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Reducing Equifinality Using Isotopes in a Process-Based Stream Nitrogen Model Highlights the Flux of Algal Nitrogen from Agricultural Streams

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Key Points:

- A process-based model to simulate the stream nitrogen budget is developed and applied to a case study
- Inclusion of nitrogen sediment stable isotopes into the model framework reduces equifinality
- Model results show algal sloughing to be on the same order of magnitude as denitrification

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Reducing equifinality using isotopes in a process-based stream nitrogen model highlights the flux of algal nitrogen from agricultural streams

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Abstract The fate of bioavailable nitrogen species transported through agricultural landscapes remains highly uncertain given complexities of measuring fluxes impacting the fluvial N cycle. We present and test a new numerical model named *T*echnology for *R*emovable *A*nnual *N*itrogen in *S*treams *F*or *E*cosystem *R*estoration (*TRANSFER*), which aims to reduce model uncertainty due to erroneous parameterization, i.e., equifinality, in stream nitrogen cycle assessment and quantify the significance of transient and permanent removal pathways. TRANSFER couples nitrogen elemental and stable isotope mass-balance equations with existing hydrologic, hydraulic, sediment transport, algal biomass, and sediment organic matter mass-balance subroutines and a robust GLUE-like uncertainty analysis. We test the model in an agriculturally impacted, third-order stream reach located in the Bluegrass Region of Central Kentucky. Results of the multiobjective model evaluation for the model application highlight the ability of sediment nitrogen fingerprints including elemental concentrations and stable N isotope signatures to reduce equifinality of the stream N model. Advancements in the numerical simulations allow for illumination of the significance of algal sloughing fluxes for the first time in relation to denitrification. Broadly, model estimates suggest that denitrification is slightly greater than algal N sloughing (10.7% and 6.3% of dissolved N load on average), highlighting the potential for overestimation of denitrification by 37%. We highlight the significance of the transient N pool given the potential for the N store to be regenerated to the water column in downstream reaches, leading to harmful and nuisance algal bloom development.

1. Introduction

Agricultural landscapes produce runoff and seepage highly concentrated in dissolved inorganic nitrogen (DIN), which can be partially self-mitigated in stream corridors via high rates of biological removal [*Seitzinger et al.*, 2002; *Alexander et al.*, 2008; *Mulholland et al.*, 2008; *Seitzinger*, 2008; *Manis et al.*, 2014]. However, researchers continue to question the transformation of DIN in agricultural streams and we now recognize that secondary processes that are commonly overlooked, e.g., algal N fate, may have a substantial role within stream nitrogen cycling [*Ford et al.*, 2015; *Webster et al.*, 2016]. In turn, these secondary processes have the potential to mask estimates of perceived dominant processes, such as denitrification. The motivation of this paper is improving the predictive capacity of nutrient pathways in agricultural streams. We develop a numerical modeling framework for stream nutrient dynamics that aims to reduce uncertainty of the fluvial nitrogen cycle assessment, and address the gap in knowledge surrounding the fate of biologically assimilated N [*Webster et al.*, 2016].

Tightly coupled physical and biogeochemical processes govern the stream nitrogen cycle in fluvial landscapes (Figure 1) [*Peterson et al.*, 2001; *Birgand et al.*, 2007; *Fox et al.*, 2010; *Sebestyen et al.*, 2014]. Benthic autotrophs assimilate dissolved nitrogen during primary production and the fate of benthic algae can follow several pathways including physical sloughing (i.e., erosion) out of the stream reach, mineralization and denitrification by heterotrophs, or mineralization and regeneration to the water column [*Peterson et al.*, 2001; *Birgand et al.*, 2007; *Webster et al.*, 2016]. Fluvial nitrogen researchers now recognize the pairing of carbon and nitrogen cycles in stream channels. Organic matter breakdown, stabilization and mineralization impact particulate C and N storage and regeneration simultaneously, while organic carbon quality and quantity impacts rates of microbial N processing, namely nitrifying and denitrifying bacteria [*Arango and*

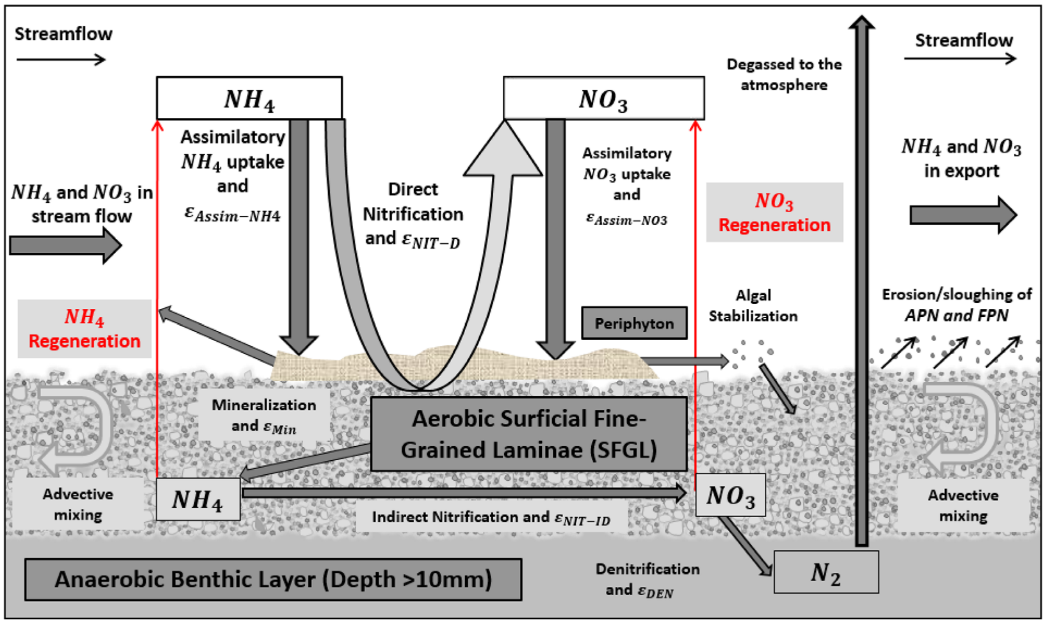

Figure 1. Perceptual model of elemental and isotope N exchanges in low-gradient agroecosystem streams (modified from *Peterson et al.* [2001]).

Tank, 2008; *Martinelli et al*., 2011; *Lane et al*., 2013; *Ford and Fox*, 2014, 2015; *Hotchkiss and Hall*, 2015]. Researchers desire continuous and integrated estimates of nitrogen fluxes for assessing the net function of the fluvial system, regardless of the apparent complexity of nitrogen dynamics [*Seitzinger*, 2008]. Nutrient concentration data from streams cannot feasibly provide continuous records of processes either because the processes are unable to be measured in situ, or because of economic constraints. Validated numerical models that simulate the stream N cycle at high temporal resolutions provide an effective tool for quantifying continuous estimates of fluxes across a range of timescales.

A precursor to confidently simulating nitrogen fluxes within the agricultural stream ecosystem is recognition of the potential for equifinality in numerical modeling results. Equifinality refers to the uncertainty of parameters in process-based numerical models that can lead to a broad range of multiple parameter sets (i.e., realities) and in turn broad range of acceptable solutions [*Beven*, 2006; *Adiyanti et al*., 2016]. Overparameterization of numerical models for stream nitrogen suggest the high potential for equifinality. Recent research suggests that these advanced model calibration and uncertainty subroutines might be coupled with ambient isotope tracers to reduce equifinality within water quality modeling [*Ford and Fox*, 2015; *Fox and Martin*, 2015; *Adiyanti et al*., 2016].

We propose several possible approaches that might address problems with equifinality within numerical modeling of stream nitrogen dynamics including: (1) the use of novel multiobjective calibration procedures with multiple response variables, (2) the use of nitrogen stable isotopes as a response variable to assist with calibration, and (3) application a robust uncertainty estimation procedure to quantify the extent of nutrient equifinality. Multiobjective calibration enables modelers to establish numerical-based criteria that considers calibration statistics for multiple model response variables or evaluation of model performance at several different timescales [*van Griensven and Bauwens*, 2003; *Rode et al*., 2007; *Ford and Fox*, 2015]. Therefore, researchers can evaluate and reduce parameter ranges based on their sensitivity to unique or multiple responses, which in turn reduces the solution space of the results. Stable nitrogen isotope signatures, typically expressed in $\delta^{15}N$ notation, are suggested as a potentially useful response variable within multiobjective calibration. Ambient $\delta^{15}N$ provide a fingerprint of the mixture of N sources and their in-stream transformations that alter the relative amount of N-15 atoms to N-14 atoms [*Kendall and McDonnell*, 1998; *Kendall et al*., 2001; *Ford et al*., 2015]. Ambient $\delta^{15}N$ signatures of a nitrogen phase (i.e., org-N, NO_3^-, and NH_4^+) provides an extra equation in the set of biogeochemical reactions being solved for the total nitrogen mass balance, and therefore shows efficacy for assisting with reducing equifinality. Finally, the Generalized

Likelihood Uncertainty Estimation (GLUE) framework provides a widely accepted approach for quantifying equifinality from numerical modeling in order that the researcher can place appropriate bounds on model results. The GLUE framework comprises Monte Carlo simulations of parameter sets with evaluation of model output against measured data to provide multiple acceptable parameterizations [*Beven and Binley*, 1992; *Dean et al*., 2009; *Gong et al*., 2011].

As researchers reduce equifinality within numerical model simulations of stream nitrogen dynamics, the potential exists for elucidating previously under-appreciated fluxes in terms of their magnitude. We find that one nitrogen flux that has received little attention is algal nitrogen sloughing and stabilization [*Ford and Fox*, 2017] and their relative comparison with permanent removal via denitrification. Agricultural streams often are rich in autotrophic production that can exert control on benthic and transported sediment organic matter composition at seasonal and longer-term timescales *via* algal stabilization, i.e., breakdown and integration of algae into benthic storage zones [*Arango and Tank*, 2008; *Griffiths et al*., 2012; *Ford and Fox*, 2017], Further, nitrogen focused studies from agricultural streams suggest that organic carbon and nitrogen transformations are tightly coupled [*Butturini et al*., 1999; *Arango et al*., 2007; *Arango and Tank*, 2008; *Newcomer et al*., 2012]. Based on our previous work, the authors recognize the potential for benthic algae fate and transport to impact nutrient processes and net nitrogen fluxes through its significance to agroecosystem C budgets [*Ford and Fox* 2014, 2015; *Hotchkiss and Hall*, 2015; *Ford and Fox*, 2017] prompting the motivation to quantify the fluxes in this paper. Further, the authors recognize the importance of algal sloughing in that under estimation of algal sloughing as a temporary sink of nitrogen could result in overestimation of denitrification, a permanent removal pathway, and therefore overestimate net nutrient attenuation by the stream. Failure to recognize the fate of algal nitrogen could result in unforeseen algal blooms in streams and downstream water bodies as delivered nitrate from agricultural streams drops yet algal nitrogen is able to mineralize and become available for primary production.

The author's objective was to reduce equifinality in numerical modeling and thereafter estimate nitrogen fluxes and removal in an agricultural stream. The authors place emphasis upon coupled carbon and nitrogen processes that stabilize algal nitrogen and slough algal nitrogen from the streambed and to compare with denitrification. Novel features of this research are: a renewed investigation of net nitrogen removal in agricultural streams by explicitly considering the algal nitrogen pool; the use of ambient-level nitrogen stable isotopes within a multiobjective calibration procedure; and the use of a numerical model and robust uncertainty analysis that tightly couples carbon and nitrogen processes in an agricultural stream; the incorporation of these features has, to our knowledge, not been reported.

2. Methods

2.1. Model Formulation

We formulate a numerical model with the intent to reduce equifinality in the stream nitrogen cycle and quantify fluxes from nitrogen pools for agriculturally impacted streams. The model simulates characteristics of agricultural streams that include: (i) the potential for upland agriculture practices that could prompt historical or current soil erosion and runoff from the land surface to the stream corridor; (ii) the potential for relatively high nutrient loading to streams due to fertilizer or manure application within the agricultural regions; (iii) relatively lowland topography that could promote the presence of fine sediments and particulate organic carbon within the stream corridor; (iv) the potential for particulate organic matter sources within the stream corridor from various allochthonous sources including soils, litter, and detritus; (v) the potential for autochthonous primary production due to benthic autotrophs within the shallow water conditions of the agricultural streams that can receive sunlight at the stream bottom; and (vi) the potential presence of an aerobic surficial layer in the benthos in which heterotrophy is pronounced.

