Linking carbon and nitrogen spiraling in streams

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Abstract: Anthropogenic activities have altered biogeochemical cycles and the fate of organic carbon (OC) and nutrients in freshwater ecosystems. To investigate coupled OC and nutrient cycling and fate in streams, we compared the spiraling lengths (S) and uptake velocities (v_f) of OC and nitrate (NO_3^-) in headwater streams ($n = 72$) across different land uses (i.e., agricultural, urban, natively vegetated) and regions ($n = 8$) in the United States. We did these comparisons with data collected for the second Lotic Intersite Nitrogen eXperiment (LINX II; Mulholland et al. 2009). OC spiraling lengths (S_{OC}) in reference (21–4180 m) were shorter than in agricultural streams (89–37,156 m) and urban streams (104–12,605 m). OC mineralization velocities (v_{f-OC}) and NO_3^- uptake velocities (v_{f-NO3}) were weakly positively correlated across all sites ($r = 0.33$, $p = 0.008$). The strongest correlations between OC mineralization and NO_3^- uptake were in streams with gross primary production (GPP) similar to ecosystem respiration (ER) and human-altered streams (all agricultural streams: $r = 0.56$, $p = 0.008$, GPP:ER = 0.6; all urban streams: $r = 0.73$, $p < 0.001$, GPP:ER = 0.5). Additionally, the distances traveled by OC and NO_3^- before they were permanently removed from the stream by mineralization (S_{OC}) or denitrification ($S_{w-\text{den}}$) were similar in magnitude, although S_{OC} was shorter than $S_{w-\text{den}}$ in most streams. This study demonstrates how OC and NO_3^- spiraling can be used to investigate how region and land use influence coupled OC-NO_3^- interactions and fate.
Key words: coupled biogeochemical cycles, organic carbon, nitrate, spiraling, uptake velocity, metabolism, LINX II, land use, region

Carbon (C) and nutrients such as nitrogen (N) are the energetic currency of ecosystems. The supply of C and other nutrients fuels ecosystem function (e.g., C metabolism and nutrient retention), shapes biotic communities and food webs, and controls downstream water quality. In recent decades, biogeochemical cycles have been altered by anthropogenic activity, especially land-use changes and nutrient loading to freshwaters (Mulholland et al. 2008). Consequently, a deeper understanding of the connection between C and nutrient cycles is needed to better assess environmental change, ecosystem function, and material fate in streams.

Energy flow and organic carbon (OC) cycling in streams is controlled by ecosystem metabolism, which is the balance between gross primary production (GPP) and respiration of autotrophic and heterotrophic organisms (ecosystem respiration, ER) (Odum 1956). OC is the primary building block for biomass in organisms and is also a byproduct of many metabolic processes. Estimates of stream metabolism and OC flux allow us to assess assimilation and mineralization of OC relative to downstream transport (i.e., OC spiraling; Newbold et al. 1982, Hall et al. 2016). Ecosystem metabolism is linked to hydrologic transport and nutrient cycling along a given stream reach (Hall and Tank 2003), and OC spiraling estimates allow C fluxes and fate to be compared both across reaches (Newbold et al. 1982, Griffiths et al. 2012, Hall et al. 2016) and with the fluxes and fate of other bioreactive solutes such as nitrate (NO_3^-), ammonium (NH_4^+), and phosphate (PO_4^{3-}) (Johnson and Tank 2009, Hall et al. 2013). OC spiraling can be used for cross-site comparisons of C fate and to assess coupled C-nutrient interactions, but these comparisons are rarely done. Moreover, C fate differs by region and degree of human disturbance, but we still lack a comprehensive understanding of how diverse environmental drivers, such as nutrient loading and land-use change, influence OC cycling in running waters (Wohl et al. 2017).

Nutrient availability can control ecosystem functions in streams and rivers. Nutrients such as inorganic N can limit

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autotrophic and heterotrophic activity and influence energy flow (Tank et al. 2018). During periods of high biological activity, headwater streams can store or transform up to ½ of the inorganic N inputs from the surrounding catchment (Peterson et al. 2001). However, changes in land use and anthropogenic N loading, particularly of NO_3^-, have led to greater N export from surface waters and eutrophication in downstream ecosystems (Mulholland et al. 2008). Land use can indirectly affect biotic NO_3^- removal and transformation through changes in light availability, NO_3^- concentration, and GPP (Mulholland et al. 2008, Hall et al. 2009). However, excess nutrient loading can saturate biotic NO_3^- removal and decrease NO_3^- uptake efficiency. In these instances, a lower proportion of N is removed in spite of increases in uptake rates, so more NO_3^- is ultimately exported downstream (Dodds et al. 2002, Bernot and Dodds 2005).

Linked measurements of C and nutrient cycling are needed to better assess ecosystem function and material fate in streams. C and nutrient cycling are coupled directly through processes such as biomass assimilation and respiratory denitrification (Fig. 1; Burgin and Hamilton 2007). Further, C and nutrient cycling may also indirectly influence each other when increases or decreases in one solute alters the stoichiometric balance of resources (Taylor and Townsend 2010, Helton et al. 2015, Rodríguez-Cardona et al. 2016). For example, the presence of biologically-reactive OC compounds can increase the assimilative demand for N in streams (Bernhardt and Likens 2002). Thus, the fate of OC is influenced by the availability of nutrients, which can enhance OC removal and loss, thereby decreasing OC transport downstream (Johnson and Tank 2009, Rosemond et al. 2015). Stoichiometric limitations affect OC and N cycles, but the extent to which OC and N transformations are linked, as well as the relationship between stream C and nutrient cycling, remain poorly understood (Fisher et al. 2004, Rodríguez-Cardona et al. 2016). Further, alterations to inland waters through anthropogenic nutrient loading, land-use change, and disturbance have changed nutrient retention capacity as well as the metabolic regimes of streams and rivers (Mulholland et al. 2008, Bernhardt et al. 2017). How alterations to stream biogeochemical cycles may change linked fluxes and fate of OC and N remains a critical unknown in stream ecosystem science.

