (Al Aamery et al., 2021). Despite a global need to understand and ensure water quality in karst, not much is known about the spatiotemporal distribution of contaminant removal within the subterranean caves that drain karst landscapes (Husic, Fox, Mahoney, et al., 2020; Simon et al., 2007).

Contaminant loading of reactive dissolved inorganic nitrogen (DIN), specifically nitrate (NO\textsubscript{3}^{-}), has increased considerably in the last few decades due to anthropogenic activity (Vilmin et al., 2018). While the removal of NO\textsubscript{3}^{-} is recognized to be driven by temperature, residence time, organic matter, and oxic conditions (Birgand et al., 2007; Mulholland et al., 2008), recent work has highlighted that given the right cocktail of field conditions, reactivity rates can be substantially greater in certain zones or time periods compared to others (Bernard-Jannin et al., 2017; Dwivedi et al., 2018; Krause et al., 2017). These conditions often occur where organic rich material and reducible nutrients interact, and residence times are long. It is plausible that subsurface karst may experience these spatiotemporal features given that karst conduits behave as an interface of surface water and groundwater dynamics (Atkinson, 1977; Husic, Fox, Adams, et al., 2020; White, 2002).

Though subsurface karst represents an extreme heterotrophic environment (Simon et al., 2003), numerous physical and biogeochemical reactions have been observed or posited (Table S1). Sediment transport occurs when sediment enters via sinks and exits at springs (Herman et al., 2008). Due to energy limitations in conduits, sediment organic carbon (SOC) and nitrogen (SN) are deposited and temporarily stored in the subsurface (Husic, Fox, Ford, et al., 2017). This stored SOC and SN decompose to carbon dioxide (CO\textsubscript{2}) and mineralize to ammonium (NH\textsubscript{4}^{+}), respectively (Musgrove et al., 2016; Simon & Benfield, 2001). Because of aphytic conditions, primary production—or the incorporation of CO\textsubscript{2} into SOC—does not occur, but immobilization of DIN into the SN biomass does (Simon & Benfield, 2002). Further, NH\textsubscript{4}^{+} may also be oxidized to NO\textsubscript{3}^{-} that can then be denitrified to dinitrogen gas (N\textsubscript{2}) (Musgrove et al., 2016). However, it is often difficult to directly measure turnover in karst conduits as they can be situated dozens of meters below the Earth’s surface at unknown geographic locations (Hartmann et al., 2014). Thus, one approach to estimating subsurface activity is to measure the quality of surficial inputs, estimate the residence time of material, and monitor the resurgence of sediment and nutrients at a spring.

Numerical modeling provides advantages in situations where data cannot feasibly be collected, such as hard-to-reach caves, and where watershed managers desire to understand how future changes will affect system functions. One method to reconcile cave inaccessibility is to measure inputs and outputs to a system and couple those measurements to calibrated physically based models that can simulate non-linear water quality processes (Chen et al., 2017; Ford et al., 2017). Further, the integration of additional data-streams, such as ambient stable isotopes (δ\textsubscript{13}C\textsubscript{Sed}, δ\textsubscript{15}N\textsubscript{Sed}, and δ\textsubscript{15}N\textsubscript{NO3}), can add even further confidence in modeled results (Jensen et al., 2018). Given the rate at which anthropogenic activity is altering the natural environment, numerical models can be used to forecast how projected activities will impact the function of streams and provide planning information to land managers (Chatterjee et al., 2018; Dosdogru et al., 2020; Melsen & Guse, 2021; Zarrineh et al., 2020). Anthropogenic environmental drivers like CO\textsubscript{2}-propelled increases to global temperature (Trimmer et al., 2012), alterations to regional-scale climate regimes (Al Aamery et al., 2016), and dynamic land use conversion (Stets et al., 2020) will alter hydrologic and biogeochemical functioning of streams. The impacts of these external drivers on nitrate removal and export in subsurface karst caves is poorly understood and further investigation is warranted given the role of karst aquifers to global water resources.

The objectives of this study were to: (a) use data and modeling to test a hypothesis for sediment biogeochemistry in karst caves, which is under-reported for these environments; (b) advance numerical modeling of internal cycling for caves using stable isotopes, and (c) provide results that advance knowledge of spatial variation, time variation, and environmental controls on N cycling in karst caves. We hypothesized that outputs from karst caves would differ from inputs due to in-conduit sediment biogeochemical activity driven by microbial-mediated reactions. We collected elemental (SOC, SN, and C:N) and isotopic (δ\textsubscript{13}C\textsubscript{Sed} and δ\textsubscript{15}N\textsubscript{Sed}) sediment data from conduit inputs and outputs as well as NO\textsubscript{3}^{-} and δ\textsubscript{15}N\textsubscript{NO3} data from system outputs. To reduce uncertainty in nitrate removal estimates, we constructed a new SN and δ\textsubscript{15}N\textsubscript{Sed} fate model and utilized it as a boundary condition for an existing model of in-conduit dynamics. We used this calibrated model to investigate spatial variation of N cycling in the cave, investigate temporal distribution of N
cycling, and simulate future climate and land use scenarios to assess sensitivity of in-conduit \( \text{NO}_3^- \) removal and export to environmental drivers.

