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Stabilization of Benthic Algal Biomass in a Temperate Stream Draining Agroecosystems

William I. Ford^{1*}, James F. Fox²

ABSTRACT

Results of the present study quantified carbon sequestration due to algal stabilization in low order streams, which has not been considered previously in carbon stream ecosystem studies. The authors used empirical mode decomposition of an 8-year carbon elemental and isotope dataset to quantify carbon accrual and fingerprint carbon derived from algal stabilization. The authors then applied a calibrated, process-based stream carbon model (*ISOFLOC*) that elicits further evidence of algal stabilization. Data and modeling results suggested that processes of shielding and burial during an extreme hydrologic event enhance algal stabilization. Given that previous studies assumed stream algae are turned over or sloughed downstream, the authors performed scenario simulations of the calibrated model in order to assess how changing environmental conditions might impact algae stabilization within the stream. Results from modeling scenarios showed an increase in algal stabilization as mean annual water temperature increases ranging from 0 to 0.04 tC km⁻² °C⁻¹ for the study watershed. The dependence of algal stabilization on temperature highlighted the importance of accounting for benthic fate of carbon in streams under projected warming scenarios. This finding contradicts the evolving paradigm that net efflux of CO₂ from streams increases with increasing temperatures. Results also quantified sloughed algae that is transported and potentially stabilized downstream and showed that benthos-derived sloughed algae was on the same order of magnitude, and at times greater, than phytoplankton within downstream water bodies.

KEYWORDS: algal stabilization, agroecosystems, fluvial carbon budget, watershed model

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1) INTRODUCTION

Highly disturbed agroecosystem streams demand scientific investigation because they have broad spatial extent, hold significant weight within the freshwater carbon budget, and partially govern downstream carbon cycling (Butman and Raymond, 2011; Griffiths et al., 2012; Ford and Fox, 2014a). Agroecosystem streams often starkly contrast forested and steep gradient systems because they have open canopies that promote high light availability to streambeds for benthic algae production, upland agriculture that produces high nutrient inputs, and low stream gradients that facilitate benthic storage of organic matter and bacterial biofilms (Rutherford et al., 2000; Griffiths et al., 2012; Ford and Fox, 2014a). In turn, low-gradient agroecosystem streams harbor autochthonous carbon within bed sediments that can cause streambeds to have a high amount of stored carbon, a wide carbon pool distribution, and a highly variable stream carbon flux across events seasonally and annually (Dalzell et al., 2005; Griffiths et al., 2012; Ford and Fox, 2014a,b; Ford et al., 2014; Fox et al., 2014). However, to our knowledge, no previous studies use long-term carbon datasets or numerical modeling to quantify C sequestration from algal stabilization in agroecosystem streams.

Algal stabilization is the coupled biotic-abiotic process by which algal biomass decomposes into more complex refractory carbon compounds in aquatic ecosystems for extended periods (Lara and Thomas, 1995; Leloup et al., 2013; Hotchkiss and Hall, 2015). At the watershed-scale, numerical models for fluvial carbon budgets broadly neglect contributions of algal stabilization to benthic carbon storage since the models treat benthic carbon as a 10 cm hydro-dynamically static anaerobic layer (DiToro, 2001; Wool et al., 2006; Chapra et al., 2008; Park and Clough, 2012). In part, scientists support this assumption with the argument that algal biomass has a net zero effect on fixation and respiration in fluvial carbon budgets due to its short

turnover lengths and a tendency for algal carbon to fuel ecosystem services (Minshall et al., 1983; Griffiths et al., 2012; Hotchkiss and Hall, 2015). However, as stated previously, few, if any studies, use long-term C records to investigate this assumption.

The effect of long-term temperature shifts and extreme hydrologic events on algal stabilization and C sequestration is of particular interest given that flow regime and water temperature are two critical drivers of algal carbon dynamics in disturbed agroecosystems (e.g., Rutherford et al., 2000; Flynn et al., 2013). Flow regime has the potential to impact algal stabilization through episodic sloughing and downstream export of benthic periphyton, fluvial erosion of stabilized algae in the benthos, and burial of stabilized algae due to depositional fluxes (Droppo and Stone, 1994; Fovet et al., 2010; Graba et al., 2013; Flynn et al., 2013; Ford and Fox, 2014a). Since temperature is the primary biotic variable controlling algal production and the microbial decomposition of coarse and stabilized algal pools, agroecosystems often have non-rate-limiting nutrient and light conditions (Rutherford et al., 2000; Griffiths et al., 2012; Ford and Fox, 2014). Recent studies suggest temperature gradient increases will promote decreases in net carbon sequestration by freshwater streams based on the argument that biotic respiration is more sensitive to temperature than autotrophic production (Yvon Durocher et al., 2010; Trimmer et al., 2012). However, these previous studies focused on short timescales and mesocosm-based experimentation due to the difficulties in obtaining long-term ambient datasets.

The objective of this study was to quantify C sequestration in agroecosystem streams as a result of algal stabilization. The authors apply an eight-year dataset of stream C elemental and isotope signatures to provide evidence of algae stabilization. Then, the authors apply a process-based stream C model to provide further evidence of algal stabilization and elucidate the physical and biological processes that govern algal stabilization. Next, the authors then use the process-

based model to simulate how changing temperature and extreme hydrologic conditions provide external control on algae stabilization. Finally, the numerical simulations allow the authors to discuss existing paradigms of temperature variability on stream CO₂ efflux and the impacts of algal sloughing to downstream ecosystems.

2) STUDY SYSTEM: AGROECOSYSTEM STREAM

The 62-km² South Elkhorn watershed (Figure 1) was chosen as a study site of algal stabilization because of the extensive knowledge of land use, flow, sediment and carbon dynamics in the system; the highly productive nature of the streambed; and the spatial and temporal variability of the dynamic benthos (Fox et al., 2010; Russo and Fox, 2012; Ford and Fox, 2014a; Ford and Fox, 2014b; Fox et al., 2014; Ford et al., 2014; Ford et al., 2015; Ford and Fox, 2015). The South Elkhorn watershed is located in the Bluegrass Region of Central Kentucky and is a mixed-use watershed that is impacted by agricultural and urban land use. During the decade-long time period of water quality data collection, the agricultural land use (57%) was primarily horse farms, while urban land use (43%) was primarily residential and commercial. Storm flows drove precipitation, and subsequently streamflow and produced 1150 mm/year of precipitation and an average stream flow rate of 1.2 m³s⁻¹. The study site had an average daily temperature of 13.1°C and experienced four distinct seasons. Silty clay loam soils in the watershed produced fine sediments ($d=20\mu\text{m}$, where d is the average particle diameter) from the watershed's landscape that were a significant component of the transported sediment load in the streams (Fox et al., 2014). During the study period, the South Elkhorn watershed had 53 perennial stream reaches: 27 first order, 13 second order and 13 third order.