Investigating the underlying mechanisms that control OC spiraling and coupled C-nutrient cycle interactions in streams is vital to creating a more integrated, multi-elemental framework of ecosystem dynamics. Here, we use data from the Lotic Intersite Nitrogen Experiment (LINX II; Mulholland et al. 2009) to answer the following questions: 1) How does OC spiraling vary in streams across different land uses and regions? 2) To what extent are OC and NO_3^- cycles in streams linked, and how do environmental factors, such as land use and heterotrophy, impact those linkages? We hypothesized OC spiraling would be less variable in human-altered streams (i.e., agricultural and urban streams) than in reference streams because environmental factors that drive metabolism and OC flux in human-altered streams experience similar changes (e.g., decreased light limitation, increased nutrient loading) regardless of the surrounding region. In streams that are more metabolically balanced (GPP ≈ ER), we predicted higher rates of GPP would lead to greater NO_3^- uptake, whereas higher ER would correspond with greater OC and NO_3^- removal via mineralization and denitrification. Thus, increased metabolic balance would result

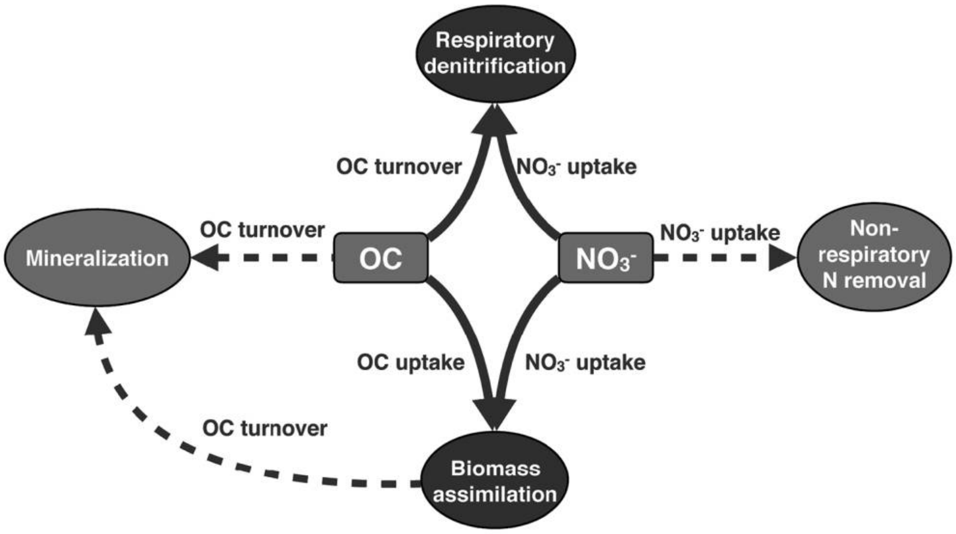

Figure 1. Coupled organic carbon (OC) and nitrate (NO_3^-) cycles. Fluxes are categorized as either uptake (assimilation, removal) or turnover (mineralization and denitrification) of OC or NO_3^-. Boxes represent solutes/materials, solid arrows represent directly-coupled fluxes, dashed arrows represent indirectly-coupled/non-coupled fluxes, and ovals represent fates. Stoichiometric balance can constrain the fates of OC and NO_3^-.

in more tightly-coupled OC and NO_3^- cycles. By linking measurements of N and OC cycling, we improve our understanding of the coupled flow and fate of energy and nutrients in streams.

METHODS

Site information

We used data from the LINX II project to calculate OC and NO_3^- uptake and spiraling (Mulholland et al. 2009) and used these data to assess regional and environmental effects on OC spiraling and coupled OC and N cycles in streams. The LINX II dataset consists of 72 second-order streams from 8 different regions spanning a range of biomes within the United States: Kansas (KS; tallgrass), Massachusetts (MA; northern deciduous forest), Michigan (MI; northern deciduous forest), North Carolina (NC; southern deciduous forest), Oregon (OR; wet coniferous forest), Puerto Rico (PR; tropical forest), Southwest (SW; desert), and Wyoming (WY; steppe) (Table 2). Here we use regional and state abbreviations for regions to remain consistent with categories used in LINX II papers (Hall et al. 2009, Mulholland et al. 2009, Bernot et al. 2010), thus allowing for easy comparison between our findings and past work based on data from these sites. Within each region, the LINX II investigators chose 3 reference (REF), 3 agricultural (AGR), and 3 urban (URB) streams to evaluate potential land-use effects on stream NO_3^- uptake, retention, and removal with stable isotope tracers (Mulholland et al. 2009). Here, we used metabolism, hydrologic (e.g., discharge, velocity, wetted width), OC concentration, and NO_3^- cycling estimates from each LINX II site to evaluate OC spiraling across different land uses and regions and to investigate the relationship between OC and NO_3^- cycling in streams.

N cycling

We used total NO_3^- uptake rates (k_{NO3}; $n = 63$ sites) and denitrification rates (k_{den}; $n = 49$ sites) from Mulholland et al. (2009) to calculate total NO_3^- uptake (S_{w-tot}) and NO_3^- uptake from denitrification (S_{w-den}) (Table 1). These metrics were measured with $^{15}N\text{-}NO_3^-$ tracer additions. Uptake length represents the distance traveled by NO_3^- before it is assimilated or permanently removed from the stream via denitrification and is calculated as the inverse of NO_3^- uptake rate ($1/k_{NO3}$ and $1/k_{den}$, respectively; Table 1; Stream Solute Workshop 1990). We also calculated NO_3^- uptake velocity (v_{f-NO3}), a mass transfer coefficient standardized for stream depth (z) and velocity (u) used to compare NO_3^- uptake among sites (Table 1; Stream Solute Workshop 1990). Finally, we calculated areal NO_3^- uptake rate (U_{tot}) and areal denitrification rate (U_{den}), which represent the relative demand for NO_3^- assimilation and denitrification by stream biota (Table 1; Stream Solute Workshop 1990, Dodds et al. 2002, Mulholland et al. 2009).