We formulated the numerical nitrogen model to simulate the stream nitrogen cycle by considering water, sediment, algae, carbon, and nitrogen transformations. The numerical model is termed *TRANSFER*. *TRANSFER* stands for *T*echnology for *R*emovable *A*nnual *N*itrogen in *S*treams *F*or *E*cosystem *R*estoration, reflecting both the transfer of nitrogen within agricultural streams and the emphasis of the model to assist with improving stream ecosystems. Figure 2 depicts major subroutines that are simultaneously processed across dissolved and particulate pools within *TRANSFER*. Specifically, new routines include a N elemental mass-balance model and N isotope mass-balance model that considers dissolved inorganic N, algal, and sediment

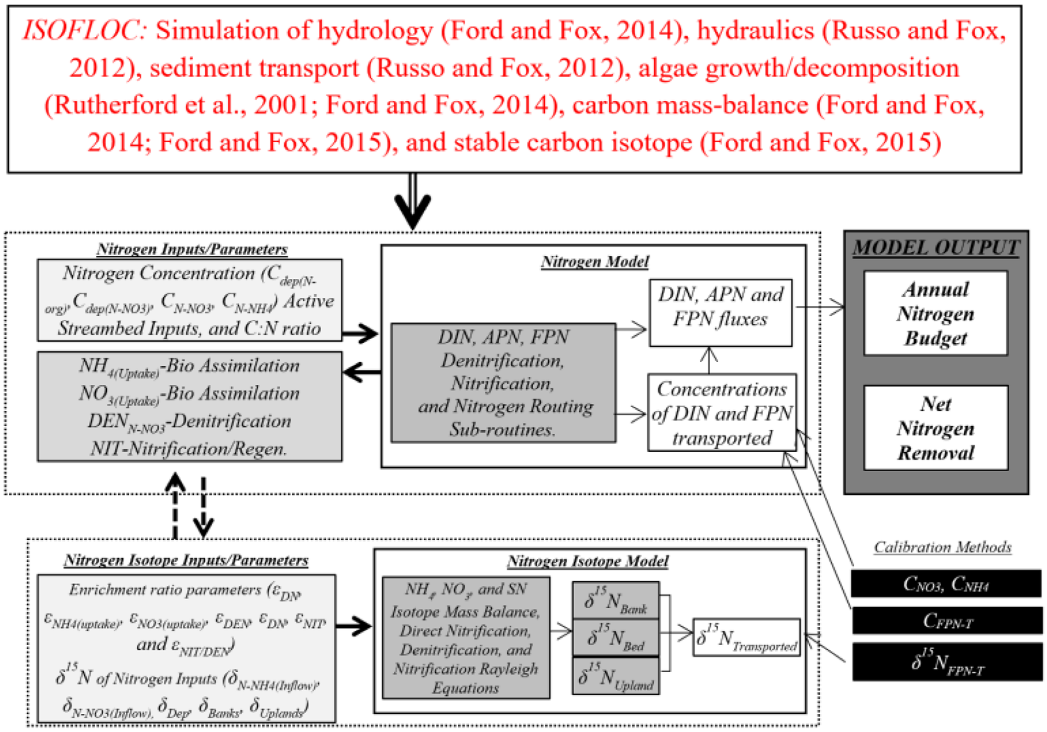

Figure 2. Flow chart for the *TRANSFER* modeling tool and model evaluation data collection needs. The ISOFLOC submodel incorporates sediment, algae, and carbon mass-balance submodels.

N species. Several of the *TRANSFER* numerical subroutines including hydraulic, sediment transport, algal mass balance, C elemental mass balance, and C isotope mass balance have been previously published elsewhere and the references are included in Figure 2 and briefly discussed below. *TRANSFER* runs within Fortran and the numerical code was compiled with the Intel Fortran Composer in Visual Studio 2015. The user manually defines spatial and temporal constraints including reach length, channel geometry, time step, timeframe and parameterization associated with input and calibration parameters.

2.1.1. ISOFLOC Model: Water, Sediment, and Carbon Subroutines

ISOFLOC simulates the hydrology-hydraulics, sediment, and organic carbon dynamics within *TRANSFER* and is based off previous publications [*Rutherford et al*., 2000; *Russo and Fox*, 2012; *Ford and Fox*, 2014, 2015]. We provide a brief explanation; however, the detailed model description, formulation, and model evaluation procedures are described elsewhere [*Ford and Fox*, 2015]. *ISOFLOC* is a reach-scale mass-balance model that simulates organic and inorganic C phases continuously including dissolved inorganic carbon (DIC), algal particulate organic carbon (APOC), and fine particulate organic carbon (FPOC). The *ISOFLOC* model includes a hydraulic and sediment transport model to assess the impact of erosion-deposition dynamics on the composition of benthic and transported particulate carbon compositions. In addition to tight coupling of the physical and biological processes, a second feature of the model is its inclusion of stable C isotopes for constraining model equifinality. The model simulates isotope mass balances of DIC, APOC, and FPOC continuously and incorporate them as a response variable in model evaluation.

2.1.2. Particulate Nitrogen Mass-Balance Subroutine

We develop the particulate nitrogen model in *TRANSFER* in the present study, which serves as the basis for physical and biological interactions of DIN with the benthic surficial fine-grained laminae (SFGL) layer by building on organic matter dynamics previously simulated in *ISOFLOC*. The SFGL is a 5–10 mm aerobic, flocculant sediment layer composed of fluvial sediment deposits, autotrophic biota and heterotrophic bacteria, and is a well-recognized feature in the benthos of low-gradient, low-order agroecosystems with cohesive sediments [*Droppo et al*., 2005; *Russo and Fox*, 2012; *Ford and Fox*, 2014; *Zahraeifard et al*., 2014]. Particulate nitrogen includes fine and coarse nitrogen pools comprised primarily of benthic algal biomass and fine particulate sediment particles and aggregates from upland sediment sources [*Ford and Fox*, 2014, 2015]. The nature of low-gradient, human disturbed systems suggests relatively minor inputs from leaf litter and detritus since they are small relative to algae and fine particulate nitrogen (FPN) [*Griffiths et al*., 2012; *Ford et al*., 2014]. *TRANSFER* simulates coarse particulate N to be composed soley of algal particulate N (*APN*). *TRANSFER* simulates the mass balance of *APN* (kgN) considering inputs from biological assimilation of DIN and outputs from mineralization, decomposition to fine particulate nitrogen and scouring due to bulk sloughing of the algal mat

$$APN_i^j = APN_{i-1}^j - Slough_{Mat-N}{}_i^j + \left(Assim_i^j - Min_{Mat}{}_i^j - DEC_{Mat}^{\mathrm{Alg}\,aej}{}_i\right) SA_{Bed} \Delta t, \tag{1}$$

where *i* is the time step identifier, *j* is the stream reach identifier, $Slough_{Mat\text{-}N}$ (kgN) is the mass of nitrogen lost from the stream reach due to algal sloughing, *Assim* (kgN m^{-2} d^{-1}) is the algal biomass assimilation rate, Min_{Mat} is the endogenous algal respiration rate (kgN m^{-2} d^{-1}), DEC_{Mat} (kgN m^{-2} d^{-1}) is the microbial breakdown rate of coarse algae to SFGL algae, *SA* (m^2) is the surface area of the bed source, and Δt is the model time step. We estimate sloughing of *APN* by dividing the sloughed algal C, from *ISOFLOC* simulations using the carbon to nitrogen mass ratio of the algal mat, $C{:}N_{Mat}$ calculated during the previous time step as

$$Slough_{Mat-N}{}_i^j = \min\left[k\left(\tau_{if}^j - \tau_{cr}^{Mat}\right)\rho_S^{Mat} SA_{Bed}\Delta t, APOC_{i-1}^j\right] / C : N_{Mat}{}_{i-1}^j, \tag{2}$$

where *k* (m^{-1}) is the erodibility coefficient, τ_f (Pa) is the shear stress of the fluid at the centroid of the erosion source, $\tau_{cr}{}^{Mat}$ (Pa) is the critical shear stress of the algal mat, $\rho_s{}^{Mat}$ (kgC m^{-3}) is the bulk density of the algal mat, and *APOC* is the algal particulate organic carbon mass in the streambed calculated in *ISOFLOC*. We assume that sloughed algae are exported from the watershed in the same time step that are eroded, since algal material is relatively neutrally buoyant and would not be expected to settle out of suspension during flow conditions that would induce sloughing. We assume nonrate-limiting conditions of DIN assimilation as follows:

$$Assim_i^j = \frac{Fix_i^j + APOC_{i\,Col}^j}{C : N_{Assim}}, \tag{3}$$

where *Fix* (kgC m^{-2} d^{-1}) is the carbon fixation rate, $APOC_{col}$ (kgC m^{-2} d^{-1}) is the algal carbon colonization rate, and $C{:}N_{Assim}$ (kgC kgN^{-1}) is the atomic carbon to nitrogen ratio of newly assimilated algae and is parameterized by the user. *TRANSFER* assumes mineralization of the coarse algal mat to occur simultaneously with endogenous respiration as follows:

$$Min_{Mat}{}_i^j = \mathrm{Re}\,s_{Mat}{}_i^j / C : N_{Min-\mathrm{Alg}\,ae}, \tag{4}$$

where Res_{Mat} (kgC m^{-2} d^{-1}) is CO_2 respired from algal endogenous respiration calculated in *ISOFLOC*, and $C : N_{Min-\mathrm{Alg}\,ae}$ (kgC kgN^{-1}) is an endogenous mineralization calibration coefficient that accounts for variable rates between algal C and N recycling. The simulated mass of algae decomposed from the algal mat to SFGL algae follows a temperature-dependent bacteria degradation rate like that simulated for C in *ISOFLOC* [*White et al*., 1991; *Ford and Fox*, 2015]

$$DEC_{Mat}^{\mathrm{Alg}\,aej}{}_i = \frac{APN_{i-1}^j * 10^\wedge\left[-c_{DEC-APN} + (0.031 * T)\right]}{SA_{Bed}\Delta t}, \tag{5}$$

where $c_{DEC\text{-}APN}$ is the APN decomposition coefficient [*White et al*., 1991]. We simulate fine particulate nitrogen composition in the SFGL as a function of erosion-deposition dynamics, production of algal FPN from APN decomposition, and mineralization of SFGL algae to ammonium as follows:

$$C_{FPN-SFGL}{}_i^j = \frac{N_{SFGL}^{Upland\,j}{}_i + N_{SFGL}^{\mathrm{Alg}\,aej}{}_i}{S_{SFGL}{}_i^j}, \tag{6}$$

where $C_{FPN\text{-}SFGL}$ (kgN kg sed^{-1}) is the sediment nitrogen concentration in the SFGL layer, S_{SFGL} (kg sed) is the supply of SFGL in the bed, N_{SFGL}^{Upland} (kgN) is the mass of nitrogen in the SFGL associated with upland sediments and is modeled as a function of deposition of upland sediments and erosion of the SFGL, and N_{SFGL}^{Algae} (kgN) is the mass of nitrogen in the SFGL associated with algal biomass and is modeled as a function of mineralization of fine algae (Min_{SFGL}), decomposition of course algae, and erosion of the SFGL. The model simulates mass of algal N mineralized, Min_{SFGL}, by microbes from the SFGL algae pool to follow similar temperature-dependent patterns to decomposition of the coarse algal mat

$$Min_{SFGL}{}_i^j = \frac{N_{SFGL}^{Alg\,ae\,j}{}_{i-1} * 10\hat{}\left[-c_{Min-SFGL-Alg\,ae} + (0.031 * T)\right]}{SA_{Bed}\Delta t}, \tag{7}$$

where $c_{Min-SFGL-Alg\,ae}$ is the algal SFGL N mineralization coefficient.

Finally, the model estimates transported FPN concentration ($C_{FPN\text{-}T}$) by multiplying nitrogen weighted fractions for the total suspended sediment load including bank ($C_{FPN\text{-}Bank}$), bed ($C_{FPN\text{-}SFGL}$), and upland ($C_{FPN\text{-}Upalnd}$) sources. We derive fractions from the sediment transport subroutine in the ISOFLOC model.