3) METHODS

3.1 Empirical Mode Decomposition of the Carbon Time-Series:

The authors monitored carbon dynamics in the agroecosystem stream using an eight-year dataset of transported sediment carbon that was subsequently post-processed with empirical mode decomposition time-series analysis (Ford and Fox, 2014a; Ford et al., 2014; Ford et al., 2015). Briefly, the authors collected transported sediment samples from the outlet of the study watershed on a weekly basis (Figure 1) utilizing *in situ* sediment traps (Phillips et al., 2000). The authors processed all samples at the University of Kentucky Hydraulics Laboratory by decanting, centrifuging, freezing, and freeze-drying to remove remaining water. The bulk freeze-dried samples were sub-sampled depending on mass; wet-sieved to retain the fines fraction; ground and weighed into silver capsules; and acidified with 6% sulfurous acid to remove carbonate phases (Verardo et al., 1990; Ford and Fox, 2014a). Transported sediment samples were analyzed for elemental compositions of fine particulate organic carbon (C_{FPOC-T}) and its isotopic signature ($\delta^{13}C_{FPOC-T}$) by combusting samples at 980°C on a Costech 4010 Elemental Analyzer and then passing the gas stream through a Gas Chromatograph (GC) column (3 m HS-Q) to a Thermo Finnigan Delta-Plus XP Isotope Ratio Mass Spectrometer (IRMS).

The authors performed empirical mode decomposition of the eight-year sediment carbon dataset using a time-series analyses outlined in Ford et al. (2015). In the present study, the authors emphasize the long-term residual trends from empirical mode decomposition (EMD), which have not been discussed previously, in order to study long-term sequestration of carbon via algal stabilization. Empirical mode decomposition is a non-linear, non-stationary time-series analysis method that decomposes a dataset into a series of intrinsic mode functions, or IMFs (Haung et al., 1998; Wu et al., 2007). IMFs are a finite series of amplitude and frequency modulated, oscillatory functions in which the lowest frequency intrinsic mode function is the

base trend and the highest frequency trend is considered noise for well-sampled datasets (Wu et al., 2007). As discussed in Ford et al. (2015), the authors performed empirical mode decomposition using a six step iterative procedure in which the dataset $X(t)$ was represented as:

$$X(t) = \sum_{i=1}^n c_i + r_n$$

where, c_i were the IMFs, and r_n was the residual noise following the coarsest frequency trend. The coarsest frequency trend was the particular trend of interest for the current work.

3.2 Deterministic Modeling of Stream Carbon Dynamics:

The authors applied the Iso¹⁸tope-based Flu¹³vial Organic Carbon, or *ISOFLC*, numerical model to further investigate the prevalence of algal stabilization and its governing processes. *ISOFLC* is a process-based stream C model that simulates both terrestrial and autochthonous carbon fate and transport within the stream corridor. Additionally, *ISOFLC* simulates multiple dissolved and particulate carbon pools and their transformations. Ford and Fox's study (2015) details *ISOFLC*'s model formulation, sensitivity, and calibration procedure and provides a detailed list of inputs and parameters with justified ranges for application of *ISOFLC* specific to the South Elkhorn watershed. In the next paragraphs, the authors provide a brief synopsis of *ISOFLC*'s description and applicability to simulate algal stabilization.

ISOFLC includes water and sediment transport subroutines that provide the basis for advective transport simulation of dissolved carbon and particulate carbon pools within the agroecosystem stream. In the present study, *ISOFLC* used carbon mass-balance equations to simulate growth and turnover of periphyton. *ISOFLC* also used numerical simulation of both the surficial fine-grained laminae (SFGL) and dissolved inorganic carbon simultaneously to

estimate coupled feedbacks between the carbon pools. Inclusion of the multiple pools within *ISOFLOC* allowed continuous simulation of autochthonous generation as well as microbial associated decomposition of the heterogeneous organic carbon mixtures within the streambed storage deposits. *ISOFLOC* continuously accounted for physical and biogeochemical processes impacting each pool, thus allowing the authors to integrate processes after simulations were complete. The authors then investigated net processes that control stream ecosystem dynamics and algal stabilization at different timescales. *ISOFLOC* continuously simulated stable carbon isotope mass balances with Rayleigh fractionations for periphyton ($\delta^{13}C_{\text{Periphyton}}$), SFGL carbon ($\delta^{13}C_{\text{SFGL}}$), and dissolved inorganic carbon ($\delta^{13}C_{\text{DIC}}$) pools. *ISOFLOC* estimated elemental signatures of transported sediment carbon ($C_{\text{FPOC-T}}$) and isotopic signatures of transported sediment carbon ($\delta^{13}C_{\text{FPOC-T}}$) using mass balances that calculate the carbon weighted average of source contributions and their associated elemental (i.e., C_{SFGL} , C_{Upland} , and C_{Bank}) and isotopic signatures (i.e., $\delta^{13}C_{\text{SFGL}}$, $\delta^{13}C_{\text{Upland}}$, and $\delta^{13}C_{\text{Bank}}$). A total of eight potentially sensitive parameters were specified for the stable isotope sub model in *ISOFLOC* including three input parameters measured at the study site ($\delta^{13}C_{\text{DIC-IN}}$, $\delta^{13}C_{\text{Upland}}$, $\delta^{13}C_{\text{Bank}}$) and five literature parameters ($\delta^{13}C_{\text{Atmosphere}}$, $\epsilon_{\text{DEC-FPOC}}$, $\epsilon_{\text{DEC-APOC}}$, $\epsilon_{\text{Assimilation-Max}}$, $\epsilon_{\text{Evasion}}$). In turn, the authors calibrated and validated the *ISOFLOC* outputs against the aforementioned eight-year organic carbon dataset ($C_{\text{FPOC-T}}$ and $\delta^{13}C_{\text{FPOC-T}}$). We refer the reader to Ford and Fox (2015) for full description of the South Elkhorn model parameterization and uncertainty analysis.