OC spiraling

We used estimates of metabolism (i.e., GPP and ER) for all LINX II sites from Bernot et al. (2010) (Table 2). These reach-scale metabolism estimates were calculated from measurements of diel dissolved oxygen taken at 5 to 15 min intervals for 24 to 48 h during summer in low flow conditions (Bernot et al. 2010). We calculated the ratio of GPP:ER to estimate metabolic balance. We calculated OC spiraling length (S_{OC}) as the ratio of downstream OC transport relative to mineralization for each LINX II site with available metabolism, hydrologic, and OC data (Table 1; Hall et al. 2016). We calculated OC concentration ([OC]) as the sum of the dissolved organic carbon concentration ([DOC])

Table 1. Organic carbon (OC) and nitrate (NO_3^-) spiraling parameters and equations. Data used to calculate OC and NO_3^- spiraling parameters are reported in Mulholland et al. (2009). Methods for metabolism estimates can be found in Bernot et al. (2010). Methods for total NO_3^- uptake rates and denitrification rates can be found in Mulholland et al. (2009). We used stream velocity (u) and depth (z) to correct NO_3^- uptake and denitrification rates. We used discharge (Q) and mean wetted width (w) in the organic C spiraling length calculations (S_{OC}). k_{NO3} and k_{den} are the longitudinal uptake rate and denitrification rate, respectively. AR_f is the fraction of gross primary production (GPP) that is respired by autotrophs. [OC] is the organic carbon concentration.

Parameter	Description	Units	Equations
S_{w-tot}	Total NO_3^- uptake length	m	$S_{w-tot} = \frac{1}{k_{NO3}}$
S_{w-den}	Denitrification length	m	$S_{w-den} = \frac{1}{k_{den}}$
v_{f-NO3}	NO_3^- uptake velocity	m/d	$v_{f-NO3} = u \times z \times k_{NO3}$
U_{tot}	Areal NO_3^- uptake rate	$g\ N\ m^{-2}\ d^{-1}$	$U_{tot} = [NO_3^-] \times u \times z \times k_{tot}$
U_{den}	Areal denitrification rate	$g\ N\ m^{-2}\ d^{-1}$	$U_{den} = [NO_3^-] \times u \times z \times k_{den}$
HR	Heterotrophic respiration	$g\ C\ m^{-2}\ d^{-1}$	$HR = ER - AR_f \times GPP$ $AR_f = 0.44 \pm 0.19$
S_{OC}	OC spiraling length	m	$S_{oc} = \frac{Q \times [OC]}{-HR \times w}$
v_{f-OC}	OC mineralization velocity	m/d	$v_{f-OC} = \frac{-HR}{[OC]}$

Table 2. Mean organic carbon concentration ([OC]), gross primary production (GPP), ecosystem respiration (ER), and GPP:ER for each land-use (AGR = agricultural, REF = reference, URB = urban) and region sub-dataset. Regions are represented by states, territories, or regions within the US where study streams were located (KS = Kansas, MA = Massachusetts, MI = Michigan, NC = North Carolina, OR = Oregon, PR = Puerto Rico, SW = Southwest, WY = Wyoming). Biome classifications for each region (as stated in Mulholland et al. 2009) are given in parentheses. Values in parentheses are the minimum and maximum value within each land use or region.

Data	# of sites	[OC] (mg/L)		GPP ($g\ O_2\ m^{-2}\ d^{-1}$)		ER ($g\ O_2\ m^{-2}\ d^{-1}$)		GPP:ER	
All sites	72	3.8	(0.3–25.9)	2.8	(0.0–16.2)	6.6	(0.4–23.0)	0.5	(0–2.0)
AGR	24	4.4	(0.7–18.8)	3.9	(0.0–16.2)	5.9	(1.0–15.7)	0.6	(0–2.0)
REF	24	3.3	(0.5–25.9)	1.2	(0.0–4.0)	6.9	(0.4–23.0)	0.3	(0–1.0)
URB	24	3.7	(0.3–13.2)	3.3	(0.1–12.0)	7.0	(0.5–17.9)	0.5	(0–1.8)
KS (tallgrass prairie)	9	3.1	(0.7–6.3)	3.3	(0.2–11.9)	3.0	(0.5–7.6)	0.8	(0.2–1.8)
MA (north deciduous forest)	9	10.5	(3.5–25.9)	1.4	(0.1–7.3)	7.5	(1.2–14.6)	0.2	(0–0.7)
MI (north deciduous forest)	9	6.0	(1.7–18.8)	0.8	(0.1–1.8)	7.0	(1.3–15.6)	0.2	(0–0.6)
NC (south deciduous forest)	9	1.6	(0.7–2.8)	0.5	(0.1–3.0)	6.4	(1.6–17.9)	0.1	(0–0.5)
OR (wet coniferous forest)	9	3.6	(1.0–10.3)	2.2	(0.0–12.0)	5.7	(1.0–14.0)	0.4	(0–1.2)
PR (tropical forest)	9	1.8	(0.5–3.3)	3.7	(0.1–9.3)	6.6	(0.4–15.7)	0.6	(0–1.4)
SW (desert)	9	2.2	(0.9–5.1)	4.4	(0.75–3.38)	8.5	(1.0–23.0)	0.6	(0.2–2.0)
WY (steppe)	9	1.1	(0.5–2.3)	6.2	(2.7–16.2)	8.5	(1.5–12.6)	0.7	(0.2–1.8)

and suspended particulate organic carbon concentration ([POC]) (Table 2). We calculated [POC] as the site mean of seston ash-free dry mass (sAFDM) multiplied by the mean %C of sAFDM of all sites with available %C data.

We used heterotrophic respiration (HR; $g\ C\ m^{-2}\ d^{-1}$) as our estimate of OC mineralization and as an areal OC removal rate comparable to U_{tot} and U_{den}. We estimated HR by assuming 44 ± 19% of daily GPP is respired by autotrophs and then calculating HR as the difference between ER and the fraction of GPP respired by autotrophs (Table 1; Hall and Beaulieu 2013). For S_{OC} calculations, we converted GPP, ER, and HR from units of $g\ O_2\ m^{-2}\ d^{-1}$ to $g\ C\ m^{-2}\ d^{-1}$, assuming a 1:1 molar relationship (as in Hall et al. 2016). Finally, we calculated $v_{f-\text{OC}}$ to compare OC spiraling among sites (Table 1). $v_{f-\text{OC}}$, similar to $v_{f-\text{NO3}}$, is a stream size-independent measure of OC mineralization relative to [OC] (Table 1; Hall et al. 2016).