2.1.3. Dissolved Inorganic Nitrogen Mass-Balance Subroutine

TRANSFER continuously simulates DIN flux and nonconservative reactions within the aerobic SFGL layer and anaerobic denitrification. *TRANSFER* models DIN flux at the outlet of each simulated reach using hydrologic inputs of volumetric water flow rate (m^3 s^{-1}), Q_i^j, for a given spatial reach, *j*, and time step, *i*, and Q_i^j can be established using data-driven, conceptual, or process-based hydrologic models calibrated for the watershed. We assume concentrations of dissolved constituents are well mixed vertically and laterally, as well as longitudinally within the discretized reach segment. Longitudinal discretization of the stream into reaches requires the potential limitation of spatial averaging nutrient concentrations and transformation rates such that streambed hot spots of N and streambed passive zones are smoothed across the discretized reach; and the potential limitation should be kept in mind when comparing point samples from the water column or streambed with reach-averaged calibration parameters. Further, we assumed reactions in the streamwater were negligible since turbidity is often low in low-order agricultural streams (i.e., periphyton is the predominant algal pool as opposed to phytoplankton) and bacterial communities are assumed to be prominent in the SFGL. *TRANSFER* estimates DIN concentration continuously for a specified nitrogen species *X* (i.e., NO_3 or NH_4) using a finite-difference approximation to the governing advection-reaction differential equation [e.g., *Webster et al.*, 2016] as

$$C_X{}_i^j = \left[C_X{}_{i-1}^j V_{i-1}^j + R_i^{jX} + \left(\underbrace{Q_{out}{}_{i-1}^{j-1} C_X{}_{i-1}^{j-1} + Q_{Trib}{}_i^j C_X^{Trib\,j}{}_i}_{Inflow} \; \underbrace{-Q_{out}{}_i^j C_X{}_i^j}_{\text{Outflow}}\right)\Delta t\right]/V_i^j, \tag{8}$$

where *C* is the concentration of a specified DIN phase (kg m^{-3}), *V* is the volume of water in the stream reach at the specified time step (m^3), *Trib* denotes tributary inputs to the stream reach, and *R* (kgN) is the net reaction flux (kg) and is modeled to include nonconservative biotic processes including assimilation, regeneration, nitrification, and denitrification as

$$R_i^{jX} = \left[Min_i^j - Assim_i^{jX}\right] SA_{Bed}\Delta t \pm NIT_i^j - DEN_i^j, \tag{9}$$

where *Min* (kgN m^{-2} d^{-1}) is the total mass of ammonium generated from organic matter mineralization and is set to zero for the nitrate reaction equation. Assimilation demands of the algae are met first by ammonium mineralized during the specified time step, ammonium in the water column, then nitrate. *NIT* (kgN) is the mass of nitrified ammonium and is added for NO_3 and subtracted for NH_4. *DEN* (kgN) is the mass of denitrified algae that is degassed from the stream channel and is set to zero for the NH_4 pool. We quantify *Min* as the sum of microbial and endogenous algal mineralization [*DePinto and Verhoff*, 1977] as

$$Min_i^j = Min_{Mat}{}_i^j + Min_{SFGL}{}_i^j. \tag{10}$$

TRANSFER considers nitrification for both direct and indirect pathways; however, for the present study, we place focus on indirect since NH_4 concentrations are typically rapidly converted to NO_3 in upper stretches of a stream reach. We assume nonrate-limiting oxygen conditions in the SFGL are satisfied for indirect nitrification, thus nitrification rates are modeled using results of *Arango and Tank* [2008] that suggest sediment exchangeable NH_4 availability and FPOC content of the SFGL ($C_{FPOC\text{-}SFGL}$) are the primary drivers. Therefore, we use a power function to simulate indirect nitrification, *NIT* (kgN kg sed^{-1} d^{-1}) as

$$NIT_i^j = \beta_{IN}\left(C_{FPOC-SFGL}{}_i^j\right)^{\alpha_{IN}} * S_{{}_{SFGL}}{}_i^j * \Delta t, \tag{11}$$

where $C_{FPOC\text{-}SFGL}$ (kgC kg sed^{-1}) is the carbon content of SFGL sediments which is calculated from *ISOFLOC* simulations, α_{IN} is the exponent calibration coefficient for indirect nitrification (assumed linear for the present study), and β_{IN} (kgN kgC^{-1} d^{-1}) is the multiplicative coefficient and has the aforementioned units to make the equation dimensionally homogenous. Since mineralized NH_4 is extremely labile and can be assimilated immediately we assume that all remaining mineralized NH_4, following satisfaction of the *IN* rates, is reuptaken by the benthos to satisfy assimilation requirements of the microbial community, with the remainder being regenerated to the water column, or stored in the pore water pool depending on flow conditions.

Denitrification is impacted by NO_3 concentration, sediment C content, and temperature; however, the functional form of how these processes covary is not well understood and *Arango and Tank* [2008] found $C_{FPOC\text{-}SFGL}$ to be the best descriptive variable in ag-disturbed streams. Therefore, we model denitrification rates, *DEN* (kgN) using a power function as

$$DEN_i^j = \beta_{Den}\left(C_{FPOC-SFGL}{}_i^j\right)^{\alpha_{Den}} \; * S_{SFGL}{}_i^j * \Delta t, \tag{12}$$

where α_{Den} is the exponent calibration coefficient for denitrification and β_{Den} (kgN kgC^{-1} d^{-1}) is the multiplicative denitrification coefficient. TRANSFER does not explicitly account for redox conditions; however, it is widely recognized that localized anoxic pockets, such as within algal mats, can be on the same order of magnitude as denitrification in the anoxic layer [*Pringle et al.*, 1988]. Therefore, we account for bulk denitrification within the stream reach at the specified time step.

As a final note, we caution that dissolved organic nitrogen (DON) is not explicitly simulated in *TRANSFER* due to a lack of seasonality in the response variable for agroecosystems. The reason for a lack of seasonality of DON in temperate agricultural stream systems is likely that autochthonous leachate is highly labile and therefore is mineralized shortly following release [*Hotchkiss and Hall*, 2015].

2.1.4. Stable Nitrogen Isotope Mass-Balance Subroutine

A novel feature of TRANSFER is the inclusion of stable isotopes as an additional model response variable to help overcome the commonly reported equifinality problem of water quality models. Stable nitrogen isotope mass balances with nitrogen advection as well as the potential for isotope fractionation during reactions are simulated in TRANSFER for APN, FPN, and DIN pools. The isotopic signature of a particular nitrogen pool given in terms of δ (‰) notation as

$$\delta_i^j = \delta_{i-1}^j X_{i-1}^j + \sum \delta_{inputs}{}_i^j \text{ X } {}_{Inputs}{}_i^j - \sum \delta_{outputs}{}_i^j \text{ X } {}_{outputs}{}_i^j - \sum \varepsilon_{frac}{}_i^j \ln\left(f_{frac}{}_i^j\right), \tag{13}$$

where *X* represents the fraction of an element in a given pool and is parameterized using outputs from the sediment model in *ISOFLOC* and the aforementioned N mass-balance model, ε (‰) is the enrichment factor during an isotopic fractionation process and Rayleigh-type models are used to simulate fractionation [*Sharp*, 2007], and *f* is the fraction of a substrate remaining after the isotope fractionation process occurs and is derived from the appropriate elemental model. In Rayleigh fractionation, $\varepsilon_{A\text{-}B}$ is defined as

$$\varepsilon_{A-B} = \left[\frac{\left({}^{15}N/{}^{14}N\right)_A}{\left({}^{15}N/{}^{14}N\right)_B} - 1\right] \times 1000, \tag{14}$$

where *A* is the product and *B* is the reactant. Implementing known inputs, outputs, and fractionation processes for APN, the isotopic submodel for APN is simulated as a weighted average of algal biomass from the previous time step, newly assimilated NH_4 and NO_3 as follows:

$$\delta^{15}N_{APN}{}_i^j = \delta^{15}N_{APN}{}_{i-1}^j X_{APN} i - 1^j + \delta^{15}N_{Assim-NO3} X_{Assim-NO3}{}_i^j + \delta^{15}N_{APN}{}_{i-1}^j X_{Assim-NH4}{}_i^j, \tag{15}$$

where $\delta^{15}N$ (‰) is the nitrogen isotopic signature of a particular pool. *TRANSFER* does not currently simulate the $\delta^{15}N_{NH4}$ due to the low levels of ammonium stemming from rapid nitrification in agricultural watersheds. Also, *TRANSFER* assumes that all mineralized SFGL algae will contribute to nitrification demands due to the proximity to nitrifying biota and thus is not likely to be available for assimilation into the algal mat. Therefore, *TRANSFER* estimates that the isotopic signature of reassimilated ammonium is the isotopic signature of the mineralized APN source (i.e., $\delta^{15}N_{APN}$ during the previous time step).

TRANSFER continuously accounts for the stable isotopic composition of nitrate ($\delta^{15}N_{NO3}$) in the stream channel as a function of nitrification, assimilation, and denitrification, and advective flux into and out of the stream reach as

$$\begin{aligned}\delta^{15}N_{NO3}{}_i^j = {} & \delta^{15}N_{NO3}{}_{i-1}^j \text{ X } {}_{NO3}{}_{i-1}^j + \delta^{15}N_{NO3-in}{}_i^j \text{ X } {}_{NO3-in}{}_i^j + \delta^{15}N_{APN}{}_{i-1}^j \text{ X } {}_{NIT-APN}{}_i^j \\ & + \delta^{15}N_{FPN-SFGL}{}_{i-1}^j \text{ X } {}_{NIT-FPN}{}_i^j - \varepsilon_{Assim-NO3}{}_i^j \ln\left(f_{Assim}{}_i^j\right) - \varepsilon_{DEN} \ln\left(f_{DEN}{}_i^j\right)\end{aligned}. \tag{16}$$

TRANSFER simulates the stable nitrogen isotope signature of FPN in the SFGL ($\delta^{15}N_{FPN\text{-}SFGL}$) as a mass balance considering deposition, decomposition of the algal mat and SFGL remaining from the previous time step. Erosion and mineralization losses are accounted for in the fraction of FPN in the SFGL from the previous time step

$$\delta^{15}N_{FPN-SFGL}{}_i^j=\delta^{15}N_{FPN-SFGL}{}_{i-1}^j\,X_{FPN-Bed}{}_{i-1}^j+\delta^{15}N_{APN}{}_{i-1}^j X_{DEC(APN)}{}_i^j+\delta^{15}N_{Upland}{}_i^j X_D{}_i^j. \tag{17}$$

Thereafter, *TRANSFER* estimates the nitrogen stable isotopic signature of suspended sediment ($\delta^{15}N_{FPN\text{-}T}$) as a weighted average of sediment nitrogen source contributions and their associated isotopic signatures (i.e., $\delta^{15}N_{FPN\text{-}SFGL}$, $\delta^{15}N_{Upland}$, and $\delta^{15}N_{Bank}$) which is derived from the sediment transport and N mass balance modeling

$$\delta^{15}N_{FPN-T}{}_i^j=\delta^{15}N_{FPN-SFGL}{}_i^j\,X_{FPN-SFGL}^{Transported}{}_i^j+\delta^{15}N_{Upland}{}_i^j\,X_{Upland}^{Transported}{}_i^j+\delta^{15}N_{Banks}{}_i^j\,X_{Banks}^{Transported}{}_i^j. \tag{18}$$

2.1.5. Multiobjective Calibration and Uncertainty Subroutine

Building on the model evaluation routines in *ISOFLOC*, *TRANSFER* consists of (1) an exploratory, global sensitivity analysis that utilizes quasi random Sobol sequences informed by nominal ranges established for site specific conditions [*Jansen*, 1999; *Sobol*, 2001; *Saltelli et al*., 2010; *Ford and Fox*, 2015] and (2) a GLUE-like calibration and validation of the model that considers equifinal solutions based on well-accepted statistical metric criteria [*Moriasi et al*., 2007; *Ford and Fox*, 2015]. Figure 3 summarizes the process and detail are described elsewhere [*Ford and Fox*, 2015].

2.2. Model Application

We apply *TRANSFER* to an 8 year simulation period in the South Elkhorn watershed (Figure 4) in order to provide a case study of the model formulation, results, and evaluation. For the region, cohesive stream

