

A test of the subsidy–stability hypothesis: the effects of terrestrial carbon in aquatic ecosystems

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Abstract. Global change is altering the movement of materials across landscapes in ways that likely have major consequences for the functioning and stability of ecosystems. For example, the export of dissolved organic carbon (DOC) from terrestrial to aquatic ecosystems is increasing globally. This browning phenomenon is expected to alter the stability of recipient aquatic ecosystems, but theory provides contrasting predictions about the form and direction of this response. We created a gradient in terrestrial DOC supply by adding humic substances on weekly basis to 10 experimental ponds (10⁶ L each) over a growing season. The manipulation of terrestrial DOC supply had strong effects on the chemical, physical, and biological properties of the pond ecosystems. Light attenuation linearly increased with terrestrial DOC supply, which created a shading effect that negatively influenced whole-pond gross primary production and respiration. Despite this, bacterial contributions to basal energy mobilization and respiration increased with terrestrial DOC supply indicating that aquatic food webs were subsidized by terrestrial inputs. After establishing the DOC gradient, we used dynamic linear models to test the subsidy–stability hypothesis by measuring the resilience and sensitivity of each pond to a pulse nutrient perturbation. We found that recovery from the perturbation decreased nonlinearly along a gradient in terrestrial DOC supply. Reciprocal transplant experiments indicated that owing primarily to its light attenuating properties and recalcitrant nature, terrestrial DOC diminished aquatic ecosystem stability by reducing nutrient turnover rates (NTR). Together, our results demonstrate that global-change-mediated alterations in the movement of material and energy between habitats can have unpredictable and dramatic impacts on the reliability of ecosystem services.

Key words: *allochthony; browning; ecosystem stability; global change; microbial processes; nutrient turnover; resilience; resistance; resource subsidy; tipping points.*

INTRODUCTION

Ecosystems are connected to each other through the cross-boundary movement of materials and energy. The delivery of nutrients, detritus, or living organisms from donor ecosystems has important consequences for the structure and function of recipient ecosystems. These allochthonous inputs serve as resource subsidies, which can regulate population densities (Sabo and Power 2002), shape community composition (Murakami and Nakano 2002), increase food chain length (Pimm and Kitching 1987), and induce trophic cascades (Leroux and Loreau 2008). Furthermore, multiple bodies of ecological theory predict that subsidies should alter the stability of recipient ecosystems. Food web models from community ecology predict that allochthonous resources should stabilize recipient habitats by dampening oscillations between consumers and their locally controlled resources (Polis et al. 1997, Huxel and McCann 1998). In contrast, ecosystem models predict that resource

subsidies can be destabilizing, especially if they slow down the turnover rates of growth-limiting nutrients (DeAngelis 1992, Cottingham and Carpenter 1994). Although critical for understanding and predicting the dynamics of spatially linked ecosystems, empirical tests of subsidy–stability theory are generally lacking (Nowlin et al. 2007).

One well-recognized class of subsidies is the transport of organic matter from terrestrial to aquatic ecosystems (Polis et al. 1997). Nearly three petagrams of carbon is exported from terrestrial to aquatic ecosystems by fluvial processes each year (Tranvik et al. 2009). A large fraction (40–90%) of this flux comes in the form of dissolved organic carbon (DOC; Mulholland 2003). Often, terrestrial DOC is considered a low-quality resource because it is composed of high-molecular weight compounds that are inaccessible to most aquatic organisms. Furthermore, terrestrial DOC chelates micronutrients (Jackson and Hecky 1980), diminishes enzymatic activity (Hättenschwiler and Vitousek 2000), and attenuates solar radiation (Thrane et al. 2014), all of which can contribute to reduced rates of primary productivity in aquatic ecosystems (Karlsson et al. 2009). Nevertheless, many studies have shown that