The authors performed model uncertainty analysis of *ISOFLOC* in order to generate a posterior solution space that was highly robust in its estimates of the fluvial sediment organic carbon budget. The authors performed 30,000 *ISOFLOC* simulations driven by semi-random Sobol sequences of the model parameter inputs (Sobol, 2001; Saltelli et al., 2010). The authors

based acceptable model performance for the posterior solution space on widely-used statistical metrics in water quality modeling, which include the root mean square error of the standard deviation of measured data (RSR), Nash-Sutcliffe efficiency (NSE), and percent bias (PBIAS) (Moriassi et al., 2007). The authors included model configurations in the posterior solution space when the following conditions were met: $RSR < 0.8$ and $NSE > 0.4$ for calibration (2/3 of the data) and validation (1/3 of the data) of the sediment carbon content signatures (C_{FPOC-T}) and $RSR < 1$ and $NSE > 0$ for calibration and validation of the sediment carbon isotopic signatures ($\delta^{13}C_{FPOC-T}$). The authors enforced a PBIAS threshold of $\pm 20\%$ to ensure model simulations did not consistently over or underestimate measured data. The specified statistics ensured that the model outputs provided superior goodness-of-fit to a mean trend suggesting that each of the acceptable model simulations reflected the underlying processes. Table 1 compiles ranges for model parameters of the scenarios meeting these criteria. It should be noted that uncertainty for the fine particulate organic carbon pool and the associated algal stabilization dynamics were fairly narrow due to the sensitivity of the model response variables (C_{FPOC-T} and $\delta^{13}C_{FPOC-T}$) to those dynamics. For this reason, the authors felt confidence in the optimized scenario that provided $RSR < 0.8$, $NSE > 0.4$, and $PBIAS < 10\%$ for all calibration and validation datasets (see Table 1).

3.3 Model Analysis of Changing Temperatures and Hydrologic Events:

The authors questioned what physical and biological controls might impact net carbon sequestration via algal stabilization. The non-linearity of physical and biogeochemical processes within the water column and surficial fine-grained laminae of ecosystems streams obfuscate the drivers of the carbon sequestration using data alone (Ford and Fox, 2015). However, the authors had the advantage of using the validated *ISOFLOC* model to test external physical and biological controls via scenario analysis. Specifically, the authors investigated temperature variation and

hydrologic extremity over the data collection and simulation periods given the prevalence of temperature and extremes to control stream processes (Peterson, 1996; Rutherford et al., 2000; Davis et al., 2013; Ford et al., 2015).

As a precursor to *ISOFLOC* model scenario analyses of temperature variation and hydrologic extremity analyses, the authors performed pre-modeling analyses of existing temperature and water flowrate data. The authors downloaded temperature data from a NOAA weather station at the Lexington Bluegrass Airport located within the center of the study basin. The authors analyzed monthly average temperatures from 1964 to the present in order to minimize effects associated with boundary conditions for the analysis. An Empirical Mode Decomposition for the 50-year time-series revealed statistically significant seasonal and decadal IMFs assuming noise greater than two standard deviations as criteria for statistically significant trends (Figure 2). Statistical criteria provided a 95% confidence interval for the temperature results. The authors also performed a storm frequency analysis using precipitation data from the basin's gauging station. A storm event in September, 2006 produced peak hourly, bihourly, and daily rainfalls of 2.1, 3.9 and 6.2 inches, respectively, which fell within the 90% confidence interval for a 100 year return interval based on the NOAA precipitation frequency data server for the Lexington Bluegrass Airport. The quantitative hydrologic analyses for the 2006 event also agreed well with the previous research by Ford et al. (2014), which showed that the 2006 storm event induced SFGL dis-equilibrium with regard to sediment carbon quality, which is likely tightly linked to SFGL algae dynamics.

Based on pre-modeling data analyses that showed residual temperature variation and hydrologic extremity in 2006, the authors tested the potential importance of the physical (hydrologic) and biological (temperature) controls upon carbon sequestration via algal

225 stabilization using *ISOFLC*. The authors used *ISOFLC* to simulate the impact of the decadal
226 residual temperature trend on surficial fine-grained laminae carbon stock and, subsequently,
227 transported sediment carbon. The fluctuation in air temperature was augmented to account for
228 the relationship between air temperature and water temperature in the study region and was then
229 removed from the input time series utilized for model simulations. Additionally, the authors
230 propagated the de-trended temperature time series through the *ISOFLC* model. The authors ran
231 additional simulations with variable temperatures to further show how temperature conditions
232 might promote carbon sequestration through chronic increase and buildup of stabilized algae in
233 the surficial fine grained laminae. The authors simulated four scenarios with a 2 and 4°C upward
234 shift in water temperature and 2 and 4°C downward shift in water temperature. The authors
235 compared results from the scenarios ran to the calibrated scenario for baseline reference. Finally,
236 to test the importance of the hydrologic control, the authors removed the 2006 storm event from
237 the input flow dataset for model simulation in *ISOFLC* by assuming base flow conditions
238 throughout the duration of the event.

239 As an aside, the authors point out to the reader that they also investigated other potential
240 drivers of carbon sequestration for the studied stream, which were subsequently marginalized
241 with respect to their influence. With regard to light availability, the authors analyzed National
242 Renewable Energy Laboratory (NREL) data for the Bluegrass airport from 1990-2010 using an
243 Empirical Mode Decomposition (EMD) analysis. No significant trends were observed at a
244 decadal timescale and the long-term residual IMF was stationary. Similarly, EMD analysis of
245 flowrate showed no distinct long-term trends that would impact carbon dynamics (Ford et al.,
246 2015). Nutrient conditions for the study site were reported elsewhere (Ford et al., 2015) and
247 showed that levels of rate-limiting macronutrients (e.g., phosphates and nitrates) were above

minimum thresholds for rate-limiting nutrient conditions (Dodds, 2007). Finally, land-use remained fairly constant in the watershed during the study time period aside from some urbanization at the upstream site of the main-stem in early 2006.