2. Materials and Methods

2.1. Study Site

The Royal Spring groundwater basin (58 km\(^2\)) is located in the highly karstified Inner Bluegrass Region of Kentucky, USA (Figure 1). The region is characterized by a temperate climate (MAT: 13.0 ± 0.7°C; MAP: 1,170 ± 200 mm), is underlain by phosphatic limestone of the Middle Ordovician period, and consists of moderately deep, well-drained soils. Land use in the basin is predominantly rural (60%) with urban (40%) headwaters. Dozens of swallets (i.e., in-stream sinkholes) line the main corridor of the surface stream and actively re-route water to the subsurface, thus leaving the surface channel dry for 80% of the year (Husic, Fox, Agouridis, et al., 2017; Figure S1a). A primary phreatic cavern, 20 m below the ground surface, transports sediment and nutrients drained from the surface and surrounding bedrock (Figure S1b). The conduit reemerges to the surface as Royal Spring (243 m a.s.l.; \( Q = 0.67 \text{ m}^3 \text{ s}^{-1} \)), which serves as the raw municipal water source for the City of Georgetown, Kentucky. The high degree of connectivity between surface contamination and subsurface transport is thought to be the primary driver for water quality deterioration at Royal Spring (UKCAFE, 2011).

2.2. Sample Collection and Analysis

Suspended sediment data (SOC, SN, \( \delta^{13}C_{\text{sed}} \), and \( \delta^{15}N_{\text{sed}} \)) were collected from two surface inputs, an urban tributary and an agricultural tributary, as well as the sub-surface output, Royal Spring, from September 2012 to August 2013 (Figure 1). The in-situ samplers collect a time-integrated sediment signal representative of the duration of sampler deployment (Phillips et al., 2000). The sediment samplers are 1-m long PVC tubes that have small inlets (5 mm) to allow fine sediment to enter, settle, and accumulate in the sampler over the period of deployment, typically ~2 weeks. In the lab, the fine sediment fraction was homogenized and acidified with 6% sulfuric acid to remove inorganics. To generate elemental (SOC, SN, and C:N) and isotopic (\( \delta^{13}C_{\text{sed}} \) and \( \delta^{15}N_{\text{sed}} \)) data, the following was performed in the University of Arkansas Stable Isotope Lab: sediment was combusted at 980°C on a Costech ECS 4010 elemental analyzer, passed through a gas chromatograph column (3 m HS-Q), and analyzed on a Thermo Finnigan Delta-Plus XP isotope ratio mass spectrometer. The elemental compositions (SOC and SN) were reported as fractions of total sediment mass whereas the isotopic (\( \delta^{13}C_{\text{sed}} \) and \( \delta^{15}N_{\text{sed}} \)) results were reported in delta notation as

\[
\delta = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000
\]

where \( R \) is the ratio of the abundance of the heavy to light isotope (e.g., \( ^{15}N/^{14}N \)) in field samples as well as reference standards of known isotopic ratio. The elemental reference was acetanilide (%C = 71.09%, %N = 10.36%) and the isotopic references were DORM (\( \delta^{13}C = -19.59, \delta^{15}N = 12.46 \)) and CCHIX (\( \delta^{13}C = -16.4\text{‰}, \delta^{15}N = 3.2\text{‰} \)). Average standard deviations of SOC, SN, \( \delta^{13}C \), and \( \delta^{15}N \) standards were 0.34%, 0.25%, 0.20‰, and 0.20‰, respectively. Average standard deviations of SOC, SN, \( \delta^{13}C \), and \( \delta^{15}N \) replicates were 0.10%, 0.01%, 0.08‰, and 0.18‰, respectively.

Discrete \( \text{NO}_3^- \) grab samples (n = 211) were collected from January 2012 to September 2013 from within the conduit using a check-valve bailer (Phreatic Conduit, Figure 1). The Kentucky Geological Survey laboratory analyzed \( \text{NO}_3^- \) samples by following U.S. EPA Method 300.0, using a Dionex ICS-3000. \( \text{NO}_3^- \) concentration was determined by retention time and peak area compared to a calibration curve generated from known
standards. Field \((n = 8)\) and lab \((n = 49)\) duplicates of \(\text{NO}_3^-\) had low standard deviations of 0.07 and 0.02 \(\text{mg N/L}\), respectively. No field or lab blanks registered above the method detection limit. The collection and analysis of other dissolved nitrogen species (\(\text{NH}_4^+\), \(\text{DON}\), and \(\delta^{15}\text{NNO}_3\)) are detailed further in the Supporting Information S1 and prior work (Husic et al., 2019; Husic, Fox, Adams, et al., 2020).