Statistical analysis

To assess how OC spiraling and fate varied across land use and region, we conducted 4 separate 2-way analyses of variance (ANOVA) on [OC], HR, $v_{f-\text{OC}}$, and S_{OC} (Table S1). We treated land use and region as fixed effects and also tested for interactions between them. Prior to ANOVA testing, we log-transformed [OC], HR, $v_{f-\text{OC}}$, and S_{OC} to improve the normality of residuals. We used Shapiro–Wilk tests to check for the normality of residuals. To relate OC and NO_3^- cycling, we calculated correlations (Pearson coefficients, r) between $v_{f-\text{OC}}$ and $v_{f-\text{NO3}}$ among all the LINX II sites, as well as among subsets of sites grouped by land-use category and region. Pearson correlation coefficients close to 1 represent tightly-coupled OC and NO_3^- cycles (i.e., mineralization of OC and uptake of NO_3^- are of similar magnitude in a given region or land-use category). To assess the relationship between metabolic balance and the link between OC and NO_3^- cycles, we compared these correlation coefficients with mean GPP:ER for each land-use category and region. Lastly, we used a power law regression to evaluate the relationship between S_{OC} and $S_{w-\text{den}}$ across sites. We used a significance level of $\alpha < 0.05$, and all analyses were done in R (R Project for Statistical Computing, Vienna, Austria).

RESULTS

GPP:ER

GPP:ER varied from 0 to 2. The mean GPP:ER for all streams was 0.4. Most sites with available GPP and ER data ($n = 69$) were heterotrophic during the study period (GPP:ER < 1; $n = 56$ sites; Table 2). Of the 13 sites with GPP:ER ≥1, which indicated net autotrophy during the study period, only 1 was a reference site. Sites in either deciduous or coniferous forest regions (MA, MI, NC, and OR) had mean GPP:ER <0.4 (0.1–0.4), whereas sites in steppe (WY), desert (SW), tropical forest (PR), and tallgrass (KS) regions had mean GPP:ER >0.49 (0.6–0.8 across WY, SW, PR, and KS) (Table 2). Mean GPP:ER across AGR and URB sites (0.6 and 0.5, respectively) were higher than mean GPP:ER across REF sites (0.3). Streams with high GPP (5.0–16.2 $g\ O_2\ m^{-2}\ d^{-1}$) occurred exclusively in URB and AGR sites. GPP in REF streams ranged from 0.0 to 4.0 $g\ O_2\ m^{-2}\ d^{-1}$ (Table 2).

OC spiraling

Interactions between land use and region were significant for [OC], indicating that patterns in [OC] across land use and region could not be interpreted separately. There were no significant interactions between land use and region for HR, $v_{f-\mathrm{OC}}$, and S_{OC} (Table S1). ANOVA residuals following transformation for all OC-spiraling metrics were normally distributed, and error variance was similar across all treatments (Table S1). We were unable to calculate [OC] and HR for 2 sites and $v_{f-\mathrm{OC}}$ and S_{OC} for 3 sites because of missing [DOC], [POC], or ER data. These missing observations led to lower residual degrees of freedom for our OC-spiraling metrics (Table S1).

We cannot interpret the main effects of land use and region on [OC] because of the significant interaction between region and land-use [OC] effects ($p = 0.036$; Table S1). Other than MA and WY, mean [OC] in REF sites was lower than mean [OC] in AGR and URB sites (Fig. S1). URB and AGR mean [OC] were similar across all regions except in MI, where mean [OC] in URB sites was twice as high as mean [OC] in AGR sites (Fig. S1). Across all sites, [OC] was mainly comprised of [DOC] (79.97 ± 18.10%). HR and $v_{f-\mathrm{OC}}$ did not differ significantly across land-use categories ($p = 0.486$ and 0.206, respectively). HR was not significantly different among the 8 regions (Fig. 2D; $p = 0.052$). $v_{f-\mathrm{OC}}$ differed significantly among regions (Fig. 2E; $p = 0.019$), with higher $v_{f-\mathrm{OC}}$ in WY sites and lower $v_{f-\mathrm{OC}}$ in KS sites relative to the other LINX II streams (Fig. 2F). S_{OC} was variable across all sites, ranging from 21 to 37,156 m (Table 3). Differences in S_{OC} across land use were not significant ($p = 0.072$; Fig. 2G). S_{OC} was similar among all regions ($p = 0.399$; Fig. 2H).

OC and NO_3^- cycling and fate comparison

For all streams with suitable LINX II data to estimate OC and NO_3^- spiraling ($n = 65$ sites), $v_{f-\mathrm{OC}}$ was positively correlated with $v_{f-\mathrm{NO3}}$ ($r = 0.33, p = 0.008$; Table S2, Fig. 3A). $v_{f-\mathrm{OC}}$ and $v_{f-\mathrm{NO3}}$ were significantly positively correlated in URB streams ($r = 0.73, p < 0.001$) and AGR streams ($r = 0.56, p = 0.008$). However, $v_{f-\mathrm{OC}}$ and $v_{f-\mathrm{NO3}}$ were not significantly correlated in any region of the LINX II dataset. Sites with significant, positive Pearson correlation coefficients between $v_{f-\mathrm{OC}}$ and $v_{f-\mathrm{NO3}}$ (URB and AGR; Fig. 4) had mean GPP:ER values of 0.5 and 0.6, respectively. These GPP:ER values were equal to or higher than mean LINX II GPP:ER (0.5) and were closer to metabolic balance (GPP:ER = 1). However, no region with GPP:ER closer to 1 had significant correlations between $v_{f-\mathrm{OC}}$ and $v_{f-\mathrm{NO3}}$ (KS, PR, SW, WY).

S_{OC} and $S_{w-\mathrm{den}}$ (the distance traveled before permanent removal of OC via mineralization and NO_3^- via denitrification, respectively) were similar in magnitude and amount of variability across all streams (Table 3, Fig. 5). S_{OC} was shorter than $S_{w-\mathrm{den}}$ in 40 of 45 sites with data available for this comparison, regardless of land use or region. The few sites where S_{OC} was longer than $S_{w-\mathrm{den}}$ were human-altered streams. However, classification by region did not predict streams where S_{OC} exceeded $S_{w-\mathrm{den}}$. Lastly, S_{OC} increased as $S_{w-\mathrm{den}}$ increased ($p < 0.001$), indicating that the relative balance between transport and removal at a given site was similar for both OC and NO_3^- (Fig. 5).