4) RESULTS

4.1 Empirical Mode Decomposition of the Carbon Time-Series:

Long-term residual trends for transported sediment carbon (C_{FPOC-T}) and transported sediment carbon isotope signatures ($\delta^{13}C_{FPOC-T}$) were non-stationary over the eight-year data collection period based on the Empirical Mode Decomposition (EMD) results (Figure 3). With regard to transported sediment carbon, results of the EMD analysis showed a net increase from a minimum transported sediment carbon (C_{FPOC-T}) value of 2.4% in 2007 to a maximum of 3.9% in 2012, resulting in a 65% enrichment in transported sediment carbon (C_{FPOC-T}) at the watershed outlet (Figure 3a). With regard to the sediment carbon isotope data, results of the EMD analysis showed a net decrease from a maximum transported sediment carbon isotope signature ($\delta^{13}C_{FPOC-T}$) of -26.3‰ in 2007 to a minimum of -27.3‰ at the end of the monitoring period, resulting in a 1‰ decrease in transported sediment carbon isotope signature ($\delta^{13}C_{FPOC-T}$) at the watershed outlet (Figure 3b).

4.2 Deterministic Modeling of Stream Carbon Dynamics:

Long-term carbon dynamics simulated in *ISOFLOC* for the South Elkhorn Watershed were consistent with non-stationary trends observed in the data driven EMD analysis. With regard to the carbon mass-balance model, *ISOFLOC* results showed an average annual transported sediment carbon (C_{FPOC-T}) value of $2.6 \pm 0.4\%$ in 2007 that increased to $3.4 \pm 0.4\%$ in 2012, resulting in a 32% enrichment in transported sediment carbon (C_{FPOC-T}) at the watershed

outlet (Figure 3a). With regard to the stable isotope model, *ISOFLC* results showed an average annual transported sediment carbon isotope signature ($\delta^{13}C_{FPOC-T}$) of $-25.7 \pm 0.2\text{‰}$ in 2007 that decreased to $-27.3 \pm 0.3\text{‰}$ in 2012, resulting in a 1.5‰ decrease in the transported sediment carbon isotope signature ($\delta^{13}C_{FPOC-T}$) at the watershed outlet (Figure 3b).

Seasonal and annual *ISOFLC* model carbon budget results, including fluxes and benthic carbon storage within periphyton and SFGL pools, quantified carbon fate and transport for the study period (see Table 2). The calibrated *ISOFLC* model showed an advective flux of $0.98 \text{ tC km}^{-2} \text{ yr}^{-1}$ for sloughed periphyton and erosion of stabilized SFGL algae equaling $0.088 \text{ tC km}^{-2} \text{ yr}^{-1}$. Of particular interest was the modelling result that $0.013 \text{ tC km}^{-2} \text{ yr}^{-1}$ of stabilized algae accrued on average in the SFGL each year. Seasonally, stabilized algae storage was highest during spring and summer when periphyton sloughing was low. SFGL stabilized algal carbon respiration outweighs stabilization of periphyton to the SFGL in fall and winter when standing stocks of periphyton became temperature limited. Annually, stabilized algal carbon storage in the SFGL was lowest in years when both periphyton sloughing and stabilized algal SFGL flux via erosion was high (i.e., 2009, 2011 and 2013). In 2006 when stabilized algae in the SFGL was low, and thus stabilized algal SFGL flux via erosion was low, net storage was above average because inputs of algal stabilization exceeded fluxes of erosion and decomposition of existing SFGL stabilized algae.

The authors integrated *ISOFLC* results and tabulated (see Table 3) annual SFGL algal carbon production and fluxes in order to assess dominant processes impacting carbon storage. *ISOFLC* results suggest that SFGL stabilized algal carbon was produced at a rate $0.19 \pm 0.02 \text{ tC km}^{-2} \text{ yr}^{-1}$ by decomposition of periphyton. Stabilized algal carbon in the SFGL decomposed at a rate of $0.086 \pm 0.013 \text{ tC km}^{-2} \text{ yr}^{-1}$ and eroded at a rate of $0.088 \pm 0.033 \text{ tC km}^{-2} \text{ yr}^{-1}$ resulting in the

aforementioned stabilized algal net storage of $0.013 \text{ tC km}^{-2} \text{ yr}^{-1}$. *ISOFLC* results suggest that physical erosion dynamics of SFGL stabilized algae have a broader range of variability (0.05 to $0.13 \text{ tC km}^{-2} \text{ yr}^{-1}$) than biological decomposition dynamics (0.06 to $0.10 \text{ tC km}^{-2} \text{ yr}^{-1}$).

4.3 Model Analysis of Changing Temperatures and Hydrologic Events:

In order to visually (see Fig 4) and quantitatively highlight the impact of algal stabilization on benthic carbon storage and fluxes, the authors ran comparative simulations with *ISOFLC* under the calibrated scenario and a scenario where algal dynamics were turned off (i.e., $P_{Max}=0$). As seen in Figure 4a, initial differences between the calibrated and no algae scenario were a result of the warmup period for the model (2005-2006) that allowed the algal carbon to build up in the SFGL. Initially, the difference in benthic carbon stock was 11 tC ; however, by the end of the model scenario the difference was nearly doubled providing visual evidence of the budget findings in Table 2. The resultant impact on the transported sediment carbon content (C_{FPOC-T}) is shown in Figure 4b. For the no algae scenario, it was visually evident that transported sediment carbon content (C_{FPOC-T}) oscillated about $2 \text{ gC}/100\text{gsed}$, while transported sediment carbon content (C_{FPOC-T}) for the calibrated scenario steadily rose to approximately $3.2 \text{ gC}/100\text{gsed}$ by the end of the eight-year simulation.

As mentioned in the methods section, the authors next performed a set of scenario analyses with *ISOFLC* to investigate if physical or temperature controls had a greater impact on stabilized algal carbon (Figure 5). First, the authors simulated the variation of the water temperature control by using a detrended temperature time-series (Figure 2). *ISOFLC* simulated results for benthic carbon stock, transported sediment carbon content (C_{FPOC-T}), and transported sediment carbon isotope signature ($\delta^{13}C_{FPOC-T}$) utilizing the detrended temperature

time-series as a model input. Removal of the temperature trend produced a simulated decrease of 1 tC in the benthos or a 6.5% decrease in the storage capacity relative to the baseline calibration simulation. As a result, the transported sediment carbon (C_{FPOC-T}) at the watershed outlet showed a residual decrease in carbon content (Figure 5b) and an increase in isotopic signature (Figure 5c) due to a larger proportion of upland soil organic carbon in the benthos.