2.3. Modeling Spatiotemporal Nitrogen Processing

We developed a numerical model to simulate physical and biogeochemical processes affecting carbon and nitrogen fate in karst caves (Figure 2). In this study, we add new SN and \(\delta^{15}\text{NSed}\) modules to an existing numerical modeling framework of SOC, \(\delta^{13}\text{CSed}\), \(\text{NO}_3^-\), \(\delta^{15}\text{NNO}_3\), \(\text{NH}_4^+\), and DON (Husic et al., 2019; Husic, Fox, Adams, et al., 2020; Husic, Fox, Ford, et al., 2017). The model simulates water, sediment, and carbon fluxes into and out of the conduit (Husic, Fox, Ford, et al., 2017), sediment particle-size distributions (Husic, Fox, Agouridis, et al., 2017), the sediment exchange between the water column and karst bed (Husic, Fox, Ford, et al., 2017), the distribution of organic matter and reactivity (from recalcitrant soil to labile algal carbon) throughout the conduit (Husic, Fox, Ford, et al., 2017), the \(\text{NO}_3^-\), \(\text{NH}_4^+\), and DON concentration of aquifer water recharging the conduit (Husic et al., 2019), and nitrification and denitrification rates within the karst conduit (Husic, Fox, Adams, et al., 2020). The model executes at an hourly timestep and at a 1-km spatial
increment to satisfy the Courant condition. The reader can find a full description of model equations, constraints, and parameterization in the Supporting Information S1.

The new SN and $\delta^{15}$N$_{sed}$ modules provide constraints on sediment nitrogen pool reactions, such as mineralization and immobilization, which can have downstream impacts to other processes, such as nitrification and denitrification that utilize the mineralization and immobilization products. A list of all relevant modeled parameters and their uncertainty can be found in Table S2. Mineralization of SN was estimated using carbon-to-nitrogen stoichiometry and first-order kinetics of SOC decomposition (Manzoni & Porporato, 2009). The production-to-respiration ratio—a proxy for immobilization-to-mineralization—was varied to estimate the immobilization of DIN into SN (Hall et al., 2016). All reactions were temperature-dependent and this relationship was modeled using Arrhenius expressions (Sheibley et al., 2003). Lastly, the cycling of nitrogen between oxidation states is recognized to discriminate in favor of lighter isotopes ($^{14}$N vs. $^{15}$N) in a process termed fractionation (Kendall et al., 2007). We model $\delta^{14}$N transfer and fractionation across particulate and dissolved nitrogen pools, which provides an additional constraint to in-conduit biogeochemistry.

A framework of model inputs, model simulation, parameter optimization, comparison to data, and statistical testing was developed (Figure 3). The hierarchal model structure was executed in the following order (a) sediment transport, (b) sediment carbon, (c) sediment nitrogen, (d) dissolved nitrogen, and (e) environmental drivers. The basic structure of evaluation, for each sub-model, involved randomly sampling parameters, executing the sub-model, and then performing statistical comparison to observed data to accept or reject the sub-model simulation. Starting with the parent model in the hierarchal set-up (i.e., sediment transport), ~100 successful parameter sets were identified. These sediment model results were then fed into the SOC and $\delta^{13}$C$_{sed}$ sub-model where ~100 successful carbon parameter sets were identified, and this process was repeated for the remaining sub-models. Therefore, modeled DIN removal satisfies the statistical criteria of all hierarchical sub-models and incorporates the uncertainty and variability present in all sub-models. The reader is referred to the Supporting Information S1 for full details of model parameterization and time-series of the model inputs for this study (Figure S2).

Regarding the new SN and $\delta^{15}$N$_{sed}$ module, 32 time-integrated sediment samples from agricultural and urban tributaries were analyzed for their elemental and isotopic nitrogen values and used as upstream inputs to the model. Internal model parameters ($k_{DEC}$, $k_{litter}$, $k_{fangle}$, $P : R$, and $P : R$) were tuned so that by the time material exited the conduit, it would not be statistically different from the 18 sediment samples collected at Royal Spring. For sediment results, we compared the measured distributions of SOC, $\delta^{13}$C$_{sed}$, SN, and $\delta^{15}$N$_{sed}$ to those modeled at Royal Spring using the non-parametric Mann-Whitney rank-sum test. Simulation results were retained if the difference between the measured and modeled distributions of all four variables were not significantly different ($\alpha = 0.05$), indicating agreement between model and data. NO$_3^-$ data were compared to NO$_3^-$ model simulations at an hourly time-step using the Nash Sutcliffe Efficiency (NSE). Half the data were used for calibration ($n = 106$) and half for validation ($n = 105$). NSE was calculated as

$$\text{NSE} = 1 - \frac{\sum_{t=1}^{T} \left( C_{m} - C_{o} \right)^2}{\sum_{t=1}^{T} \left( C_{m} - \bar{C}_{o} \right)^2}$$

where $T$ is the total number of observations, $C_{o}$ is the observed value at time $t$, $C_{m}$ is the modeled value at time $t$, and $\bar{C}_{o}$ is the mean of observed values. The NSE metric ranges from $-\infty$ to 1, with 1 indicating a perfect match of the model to data and 0 indicating the model performs no better than the mean of the data (Moriasi et al., 2007). Nitrate simulation results were retained if the modeled NSE was greater than 0.3, which given the hourly evaluation timestep should adequately capture mean conduit behavior and seasonal trends (Wellen et al., 2015).