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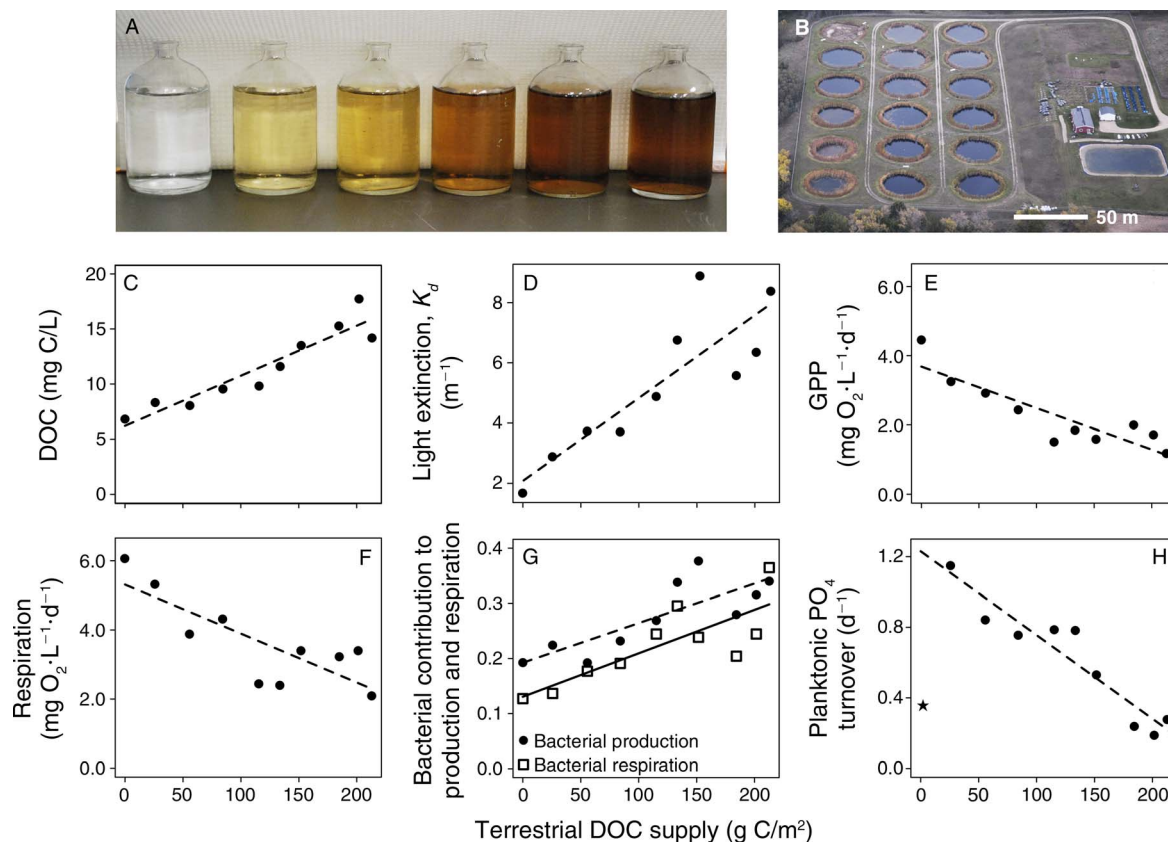


FIG. 1. Effects of experimental browning on the chemical, physical, and biological properties of pond ecosystems. (A) Water samples taken from ponds along the terrestrial dissolved organic carbon (DOC) supply gradient. (B) Aerial photo of the experimental ponds (30 m diameter; 2 m maximum depth). Pond (C) DOC concentration and (D) light extinction coefficient (K_d) linearly increased with DOC supply during the first 80 d of the study. Both (E) whole-pond gross primary productivity (GPP) and (F) respiration (R) were negatively influenced by DOC supply. However, (G) the proportional contribution of bacteria to ecosystem productivity (BP) and respiration (BR) increased with DOC supply. Finally, (H) phosphorus turnover rates in the water column declined with increasing DOC supply. The regression line for phosphorus turnover rates does not include the observation from the reference pond (star symbol). However, we supply parameter estimates and summary statistics for the complete data set along with other response variables in Table 1.

terrestrial DOC inputs support the catabolic and anabolic processes of heterotrophic bacteria (Jansson et al. 2008). Ultimately, terrestrial carbon accounts for >50% of biomass production at higher trophic levels (e.g., zooplankton and fish) in systems with high concentrations of terrestrial DOC relative to the supply of autochthonous resources (Carpenter et al. 2005). Thus, terrestrial DOC inputs have the potential to reroute energy flow in aquatic ecosystems in ways that may enhance or diminish ecosystem stability (Wetzel 1995).