DISCUSSION

Despite the strong links between OC and NO_3^- cycles in streams, the underlying mechanisms of coupled OC-NO_3^- dynamics and the influence of land use and region on OC-NO_3^- interactions remain poorly understood (Helton et al. 2015, Rosemond et al. 2015, Rodríguez-Cardona et al. 2016). We found that both OC fate and the link between OC and NO_3^- cycles are influenced by land use, region, and GPP:ER. The distances traveled by OC and NO_3^- before removal (S_{OC} and $S_{w-\mathrm{den}}$) are similar in magnitude. However, OC is removed via mineralization faster than NO_3^- is removed via denitrification in most sites. By coupling NO_3^- and OC spiraling, we were able to quantify the linked fate of energy and nutrients in streams and rivers.

Land use and region influence on OC fate

The significant interaction between regional and land-use effects on [OC] prevented us from interpreting these effects separately (Table S1). However, in general, REF sites had lower [OC] across all regions except MA (Fig. S1). $v_{f-\mathrm{OC}}$ varied by region. We found no significant differences in HR or $v_{f-\mathrm{OC}}$ across land uses. However, mean, minimum, and maximum S_{OC} were longer in AGR and URB sites than in REF sites, indicating that OC is transported further prior to mineralization in human-altered streams than in reference streams (Table 3, Fig. 2G). Additionally, within-group variability of HR (i.e., OC mineralization) across sites within each land-use category was much greater than variability in mean HR among land-use categories (Fig. 2C). The large variability in HR within each land-use category is likely the result of variation in the controls on HR among different sites and regions (Fig. 2D). Hydrology, temperature, inorganic nutrients, GPP, and organic matter can all regulate OC mineralization, and these variables change with land use and region (Bernot et al. 2010). For example, OC mineralization in forested regions (MA, MI, NC, and OR) is expected to be more subsidized by terrestrial OC inputs than open canopy streams (Tank et al. 2010). Higher light and temperature in open canopy streams (WY, KS, and SW) can lead to higher rates of metabolism and subsequently increase OC mineralization (Bernot et al. 2010, Mulholland et al. 2001).

Spiraling lengths of OC were longer in human-altered sites than in reference sites. There were no significant differences in HR among the 3 land-use categories but discharge, OC:NO_3^-, and other environmental factors linked to land use can also affect OC spiraling and fate. In agricultural streams, higher light and nutrients as well as higher quality organic matter inputs (i.e., algae, low C:N detritus) often increase OC mineralization rates (Griffiths et al. 2009, 2012).

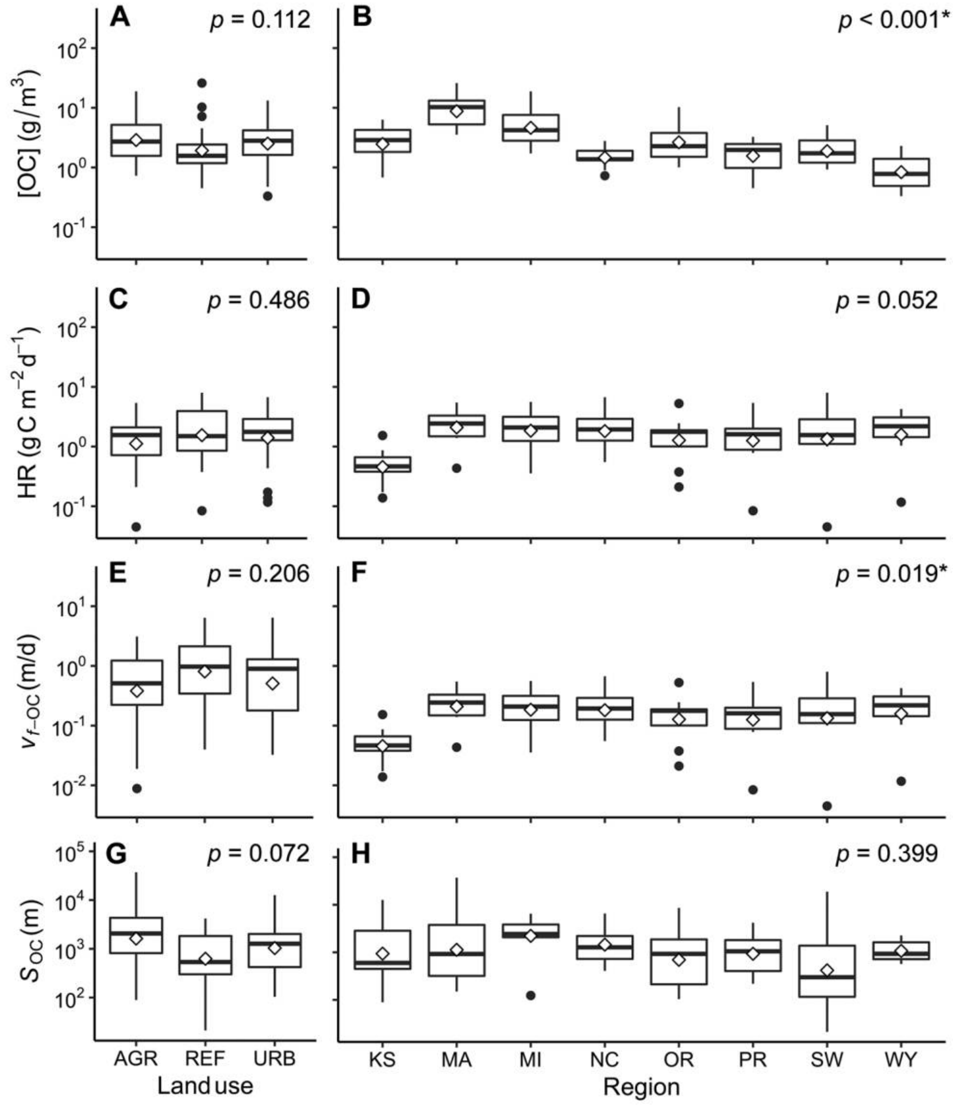

Figure 2. Left panel: Organic carbon concentration ([OC]) (A), OC mineralization rate (as heterotrophic respiration, HR) (C), OC mineralization velocity ($v_{f-\mathrm{OC}}$) (E), and OC spiraling length (S_{OC}) (G) in agricultural (AGR), reference (REF), and urban (URB) streams. Right panel: OC (B), HR (D), $v_{f-\mathrm{OC}}$ (F), and S_{OC} grouped by region (H). Regions are represented by states, territories, or regions within the USA where study streams were located (KS = Kansas, MA = Massachusetts, MI = Michigan, NC = North Carolina, OR = Oregon, PR = Puerto Rico, SW = Southwest, WY = Wyoming). White diamonds represent the mean value within a given land use or region. Asterisks (*) denote significance within $p < 0.05$ based on a 2-way analysis of variance with fixed effects.