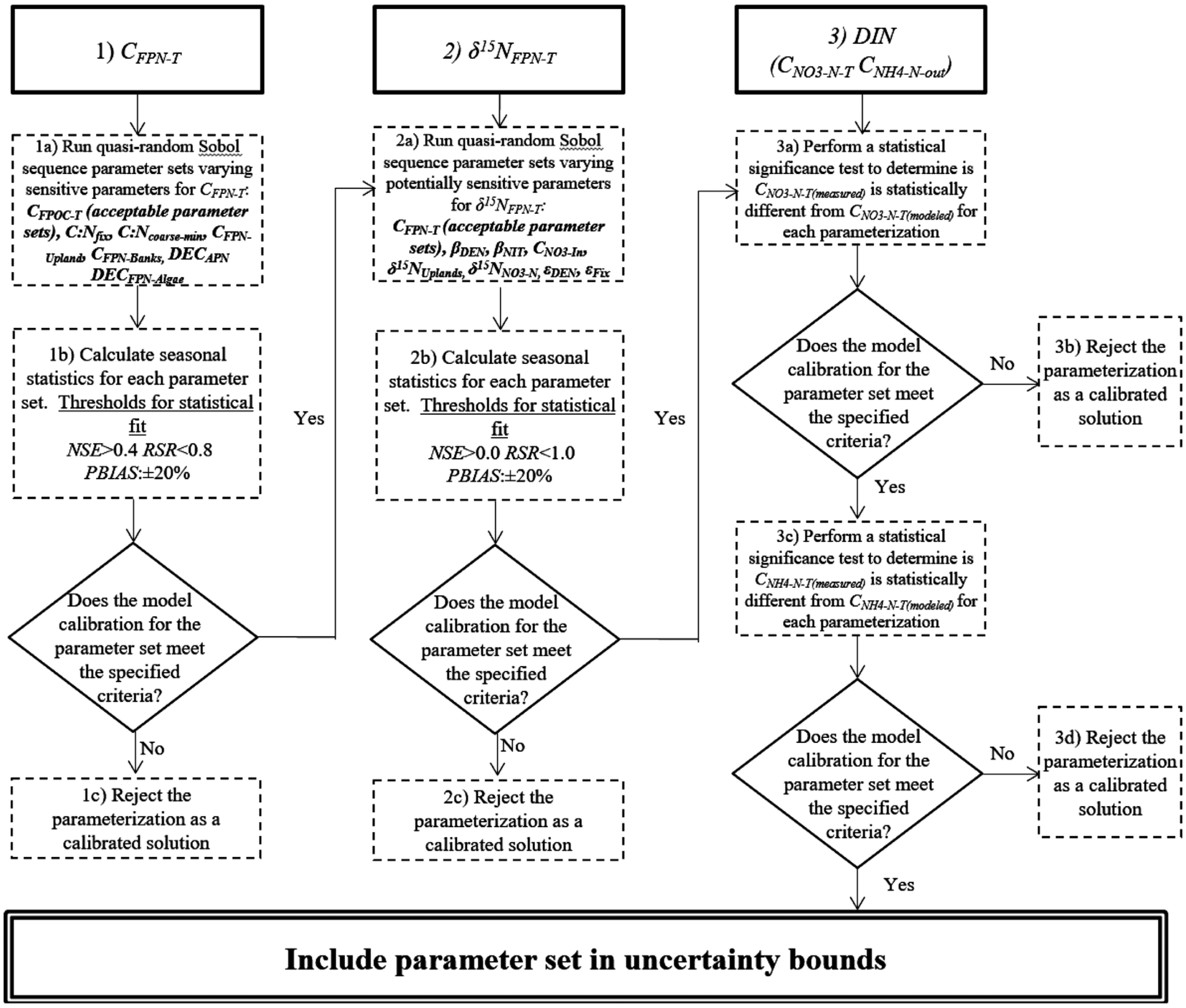

Figure 3. Model calibration and uncertainty analysis procedure for evaluating TRANSFER in low-gradient ag-disturbed ecosystems.

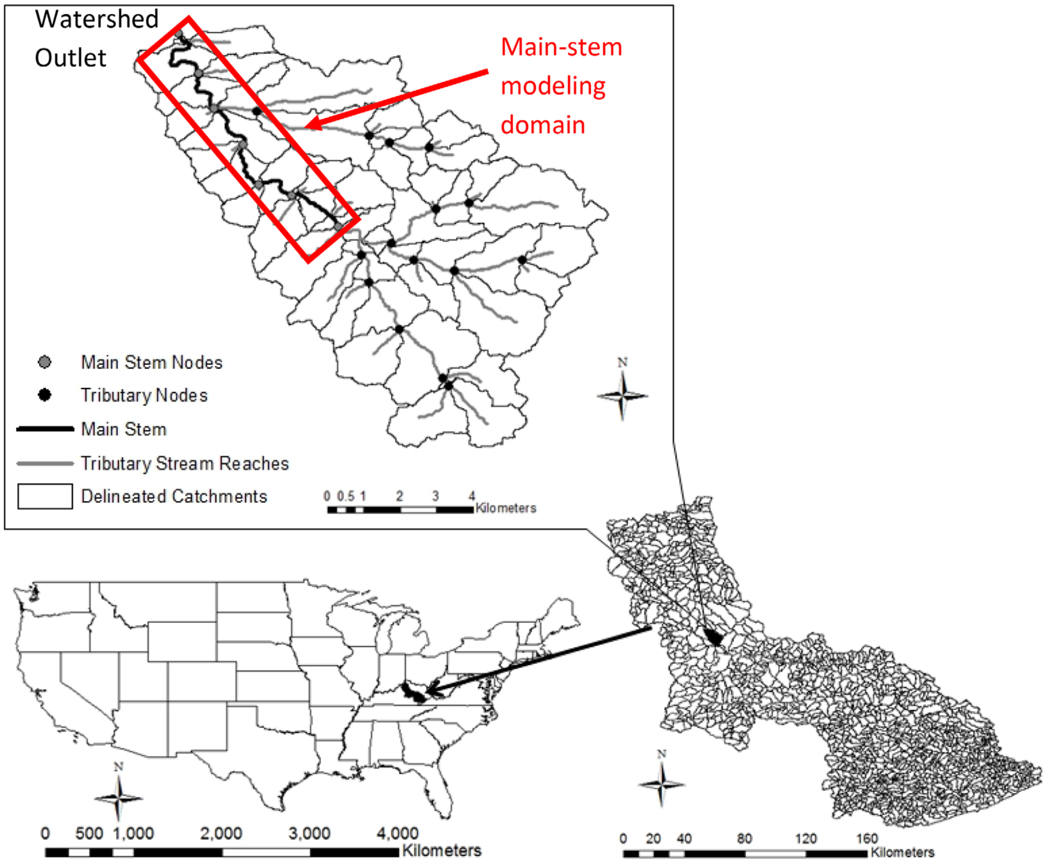

Figure 4. Model domain for the South Elkhorn watershed in the Bluegrass Region of Central Kentucky, USA.

banks coupled with densely compacted fine legacy sediments overlying a bedrock controlled streambed limit the prominence of hyporheic flow, and hence, we do not explicitly consider in the current model [*Ford and Fox*, 2014]. We qualify the aforementioned exclusion of dissolved organic nitrogen (DON) for the current case-study based on previous measurements of dissolved organic carbon in the watershed that hovered around 1.5 mg/L and did not show distinct seasonality. Based on a conservatively low Redfield ratio equal to 6.6:1 [*Martiny et al*., 2014], we estimate the DON concentration is 0.23 mg/L. Thus, DON is an order of magnitude smaller than DIN for this system. This result corroborates well with other temperate agricultural streams we have been working in where nitrate dominates N signatures and seasonality for TN year-round. The *TRANSFER* application was simulated at a 30 min time step in six equivalently sized reaches over the 8 year period that builds on previous modeling work in the watershed [*Fox et al*., 2010; *Russo and Fox*, 2012; *Ford and Fox*, 2015]. We refer the reader to the previous work for detailed site description and supporting model work. Table 1 shows parameterization of the South Elkhorn *TRANSFER* application, including parameter IDs, parameter descriptions, references, units, and nominal ranges, which was accomplished through field-based measurements and literature parameterization.

We parameterize carbon to nitrogen atomic ratio of assimilated algal biomass ($C{:}N_{Assim}$) and initial isotopic signatures of the algal mat based on point sample measurements within the stream channel, which were subsequently ground and combusted on an elemental analyzer interfaced with an Isotope Ratio Mass Spectrometer (IRMS) [*Ford et al*., 2015]. We assumed the factor to account for mineralization of coarse algal biomass ($C{:}N_{Coarse\text{-}min}$) had the same range as assimilated algae in order to ensure the C:N ratio of the algal mat did not exceed reasonable bounds. Sediment N content ($C_{FPN\text{-}Banks}$ and $C_{FPN\text{-}Upland}$) and isotopic signatures ($\delta^{15}N_{Banks}$ and $\delta^{15}N_{Uplands}$) of potential sources were measured in the watershed using transported sediment samples collected at high flows and grab samples from scouring banks, respectively, and were analyzed on the IRMS [see *Ford and Fox*, 2015; *Ford et al*., 2015]. We derived concentrations ($C_{NO3\text{-}In}$ and $C_{NH4\text{-}In}$) and N isotope values of stream water nitrate from tributary measurements over the course of a 14 month

Table 1. Inputs and Parameterization for the South Elkhorn Application of TRANSFER Including Parameters Associated With the Nitrogen Mass Balance and Isotope Mass Balance Including Parameter IDs, Descriptions, Optimal Ranges, Sources, and Units

Parameter ID	Parameter Description	Range Simulated in Model	Range Calibrated in Model (C_{FPN})	Range Calibrated in Model ($\delta^{15}N_{FPR}$)	Range Calibrated in Model ($C_{NO3\text{-}out}$)	Range Calibrated in Model ($C_{NH4\text{-}out}$)	Source for Selected Range	Units
Nitrogen mass-balance model								
$C{:}N_{Assim}$	Carbon to nitrogen ratio for algae fixation	8.0–15.0	8.11–13.41	8.11–13.41	8.11–13.41	8.1–10.8	Measured at site	
$C{:}N_{Min\text{-}Algae}$	Respiration: Mineralization factor	8.0–15.0	9.58–14.50	9.58–14.50	10.4–14.5	10.4–14.4	Measured at site	
$C_{FPN\text{-}Upland}$	Nitrogen content of hillslope sediments	0.17–0.29	0.18–0.28	0.18–0.28	0.19–0.28	0.19–0.28	Measured at site	%N
$C_{FPN\text{-}Bank}$	Nitrogen content of bank sediments	0.12–0.24	0.12–0.23	0.12–0.23	0.12–0.23	0.12–0.23	Measured at site	%N
α_{IN}	Exponent coefficient for nitrification function	1	1	1	1	1	*Arango and Tank* [2008]	
α_{DEN}	Exponent coefficient for denitrification function	1	1	1	1	1	*Arango and Tank* [2008]	
β_{IN}	Multiplicative coeff. for nitrification function	5×10^{-10}–5×10^{-7}		2.2×10^{-9}–4.8×10^{-7}	2.2×10^{-9}–4.8×10^{-7}	8.7×10^{-8}–4.8×10^{-7}	*Mulholland et al.* [2008]	kgN m^{-2} s^{-1}
β_{DEN}	Multiplicative coeff. for denitrification function	5×10^{-10}–5×10^{-7}		1.1×10^{-8}–5×10^{-7}	1.6×10^{-8}–4.8×10^{-7}	3.8×10^{-8}–4.8×10^{-7}	*Mulholland et al.* [2008]	kgN m^{-2} s^{-1}
$C_{NO3\text{-}N\text{-}In}$	Inflowing nitrate concentration	1.0–5.0		1.09–4.98	3.0–4.96	3.0–4.96	Measured at study site	mgN L^{-1}
$C_{NH4\text{-}N\text{-}In}$	Inflowing ammonium concentration	0.01	0.01	0.01	0.01	0.01	Measured at study site	mgN L^{-1}
$c_{DEC\text{-}APN}$	APN decomposition coefficient	2.2–3.8	2.85–3.58	2.85–3.58	2.85–3.58	2.84–3.58	*Ford and Fox* [2015]	d^{-1}
$c_{Min\text{-}SFGL\text{-}Algae}$	SFGL algae mineralization coeff.	2.2–3.8	3.21–3.79	3.21–3.79	3.33–3.79	3.33–3.78	*Ford and Fox* [2015]	d^{-1}
ISOFLOC Uncertainty	Equifinal parameter sets for the FPOC model						*Ford and Fox* [2015]	
Stable nitrogen isotope mass-balance model								
ε_{DEN}	Denitrification fractionation factor	2.0–18.0		2.1–17.5	3.8–16.3	3.8–16.2	*Kendall et al.* [2007] (ref within)	‰
ε_{Assim}	Fixation fractionation factor	0.0–13.0		0.2–12.8	0.4–12.8	0.4–12.8	*Kendall et al.* [2007] (ref within)	‰
$\delta^{15}N_{NO3\ (06\text{–}07)}$	Nitrate-N isotope signature	2.0–16.0		2.9–16.0	2.9–16	2.9–16	Measured at site	‰
$\delta^{15}N_{NO3\ (08)}$	" "	−6.0–16.0		−2.2–10.8	−2.2–10.8	−1.6–10	Measured at site	‰
$\delta^{15}N_{NO3\ (09\text{–}13)}$	" "	−2.0–16.0		2.0–14.9	2.0–14.9	2–14.9	Measured at site	‰
$\delta^{15}N_{Banks}$	Bank sediment N isotope signature	5.9–7.9		5.9–7.8	5.9–7.8	5.9–7.7	Measured at site	‰
$\delta^{15}N_{Uplands}$	Upland sediment N isotope signature	4.0–6.0		4.1–5.9	4.1–5.9	4.2–5.9	Measured at site	‰

sampling period [*Ford et al*., 2015]. Nitrate concentration grab samples were analyzed at the Kentucky Geological Survey on a Dionex Ion Chromatograph using standard EPA protocol [*ASTM*, 1996].

As shown in Table 1, we use a variable nitrate isotope signature from 2006–2007, 2008, to 2009–2013. Briefly we provide the following justification for this parameter modification. For 2006–2007, we have low SFGL algal carbon (and thus low mineralization) due to the reset of the bed from a 100 year storm event [*Ford and Fox*, 2017]. For this reason, most of the DIN transported in the main stem of the watershed will be a mix of upland fertilizers and soils. During 2008, the watershed had extensive drought conditions during summer and early fall, thus minimizing the ability of the upland sources to deliver NO_3 to the stream [*Ford and Fox*, 2014; *Ford et al*., 2014]. For this reason, we suspect connectivity of DIN to upland soils and fertilizers is diminished and mineralization from upland tributaries becomes prominent. From 2009 to 2013, we have an enriched SFGL algal N source coupled with upland connectivity suggesting a slight dampening of the DIN pool [*Ford and Fox*, 2017].