Testing the subsidy–stability hypothesis at the land–water interface is important given the growing evidence that DOC concentrations in aquatic ecosystems are rising around the globe (Roulet and Moore 2006, Monteith et al. 2007). For example, DOC levels increased in >70% of the lakes, streams, and rivers surveyed throughout Scandinavia, the United Kingdom, and North America over the last 25 years (Monteith et al. 2007). While the cause of this browning trend

remains to be determined, it has been hypothesized that elevated temperatures, altered hydrology, land-use change, or reduced acid deposition may be contributing to the increased flux of DOC from terrestrial to aquatic ecosystems (Garnett et al. 2000, Roulet and Moore 2006, Monteith et al. 2007). Far less attention, however, has focused on the consequences of this widespread environmental change for recipient aquatic ecosystems and the stability of the ecosystem services they provide (Brothers et al. 2014; Solomon et al. 2015).

We investigate how ecosystem connectivity via cross-system exchange of resources affects recipient ecosystem stability. We tested theoretical predictions about subsidy–stability relationships by manipulating the supply of terrestrial DOC to 10 large (10⁶ L) experimental ponds (Fig. 1A, B). Our regression-based design created a DOC supply gradient that spanned and slightly exceeded what is reported for north temperate lakes (Mulholland 2003, Hanson et al. 2004). After establishing the DOC gradient, we tested the subsidy–stability hypoth-

esis by measuring the resilience and sensitivity of each pond to a pulsed nutrient perturbation. Finally, we conducted a reciprocal transplant experiment to identify key mechanisms regulating the response of aquatic food webs to the pulse perturbation. The combination of whole-pond manipulation, time-series modeling, and mechanistic experiments provides novel insight into the role of terrestrial subsidies in regulating the stability of recipient aquatic ecosystems. The results of our study highlight the importance of ecosystem connectivity for the reliability of ecosystem services under global change scenarios (e.g., browning of aquatic ecosystems).

METHODS

Ponds and terrestrial DOC manipulation

We conducted our studies at the Michigan State University W. K. Kellogg Biological Station Experimental Pond Facility in Hickory Corners, Michigan, USA. Each of the ponds at the facility is 30 m in diameter with a maximum depth of 2 m for an estimated operating volume of 10^6 L (Fig. 1B). Following construction in 1971, the bottom of each pond was lined with vinyl plastic and covered with sand. Since then, organic sediments have become well developed owing to the deposition of in situ production. In early spring of 2009, we filled 10 ponds from a groundwater well and stocked them with 30 juvenile bluegill sunfish (*Lepomis macrochirus*) from a nearby lake. Following a 30-d acclimation period, we determined that the ponds were oligo-mesotrophic (total phosphorus, 12 $\mu\text{g/L}$; total nitrogen, 200 $\mu\text{g/L}$), had relatively low DOC concentrations (4 mg/L), and had established a *Daphnia*-dominated zooplankton community. We then created a terrestrial DOC gradient by adding humic substances to ponds using a 5-horsepower trash pump on a weekly basis. One of the ponds received no terrestrial DOC and served as a reference. For the remaining ponds, humic substances were added to achieve supply rates that were equally spaced between 25 and 210 g C/m² over the 110-d experiment.

The humic substances used in our study came from a product called Super Hume, which is marketed by CropMaster, United Agricultural Services of America (Lake Panasoffkee, Florida, USA). Super Hume is extracted from Leonardite shale, a naturally occurring mineral found in North America and other parts of the world. Commercially available humic substances are commonly used as an agricultural amendment to improve soil water retention, reduce soil erosion, buffer fluctuations in pH, and stimulate the activity of microorganisms (Quilty and Cattle 2011). Super Hume has a high organic carbon content (40 g C/L) and is composed of 17% humic acid and 13% fulvic acid by dry mass. We thoroughly characterized Super Hume to assess its suitability as an analog for terrestrial DOC (Lennon et al. 2013). From this, we learned that (1) the light-absorbing properties of Super Hume are comparable to those of terrestrial DOC found in aquatic

ecosystems, (2) Super Hume has low concentrations of nitrogen and phosphorus (6.5 mg/L and 125 $\mu\text{g/L}$, respectively), but is enriched in lignin, protein, and quinone, which is typical of soil-derived organic matter entering aquatic ecosystems, (3) aquatic bacteria are capable of using Super Hume as a carbon and energy source for growth and respiration, and (4) the direct ingestion of Super Hume increased the fitness of a common zooplankton grazer isolated from the experimental ponds (*Daphnia pulex* \times *pulicaria*).