Despite this, agricultural streams can be less efficient at removing OC at baseflow than forested streams (Griffiths et al. 2012), which suggests that changes in OC flux, rather than biology, may increase OC spiraling lengths. Furthermore, altered hydrology through stream channelization and decreased transient storage can increase S_{OC} in agricultural streams (Bernot et al. 2010, Griffiths et al. 2012). Our results are consistent with these findings because agricultural streams acted as better OC transporters than did reference streams. Similar to agricultural streams, urban streams have disrupted OC cycles with GPP:ER closer to 1 and also tend to be OC-limited (Kaushal and Belt 2012, Larsen and Harvey 2017). Increased light availability and temperature along with lower $\mathrm{OC}:\mathrm{NO}_3^-$ can lead to higher rates of GPP in urban streams (Bernot et al. 2010, Kaushal et al. 2014). However, human alterations to urban streams and catchments (e.g., channelization, increases in impervious surface area, and decreases in transient storage zones) can lead to shorter water residence times, increased hydrologic variability, and greater export of OC relative to OC removal (Smith and Kaushal 2015). Longer S_{OC} in the LINX II urban streams throughout the US suggests urban streams remove OC less efficiently than reference streams and, similar to agricultural streams, transport more OC downstream.

Table 3. Mean organic carbon (OC) spiraling length (S_{OC}), OC mineralization velocity ($v_{f-\mathrm{OC}}$), total nitrate ($\mathrm{NO_3}^-$) uptake length ($S_{w-\mathrm{tot}}$), $\mathrm{NO_3}^-$ uptake velocity ($v_{f-\mathrm{NO3}}$), and denitrification length ($S_{w-\mathrm{den}}$) for each land use (AGR = agricultural, REF = reference, URB = urban) and region sub-dataset. Regions are represented by states, territories, or regions within the US where study streams were located (KS = Kansas, MA = Massachusetts, MI = Michigan, NC = North Carolina, OR = Oregon, PR = Puerto Rico, SW = Southwest, WY = Wyoming). Values in parentheses are the minimum and maximum values within each land use or state.

Data	S_{OC} (m)		$v_{f-\mathrm{OC}}$ (m/d)		$S_{w-\mathrm{tot}}$ (m)		$v_{f-\mathrm{NO3}}$ (m/d)		$S_{w-\mathrm{den}}$ (m)	
All sites	2584	(21–37,156)	1.17	(0.01–6.43)	2102	(20–18,332)	2.37	(0.04–25.83)	22,330	(99–183,486)
AGR	4561	(89–37,156)	0.82	(0.01–3.10)	2627	(49–18332)	3.21	(0.07–25.83)	24,497	(172–167,785)
REF	1169	(21–4180)	1.50	(0.04–6.39)	1445	(20–12619)	2.39	(0.04–8.30)	12,884	(99–66,225)
URB	2060	(104–12,605)	1.16	(0.03–6.43)	2203	(25–6757)	1.56	(0.04–14.43)	22,118	(364–183,486)
KS	2479	(89–12,605)	0.31	(0.03–1.05)	301	(20–777)	2.39	(0.14–8.02)	14,796	(364–36,101)
MA	5473	(150–37,156)	0.38	(0.04–0.93)	2301	(356–4608)	0.31	(0.04–1.04)	5363	(1869–13,405)
MI	3112	(124–6476)	0.89	(0.02–3.25)	2715	(406–8811)	0.72	(0.17–2.68)	41,297	(2532–167,785)
NC	2196	(408–6537)	1.53	(0.41–4.13)	7343	(424–18,332)	0.68	(0.04–1.88)	56,192	(1362–183,486)
OR	1736	(104–8596)	1.04	(0.02–3.48)	1239	(25–4247)	1.06	(0.14–3.31)	10,447	(4255–17,301)
PR	1370	(218–4180)	1.24	(0.04–3.56)	2340	(315–8480)	0.61	(0.19–1.35)	19,520	(990–66,225)
SW	2811	(21–18,838)	1.77	(0.01–6.39)	695	(40–5155)	3.46	(0.10–9.35)	4716	(99–9346)
WY	1213	(568–2263)	2.56	(0.24–6.43)	557	(85–2500)	9.15	(0.60–25.83)	14,816	(172–36,232)

Coupled OC and $\mathrm{NO_3}^-$ cycles linked to metabolic balance

Measurements of OC and $\mathrm{NO_3}^-$ spiraling allow us to compare OC and $\mathrm{NO_3}^-$ removal and test potential drivers of coupled OC-$\mathrm{NO_3}^-$ cycles. We investigated the relationship between GPP:ER and coupled OC-$\mathrm{NO_3}^-$ removal because OC mineralization contributes to ER (Hall et al. 2016) and because N assimilation and removal, particularly of $\mathrm{NO_3}^-$, depend on GPP (Hall and Tank 2003, Heffernan and Cohen 2010). Overall, the lack of a consistent trend between $v_{f-\mathrm{OC}}$ and $v_{f-\mathrm{NO3}}$ correlations and GPP:ER suggests that environmental factors beyond ecosystem metabolic balance influence OC-$\mathrm{NO_3}^-$ cycle coupling.