Second, the authors considered hydrologic control through removal of the September 2006 event (Figure 5d-f). *ISOFLOC* results showed seasonal fluctuations of benthic FPOC, consistent with the baseline model run. However, the benthic stock gradually decreased resulting in a net loss—rather than accrual—of stabilized algal carbon to the SFGL (Figure 5d). *ISOFLOC* results suggest that transported sediment carbon content (C_{FPOC-T}) and transported sediment carbon isotope signature ($\delta^{13}C_{FPOC-T}$) were relatively static over long timescales due to the loss of stabilized algae (see Figure 5e-f).

Finally, the authors performed another set of scenarios with *ISOFLOC* to investigate to what extent more pronounced temperature shifts might have impacted stabilized algal accrual. The increasing shifts in simulated water temperature resulted in a net increase in storage relative to the calibrated scenario. A downward shift in simulated water temperature resulted in a net decrease in storage relative to the baseline calibration scenario (see Figure 6). Similarly, when comparing the downward shifts in simulated water temperature, the 2°C shift had a smaller decrease in net storage relative to the 4°C shift. Somewhat unexpectedly, the 2°C scenario produced a higher net storage of stabilized algal carbon compared to the 4°C shift. Furthermore, differences between stabilized algal carbon storage for the decreasing temperature scenarios were much greater than net differences between increasing temperature scenarios.

5) DISCUSSION

5.1 Algal Stabilization in Streams Draining Agro-ecosystems:

Results of the study provide the first watershed-scale evidence of algal stabilization to produce long-term net sequestration of benthic algal biomass in streams draining agro-ecosystems. Both the data-driven results from empirical mode decomposition and the process-based numerical modeling results from *ISOFLOC* showed a net increase in carbon from early years (2006-2008) to later years (2010-2013) over the eight-year period transported sediment carbon (C_{FPOC-T}) was studied (Figure 3a). The data and *ISOFLOC* results suggest a gradual increase in the carbon source that delivered sediment carbon to the water column. The net increase in fluvial carbon was not explained by annual benthic functioning controlled by seasonal autochthonous growth followed by heterotrophic turnover that would favor a multi-year equilibrium carbon signal (Ford and Fox, 2015). Nor was the net fluvial carbon increase attributed to a shift in allochthonous carbon provenance because external carbon sources, including agricultural surface soils and bank sediments, have relatively low carbon density ($\sim 2 \text{ gC } 100\text{gsoil}^{-1}$) in this basin (Ford et al., 2015). Rather, carbon isotope results reflect an accrual of stabilized algal-originated carbon in the benthos, which was evident from the net decrease in the sediment carbon isotope signature ($\delta^{13}C_{FPOC-T}$) from early years (2006-2008) to later years (2010-2013) for the eight year transported sediment carbon isotope dataset (Figure 3b). The algal stabilization process is further supported based on literature comparison, in which the long-term isotopic trend (Figure 3b) suggests a shift from terrestrial soil organic carbon (-25 to -27‰) to a mixture of terrestrial soil organic carbon and algal biomass (-30 to -35‰) (Kendall et al., 2001; Jacinthe et al., 2009; Ford et al., 2015). Interestingly, the accumulation of stabilized algal carbon within the benthic sediment carbon pool known as the surficial fine grained laminae

(SFGL) explains the downward shift in the transported sediment carbon isotope signature ($\delta^{13}C_{FPOC-T}$). As was highlighted in the results, carbon sequestration via algal stabilization occurs in the South Elkhorn watershed because annual input of algal carbon byproducts to the streambed outweighed losses from particulate carbon erosion and microbial respiration (Tables 2-3). In summary, multiple lines of evidence point towards the prevalence of algal stabilization with the agroecosystem stream over the study period.

Our deterministic modeling and time-series findings of net benthic carbon sequestration due to algal stabilization is partially supported by recent studies on contemporary fluvial carbon cycling and laboratory-scale investigations of algal stabilization (Lara and Thomas, 1995; Regnier et al., 2013; Hotchkiss and Hall, 2015). Lara and Thomas (1995) used radio-labeled carbon on batch cultures of an oceanic diatom to quantify stabilization dynamics and found approximately 5% of the maximum carbon biomass was stabilized to recalcitrant humic substances in the first month. Regnier et al. (2013) utilized a synthesis of published studies focusing on the impact of anthropogenic disturbance to alter sequestration and fluxes of fluvial organic carbon and showed that autochthonous C from inland waters is likely stored or exported from inland freshwater systems at a rate of ~0.3 PgC/year. Hotchkiss and Hall (2015) utilized a carbon isotope tracer-based experiment to show that autochthonous C in a low order stream has two distinct fates: either short-term exudation and respiration or longer-term storage and downstream export. Despite the previous evidence of algal stabilization, no studies to our knowledge have included this effort in watershed-scale fluvial organic carbon budgeting; moreover, numerical models broadly neglect the fate of stabilized algae in the SFGL (Ford and Fox, 2015). Findings from the present study suggest the potential importance of algal stabilization to the fluvial carbon budget. Furthermore, the authors suggest numerical models,

such as *ISOFLUC*, are effective tools for in-stream fluvial carbon budgeting including quantification of algal carbon fate and transport.