### 2.4. Forecasting Environmental Drivers

We investigated how environmental drivers—defined as shifts to climate and land use by the year 2057—may increase or decrease spatiotemporal NO$_3^-$ removal in the karst conduit (Table S3). Downscaled Global Climate Model (GCM) results were used to forecast relative shifts in mean monthly temperature and flow-rate (Table S3). The GCM design is based on work performed in a neighboring basin in the Inner Bluegrass Region, Kentucky, USA (Al Aamery et al., 2016). Specifically, Al Aamery et al. (2016) ran an ensemble of...
112 GCM models through a calibrated SWAT model to relate future climate changes to monthly streamflow variability. Discharge projections (ΔQ) are variable and range from −11.0% in June to +33.8% in December, indicating drier summers and wetter winters (Table S3). An empirical relationship was created to relate monthly air temperature in the region to conduit water temperature (Tcond = 0.35 × Tair + 10.59, R² = 0.54, p < 0.05). From there, GCM forecasts were used to relate future air temperature changes to related conduit temperature changes (ΔT; Table S3). United States Geological Survey EROS spatially explicit simulations were used to forecast land cover change (Sohl et al., 2007). Depending on the scenario (A2, B1, or A1B), by 2057 the present-day 40% urban land use will increase to 84% (A2), 88% (B1), or 93% (A1B), which will impact the loading of sediment from urban and agricultural tributaries (Table S3). In addition to sediment,
land use change also alters landscape nitrate loading. We used recent results from a SWAT model (conducted in a basin with similar land use and climate impacts) to simulate seasonal nitrate concentration changes ($\Delta N$) related to land use and stress (Zarrineh et al., 2020). For a full description of climate and land use projection modeling, the reader is referred to the Supporting Information S1.

Within the numerical model, the effects of temperature changes are realized in all biogeochemical transformations; flow rate changes are reflected in quantity of inputs, erosion intensity, and sediment residence time; and land use changes impact the quality of SOC and SN supplied to the subsurface. Using present-day calibration of the sediment, carbon, and nitrogen dynamics, we simulated 12 future scenarios using mean changes in temperature ($\Delta T$), discharge ($\Delta Q$), and land use ($\Delta N$, A2, B1, A1B) as variable inputs to the model. For the first 9 scenarios, we adjusted only a single variable ($T$, $Q$, or land use) using the projected mean ($\Delta$) plus or minus one standard deviation ($\pm 1 \sigma$) while keeping the other two variables at present-day values: (a) $\Delta T$, (b) $\Delta T - 1 \sigma$, (c) $\Delta T + 1 \sigma$, (d) $\Delta Q$, (e) $\Delta Q - 1 \sigma$, (f) $\Delta Q + 1 \sigma$, (g) B1 plus $\Delta N$, (h) A2 plus $\Delta N - 1 \sigma$, (i) A1B plus $\Delta N + 1 \sigma$. For the final three scenarios, we varied all three variables together to see cumulative impacts of multiple drivers: (j) $\Delta T$, $\Delta Q$, B1 plus $\Delta N$; (k) $\Delta T - 1 \sigma$, $\Delta Q - 1 \sigma$, A2 plus $\Delta N - 1 \sigma$; and (l) $\Delta T + 1 \sigma$, $\Delta Q + 1 \sigma$, A1B plus $\Delta N + 1 \sigma$. Thereafter, for each scenario, we compared the percent change in a number of yields in a two-year period relative to present-day conditions: sediment carbon input and decomposition in the conduit (SOC$_{IN}$ and SOC$_{DEC}$); sediment nitrogen input, mineralization, and immobilization in the conduit (SN$_{IN}$, SN$_{MIN}$, and SN$_{IMM}$); and nitrate export and removal via denitrification (NO$_3^-$$_{OUT}$ and NO$_3^-$$_{DEN}$).

### 3. Results and Discussion

As will be shown, results support the hypothesis that N exports from the karst cave differ from inputs due to sediment-mediated internal cycling. Data results provide initial evidence, which is bolstered by calibrated numerical model simulations. Stable N isotope modeling explicitly quantifies the magnitudes of mineralization and immobilization, which are otherwise overlapped in elemental N results alone. The successful model performance allows for investigation of time distribution, spatial variation, and environmental controls on N cycling in the karst cave, as well as discussion and comparison with other systems. As mentioned in the methods, this main-text discussion focuses on the new SN, $\delta^{15}$N$_{sed}$ and DIN evaluation, whereas a discussion of the other sub-models can be found in Figure S3 and Table S4.

#### 3.1. Inferring In-Conduit Nitrogen Hypothesis From Elemental and Isotope Data

Elemental (SN, SOC, and C:N) and isotopic ($\delta^{13}$C$_{sed}$ and $\delta^{15}$N$_{sed}$) sediment data showed substantial differences from tributary inputs to the spring output, which allowed inference as to the net function of internal conduit biogeochemistry (Table 1). Spring outputs were relatively depleted in nitrogen content (SN = 0.36 ± 0.09%) when compared to tributary inputs (SN = 0.43% ± 0.07%) ($p < 10^{-2}$), but not as depleted as carbon inputs (SOC = 5.23% ± 1.24%) to outputs (SOC = 3.35% ± 0.60%) ($p < 10^{-5}$). Additionally, sediment discharged from Royal Spring was significantly enriched in 15N ($\delta^{15}$N$_{sed}$ = 6.45‰ ± 0.71‰) compared to tributary inputs ($\delta^{15}$N$_{sed}$ = 5.07‰ ± 1.01‰) ($p < 10^{-5}$). To exclude the possibility of unaccounted sources of SOC and SN, we evaluated $\delta^{13}$C$_{sed}$ and particle size distribution results (common sediment tracers; Davis & Fox, 2009) and found no significant difference between inputs and outputs, which suggests the same particulate matter throughout. Taken together, these results suggest that the temporary residence