Regarding literature-based parameterization, we utilized uncertainty bounds from the previous *ISOFLOC* application in the watershed to propagate uncertainty of sediment and C submodules and breakdown of organic N [*Sinsabaugh et al*., 1994; *Webster et al*., 1999; *Alvarez and Guerrero*, 2000; *Jackson and Vallaire*, 2007; *Yoshimura et al*., 2008; *Ford and Fox*, 2014, 2015]. We assumed losses from the FPN pool were associated with mineralization and we neglect the contributions of decomposition of fine particulate nitrogen associated with soil nitrogen since these rates are typically orders of magnitude lower and were insensitive for C dynamics in the aforementioned modeling studies. Exponent coefficients for the nitrification and denitrification models (α_{IN} and α_{DEN}) were set to one since previous studies of nitrification and denitrification in agricultural based streams in Michigan have shown that processes vary linearly with sediment organic carbon content [*Arango and Tank*, 2008]. Rates of nitrification and denitrification were assumed to have comparable ranges, and vary over three orders of magnitude (10^2–10^4 μgN m^{-2} h^{-1}), which is consistent with rates in ag-streams [*Arango and Tank*, 2008; *Mulholland et al*., 2008]. Isotopic enrichment values associated with assimilation of nitrate ($\varepsilon_{Assim\text{-}NO3}$) were parameterized from broad ranges found for algal and bacterial uptake, while fractionations associated with denitrification (ε_{DEN}) were broadly parameterized to account for benthic and riparian denitrification potential [*Wada*, 1980; *Heaton*, 1986; *Montoya et al*., 1991; *Kendall and McDonnell*, 1998; *Needoba et al*., 2004; *Kendall et al*., 2007; *Fox et al*., 2010].

For multiobjective calibration, we collected 8 years of transported sediment samples using in situ sediment traps [*Phillips et al*., 2000] that were analyzed for sediment N elemental ($C_{FPN\text{-}T}$) and isotopic ($\delta^{15}N_{FPN\text{-}T}$) signatures. Descriptions of sample processing and analysis are detailed elsewhere [*Ford and Fox*, 2014, 2015; *Ford et al*., 2015]. In total, 205 samples were available for model evaluation, of which two-thirds were used for model calibration and one-third was used for validation.

3. Results

3.1. Sensitivity Analysis Results

The sensitivity analysis results showed that the individual response variables (i.e., see $C_{FPN\text{-}T}$, $\delta^{15}N_{FPN\text{-}T}$, and *DIN* in Figure 4) were sensitive to different model parameters, which in turn provided efficacy to the multiobjective calibration procedure applied in this study (Table 2). Further, we found that individual response variable dependence upon sensitive parameters was well explained with respect to our understanding of carbon and nitrogen processes within the stream, the details of which are explained below.

Results of the sensitivity analysis for the sediment nitrogen elemental ($C_{FPN\text{-}T}$) response variable showed sensitivity to model parameters that have a direct impact on SFGL nitrogen composition (see Table 2). Parameters associated with coupling of carbon and nitrogen dynamics, including $C{:}N_{Algae}$, $C{:}N_{Coarse\text{-}min}$, DEC_{APN}, and $DEC_{FPN\text{-}Algae}$, accounted for 68% of the variance of the sediment nitrogen content ($C_{FPN\text{-}T}$) response variable. The dependence of sediment nitrogen content upon coupled carbon and nitrogen dynamics is further evidenced by the sum of first-order indices of 0.32 and total-order indices of 1.61 for the sensitive parameters [see *Saltelli et al*., 2010]. This finding highlights the need to calibrate the carbon-nitrogen parameters in concert to reduce equifinality associated with sediment nitrogen dynamics. $C_{FPN\text{-}T}$ was not sensitive to inflowing nitrate concentration ($C_{NO3\text{-}in}$), denitrification rates (β_{DEN}), and nitrification rates (β_{IN}) suggesting they were insignificant in calibration. The reason for lack of sediment nitrogen dependence on the nitrate variables is related to nonrate-limiting nitrate conditions for the agricultural stream

Table 2. First-Order and Total-Order Sensitivity Indices for $C_{FPN\text{-}T(av)}$ and $\delta^{15}N_{FPN\text{-}T(av)}$ *for* Each of the Sensitive Parameters in the *FPN* and ^{15}N Mass Balances[a]

	First-Order Index S_i		Total-Order Index S_{Ti}	
Parameter	$C_{FPN\text{-}T(av)}$	$\delta^{15}N_{FPN\text{-}T(av)}$[b]	$C_{FPN\text{-}T(av)}$	$\delta^{15}N_{FPN\text{-}T(av)}$[b]
ISOFLOC Uncertainty	0.050	0.055	0.264	0.055
$C{:}N_{Assim}$	0.086	*0.000*	0.373	0.204
$C{:}N_{Min\text{-}Algae}$	0.033	*0.000*	0.197	1.270
$c_{DEC\text{-}APN}$	0.097	0.036	0.477	0.097
$c_{Min\text{-}SFGL\text{-}Algae}$	0.057	*0.000*	0.297	0.042
$C_{FPN\text{-}Upland}$	0.001	0.012	0.000	0.002
$C_{FPN\text{-}Bank}$	0.000	*0.000*	0.000	0.000
β_{DEN}	0.000	*0.000*	0.000	1.429
β_{IN}	0.000	*0.000*	0.000	0.019
$C_{NO3\text{-}N\text{-}In}$	0.000	0.030	0.000	0.095
$C_{NH4\text{-}N\text{-}In}$	0.000	0.000	0.000	0.000
ε_{DEN}		*0.000*		0.016
ε_{Assim}		0.183		0.305
$\delta^{15}N_{Uplands}$		0.000		0.014
$\delta^{15}N_{Banks}$		0.000		0.000
$\delta^{15}N_{NO3}$		0.190		0.623

[a] Values that were less than zero and associated with numerical integration of the Monte Carlo method in the sensitivity analysis are assumed to have no impact on the model and thus are assumed equal to zero.

[b] Results for the isotope sensitivity analysis only include 20% of simulations that provided plausible results. *Italicized numbers reflect negative values due to errors induced from subsampling of Sobol sequence.*

[*Ford et al*., 2015]. In summary, we found the sediment nitrogen content ($C_{FPN\text{-}T}$) response variable was more sensitive to growth and decomposition of algal nitrogen as opposed to dissolved inorganic nitrogen.

Results of the sensitivity analysis for the sediment isotope ($\delta^{15}N_{FPN\text{-}T}$) response variable showed high dependence upon dissolved inorganic nitrate transformation rates including algal uptake/regeneration, denitrification, and inflowing DIN concentrations (see Table 2). $\delta^{15}N_{FPN\text{-}T}$ was sensitive to the nitrate concentration of the water column (N-NO_3) due to the fact that the total nitrogen pool size (i.e., reservoir) impacts the isotopic composition of the product ($\delta^{15}N_{FPN\text{-}T}$) during fractionation. Further, the isotopic composition of the nitrate pool ($\delta^{15}N_{NO3}$), enrichment during uptake ($\varepsilon_{Fix\text{-}NO3}$) as well as rate of denitrification (β_{DEN}) following mineralization of organic nitrogen all can impact the isotopic composition of the sediment during fractionation. We note that the isotope response variable only utilized 20% of simulations since many scenarios produced unrealistic conditions, therefore we caution that these indices are more qualitative as opposed to quantitative representation of variance [*Saltelli et al*., 2010]. Nitrogen content and isotopic signatures of bank and upland sediments were not highly sensitive to the ($\delta^{15}N_{FPN\text{-}T}$) response variable, and nor was the nitrification parameter. Reasons for the result were that $\delta^{15}N$ of soils tend to have a fairly narrow range in the uplands of the watershed [*Fox et al*., 2010; *Ford*, 2014] and isotope enrichment during nitrification tends to be small so long as ammonification is rate limiting [*Kendall et al*., 2007].

3.2. Multiobjective Calibration Results

The sensitivity of $C_{FPN\text{-}T}$ and $\delta^{15}N_{FPN\text{-}T}$ to different sets of model parameters point toward the sequence used for phases of the multiobjective calibration as shown in Figure 4. First, we calibrated sediment nitrogen content ($C_{FPN\text{-}T}$) for organic matter variables during phase one because N is nonrate limiting in the agricultural stream. Then, we calibrated sediment N isotope response variable during phase two because the isotopic variable of the product, and later the substrate for mineralization reactions, is sensitive to the dissolved nitrogen concentration and its isotopic values and rates. Finally, we checked net dissolved inorganic nitrogen content in the water column (DIN: $C_{NO3\text{-}N\text{-}T}$ and $C_{NH4\text{-}N}$) to ensure that the parameter space produces results consistent with the measured nitrate and ammonium. The results of the sensitivity that lead to the calibration method is noteworthy because few studies have reported use of sediment substrate nitrogen variables for calibration purposes. Therefore, the sensitivity results reveal a new way to help calibrate and validate numerical models for nutrient dynamics, which is discussed further below.

The ability of the multiobjective calibration subroutine to reduce equifinality of nitrogen dynamics solution space is illustrated by observing the output histograms following each stage of calibration. First, we consider algae dynamics in modeling (Figure 5, column one). The nonuniform, right-skewed distribution of sloughed algae reflects the uncertainty output distribution of the ISOFLOC model [*Ford and Fox*, 2015]. From the raw parameterization results through Phase 2 of calibration, there is minor change in the shape of the distribution; however, we see a shift in the minimum value following Phase 1 which is due to the sensitivity of the $C_{FPN\text{-}T}$ response variable to algal sloughing parameters. Following Phase 3 of calibration, the distribution favors a slightly more bimodal distribution in which modes occur at 0.1 and 0.3 tN km^{-2} yr^{-1}.

Equifinality is also reduced for the denitrification flux (Figure 5, column two) highlighting the efficacy of the multiobjective calibration tool. The posterior solution space was uniformly distributed for raw results (reflecting the Sobol sampling scheme) and remained uniformly distributed following Phase 1 of the calibration due to insensitivity of denitrification parameters on the $C_{FPN\text{-}T}$ response variable. Phase 2 ($\delta^{15}N_{FPN\text{-}T}$) reduced equifinality because several parameterizations that produce denitrification fluxes in the 0.2–0.6 tN km^{-2} yr^{-1} were rejected and we found development of a right-skewed histogram. Further, following Phase 3 of calibration (DIN) we found further constraint and development of a bimodal distribution similar to that of sloughed algae.

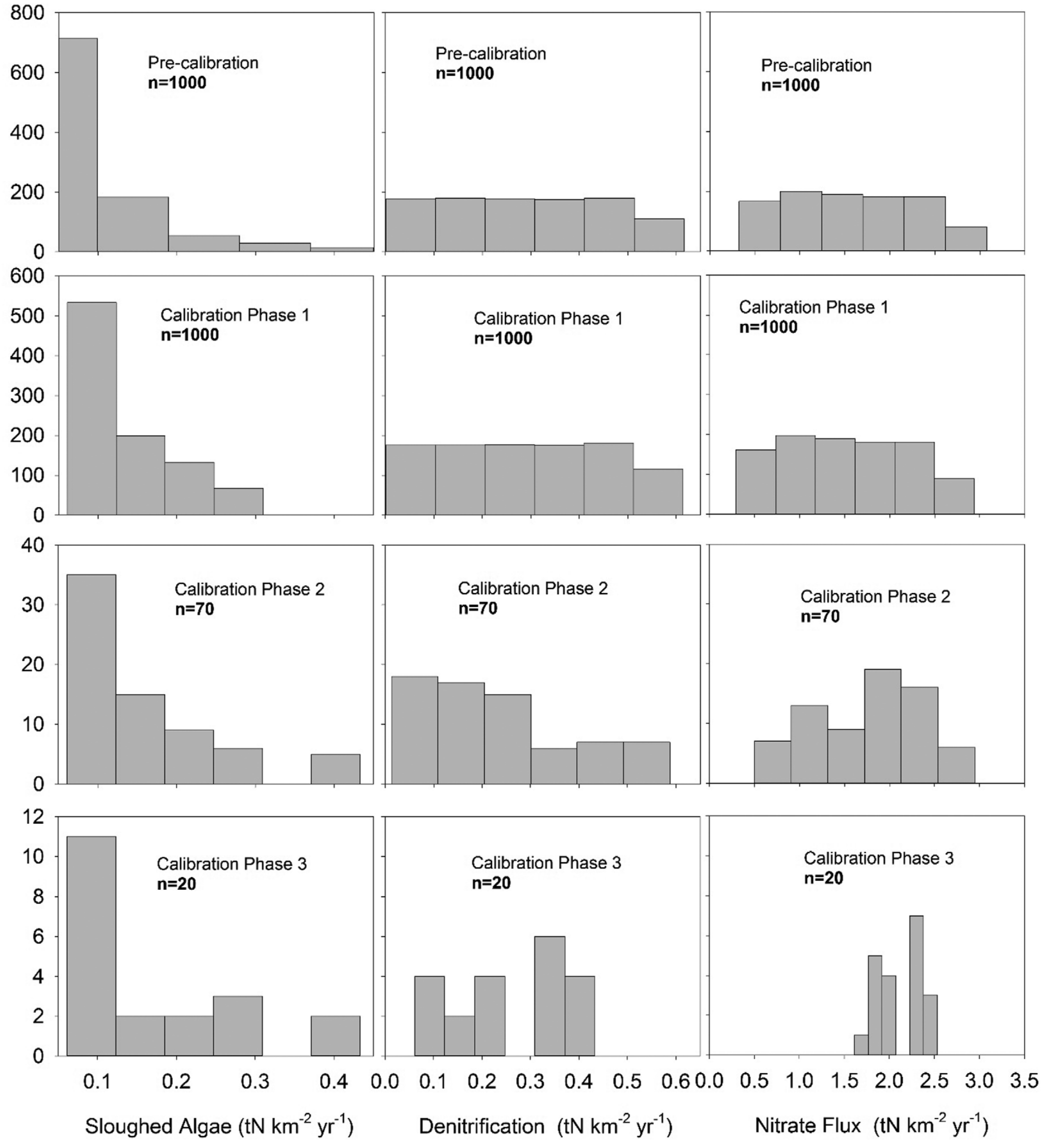

Figure 5. Histograms of the posterior solution space for TRANSFER during phases of model calibration. The histograms illustrate the ability of sequential model calibration to reduce equifinality for N fluxes for the South Elkhorn application.