5.2 Process and External Factors controlling Algal Stabilization:

Results of the *ISOFLUC* scenario analysis for the South Elkhorn watershed provided insight into the role of biological and physical processes and how they impacted carbon sequestration due to algal stabilization at an annual timescale in streams draining agroecosystems. The small standard deviation of stabilized algae decomposition (Table 3) reflected the small deviation in temperature gradients from year to year, while the large standard deviation for stabilized algal erosion reflected two primary mechanisms. First, stabilized algal erosion can be shear limited and is thus directly impacted by the flow regime with higher flows having higher capacity to induce incipient motion. For instance, years with high annual precipitation (e.g., 2009) had highly stabilized algal erosion fluxes (e.g., $0.121 \text{ tC km}^{-2} \text{ yr}^{-1}$) whereas years with low precipitation (e.g., 2007) had substantially smaller algal FPOC fluxes (e.g., $0.054 \text{ tC km}^{-2} \text{ yr}^{-1}$). Second, shielding of algal biomass due to large depositional fluxes during high magnitude storm events (i.e., the 100-year precipitation event in 2006) minimized stabilized algal erosion. *ISOFLUC* simulations showed low stabilized algae erosion during 2006, despite having high annual rainfall, which was a result of the large depositional flux during the September event. As a result, the stabilized algae was shielded from fluvial erosion because it was well mixed within a larger SFGL layer. Thus, the authors suggest that physical-hydrologic controls govern the variability of watershed source-sink capacity of carbon via algal stabilization at an annual scale. The yearly scale is particularly notable given that stream ecosystem and carbon transport studies often only consider seasonal and event variations of biologic and physical processes.

Following from the controlling processes, at least two external factors controlled algal stabilization in the stream and its maximum capacity, including high magnitude stochastic storm events and subtler deterministic temperature shifts. As seen in Figure 5, removal of the subtle temperature trend shown in Figure 2 resulted in less favorable conditions for algal stabilization and thus a net decrease in SFGL C storage. For a residual temperature increase, both algae and heterotrophic bacteria populations increased up to maximum saturation levels, e.g., population limitations (White et al, 1991; Rutherford et al., 2000). Therefore, a residual temperature increase promoted favorable conditions over longer timeframes for bacterial and algal populations resulting in population maximums. Hence, when conditions for algal sequestration occur for longer periods of the year, a chronic increase and buildup of stabilized algae in the SFGL can occur. However, we note that this will approach a maximum due to a higher rate of SFGL algal decomposition as compared to inputs from coarse algae as evidenced by the 2-4°C increase scenario in Figure 6. With regard to high magnitude, stochastic events, heavy depositional fluxes provide a supply of terrestrial fine particulate organic carbon (Russo and Fox, 2012; Ford et al., 2014). Results in Figure 5d-f showed that removal of the 100-year precipitation event resulted in a long-term residual decrease in stabilized algal carbon storage in addition to a fairly stable annual transported sediment carbon content (C_{FPOC-T}) and transported sediment carbon isotope signature ($\delta^{13}C_{FPOC-T}$) when compared with the baseline (calibrated) condition. Collectively, findings from this study highlight the ability of external factors to control maximum algal stabilization in the SFGL in which stochastic storm events governed how far the SFGL deviates from maximum conditions (i.e., net zero carbon storage due to algal stabilization), and long-term residual temperature changes provided a migrating maximum capacity target.

5.3 Broader Implications:

5.3.1 Sensitivity to Temperature Shifts

The findings of increased algal stabilization, when subjected to long-term temperature increases, contradict the emerging paradigm for response of stream ecosystem C dynamics to temperature changes. Broad-scale fluvial carbon research has shown the efficacy of fluvial systems to degass CO₂ (Cole et al., 2007; Butman and Raymond, 2011). For example, US rivers are supersaturated with carbon dioxide when compared with the atmosphere, emitting 97±32 Tg carbon each year (Butman and Raymond, 2011). Following with this assumption, recent results suggest that in the future, increasing temperature gradients will result in decreases of net ecosystem productivity by 13% since respiration by biota is suggested to be more sensitive to temperature than autotrophic production (Yvon Durocher et al., 2010). Conventional wisdom tends to neglect the ability of surface fine grained laminae to trap newly generated carbon within benthic sediments since studies typically assume fresh autochthonous carbon has short turnover lengths (Griffiths et al., 2012). Contradictory to conventional wisdom, results in Figure 6 suggested that the SFGL would sequester between 0 and 0.04 tC km⁻² °C⁻¹ when subjected to a change in MAT. The range was due to the non-linearity of the benthic SFGL dynamics in which high temperature scenarios were approaching maximum sequestration capacity, and lower MAT subjected to temperature change was further from max capacity and hence stabilized algae at a higher rate.

The increased sequestration rate per °C temperature increase has significant implications for C budgets under potential warming scenarios projected by RCP simulations, which conservatively estimate a global 2°C temperature shift with a high of 4°C by 2100. While studies have traditionally assumed a net zero effect of algal fixation due to high rates of turnover, recent

estimates suggest ~0.3 Pg autochthonous C/year is either buried or exported from inland waters (Cole et al., 2007; Regnier et al., 2013; Hotchkiss and Hall, 2015). To place our results in a broader context, we extrapolated our findings globally assuming that agricultural systems have non-rate limiting nutrient conditions, open canopies, and have comparable stream and hillslope gradients, resulting in prominence of the SFGL layer. Assuming that there are approximately 60 million km² of agricultural land, we find that changes in sequestration with respect to increases in temperature could range from zero Pg/°C for tropical climates where maximum stabilization capacity of algae has been met or exceeded to upwards of 0.003 PgC/°C for cooler climatic regions that currently have low algal stabilization. Thus, under a 4°C shift we could see the balance of autochthonous C storage and burial dynamics increase by upwards of 4% as opposed to the decrease in NEP of 13% that was observed in Trimmer et al. (2012). The finding highlights the need for continued study of these disturbed systems in terms of in-stream C fate and transport.

5.3.2 Algal Sloughing to Downstream Ecosystems:

While our findings to this point have focused on the role of stabilization for sequestering sediment carbon at the small watershed scale (e.g., 100km²), these findings were conservative for total sequestration because the fate of the sloughed algal pool in receiving waterbodies had not been explicitly accounted for. Specifically, we were interested in how periphyton sloughing impacted the net annual storage of carbon due to algal stabilization and to assess the resultant implications for downstream waterbodies (Figure 7). In general, we found that algal storage and sloughing were negatively correlated ($R^2=0.6$) with years of high sloughing (2006, 2009, 2011, and 2013) generally resulting in a net C storage near or below zero. Conversely, in years with low sloughing (2007, 2008, 2010, and 2012), net C storage reached upwards of 0.1 tC km⁻²yr⁻¹

which equated to approximately 15% of the sloughing flux in low sloughing years. This finding suggested significant potential for sloughed periphyton stabilization to sequester carbon in downstream reaches.