### Table 1

<table>
<thead>
<tr>
<th>Site</th>
<th>No. of samples</th>
<th>SOC (%)</th>
<th>SN (%)</th>
<th>C:N (−)</th>
<th>$\delta^{13}$C$_{sed}$ (%)</th>
<th>$\delta^{15}$N$_{sed}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban tributary</td>
<td>24</td>
<td>5.70 (±1.06)</td>
<td>0.44 (±0.08)</td>
<td>12.99 (±1.37)</td>
<td>−26.36 (±0.67)</td>
<td>4.65 (±0.74)</td>
</tr>
<tr>
<td>Rural tributary</td>
<td>8</td>
<td>3.83 (±0.26)</td>
<td>0.41 (±0.05)</td>
<td>9.43 (±0.51)</td>
<td>−27.51 (±0.44)</td>
<td>6.33 (±0.50)</td>
</tr>
<tr>
<td>All tributaries</td>
<td>32</td>
<td>5.23 (±1.24)</td>
<td>0.43 (±0.07)</td>
<td>12.10 (±1.98)</td>
<td>−26.64 (±0.80)</td>
<td>5.07 (±1.01)</td>
</tr>
<tr>
<td>Royal Spring</td>
<td>18</td>
<td>3.35 (±0.60)</td>
<td>0.36 (±0.09)</td>
<td>9.47 (±0.80)</td>
<td>−26.61 (±0.86)</td>
<td>6.45 (±0.71)</td>
</tr>
</tbody>
</table>

*Note. Results are presented as a mean ± standard deviation.*
of sediment within the karst cave contributes significant biogeochemical transformations to transported carbon and nitrogen.

Sediment data results indicated decomposition of SOC and net-mineralization of SN during temporary residence in the karst cave. However, the extent of SOC decomposition (36% decrease from 5.23% to 3.35%) outpaced SN mineralization (16% decrease from 0.43% to 0.36%). To explain the non-linearity between observed SOC and SN trends, we investigated δ\textsuperscript{13}C\textsubscript{sed} and δ\textsuperscript{15}N\textsubscript{sed} transformations. While δ\textsuperscript{13}C\textsubscript{sed} data showed non-significant differences (−26.64‰ ± 0.80‰ vs. −26.61‰ ± 0.86‰), which was expected given negligible 13C enrichment during SOC decomposition (Kendall et al., 2001), the δ\textsuperscript{15}N\textsubscript{sed} data showed substantial enrichment (5.07‰ ± 1.01‰ vs. 6.45‰ ± 0.71‰). The nearly 1.5‰ enrichment in δ\textsuperscript{15}N is indicative of a biogeochemical process that prefers lighter \textsuperscript{14}N to heavier \textsuperscript{15}N (Jensen et al., 2018). Based on our conceptual model for subsurface fate (Figure 2), δ\textsuperscript{15}N\textsubscript{sed} enrichment is either the result of SN mineralization or DIN immobilization. It is unlikely that SN mineralization alone can explain the observed increase because isotope enrichment from fractionation is small during mineralization (Kendall et al., 2007). Therefore, data suggest that immobilization plays a role in sediment biogeochemistry, which is corroborated by the fact that δ\textsuperscript{15}N of sediment inputs trended toward that of conduit nitrate (cave water δ\textsuperscript{15}N\textsubscript{NO\textsubscript{3}} = 11.25‰ ± 6.96‰, n = 23; unpublished data, Gerlitz, 2020). Incorporation of heavier N-15 of nitrate into the sediment biomass would cause an increase in the δ\textsuperscript{15}N\textsubscript{sed} signature. Further evidence for the presence of N immobilization was given by the decrease in C:N from tributary inputs (12.10 ± 1.98) to Royal Spring (9.47 ± 0.80), which approaches the fairly ubiquitous microbial biomass ratio (8.60 ± 0.30) (Cleveland & Liptzin, 2007). However, without numerical modeling of the transformations of nitrogen species, it is difficult to estimate the extent to which particular processes dominate. Therefore, in the next section, we establish the efficacy of a numerical model to evaluate processes and identify spatiotemporal variability in nitrogen processing.