Regardless of region, human-altered streams (agricultural and urban) had higher GPP:ER and tighter OC and $\mathrm{NO_3}^-$ cycle coupling than did reference sites (Fig. 3A–B). $v_{f-\mathrm{OC}}$ and $v_{f-\mathrm{NO3}}$ were not correlated, and GPP:ER was below the mean overall LINX II GPP:ER in reference sites (Table S2, Fig. 4). Together, these findings show that environmental factors that differ across regions such as light availability, hydrologic regime, and OC and nutrient availability, are important drivers of regional OC and $\mathrm{NO_3}^-$ fate differences in reference streams (Hall et al. 2009). Further, these results show that GPP:ER and OC-$\mathrm{NO_3}^-$ coupling is higher in human-altered streams. Urbanization and agricultural development often impact streams similarly, regardless of region, leading to decreased canopy cover, greater nutrient and sediment loading, changes in the organic matter pool and sources, and channelization (Mulholland et al. 2008, Tank et al. 2018). These physiochemical changes to urban and agricultural streams influence the demand for energy and nutrients (Wymore et al. 2016, Covino et al. 2018) and can make urban and agricultural streams behave similarly across regions (i.e., urban homogenization; Groffman et al. 2014). Physical and chemical shifts in human-altered streams disrupt the fate of $\mathrm{NO_3}^-$ by decreasing $\mathrm{NO_3}^-$ uptake efficiency (Dodds et al. 2002, Mulholland et al. 2008). Our work builds on this result by indicating that the human-induced alterations to hydrology and metabolism also change the fate of OC and increase the coupling of OC-$\mathrm{NO_3}^-$ cycles (Fig. 4).

$v_{f-\mathrm{OC}}$ and $v_{f-\mathrm{NO3}}$ were not significantly correlated in any region. Differences in OC and $\mathrm{NO_3}^-$ cycling between reference and human-altered streams within each region may confound regional assessments of OC-$\mathrm{NO_3}^-$ coupling. This result supports the idea that GPP:ER alone cannot predict regional coupling of in-stream OC and $\mathrm{NO_3}^-$ removal. We found no relationship between light and coupled OC-$\mathrm{NO_3}^-$ removal, but we expect that differences in light regimes, OC and N sources, and biotic communities can influence the regional coupling of OC and $\mathrm{NO_3}^-$ cycles (Hall et al. 2009, Wymore et al. 2016). Availability and demand for $\mathrm{NH_4}^+$ relative to $\mathrm{NO_3}^-$ may also decouple OC and $\mathrm{NO_3}^-$ cycles, especially in low $\mathrm{NO_3}^-$ and low GPP:ER streams (Peterson et al. 2001, Hall and Tank 2003).

Downstream fate of OC and $\mathrm{NO_3}^-$

OC and $\mathrm{NO_3}^-$ travel similar distances downstream before being removed from transport through OC mineralization or denitrification. For most sites, S_{OC} was shorter than $S_{w-\mathrm{den}}$, suggesting that headwaters mineralize OC to $\mathrm{CO_2}$ more efficiently than they denitrify $\mathrm{NO_3}^-$ to $\mathrm{N_2}$ (Fig. 5). Further, the relationship between S_{OC} and $S_{w-\mathrm{den}}$ was statistically significant, suggesting that the removal and downstream fate of OC and $\mathrm{NO_3}^-$ were linked across the LINX II sites. These findings are consistent with previous findings of a strong, significant relationships between ER and areal denitrification rate (Fig. S2; Mulholland et al. 2009). Nutrient loading

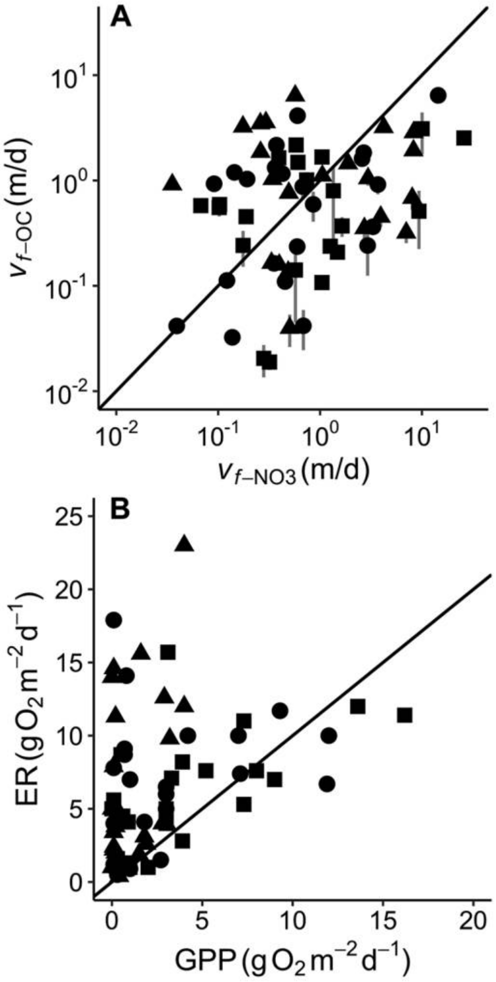

Figure 3. Organic carbon mineralization velocity ($v_{f\text{–OC}}$) vs nitrate (NO_3^-) uptake velocity ($v_{f\text{–NO3}}$) (A) and ecosystem respiration (ER) vs gross primary production (GPP) (B) in all streams. Sites are also categorized by land use (agricultural sites are squares, urban sites are circles, and reference sites are triangles). Solid lines in each panel are 1:1 relationships and represent tightly-coupled OC-NO_3^- cycling ($v_{f\text{–OC}} = v_{f\text{–NO3}}$) or metabolic balance (GPP = ER). Vertical bars represent the uncertainty of $v_{f\text{–OC}}$ based on heterotrophic respiration (HR) estimates from Hall and Beaulieu (2013). A color version of this figure with state and regional delineations can be found in the supplemental materials (Fig. S3).

to freshwaters is widespread, so NO_3^- is not limiting in many urban and agricultural ecosystems (Mulholland et al. 2008). NO_3^- loading can increase heterotrophic demand of OC (Lutz et al. 2011), resulting in higher retention and removal of OC in streams and, thus, shorter S_{OC}. In addition to OC demand being higher in environments with low OC:N, NO_3^- is not the most energetically-favored form of N (Taylor and Townsend 2010, Helton et al. 2015). Thus, spiraling lengths and export of NO_3^- are longer than spiraling lengths and export of NH_4^+ (Hall et al. 2013). Assimilatory demand of NH_4^+ can account for a large portion of total N uptake, especially in low NO_3^- streams (Peterson et al. 2001, Dodds et al. 2002, Hall and Tank 2003). NO_3^- removal by denitrification can also be limited by the availability of labile OC (Baker et al. 1999, Zarnetske et al. 2011).