Pond responses along the DOC gradient

We measured a suite of chemical, physical, and biological response variables over the course of the experiment. We sampled at the center of each pond in a kayak three times a week from 8 June 2009 to 25 September 2009. With depth-integrated water samples (0–0.5 m), we measured phosphate concentrations on 0.7- μm filtrates using a molybdenum blue analysis (Wetzel and Likens 1991), DOC concentrations on 0.7- μm filtrates via oxidation and subsequent nondispersive infrared (NDIR) detection with a Shimadzu TOC-V (Shimadzu, Kyoto, Japan), and algal biomass by quantifying chlorophyll *a* with a fluorometer after cold-extracting plankton from 0.7- μm glass fiber filters in 95% ethanol (Welschmeyer 1994). We also quantified the metabolic activity of heterotrophic bacteria by measuring bacterial productivity (BP), bacterial respiration (BR), and bacterial growth efficiency (BGE). BP was estimated based on the uptake and incorporation of ³H-leucine into bacterial protein (Smith and Azam 1992), BR was measured as the decline in dissolved oxygen on 2.7- μm pond filtrate (Whatman GF/D; Whatman, Piscataway, New Jersey, USA) using a Presens SensorDish Reader (Presens, Regensburg, Germany; Briand et al. 2004), and BGE was calculated as BP/(BP + BR). Nutrient turnover rates (NTR) were estimated from surface water samples as (P uptake – P regeneration)/P biomass, where P uptake and P regeneration rates were measured on unfiltered pond water using ³³P pulse-chase methods (Hudson and Taylor 1996) and P biomass was measured as particulate phosphorus on 0.7- μm retentates. We quantified zooplankton biomass as the dry mass of samples obtained using a 10-L Schindler-Patalas sampler with a 73- μm mesh. Whole-pond gross primary production (GPP) and respiration (R) were estimated using a model fit to open water dissolved oxygen concentrations collected every 10 min using YSI 6600V2 sondes (YSI, Yellow Springs, Ohio) positioned at the center of each pond and a depth of 0.4 m (Solomon et al. 2013). Last, light extinction (K_d) was estimated in each pond on a weekly basis from depth profiles of photosynthetically active radiation (PAR). Using data from the first 80 d of the study, we used general linearized models (GLM) in R version 2.14 (R Development Core Team) to evaluate how time-averaged response variables responded to DOC loading rates.

Quantifying stability

Ecologists measure stability in various ways. In this study, we estimated stability based on the response of food web and ecosystem properties to a pulse of inorganic nutrients, a type of perturbation that is common in aquatic habitats (Nowlin et al. 2008). Using a 5-horsepower trash pump, we added 500 L of an inorganic nutrient solution ($\text{NH}_4\text{NO}_3 + \text{Na}_2\text{HPO}_4$) to each pond on 27 August 2009, which was day 81 of experiment (day of year, 239). This nutrient addition elevated inorganic nitrogen and phosphorus concentrations by 565 $\mu\text{g/L}$ and 50 $\mu\text{g/L}$, respectively. We targeted these concentrations based on the mean N:P ratio of surface water samples prior to the nutrient perturbation (11:1 by mass) in an effort to maintain the phosphorus-limited state of ponds. We then used dynamic linear models (DLM) to quantify stability based on time-series data spanning the 30-d period following the nutrient perturbation (Pole et al. 1994, Cottingham and Schindler 2000). When using DLM, the probability distribution of a system state variable is serially updated using a Bayesian framework, but the contributions of past measurements are discounted based upon a statistically inferred memory parameter (Pole et al. 1994, Cottingham and Schindler 2000). We used a dynamic linear model with a single parameter, μ , to iteratively make one-step-ahead predictions of a given response variable at time i based upon the posterior of μ for time $i - 1$ and then used our i th observation and Bayes' rule to estimate the posterior of μ at time i . This process was repeated across the pre-perturbation period (36 time points sampled over 12 weeks) for all response variables, including phosphate concentration, phytoplankton biomass, zooplankton biomass, and whole-pond gross primary production and respiration. Following the perturbation, we quantified stability from each time series based on the extent to which the system deviated from the projections from the pre-perturbation posterior probability distribution. We defined resilience as the inverse of return time, which was estimated as the amount of time a given response variable remained outside of our 90% posterior density intervals following the nutrient perturbation. We also estimated sensitivity as the maximum displacement, which is calculated as the difference between post-perturbation observations and the mean of the final pre-perturbation posterior distribution of each response variable. All DLM fits were conducted using custom scripts written in R (version 2.14). Nonlinear statistical models describing the relationship between terrestrial DOC supply and resilience or sensitivity were fit by maximum likelihood using custom likelihood functions and the `optim()` function in R. Code for DLM and nonlinear statistical models are available online.⁴