3.2. Modeling Spatial and Temporal Nitrate Removal

3.2.1. Model Evaluation and Uncertainty

Sediment biogeochemistry model results successfully simulated the data-observed trends in SOC, SN, δ\textsuperscript{13}C\textsubscript{sed}, and δ\textsuperscript{15}N\textsubscript{sed} transport through the karst conduit (histogram comparison, Figure 4; time-series plot, Figure S3). The model doesn’t capture the width of the observed data distribution as the physics and biogeochemistry in the model are averaged over 1-km reaches, which has the impact of averaging out simulation extremes. Recall, successful model performance was determined if data and modeled distributions of δ\textsuperscript{13}C\textsubscript{sed} and δ\textsuperscript{15}N\textsubscript{sed} were not significantly different as evaluated by the Mann Whitney test (Table S4). In general, numerical modeling indicates that SOC and SN are deposited to the karst conduit bed where they undergo decomposition (63.6 ± 10.9 mg C m\textsuperscript{-2} d\textsuperscript{-1}) and mineralization (8.9 ± 3.1 mg N m\textsuperscript{-2} d\textsuperscript{-1}), respectively. In addition to mineralization, the SN pool also experiences immobilization (8.0 ± 2.9 mg N m\textsuperscript{-2} d\textsuperscript{-1}), as was suggested by data results. Our modeled immobilization rates were greater than those observed in an accessible cave in West Virginia, USA (0.32 ± 0.2 mg N m\textsuperscript{-2} d\textsuperscript{-1}; Simon & Benfield, 2002). This difference in rates could be due to the low organic matter stocks in the West Virginia cave relative to our site. Compared to surface-streams, however, both cave systems are on the lower-end of literature-reported uptake values (13.82–328.32 mg N m\textsuperscript{-2} d\textsuperscript{-1}) likely due to the lack of photic primary production (Peterson et al., 2001; Simon & Benfield, 2002).
The sediment biogeochemistry model, calibrated with stable nitrogen isotope subroutines, was then applied as a boundary condition for the in-conduit NO$_3^-$ model, greatly improving performance (Figure 5 and Table S4). During calibration, if we turn off sediment-mediated reactions, the NO$_3^-$ model performs poorly (NSE = 0.06), whereas including reactions increases performance substantially (NSE = 0.68) (Figure S4). During the validation period, which was characterized by more variable hydrology, the NSE was lower (NSE = 0.18), but given that our DIN model is evaluated at an hourly time-step, and statistics are recognized to worsen as temporal resolution increases (Moriasi et al., 2007), we considered the model performance to be satisfactory. Further, our model performs comparably to other recent NO$_3^-$ studies that utilized much lower temporal resolutions for evaluation, such as monthly (e.g., Fonseca et al., 2014) and daily (e.g., Bauwe et al., 2015). The impact of including sediment-mediated reactions was most helpful during the dry season (June to November) when conduit recharge from the surrounding bedrock is high in nitrate concentration, but Royal Spring concentrations are lower. To reconcile this concentration gradient, sediment-facilitated denitrification ($29.7 \pm 5.3$ mg N m$^{-2}$ d$^{-1}$) and immobilization ($8.0 \pm 2.9$ mg N m$^{-2}$ d$^{-1}$) reduce the higher aquifer concentrations to those observed at the spring.

Sensitivity analysis of the model parameters highlights the importance of adequately parameterizing the immobilization rate and organic matter reactivity (Table S2). The SN and $\delta^{15}$N$_{sed}$ model results were sensitive to the ratio of immobilization-to-mineralization (median value = 0.88), indicating that decomposition of the SN pool slightly outpaces growth and is a controlling process. Further, the model was sensitive to the rate at which labile (litter and algae) and recalcitrant (soil) organic stocks are decomposed. In general, the rate of turnover for labile pools ($\sim$10$^{-3}$ d$^{-1}$) was two orders of magnitude greater than that of the recalcitrant pool ($\sim$10$^{-5}$ d$^{-1}$). The SN pool also provided an additional constraint on DIN reactions as material fluxes between the SN and DIN pools limited the availability for turnover. Regarding DIN model sensitivity, the model was most sensitive to the rate of denitrification of NO$_3^-$, followed by the rate of nitrification of NH$_4^+$, and relatively insensitive to mineralization of DON. Further details regarding sensitivity and performance of all model components (sediment and carbon) are included in Table S4. Coupling multi-objective calibration during modeling assisted with fine-tuning reaction rates and reducing overall uncertainty, which corroborates with a growing number of studies showing utility of combining stable isotopes with numerical modeling to infer processes in environmental water systems (Adiyanti et al., 2016; Ford et al., 2017; Husic, Fox, Adams, et al., 2020). With confidence in the ability of our model to simulate trends in nitrogen physics and biogeochemistry, we proceeded to investigate temporal and spatial variations of N cycling in the cave and simulate future climate and land use scenarios to assess response of in-conduit nitrate removal to environmental drivers.

### 3.2.2. Temporal Variability in Nitrate Removal

Numerical modeling of temporal NO$_3^-$ removal shows lower removal in wet seasons contrasted by higher removal in dry seasons (Figure 6). Wet season behavior in the conduit appears to be relatively chemo-static with only slight changes in the NO$_3^-$ concentration of outflow at Royal Spring compared to aquifer...

![Figure 5. Timeseries of nitrate from the karst conduit and discharge at Royal Spring. Nitrate model bounds contain 95% of the solution space. Three data outliers are beyond the y-axis limits (January ’13) and are enumerated in a box to not distort the axis. Gray-shaded regions indicate dry seasons.](image-url)
recharge to the conduit. During wet periods, which account for 75% of spring discharge, residence time of water in the 16 km-long conduit can be less than 24 hr (Husic, Fox, Agouridis, et al., 2017) thus not allowing for substantial interaction with denitrifying and immobilizing bacteria in the sediment bed. On the other hand, dry season behavior indicates stark differences between aquifer recharge and spring discharge, averaging 17.7 ± 21.8% removal (with a max of 90%) of conduit inputs. Rates of modeled denitrification (29.7 ± 5.3 mg N m⁻² d⁻¹) within the sediment bed are on the lower-end reported in surface rivers (100–1,000 mg N m⁻² d⁻¹; Piña-Ochoa & Álvarez-Cobelas, 2006), but given the long residence times, high temperatures, and bioavailable carbon source, denitrification cumulatively removes considerable NO₃⁻. Thus, temporal moments of elevated NO₃⁻ removal are mediated by hydrologic residence time and the seasonality of DIN recharge to the conduit.