Table 3. Quantification of Equifinality Reduction From Multiobjective Calibration of the *TRANSFER* Model

Equifinality Reduction Metric	Precalibration Size (%)	Phase I (% Size of Original Space)	Phase II (% Size of Original Space)	Phase III (% Size of Original Space)
Solution space—model calibration from Figure 3				
Total solution space for algae	100%	69%	69%	69%
Total solution space for DEN	100%	100%	93%	88%
Total solution space for DIN flux	100%	96%	89%	33%
Solution space—model calibration from DIN (no sediment variables)				
Total solution space for DEN	100%			100%
Total solution space for DIN flux	100%			56%

Finally, we highlight equifinality reduction for nitrate fluxes (see Figure 5, column three). Like denitrification fluxes, nitrate fluxes were uniformly distributed for precalibration and Phase 1 of the calibration which reflects insensitivity to the $C_{FPN\text{-}T}$ response variable. We found nitrate fluxes to take on a unimodal distribution following Phase 2 of calibration, in which the range was narrowed by $>$0.1tN km^{2} yr^{-1}. The decrease in outputs on the left side of the distribution reflected the decrease in rejection of high-end denitrification estimates. When considering realities of DIN concentrations, Phase 3 further restricted the range of the histogram by more than 1 tN km^{-2} yr^{-1} which removed a number of equifinal parameterizations with low denitrification rates (and low influent nitrate concentrations) that would meet statistical significance tests of the sediment response variable.

To further quantify the equifinality reduction associated with the multiparameter calibration, we summarize results of the sequential model phases in the top half of Table 3, and we summarize equifinality reduction for model calibration that uses only the DIN response variables in the bottom half of Table 3. The effects of the carbon modeling component to reducing algal sloughing uncertainty for C dynamics has been previously characterized as an 80% reduction in the solution parameter space using C elemental and isotope signatures [*Ford and Fox*, 2015]. A similar finding is shown in Table 3 for the TRANSFER model. We find for nitrogen that the additional sediment elemental response variable reduces uncertainty by 31%. As can be seen, no additional calibration bonus was observed between Phase I and Phase III, so we did not consider improvement of the solution space when calibrating with DIN alone. For denitrification, we find that the multiresponse framework including stable N isotopes and DIN reduces uncertainty ranges of DEN by 12%; however, a 0% improvement was found when calibrating only with the DIN pool. Likewise, the combined uncertainty reduction of algae sloughing by the elemental model and denitrification resulted in 67% equifinality reduction for nitrate flux using our proposed model calibration framework, and a 44% reduction when using solely DIN. The results show substantial reductions in the solution space (equifinality) when utilizing sediment elemental and isotope response variables.

Table 4. Goodness of Fit Statistics for the *TRANSFER* South Elkhorn Application Following Multiobjective Calibration Using (a) Seasonally Averaged Model Evaluation of Response Variables and (b) Event-Based Model Evaluation of Response Variables

	C_{FPN}	$\delta^{15}N_{FPN}$ (*Variable* $\delta^{15}N_{NO3}$)
(a) Seasonal		
Calibration		
NSE	0.409–0.537	0.010–0.334
RSR	0.680–0.770	0.816–0.995
PBIAS	−4.629–3.958	−4.093–7.811
Validation		
NSE	0.4160.782	0.008–0.641
RSR	0.466–0.764	0.599–0.996
PBIAS	−6.690–1.374	−3.946–4.986
(b) Event-Based		
Calibration		
NSE	0.248–0.350	−0.307–0.035
RSR	0.806–0.867	0.983–1.144
PBIAS	−8.675–0.392	−4.593–7.696
Validation		
NSE	0.100–0.337	−0.809–0.188
RSR	0.814–0.949	1.090–1.345
PBIAS	−6.682–−0.691	−3.181–5.865

3.3. Model Goodness of Fit Results

The multiobjective calibration allowed us to gain confidence in the results from *TRANSFER*. Results of the multiobjective calibration and uncertainty analyses showed the ability of *TRANSFER* to capture SFGL nitrogen dynamics at seasonal to multiyear timescales (see Table 4 and Figure 6). Comparison with measured $C_{FPN\text{-}T}$ data showed acceptable statistics with optimum seasonally averaged NSE values of 0.54 and 0.78 and RSR values of 0.68 and 0.47 for calibration and validation, respectively. Regarding event-to-event dynamics, the model captures dynamics fairly well as evidenced by the slight drop off in calibration statistics, with optimum NSE

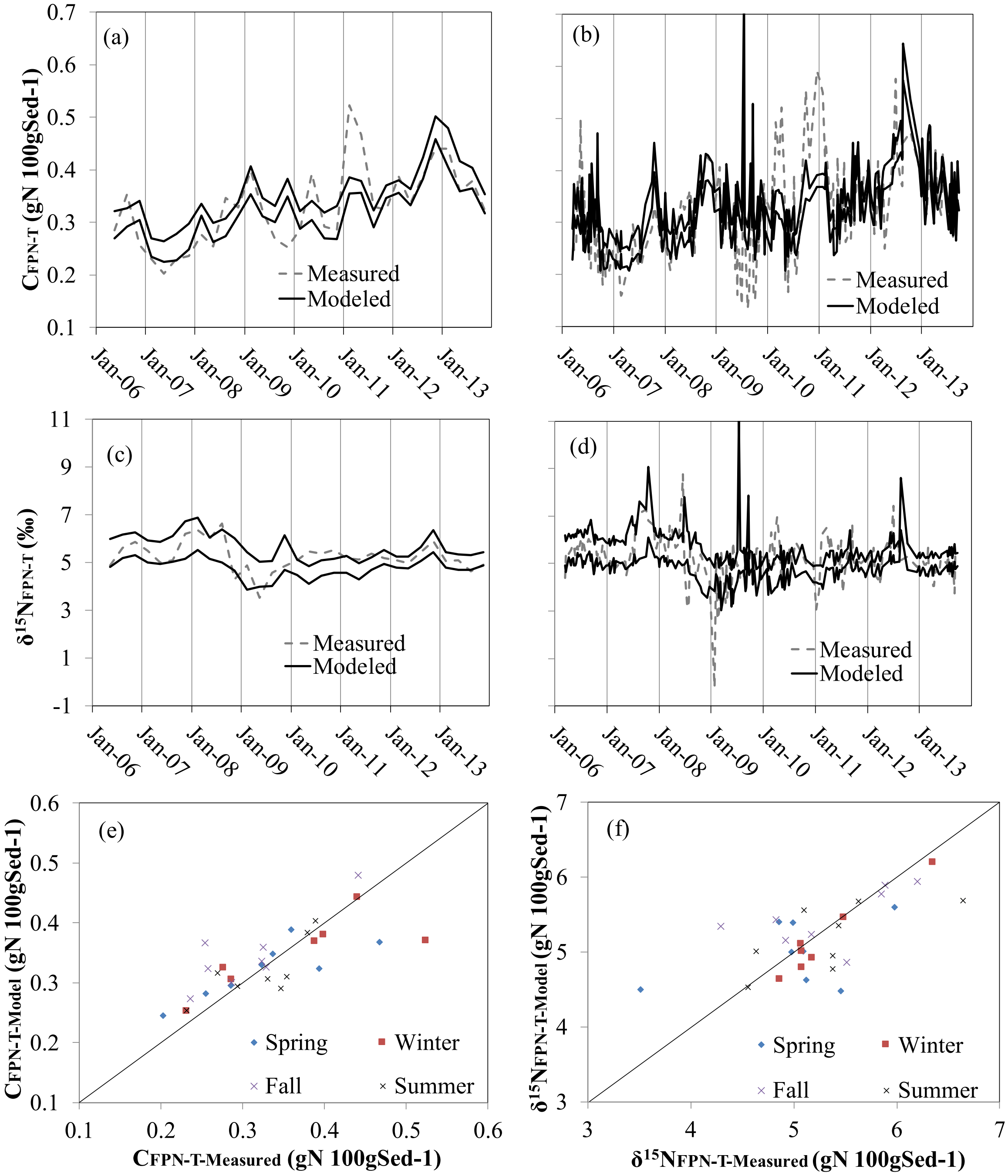

Figure 6. Calibration for (a and b) $C_{FPN\text{-}T}$ and (c and d) $\delta^{15}N_{FPN\text{-}T}$ for both (a, c) seasonal averaging and (b, d) event-based calibrations. Seasonally averaged modeled versus measured values are also plotted in aggregate and by season for (e) $C_{FPN\text{-}T}$ and (f) $\delta^{15}N_{FPN\text{-}T}$ with reference 1:1 line. The average of the maximum-minimum range in a-c is plotted in Figures 6e and 6f.

values of 0.35 and 0.34 for calibration and validation, respectively. The ability to capture event-based dynamics reflects the ability of the model to capture timing of benthic biological and physical processes and partitioning of source contributions for the bulk sediment nitrogen pool. Seasonal and event-based time series of measured and modeled data (with uncertainty bounds) are found in Figure 6. In general, we found that seasonal and long-term trends were captured with maximums occurring in late-fall and minimums in early spring, consistent with trends observed for C dynamics [*Ford and Fox*, 2014, 2015]. Further, long-term increases in $C_{FPN\text{-}T}$ were consistent between measurements and simulations. For the measured and modeled data, we found the average increased from 0.25 gN/100 gsed in 2006 to 0.35 gN/100 gsed in 2013. Similarly, model results suggested an increase from 0.25 gN/100 gSed in 2006 to 0.35 gN/100 gSed in 2013.

Results of the calibration and uncertainty analysis for $\delta^{15}N_{FPN-T}$ highlight the ability of the model to capture seasonal and long-term dynamics of N fate and transport; however, some weaknesses in model predictions were observed during winter and the event-based timescale. Seasonal peaks and valleys (e.g., 2006–2007), abrupt changes due to a change in NO_3 isotope source signature (e.g., 2008–2009) and reestablishment of equilibrium were accurately reflected in model calibration for both sediment elemental and isotope response variables, as evidenced by visual agreement in Figure 6 and seasonal statistics in Table 4. We found deficiencies in the ability of the model to capture peaks in early to midwinter in 2010 and 2011 and during the wet summer of 2009. In 2010 and 2011, measurements of C_{FPN-T} at the watershed outlet showed secondary peaks (i.e., following peaks in late fall) that were not captured by the numerical model. Secondary peaks could be attributed to unforeseen abiotic processes (e.g., adsorption to variably charged sesquioxides) in the SFGL [*Ford et al*., 2015]. Further, overprediction of measured data in spring-fall of 2009 likely reflected deeper gully or bank erosion and transport from the watershed since the events occurred during a wet summer with a high number of high intensity, short-duration storms [*Ford and Fox*, 2014]. Regarding event-based timescales, statistical metrics suggest inferior predictions to a mean model for $\delta^{15}N_{FPN-T}$ (i.e., $RSR>1$ and $NSE<0$), even for optimum statistics. In addition to the aforementioned anomalies, the poor statistical metrics during event-based timescales are likely attributed of the inability of the model to capture rapid fluctuations of $\delta^{15}N_{FPN-T}$ especially in 2008 and early 2009 resulting from a combination of measurement error and inadequate simulation of spatiotemporal variability of processes [*Fox et al*., 2010].