⁴ https://github.com/LennonLab/Subsidy_Stability

Reciprocal transplant experiment

We conducted a reciprocal transplant experiment to gain insight into the mechanisms driving patterns of pond stability to the nutrient perturbation observed at the whole-pond scale. Specifically, the experiment was designed to tease apart two important properties of terrestrial DOC: its lability (i.e., resource quality) and its ability to attenuate photosynthetically active radiation. We deployed a total of 32 microcosms (1-L bottles) in a low-DOC pond (no Super Hume) and a high-DOC pond (225 g/m^2 Super Hume). The replicated ($n = 4$) three-way, full-factorial design manipulated labile carbon, light availability, and water source. First, microcosms were filled with unfiltered source water from the low-DOC pond or water from the high-DOC pond. Then, half of the microcosms received labile carbon (2.9 mg of glucose). Last, light was manipulated by incubating microcosms in either the low-DOC pond (high light) or high-DOC pond (low light). We initiated the experiment by suspending the microcosms in the middle of each pond at 0.5 m after adding 0.05 mg of phosphorus (Na_2HPO_4) and 0.95 mg of nitrogen (NH_4NO_3) to each microcosm to mimic the nutrient perturbation applied to each pond. Phosphate was quantified prior to and after 48 h of in situ incubation using the limnological procedures described in *Methods: Pond responses along the DOC gradient*. From this, we estimated phosphate uptake rates for each microcosm and evaluated the three main effects (labile carbon, light availability, and source pond) and their interactions using a three-way analysis of variance (ANOVA).

RESULTS

Pond responses to terrestrial DOC supply

The manipulation of terrestrial DOC supply had strong effects on the chemical, physical, and biological properties of the pond ecosystems (Fig. 1). There was a positive correlation between terrestrial DOC supply and the concentration of DOC in the ponds (Fig. 1C). Light attenuation linearly increased with terrestrial DOC supply (Fig. 1D), which created a shading effect that negatively influenced whole-pond gross primary production and respiration (Fig. 1E, F). Across the terrestrial DOC supply gradient, we observed a 74% decline in gross primary productivity (GPP; Fig. 1E) and a 65% decline in whole-ecosystem respiration along the DOC gradient (Fig. 1F). Despite this, bacterial contributions to basal energy mobilization (GPP + BP) and respiration increased with terrestrial DOC supply (Fig. 1G). Finally, nutrient turnover rates in the water column declined with increasing terrestrial DOC supply (Fig. 1H). Table 1 provides parameter estimates and errors, coefficient of determinations (R^2), and P values associated with the linear models describing the pond responses to terrestrial DOC supply prior to the pulse nutrient addition.

TABLE 1. Parameter values and summary statistics describing the relationships between terrestrial dissolved organic carbon (DOC) supply (g C/m^2) and limnological response variables in the 80-d period preceding the pulse nutrient perturbation.

| Response variable | Intercept | Slope | R^2 | P |
|---|-----------------|---------------------|-------|---------|
| DOC (mg/L) | 6.2 (0.82) | 0.045 (0.006) | 0.88 | <0.0001 |
| Light extinction, K_d (m^{-1}) | 2.1 (0.80) | 0.028 (0.006) | 0.73 | 0.0016 |
| Gross primary productivity, GPP ($\text{mg O}_2\text{-L}^{-1}\text{-d}^{-1}$) | 3.7 (0.3) | -0.012 (0.002) | 0.79 | 0.0006 |
| Ecosystem respiration, R ($\text{mg O}_2\text{-L}^{-1}\text{-d}^{-1}$) | 5.3 (0.48) | -0.014 (0.035) | 0.67 | 0.004 |
| Bacterial productivity | 0.19 (0.025) | 0.007 (0.0002) | 0.66 | <0.0001 |
| Bacterial respiration | 0.13 (0.027) | 0.0008 (0.00020) | 0.66 | 0.005 |
| Nutrient turnover rate, pond 10 removed (h^{-1}) | 1.23 (0.08) | -0.005 (0.0006) | 0.89 | <0.0001 |
| Nutrient turnover rate, all ponds (h^{-1}) | 0.80 (0.205) | -0.002 (0.0015) | 0.47 | 0.021 |

Notes: Bacterial productivity represents the amount of bacterial productivity divided by the sum of gross primary productivity and bacterial productivity, while bacterial respiration represents the amount of bacterial respiration divided by ecosystem respiration. Standard errors of the parameter estimates are reported in parentheses. Values in this table correspond with panels in Fig. 1.