The significance of algal POC fluxes from small streams to downstream rivers and reservoirs has received little attention in the scientific literature but has significant implications not only for carbon sequestration, but also nutrient release and oxygen uptake during turnover. As a first attempt at quantifying the respective downstream fluxes, we upscaled our results and compared periphyton produced in small streams to phytoplankton produced in the Kentucky River, which is a series of slack water pools similar to other large rivers in the Midwest USA. To compare the drainage area normalized sloughed periphyton fluxes to the fluxes of phytoplankton, we utilized measurements of phytoplankton cell concentrations in the HUC 6 Kentucky river shown in Figure 1 (Stevenson and White, 1995), converted those concentrations to organic C equivalents (Menden-Deurer and Lassard, 2000), and estimated drainage area normalized fluxes to downstream waterbodies utilizing a USGS gauging station near the confluence of the Kentucky and Ohio rivers (USGS 03290500: Kentucky River at Lock 2, Lockport, KY). The comparisons in Figure 7b suggested that sloughed algal carbon from the small agroecosystem streams are on the same order of magnitude, and in some years greater, than *in situ* phytoplankton production on the Kentucky River. Despite the lack of study of stabilization dynamics for periphyton that settles out in downstream waterbodies, the process' significance is partially supported by large riverine studies where the deposition of labile organic matter has been suggested to promote heterotrophic induced oxygen depletion (Ohte et al., 2007; Stringfellow et al., 2009). While Ohte's and Stringfellow's studies focused on phytoplankton turnover, their findings highlight the sensitivity of receiving water bodies to upstream algal

dynamics. As such, future studies should consider implications of the periphyton stabilization process. Future riverine models quantifying fluvial carbon budgets across scales need to consider stabilization rates of both periphyton and phytoplankton algal pools jointly to better constrain broad-scale estimates of inland autochthonous burial and transport.

Further, future studies that utilize *ISOFLoc* to determine downstream sloughing of algal biomass should consider alternative mechanisms that could potentially impact benthic periphyton stock and the fluvial carbon cycle. Of specific interest to periphyton sloughing is the C flux associated with periphyton grazing by macroinvertebrates. Grazing has been highlighted as a mechanism that can exert top-down control on periphyton, especially during mild temperature, low biomass conditions (Rutherford et al., 2000). Since *ISOFLoc* does not explicitly consider the impact of macroinvertebrate grazers on periphyton standing stocks, we find our estimates to likely represent a high end of sloughing. However, for many streams draining agroecosystems, we expect this impact to be low since the majority of sloughing occurs during high biomass and high temperatures when grazers have been found to have minimal effects on the standing stock of periphyton (Rutherford et al., 2000).

CONCLUSION

Findings from the South Elkhorn application of *ISOFLoc* and empirical mode decomposition (EMD) of the eight-year dataset provided significant evidence of algal stabilization to produce net sequestration of organic carbon in the surficial fine-grained laminae. Based on *ISOFLoc* modelling results, the magnitude of stabilization is likely governed by erosion-deposition dynamics and, to a lesser degree, temperature gradients. *ISOFLoc* scenario

analysis of hydrologic controls suggested that significant deposition events had a shielding effect, thereby minimizing the flux of stabilized algal carbon to downstream reaches. Scenario analysis of biotic controls implied that increasing temperature gradients resulted in increases in algal stabilization, which contradicts existing paradigms that point to decreases in net ecosystem productivity due to microbial respiration. The authors finding of upwards of 15% of sloughed periphyton to be stored during high residence time has significant implications for downstream stabilization of algal biomass in large rivers and reservoirs. In conclusion, the authors suggest algal stabilization has potentially significant implications for carbon sequestration in large-scale carbon budgets and tools such as *ISOFLOC* can be used to successfully quantify this stabilization.

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Figure 1) The figure displays (a) the South Elkhorn study site and ISOFLC model domain located in Kentucky, USA within the Kentucky River Basin, (b) microscopy analysis of transported sediment aggregates at the watershed outlet highlighting the prominence of autotrophic organisms in transport, and (c) study site image of periphytic algal mats commonly observed in the benthos of the South Elkhorn.

Figure 2. EMD Analysis of 40 year Time-series of monthly temperature at the Bluegrass Regional Airport in Lexington, KY. Chart includes raw timeseries, statistically significant seasonal IMFs and statistically significant decadal IMFs. Long-term residual trend is relatively stationary.

Figure 3. Time-series of monthly averaged data ± 1 S.D., calibrated model output, and the residual IMFs from the empirical mode decomposition for a) transported sediment carbon content (C_{FPOC-T}) and b) stable carbon isotopic signature of transported sediment carbon ($\delta^{13}C_{FPOC-T}$).

Figure 4) (a) Benthic carbon stock and (b) transported C_{FPOC-T} for the optimized model scenario and a secondary simulation which set maximum algal biomass (P_{Max}) to zero (i.e., no algae simulations).

Figure 5. ISOFLC parameterization for the optimized model run (e.g., NSE>0.4, RSR<0.8, PBIAS <10%) for both calibration and validation was utilized to run a suite of tests to tease out causes for long-term increases in SFGL carbon. Results are included for the response of SFGL carbon stock (a,d) and the response variable (C_{FPOC-T}) (b,e) and the stable carbon isotope response variable transported sediment carbon isotope signature ($\delta^{13}C_{FPOC-T}$) (c,f). Further, results are included for two suggested drivers of long-term variability of long-term residual temperature increases (a-c) and extreme events (d-f).

Figure 6. Benthic carbon accrual in response to shifts in mean annual temperature assuming a 2 and 4 degree increase (decrease) in temperature. The analysis eludes to the potential sensitivity of processes subjected to different climatic conditions and subjected to shifts in mean annual temperatures for the local watershed (assuming other calibrated parameters hold).

Figure 7) (a) Sloughing of periphyton vs. storage of algal carbon for optimum model scenario and (b) comparison of sloughed periphyton vs. phytoplankton in the Kentucky River utilizing results from *ISOFLC*

726 Table 1) Model parameterization table with parameter identifiers, parameter descriptions, calibrated values, and uncertainty ranges
727 meeting statistical thresholds. The optimized calibration parameterization used for the results of this study (RSR<0.8, NSE > 0.4, and
728 PBIAS<10% for all calibration/validation scenarios) is highlighted in bold.