For nitrate, we compared distributions of C_{NO3-N} measured at the watershed outlet to the modeled distribution using a statistical *t* test. Of the 65 parameter sets that provided sufficient statistical fit for the sediment calibration, only 23 were found to be statistically equivalent to the measured distribution for NO_3 (two-tailed *P* value <0.05). Figure 7 compares visual time series of the continuous modeled concentrations of nitrate with sparsely collected data from the watershed outlet from 2010 to 2013. Somewhat surprisingly, model simulations visually capture dynamics observed in the nitrate data without using a calibration procedure focused on seasonality of the DIN pool, providing additional confidence in uptake and denitrification estimates. While the calibration would be strengthened with additional nitrate data, we find this to be a promising result considering the high cost in monitoring and analyzing nitrate concentrations due to high temporal variability. Since ammonium concentrations were below detection limits, no graphical representation of ammonium dynamics was included.

3.4. Nitrogen Flux and Nitrate Removal Results

The posterior solution space from the final stage of the uncertainty analysis provided uncertainty estimates for the annual fluvial nitrogen budget including downstream advective fluxes and net nitrogen removal (see Table 5). Advective nitrate transport was the largest flux while sloughed algae, nitrification, fixation,

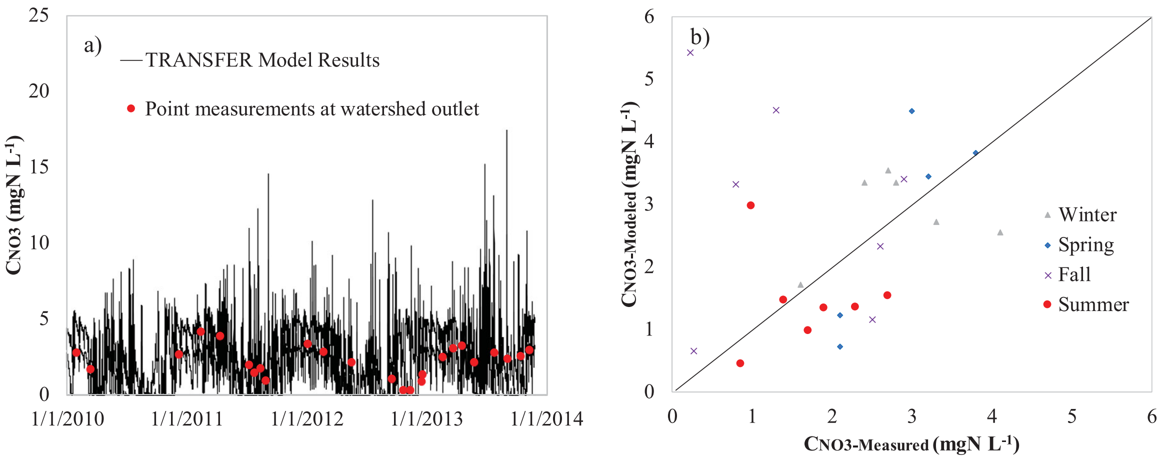

Figure 7. Time series (a) of modeled nitrate concentration results at the watershed outlet are compared with sparse grab samples that were collected and analyzed for NO_3-N at the Kentucky Geological Survey Laboratory. Daily averaged modeled results are compared with measured grab samples and plotted in aggregate and by season.

Table 5. Annual Fluvial Nitrogen Budget for Sediment and Dissolved Nitrogen Pools Including Algae, Fine Particulate Nitrogen, NO_3, and NH_4[a]

Nitrogen Flux (tN km^{-2} yr^{-1})	2006	2007	2008	2009	2010	2011	2012	2013	Average
Advective downstream fluxes									
Q (m^3 yr^{-1})	1.53	0.93	1.19	1.55	0.81	1.58	0.78	1.38	1.23
$NO_{3\text{-IN}}$	2.34–3.87	1.41–2.33	1.81–3.00	2.36–3.90	1.23–2.03	2.40–3.98	1.19–1.97	2.10–3.48	1.88–3.11
$NO_{3\text{-out}}$	2.02–3.18	1.19–1.85	1.62–2.55	2.05–3.25	1.00–1.54	2.12–3.35	0.96–1.43	1.79–2.84	1.61–2.53
$NH_{4\text{-IN}}$	0.01–0.01	0.00–0.00	0.01–0.01	0.01–0.01	0.00–0.00	0.01–0.01	0.00–0.00	0.01–0.01	0.01–0.01
$NH_{4\text{-Out}}$	0.01–0.01	0.00–0.01	0.01–0.01	0.01–0.01	0.00–0.00	0.01–0.01	0.00–0.01	0.01–0.01	0.01–0.01
Transported FPN	0.05–0.07	0.02–0.02	0.03–0.04	0.04–0.04	0.02–0.02	0.04–0.04	0.01–0.01	0.03–0.04	0.03–0.04
Sloughed algae	0.10–0.60	0.05–0.41	0.03–0.33	0.10–0.49	0.03–0.25	0.07–0.55	0.03–0.37	0.08–0.41	0.06–0.43
Benthic transformation fluxes									
Nitrification	0.07–0.33	0.07–0.35	0.07–0.33	0.07–0.31	0.08–0.37	0.08–0.31	0.10–0.41	0.07–0.34	0.08–0.35
Fixation	0.16–0.71	0.05–0.54	0.00–0.48	0.14–0.68	0.03–0.59	0.12–0.60	0.03–0.63	0.19–0.76	0.09–0.63
Denitrification	0.04–0.56	0.04–0.52	0.04–0.58	0.04–0.60	0.04–0.58	0.05–0.61	0.05–0.64	0.04–0.56	0.04–0.59

[a] Further, uptake and transformations of N species are quantified directly each year, and on average, to partition permanent and transient removal pathways.

and denitrification fluxes were generally an order of magnitude lower (approximately 10% of DIN). Fine particulate nitrogen and ammonium fluxes were two orders of magnitude lower. As expected, years with flow rates above the 8 year average (i.e., 2006 and 2009, 2011, and 2013) had the highest downstream advective fluxes of nitrate, sloughed algae, and sediment nitrogen. Less intuitive was the result that fixation was generally higher for high flow years, which we attribute to higher sloughing, reducing rate-limiting conditions for population saturation. No distinct trends were observed for nitrification, denitrification, and fixation from year to year.

We placed emphasis on algal sloughing at weekly to seasonal timescales given that few, if any, studies have highlighted the flux and interestingly it was the same order of magnitude as denitrification (Figure 8). Sloughed algae flux estimates from *TRANSFER* were lowest in spring and winter with moderate fluxes in summer and high fluxes in fall. As a result, sloughed algae fluxes varied from 4% of the total downstream N flux in winter to 14% in summer. On an event basis, sloughed algae fluxes had high variability; however, periodically they accounted for upward of 45% of the downstream N-flux for a given event, with the highest fluxes in late fall-early winter when large algal mats are subjected to high shear stresses from large storm events (Figure 8a). More moderate flow conditions in summer and early fall (e.g., 2009) and nonrate-limiting conditions for algal growth promoted smaller, yet more consistent contributions of algal sloughing on the order of 20% of the downstream N flux (Figure 8b). Despite high flow conditions in winter and early spring, little to no sloughing flux occurred due to nonfavorable growth conditions for algae following the mass removal that occurred during high flows in the fall (Figure 8b).

We used *TRANSFER* results to quantify net N removal by the stream channel and specifically partitioning between permanent (denitrification) and transient (sloughing) removal pathways (Table 5). Results from the uncertainty analysis showed an average net nitrate removal of 17% of inflowing nitrogen in the main stem of the watershed. Net nitrogen removal varied widely under uncertainty predictions from 7% to 23% nitrate removal annually. Of the fraction that is removed, an average estimate of 63% was removed via denitrification and 37% was removed through algal sloughing (i.e., erosion). Seasonal partitioning between permanent (denitrification) and temporary (sloughed algae) nitrate removal pathways (Figure 8c) yielded similar results. Based on flux estimates, summer and fall had the highest net nitrate removal rates with 38% and 51% of the removal originating from algal sloughing and the remainder associated with denitrification. Winter and Spring had less favorable removal rates with <30% of losses from sloughed algae in each season. Results show that on an event basis, algal sloughing can constitute >90% of the net nitrogen removal, especially in late fall and early winter if large algal mats are still present. As evidenced by comparison of Figures 8a and 8c, sloughing consistently represents 50% or more of the net nitrogen flux on a weekly basis during high flow conditions.

4. Discussion

4.1. Equifinality Reduction Using TRANSFER

Our findings highlight *TRANSFER's* efficacy to reduce equifinality of nitrogen model results by coupling a multiobjective calibration subroutine with a unique set of calibration variables. Three features are note-

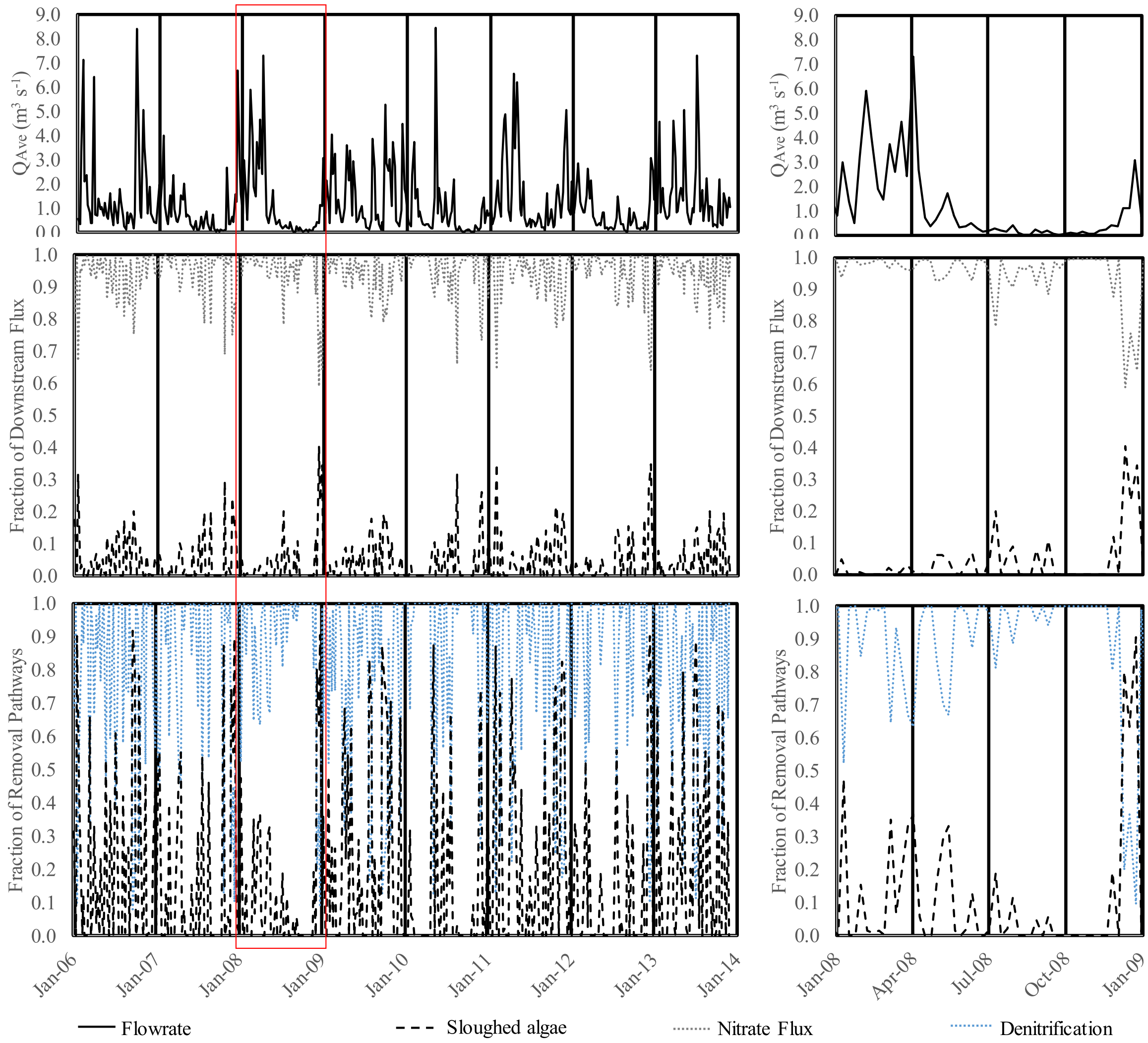

Figure 8. Time series of observed average weekly flow rate at the watershed outlet, fractions of sloughed algae, and nitrate contributing to downstream N fluxes, and fractions of sloughed algae and denitrification contributing to permanent and transient nitrate removal pathways. Fluxes from 2008 are enhanced to highlight seasonal and event to event dynamics.

worthy including: (1) the ability of sediment nitrogen to calibrate algal nitrogen dynamics; (2) the ability of the stable isotope signature of sediment to calibrate dissolved nitrogen transformation; and (3) the importance of the ordering of stages within the multiobjective calibration subroutine.