Subsidy–stability relationship

Terrestrial DOC supply had a strong effect on food web and ecosystem stability in response to the nutrient perturbation. Using dynamic linear modeling (DLM), we estimated resilience from the phosphorus time-series data and observed an abrupt transition in aquatic ecosystem stability along the terrestrial DOC gradient. Ponds receiving low inputs of terrestrial DOC ($0\text{--}100 \text{ g C/m}^2$) were resilient and removed phosphate from the water column in less than 24 h (Fig. 2). However, ponds receiving elevated inputs of terrestrial DOC ($100\text{--}210 \text{ g C/m}^2$) recovered slowly from the nutrient pulse ($>10 \text{ d}$ in some cases), which led to the accumulation of bioavailable phosphorus in the water column (Fig. 2). The nonlinear relationship between terrestrial DOC supply and resilience was well described by a sigmoidal model (Fig. 2, Table 2).

Terrestrial DOC supply also determined how higher trophic levels and whole ecosystem metabolism responded to the nutrient pulse. The sensitivity of algal biomass, zooplankton biomass, gross primary productivity, and ecosystem respiration exhibited complex, nonlinear responses along the DOC gradient (Fig. 3, Table 3). Interestingly, the maxima of the hump-shaped sensitivity relationships all occurred near the DOC supply threshold ($50\text{--}100 \text{ mg C/m}^2$) that was observed for ecosystem resilience (Fig. 2). To facilitate comparison of sensitivities among food web and ecosystem properties, we scaled the maximum displacement by the pre-perturbation mean for each response variable in each pond. This approach revealed that mean scaled displacement was highest for algal biomass (3.1) followed by zooplankton biomass (0.19), gross primary productivity (0.05), and respiration (0.04; see Appendix).

Inferences from reciprocal transplant

The results from our reciprocal transplant experiment confirmed the patterns that we observed in the whole-pond experiment. Specifically, we identified strong two-way interactions between the light environment and carbon quality and the source pond and carbon quality (Table 4). When microcosms were incubated in their home environment, phosphorus uptake rates were 90% greater in the low terrestrial DOC pond than in the high terrestrial DOC pond. Our results suggest that light limitation contributed appreciably to this pattern. This was evidenced by a 21% reduction in phosphorus uptake rates when microcosms with source water from the low terrestrial DOC pond were incubated in the pond with high terrestrial DOC, and conversely by a 71% increase in phosphorus uptake rates when microcosms with source water from the high terrestrial DOC pond were incubated in the pond with low terrestrial DOC (Fig. 4).

In addition to the effects of light limitation, our results indicate that phosphorus uptake rates were influenced by the quality of terrestrial DOC (Table 4). The suppression of phosphorus uptake by terrestrial DOC could almost entirely be alleviated through the addition of labile carbon (Fig. 4). For example, in microcosms with source water from the high terrestrial DOC pond, phosphorus uptake rates increased by 61–88% depending on the light environment. In contrast, labile carbon had a much smaller effect (3–23%) on phosphorus uptake rates in microcosms with source water from the low terrestrial DOC pond.

DISCUSSION

Inputs of terrestrial DOC significantly altered the flows of energy and nutrients in the recipient aquatic ecosystems. Despite overall reductions in gross primary productivity (GPP) and respiration (R), heterotrophic