The conduit sediment bed shows similarities and differences with surface streams draining mixed-use landscapes. Surface systems show high temperature dependence with low winter temperatures reducing production and transformation rates by orders of magnitude (Veraart et al., 2011). Further, enhanced removal during dry periods is similar to slow-moving surface waters, overlying agriculturally derived sediments, where denitrification dominates NO₃⁻ fate (Birgand et al., 2007; Comer-Warner et al., 2019, 2020; Zarnetske et al., 2011). On the other hand, light-absent karst caves do not have light-dependent autotrophy and temperature dependence on reactions is mediated due to the stability of subsurface conduit water temperatures (T = 14.1 ± 3.5°C). Further, as karst caves are dependent upon external sources of organic matter to fuel reactions (Simon et al., 2007), hydrologic delivery of SOC becomes a greater control on elevated periods of removal in the subsurface whereas surface streams can generate autochthonous carbon to fuel removal. Our results indicate that moments of elevated removal occur during dry conditions when organic matter is temporarily trapped in the subsurface, water residence times are long, and temperatures are high. These results suggest that other mature karst systems characterized by seasonal hydrologic behavior, connections to surface inputs, and export of sediment at springs could experience similar behavior.

### 3.2.3. Spatial Variability in Nitrate Removal

Numerical modeling of spatial NO₃⁻ removal shows greater removal in the upper conduit reaches near entrances where sediment is delivered with lower removal downstream (Figure 7a). Reach-averaged rates (in 4-km segments), from upstream to downstream, were 34.1 ± 7.6, 32.4 ± 5.1, 30.5 ± 5.6, and 28.0 ± 5.5 mg N m⁻² d⁻¹. Elevated removal was primarily driven by greater SOC content in upstream sediment (Figure 7b). Urban tributaries, located in the headwaters of Royal Spring (Figure 1), are highly concentrated in SOC and thus supply the upstream conduit reaches with labile organic inputs (Table 1). Once in the conduit, energy constraints on transport cause substantial deposition of surface inputs (Husic, Fox, Agouridis, et al., 2017; Husic, Fox, Ford, et al., 2017), providing the carbon needed to sustain elevated denitrification (Hall et al., 2016). On the other hand, the downstream portions of the conduit are largely disconnected from the surface and rely on upstream transport of already-processed material to fuel weaker denitrification. Thus, spatial NO₃⁻ removal is controlled by the cyclical delivery of labile SOC from the surface during the wet season followed by elevated denitrification in the well-connected upstream cells during the warmer dry season.

Spatial gradients of NO₃⁻ removal in cave streams have not been discussed extensively, though the need has been stated (Simon et al., 2007), partly due to the difficulty in accessing subterranean field sites (Husic, Fox, Adams, et al., 2020). Our results show three-fold increase in removal near sediment-input zones during the summer (~60 mg N m⁻² d⁻¹) relative to disconnected reaches (~20 mg N m⁻² d⁻¹). Our numerical modeling results show some parallels and differences when viewed beside surface stream studies. While quasi-seasonal organic matter fluctuations in surface streams are based on autotrophy and heterotrophy (Arango et al., 2007; Hall et al., 2016), the subsurface stream behavior is more heavily influenced by hydrodynamic

![Figure 6. Numerical modeling of temporal variability in nitrate removal. Time-series of modeled NO₃⁻ concentrations of recharge into the conduit (inflow) and discharge exiting the conduit at the spring (outflow). Discharge at Royal Spring is plotted on the secondary y-axis. Gray-shaded regions indicate dry seasons.](image-url)
delivery of SOC coupled to longitudinal sediment transport within the conduit. Further, it has been suggested by Hedin et al. (1998) that the convergence of multiple groundwater pathways form zones of high denitrification due to the favorable redox conditions. Given that subsurface karst rivers drain multiple groundwater pathways, ranging from matrix-, fracture-, and conduit-sized, it is conceivable that favorable conditions may arise although further field data is needed. These results suggest that the study of water and sediment transport—coupled to SOC and SN biogeochemistry—is vital for understanding NO$_3^-$ removal in subsurface as well as surface systems.