We find that simulation of algal nitrogen fate is constrained using the sediment nitrogen response variable ($C_{FPN\text{-}T}$). Results highlight the sensitivity of algal nitrogen growth and decomposition parameters to the sediment nitrogen response and a lack of sensitivity to dissolved nitrogen parameters, e.g., concentrations, denitrification, and ammonification (Table 2 and Figure 5). This finding occurs because algae growth and decomposition dynamics control seasonal and annual variability of sediment nitrogen [*Ford et al.*, 2015]. Biodegraded algae become integrated into the SFGL, which retains the algal nitrogen fingerprint to some degree [*Ford and Fox*, 2014, 2015, 2017]. Algal nitrogen is not rate-limited by dissolved inorganic nitrogen in the water column and thus we see insensitivity to these reactions. Therefore, sediment nitrogen is a unique calibration tool for benthic algal nitrogen dynamics in streams.

We highlight that use of the ambient stable nitrogen isotopic signature of sediment as a model response variable constrains dissolved nitrogen dynamics. Nitrogen integrated into the sediment algal pool will reflect properties of nitrogen from its DIN source (NO_3 and NH_4), and the isotopic signatures have been found to be sensitive to remineralization, nitrification, denitrification, preferential assimilation, and uptake

[*Peterson et al*., 1997; *Kendall and McDonnell*, 1998; *Wollheim et al*., 1999; *Kendall et al*., 2007; *Hall et al*., 2009]. Our findings highlight that inaccurate estimates of dissolved nitrogen parameters (e.g., denitrification) will impact the isotopic signature of the nitrate source as well as transported sediment N and hence provide unrealistic Sobol parameter sets in simulation. The stable isotope signature effectively discriminates between those unrealistic conditions as compared to plausible equifinal solutions that meet criteria for acceptance of model solutions. This idea, reflected in the sensitivity and calibration results of Figures 5 and 6 and Table 5, highlights the significance of sediment nitrogen isotope signatures in agroecosystems for constraining dissolved nitrogen fluxes and thus reducing numerical model equifinality. We highlight the feasibility of collection and analysis of ambient sediment N isotopes into routine sampling. Samples can be collected on a weekly basis, stabilized and processed using accepted methods, and stored for extended periods of time until ready for batch analysis [*Phillips et al*., 2000; *Ford and Fox*, 2015; *Ford et al*., 2015]. Unlike dissolved N isotope measurements, sediment N isotope analysis is relatively inexpensive and is commonly performed in stable isotope laboratories.

As a final innovative feature of the numerical modeling performed here, we highlight the importance of the ordering of the multiobjective calibration approach. The sediment nitrogen and sediment nitrogen isotope response variables impact the distribution of equifinal outputs of nonconservative algal sloughing, nitrification, and denitrification. Without these intermediate calibration steps (i.e., purely calibrating with nitrate), we would find a broad range of rates that would satisfy the statistical significance test used for nitrate and ammonium because NO_3 and NH_4 are not very sensitive to mineralization rates, denitrification rates, and uptake rates. The DIN calibration was critical for reducing the broad uncertainty in nitrate and ammonium fluxes solution space, as well as further constraining denitrification and sloughing equifinality of parameter spaces.

4.2. Algal Sloughing Within Freshwater Nitrogen Dynamics

Results of the *TRANSFER* application reveal the significance of algal sloughing relative to presumed important removal pathways of DIN. Agricultural runoff with high dissolved nitrogen loads places strain on the water bodies yet it is accepted that internal stream cycling may help to self-mitigate and remove nutrient loads [*Seitzinger*, 2008]. For example, in the Mississippi River Basin for which this study is located, past research has estimated that on the order of 50% of agricultural-associated nutrient runoff is removed by the fluvial network prior to the water reaching the Gulf of Mexico [*Alexander et al*., 2008]. The present study suggests that denitrification removes 10.7% of the dissolved nitrogen load within the third-order stream system. Algal sloughing removes a slightly lower level of dissolved nitrogen from the stream system (6.3% of dissolved N load annually). The remaining 83% is transported downstream as nitrate. TRANSFER results predict sloughed algal nitrogen fluxes to be sometimes greater than traditionally important denitrification and advective downstream nitrate transport (Table 5). Few previous studies have explicitly considered the potential role of algae to temporarily or permanently store nitrogen within the fluvial system.

We highlight a need to quantify the fate of sloughed algal nitrogen within the fluvial network and in downstream water bodies. Algae is a labile pool of organic matter and is recognized as carbon-rich and composed of highly labile neutral sugars [*Vieira and Myklestad*, 1986; *Waite et al*., 1995; *Lane et al*., 2013]. One end-member assumption based on traditional spiraling concepts is that all benthic-derived sloughed algae has short turnover lengths within the fluvial network and algal nitrogen is remineralized to dissolved nitrogen. However, such an assumption neglects the potential for long-term sequestration of algal nitrogen, analogous to findings for algal carbon in recent C isotope-tracer studies [*Hotchkiss and Hall*, 2015; *Ford and Fox*, 2017]. Ultimately, the transient pool of nitrogen can be denitrified or rereleased as dissolved inorganic nitrogen in downstream water bodies. The algal nitrogen fate question seems particularly relevant given that recent carbon cycling research showed that benthic-derived sloughed algae was on the same order of magnitude, and at times greater, than phytoplankton within downstream water bodies [*Ford and Fox*, 2017].

The fate of algal nitrogen as it leaves agricultural watersheds is likely intertwined with physical processes as well as dissolved nitrogen and sediment nitrogen also exported from the watershed. Turbulent action of small to intermediate streams have the high potential to disaggregate benthic algae [*Jarvis et al*., 2005] to colloidal and dissolved size fractions that allows greater surface area for mineralization and possible denitrification occurring on suspended particles in the water column. At the same time, the high amount of exopolymeric substances (i.e., mucilage) associated with algae promotes the potential for flocculation in larger slower moving water bodies [*Worner et al*., 2002]. Settling of flocs in rivers, lakes, and estuaries allow for the building of hybrid sediments when the autochthonous material combines with terrestrial derived particulates [*Droppo et al*., 2005]. The net fate of algal-nitrogen within such hybrid sediments is not well known although it is well recognized that both rerelease and denitrification both occur [*Revsbech et al*., 2005]. For example, high dissolved nitrogen loads associated with agricultural runoff will be expected to decrease during summer periods, such as in the Mississippi River Basin. In turn, stored algal-nitrogen in the beds of large rivers, lakes, and estuaries provides bioavailable organic matter and a source of nitrogen for nitrifying bacteria [*Revsbech et al*., 2005] which could increase water-borne nitrate for blue-green algal blooms in late summer. As another example, further decreases of nitrate in the water column ($<$150 μg/L) [*Seitzinger et al*., 2006; *Arango and Tank*, 2008] in late summer and fall would promote the onset of coupled nitrification-denitrification in the beds of lakes, rivers and estuaries and provide permanent removal pathway of the benthic-derived algal nitrogen from the fluvial system.

Taken together, some portion of the sloughed algal nitrogen is permanently removed from the fluvial network while another portion is remineralized potentially promoting a decrease in downstream water quality. Given that the magnitude of algal nitrogen leaving the watershed is on the order of 10%, further elucidating its fate remains an open question for scientists focused on nutrient cycling and water quality (i.e., hazardous algal blooms in late summer, early fall).

4.3. Applicability and Limitations of TRANSFER and Its Results

We find that the results of this study and model evaluation technique are transferable to other agroecosystems with prominence of an active SFGL layer and nonrate-limiting nutrient conditions. We developed *TRANSFER* based on a synthesis of nitrogen cycling perceptions in agroecosystems [*Birgand et al*., 2007; *Mulholland et al*., 2008] and findings of coupled C and N dynamics including importance of C quality for biotic cycling, SFGL dynamics, and algal fate and transport [*Arango and Tank*, 2008; *Ford and Fox*, 2014, 2015]. The results of the calibration (Figures 5–7 and Table 4) provide confidence in our conceptual model of nitrogen cycling in streams and show the effectiveness of multiobjective calibration and uncertainty using ambient sediment nitrogen elemental and isotopic signatures to reduce equifinality for nutrient cycling problems. The flux rates parameterized through calibration in *TRANSFER* that are consistent with rates commonly reported for fluvial agroecosystems further support our confidence in the model. As an example, uncertainty bounds that show denitrification rates and fixation rates that range from 10^3 to 10^4 μg m^{-2} h^{-1} fall in the middle to high end of rates reported in the literature for riverine systems [*Mulholland et al*., 2008]. High rates of fixation are reflective of the highly productive nature of open-canopy, high nutrient streams in the watershed that create nonrate-limiting conditions for algal growth [*Griffiths et al*., 2012; *Ford and Fox*, 2014]. High rates of denitrification are likely supported by high quality carbon pools associated with algal biomass and detritus accrued in the SFGL of the agroecosystem streams [*Martinelli et al*., 2011; *Lane et al*., 2013; *Ford et al*., 2014].

While we find results and transferability of the model to nutrient-rich urban and agroecosystem streams with low hyporheic exchange, we highlight limitations for applicability in contrasting landscapes. *TRANSFER* was developed, primarily for agroecosystem streams, which tend to be fueled by autochthonous C sources as opposed to allochthonous leaf litter and detritus due to open canopy cover, high nutrient conditions, and low-gradient streambeds [*Rutherford et al*., 2000; *Griffiths et al*., 2012; *Ford and Fox*, 2014]. Nitrogen dynamics in forested landscapes are heavily regulated by upland leaf litter and detritus and low nutrient thresholds that can produce rate-limiting conditions for algal growth. In steep-gradient systems, it is perceived that SFGL storage is small relative to its low-gradient counterparts, which will limit the ability of algal stabilization to integrate DIN fingerprints across timescales. As a result, model applications in these landscapes may show lower sensitivity to the algal pool. Nevertheless, it is perceived that sediment isotopes will be highly sensitive to processing of coarse leaf litter and detritus, and nutrient regeneration, which highlights their potential utility in such landscapes. Certainly, in organic rich catchments (e.g., peat), or phytoplankton dominated rivers, such as larger systems downstream, organic N in the water column may vary substantially seasonally and may show high utility for informing in-stream models and reducing equifinality. We suggest considering this response variable in future work to extend the current modelling framework to such systems. Finally, TRANSFER does not explicitly simulate hyporheic exchange, which stems from the current application in fine-textured soils with bedrock controlled streambeds. Nevertheless, this process can become important in porous sandy and gravel bed rivers [*Trimmer et al*., 2012]. The reactions and interactions of groundwater and surface water could be easily integrated through addition of a subsurface nutrient pool and lateral and vertical exchange terms between the surface and subsurface pool. We foresee this as an exciting extension of the model and a promising area for future work.

While in general the *TRANSFER* model confirms our perceptions of nitrogen cycling, some of the event based results show deficiency in model evaluation statistics and highlight areas for further research and refinement. We surmise that the deficiency is likely reflective of epistemic uncertainty in model structure and our lack of understanding of the anomalous mobilization of soil and sediment nitrogen and the anomalous demobilization of dissolved N, such as occurring under stresses associated with drought followed by hydrologic events or as occurring after rather inert sediment has blanketed the streambed during winter months, respectively. We highlight that N fate and transport could be influenced by both biotic and abiotic processes [see *Ford et al*., 2015] prompting the need for mesoscale laboratory experimentation. Related, the (dis)connectivity of the stream corridor and its uplands can be heterogeneous in space and time. Epistemic uncertainty in biogeochemical simulation of DIN transformation could also contribute to model deficiencies. For instance, our model structure does not explicitly simulate redox conditions and explicit discretization of zones for denitrification to occur. A recent study by *Reisinger et al*. [2016] highlighted the importance of water column denitrification in large riverine systems to be on the same order of magnitude as benthic sediment denitrification, which tends to dominate in headwater streams. Likewise, localized anoxic patches can govern denitrification in benthic biofilms and sediment layers [*Pringle et al*., 1988]. Therefore, we foresee several opportunities improve the perceptual and numerical model in *TRANSFER*.

To overcome some of these existing limitations, we expect that the model can be coupled with other existing field-based parameter quantification methods as well as innovative and smart high-resolution data collection sensing systems. We expect that isotope injection studies could improve the parameterization of the model, especially for autotrophic and heterotrophic productive time periods in which one might run such studies. For example, stream augmentation via 15-N labeling allows quantification of biotic assimilation, denitrification as well as regeneration within streams [*Peterson et al*., 2001; *Mulholland et al*., 2008] and augmentation using 13-C labeling allows assessment of short-term and long-term algal fate [*Hotchkiss and Hall*, 2015]. Temporally varying parameterization of the model based on results from injection studies could perhaps provide stronger estimates and prediction potential for TRANSFER. In addition, high resolution sensing of nitrate [e.g., *Miller et al*., 2016], for example, could provide enriched calibration data that could allow for temporal variation of parameterization.

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