The few LINX II streams with $S_{OC} > S_{w\text{–den}}$ were human-altered sites, but $S_{OC} > S_{w\text{–den}}$ was not explained by region or GPP:ER. Ultimately, the relative demand for and supply of both OC and NO_3^- control the removal, fate, and coupling of OC and NO_3^- in streams (Rodríguez-Cardona et al. 2016, Wymore et al. 2016, Wollheim et al. 2018). Stream OC:NO_3^- may be a useful metric to infer the balance between energy and nutrient demands. Indeed, in previous studies OC:NO_3^- was a better predictor of NO_3^- uptake than was NO_3^- concentration alone (Dodds et al. 2004, Rodríguez-Cardona et al. 2016, Wymore et al. 2016). Both OC:NO_3^- and GPP:ER influence the cycling and fate of OC and NO_3^- in streams, but spatially-explicit drivers linked to region and land use contribute to the variability in OC and NO_3^- spiraling among sites. Further research on coupled OC-NO_3^- fate should recognize the importance of local and regional controls on ecosystem function and be guided by region-specific drivers of C and nutrient dynamics.

Conclusion

From our analyses, we concluded that land-use change likely influences OC spiraling, C fate, and linked OC-NO_3^-

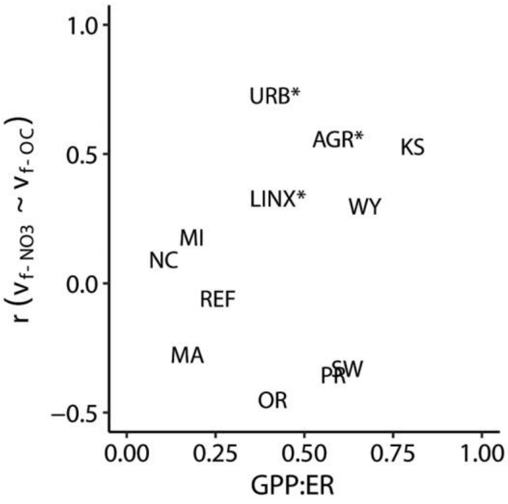

Figure 4. The relationship between correlations (*r*) between organic carbon mineralization velocity ($v_{f\text{–OC}}$) and nitrate (NO_3^-) uptake velocity ($v_{f\text{–NO3}}$) and the ratio of gross primary production (GPP) and ecosystem respiration (ER). Regions are represented by states, territories, or regions within the US where study streams were located (KS = Kansas, MA = Massachusetts, MI = Michigan, NC = North Carolina, OR = Oregon, PR = Puerto Rico, SW = Southwest, WY = Wyoming). Sites are also categorized by land use (AGR = agricultural sites, URB = urban sites, REF = reference sites). Asterisks (*) denote regions or land use categories with significant ($p < 0.05$) correlations (*r*) between $v_{f\text{–NO3}}$ and $v_{f\text{–OC}}$.

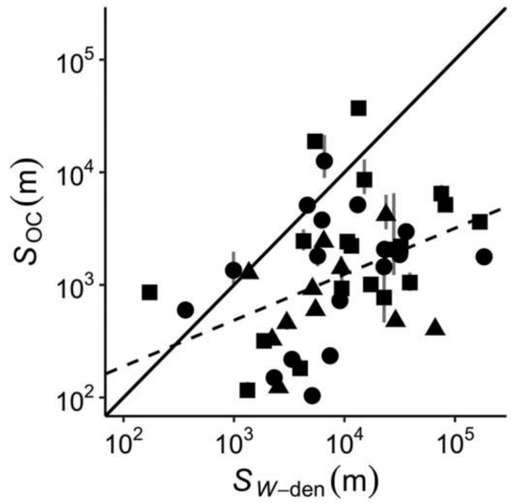

Figure 5. Organic carbon spiraling length (S_{OC}) vs denitrification length ($S_{w-\text{den}}$). Sites are also categorized by land use (agricultural sites are squares, urban sites are circles, and reference sites are triangles). The solid line represents $S_{OC} = S_{w-\text{den}}$. The dashed line represents the power-law relationship ($\log_{10} S_{OC} = 1.43 + 0.42 \times \log_{10} (S_{w-\text{den}})$; $r^2 = 0.21$; $r = 0.48$; $p < 0.001$). Vertical bars represent the uncertainty of S_{OC} based on heterotrophic respiration (HR) estimates from Hall and Beaulieu (2013). A color version of this figure with state and regional delineations can be found in the supplemental materials (Fig. S4).

interactions in streams. Human-altered streams had lower rates of OC mineralization, which led to greater transport of OC downstream before permanent removal. Further, while metabolic balance (GPP:ER) was common in regions with tightly-linked OC and NO_3^- cycles, it did not serve as a clear, universal predictor of OC-NO_3^- interactions and fate. Rather, the variation in metabolism and OC-NO_3^- spiraling among region and land-use groups suggests that spatial patterns in environmental drivers exert greater control over coupled OC-NO_3^- interactions than OC:NO_3^- (Taylor and Townsend 2010, Helton et al. 2015, Wymore et al. 2016) or GPP:ER. Moving forward, we expect that longer time series of stream metabolism estimates with dissolved organic matter and NO_3^- sensor data will lead to improved understanding of both the temporal (e.g., annual, seasonal, diel) variability in OC-NO_3^- transformations and the influence of different hydrologic conditions on OC and NO_3^- fluxes and fate through stream networks (Heffernan and Cohen 2010, Snyder et al. 2018). Currently, the use of sensors is limited to sites with higher OC and NO_3^- concentrations, but we see great opportunity in the use of sensor data to understand high-frequency patterns in OC and NO_3^- dynamics and OC-NO_3^- interactions over space and time that cannot be captured with whole-stream experiments alone. Linking land-use and region-specific influences on coupled OC-NO_3^- interactions across a broad range of hydrologic conditions will improve our ability to assess the flow and fate of C and nutrients throughout stream networks.

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Author contributions: ERH and SP conceived the study, and all authors collaborated on the framework for analyses given previous LINX II studies. SP prepared and analyzed data and performed statistical analyses. SP and wrote the manuscript with ERH, BMO, and MTG.

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