<i>Parameter ID</i>	<i>Parameter Description</i>	<i>Calibrated Value</i>	<i>Uncertainty Range</i>	<i>Units</i>
τ_{cr}^{algae}	Algal critical shear stress	1.12	0.76 - 1.63	Pa
P_{col}	Algal colonization rate	6.73E-05	1.49E-05 – 6.73E-05	kgC m ⁻² d ⁻¹
P_{Max}	Max algae fixation rate	2.34E-03	5.25E-04 – 3.12E-03	kgC m ⁻² d ⁻¹
DEC_{APOC}	Decomposition of coarse to fine algal carbon	3.1	2.40 - 3.34	d ⁻¹
$DEC_{FPOC-Algae}$	Decomposition of fine to dissolved algal carbon	3.44	3.26 - 3.77	d ⁻¹
d_{SFGL}	Depth of the SFGL	9.74E-03	5.44E-03 – 9.98E-03	m
C_{Upland}	Carbon content of hillslope sediments	2.68E-02	0.023 - 0.031	gC gSed ⁻¹
C_{Bank}	Carbon content of bank sediments	1.77E-02	0.010 – 0.019	gC gSed ⁻¹
C_{DIC-IN}	Concentration of dissolved inorganic carbon in tributaries	8.21E-02	0.029 – 0.084	mgC L ⁻¹
$\delta^{13}C_{DIC-IN}$	Isotopic signature of inflowing dissolved inorganic carbon	-14.7	-14.7 – -10.7	‰
$\delta^{13}C_{Upland}$	Isotopic signature of hillslope sediment carbon	-27	-27.3 – -26.1	‰
$\delta^{13}C_{Bank}$	Isotopic signature of bank sediment carbon	-24.1	-25.7 – -23.9	‰
$\delta^{13}C_{Atmosphere}$	Isotopic signature of atmospheric CO ₂	-7.09	7.11 – 8.54	‰
$\epsilon_{DEC-FPOC}$	Decomposition of fine sediment carbon isotope fractionation	0.95	0.01 – 1.99	‰
$\epsilon_{DEC-APOC}$	Fractionation associated with decomposing algae	1.73	0.08- 1.76	‰
$\epsilon_{Assimilation-Max}$	Maximum fractionation associated with algal carbon fixation	19	16.5 – 23.8	‰
$\epsilon_{Evasion}$	Fractionation associated with stream channel evasion to the atmosphere	1.03	0.03 – 1.90	‰
pH	Streamwater pH	7.4	7.38 – 8.36	-----
w_{CO2}	Gas transfer velocity of CO ₂	3.84E-05	3.75E-05 – 6.87E-05	m s ⁻¹
Pre	Precipitation of particulate inorganic carbon	3.31E-08	8.7E-09 – 6.02E-08	kgC m ⁻² s ⁻¹
Dis	Dissolution of particulate inorganic carbon	1.18E-08	1.3E-09 – 1.54E-08	kgC m ⁻² s ⁻¹
P_{resp}	Respiration rate of the algal mat	0.12	0.04 - 0.15	d ⁻¹

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Table 2) Fluvial organic carbon budget results for the eight year ISOFLOC simulation in the South Elkhorn Watershed. Fluxes and storage terms are normalized by watershed area. Values are provided for the optimum scenario.

Net Humified Algal Carbon Storage (tC km⁻²)					
	Winter	Spring	Summer	Fall	Annual
2006	-0.033	0.038	0.039	-0.021	0.023
2007	-0.013	0.049	0.066	-0.022	0.080
2008	-0.056	0.023	0.069	-0.005	0.032
2009	-0.034	0.005	0.028	-0.046	-0.046
2010	-0.021	0.025	0.061	0.000	0.065
2011	-0.047	-0.020	0.057	-0.036	-0.045
2012	-0.031	0.046	0.044	-0.018	0.042
2013	-0.046	0.000	0.007	-0.003	-0.043
Average	-0.035	0.021	0.046	-0.019	0.013
Humified Algal Carbon Flux (tC km⁻²)					
	Winter	Spring	Summer	Fall	Annual
2006	0.029	0.013	0.030	0.019	0.091
2007	0.011	0.007	0.007	0.029	0.054
2008	0.043	0.015	0.003	0.012	0.073
2009	0.024	0.029	0.030	0.038	0.121
2010	0.014	0.019	0.008	0.006	0.047
2011	0.034	0.045	0.010	0.035	0.125
2012	0.025	0.004	0.018	0.020	0.067
2013	0.033	0.034	0.047	0.014	0.129
Average	0.027	0.021	0.019	0.022	0.088
Periphyton Carbon Flux (tC km⁻²)					
	Winter	Spring	Summer	Fall	Annual
2006	0.294	0.185	0.548	0.365	1.393
2007	0.173	0.142	0.071	0.253	0.639
2008	0.174	0.117	0.018	0.085	0.395
2009	0.126	0.496	0.576	0.464	1.197
2010	0.038	0.446	0.101	0.058	0.644
2011	0.128	0.467	0.098	0.380	1.073
2012	0.232	0.024	0.093	0.175	0.524
2013	0.217	0.485	0.672	0.153	1.526
Average	0.173	0.295	0.272	0.242	0.982

Table 3) ISOFLOC simulation of algal FPOC dynamics in the South Elkhorn watershed including fluxes into (periphyton decomposition) and out (algal SFGL decomposition and erosion) of the SFGL. Results are provided for the calibrated scenario.

Year	Periphyton C Decomposition (tC km⁻²)	Humified Algal C Decomposition (tC km⁻²)	Humified Algal C Erosion (tC km⁻²)	Net Humified C Storage (tC km⁻²)
2006	0.172	0.058	0.091	0.023
2007	0.215	0.082	0.054	0.080
2008	0.191	0.086	0.073	0.032
2009	0.164	0.090	0.121	-0.046
2010	0.205	0.093	0.047	0.065
2011	0.173	0.094	0.125	-0.045
2012	0.210	0.102	0.067	0.041
2013	0.170	0.083	0.129	-0.042
Total	1.500	0.687	0.706	0.107
Average	0.188	0.086	0.088	0.013
Std Dev	0.020	0.013	0.033	0.051