3.3. Forecasting Environmental Drivers of Nitrate Removal

Projections of our stable-isotope-calibrated model to the year 2057 predict that environmental drivers will increase the degree of NO$_3^-$ removal in the karst conduit (Figure 8). The average increase, relative to present-day removal, depends on the forecasted driver: +5.2% for temperature, −2.0% for discharge, +12.3% for land use, and +14.1% for a combination of all three drivers. Forecasted temperature increases have the net result of accelerating the denitrification process. Increases to spring discharge slightly decrease NO$_3^-$ removal due to a reduction in residence time of NO$_3^-$ and a scouring of organic material in the cave bed. NO$_3^-$ removal is partly offset by increased deposition of terrestrial organic material, which fuels denitrification. The doubling of urban land use (by 2057) is anticipated to increase NO$_3^-$ removal primarily because urban tributaries in the basin are more concentrated in organic-rich sediment. However, the benefits of increased NO$_3^-$ removal are largely offset by a coincident increase in NO$_3^-$ loading to the spring (+28.1%). This loading primarily occurs because our wet temperate region is projected to get wetter with more well-connected nitrate leaching flow paths (Al Aamery et al., 2016, 2018, 2021). Our results, indicating increased nitrate removal, show how human-induced changes in land use can drive significant nitrate removal in karst systems.
flux from wetter forecasted conditions, have been suggested more broadly in past studies (Donner & Scavia,, 2007; Zarrineh et al., 2020). However, exact rainfall and discharge projections are difficult to constrain as they are derived from precipitation which is fraught with uncertainty (Hawkins & Sutton,, 2011), nevertheless the majority of global climate models suggest increased mean and ensemble rainfall in this region (e.g., ΔMAP on the order of 10%–12% for GCM ensemble averages, Al Aamery et al., 2016, 2018).

Notwithstanding uncertainties, our results broadly agree with studies conducted across a broad range of climates and land uses. In agricultural catchment in the Chesapeake Bay, USA, climate change simulations project an increase NO\textsubscript{3}\textsuperscript{−} and sediment discharge, but only during the spring and winter with a decrease during the summer (Wagena et al., 2018), highlighting that the effect of seasonal processes may become exacerbated as weather extremes prevail. Most studies reviewed by the authors showed that, in the absence of drastic and proactive land management and regulation (Bartosova et al., 2019; Gabriel et al., 2018), NO\textsubscript{3}\textsuperscript{−} export will likely increase either as a result of hydrologic leaching of NO\textsubscript{3}\textsuperscript{−} or accelerated mineralization of SN brought on by increased soil temperatures (Olesen et al., 2019; Trolle et al., 2019). Regarding land use, the uncertainties associated with forecasting land use, management, and regulation are recognized to dominate projected fluxes more so than any feasible changes to the climate (Bartosova et al., 2019; Trolle et al., 2019). Thus, while land use seems to be the most impactful determinant of future water quality it also offers an opportunity for land managers and stakeholders to put into place sustainable practices, informed by process-based modeling tools, to advise decision-making and improve water quality.

4. Conclusion

This contribution reports sediment biogeochemistry and its impact nitrogen cycling in a karst cave, which was under-reported previously for this environment. Elemental and stable isotope data and modeling support the hypothesis that N exports from the karst caves differ from inputs due sediment-driven internal

Figure 8. Impacts of forecasted environmental drivers on model yields. Definition of model yields: sediment carbon input and decomposition in the conduit (SOC\textsubscript{IN} and SOC\textsubscript{DEC}); sediment nitrogen input, mineralization, and immobilization in the conduit (SN\textsubscript{IN}, SN\textsubscript{MIN}, and SN\textsubscript{IMM}); and nitrate export and removal via denitrification (NO\textsubscript{3}\textsuperscript{−}\textsubscript{OUT} and NO\textsubscript{3}\textsuperscript{−}\textsubscript{DEN}). The “Combined Drivers” projection reflects the effects of concurrent changes to temperature, discharge, and land use. Note: x-axes ranges differ between subplots and some uncertainty bands are obscured by mean marker.
cycling. Stable N isotope data of cave sediment and nitrate were used together with numerical modeling to estimate N mineralization and immobilization, which are otherwise over-printed in element N results and difficult to separate. For example, the NSE of NO$_3$~$^-$ simulation increases from 0.06 to 0.68 when stable isotope subroutines are included. Key findings of the data and modeling were as follows:

1. Modeled mineralization of sediment N and immobilization of dissolved N occur at rates of 8.9 ± 3.1 mg N m$^{-2}$ d$^{-1}$ and 8.0 ± 2.9 mg N m$^{-2}$ d$^{-1}$, respectively. The seldom reported N immobilization in the cave is prominent despite the lack of photic primary production.

2. Modeled temporal gradients of nitrate removal indicate that as much as 90% of nitrate inputs are removed during the dry season. On the other hand, during wet conditions, negligible removal occurs as residence time of material in the turbulent conduit are low.

3. Modeled spatial gradients of nitrate removal are controlled by the cyclical delivery of labile SOC from the surface during the wet season. Nitrate removal appears greatest near cave entrances where deposition of labile SOC fuels elevated denitrification relative to disconnected sections of the cave.

4. Projected effects of environmental drivers suggest that rainfall and urban land use changes by 2057 will elevate denitrification (+14.1%), but that this nitrate removal will largely be offset by rainfall-driven increases in soil nitrate leaching (+28.1%) as our region gets wetter.

Conflict of Interest

The authors declare no conflicts of interest relevant to this study.

Data Availability Statement

Calibration data, computer code, and model results will be stored and publicly available on Open Science Framework (at: http://doi.org/10.17605/OSF.IO/MG867).

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References From the Supporting Information