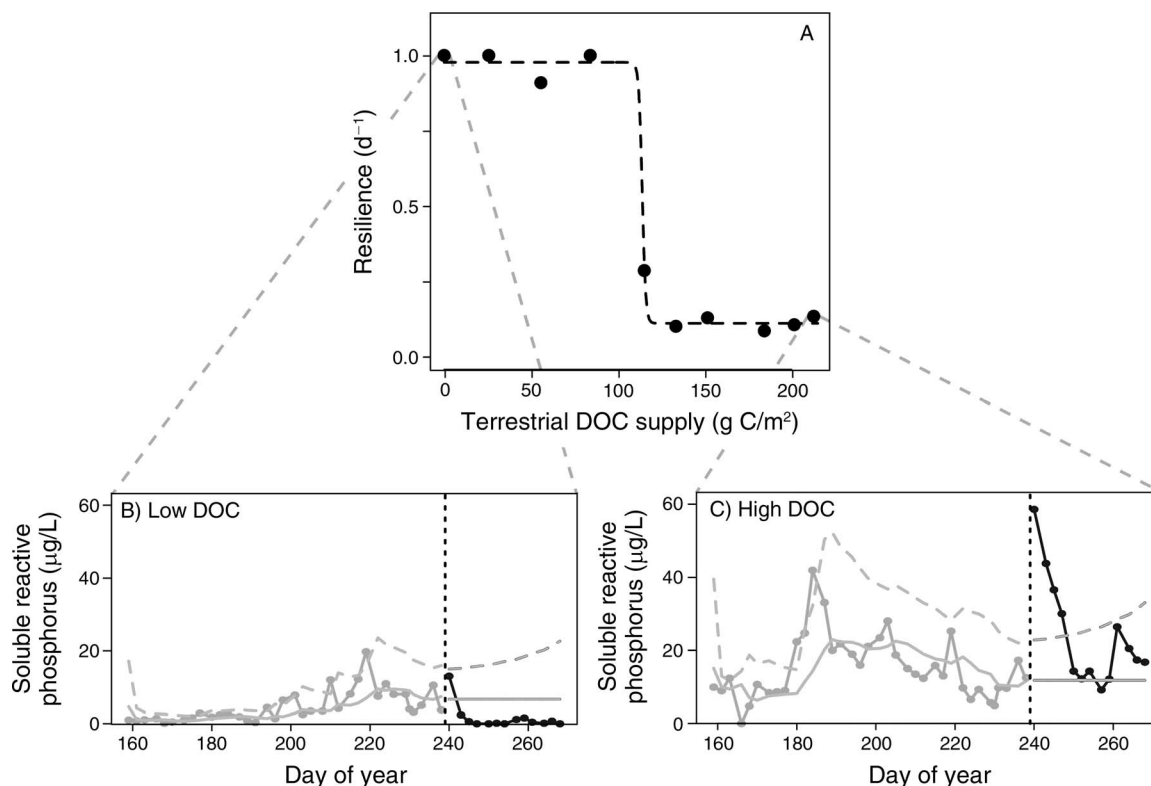


FIG. 2. (A) Pond resilience (measured as inverse of return time) to a nutrient pulse decreased nonlinearly along the DOC supply gradient. Panels (B) and (C) show the time courses for phosphate concentrations in (B) a pond with low terrestrial DOC supply and (C) a pond with high terrestrial DOC supply. The inorganic nutrient perturbation was conducted on day 81 of the experiment (day of year, 239) as indicated by the vertical dashed lines in the lower panels. Dynamic linear model (DLM) predictions and 90% posterior density intervals are shown for the pre-perturbation period (gray lines) where model parameters were updated after each observation. Black lines indicate prediction intervals for the post-perturbation period. See Table 2 for the statistical model, parameter estimates (dashed line), and summary statistics.

bacteria were subsidized by terrestrial carbon, and their relative contribution to ecosystem processes increased along the DOC gradient (Fig. 1). These responses to terrestrial DOC supply led to a strong, nonlinear decline in aquatic ecosystem resilience (Fig. 2), while also altering the sensitivity of other food web and ecosystem properties (Fig. 3). Our results suggest that the light-attenuating properties of terrestrial DOC destabilized pond ecosystems by directly and indirectly reducing nutrient turnover rates of planktonic communities (Fig. 4). Although terrestrial DOC is often considered a resource subsidy for aquatic food webs, our results suggest that it strongly modifies the chemical and physical environment in ways that alter the stability of aquatic ecosystems. As such, the browning of inland water bodies may have consequences for the reliability of aquatic ecosystem services.

Effects of terrestrial DOC supply on aquatic ecosystems

There has been a long-standing debate regarding the effect of terrestrial DOC on the structure and function of aquatic ecosystems. Historically, aquatic habitats with high concentrations of humic substances have been

classified as dystrophic (i.e., ill-fed; Williamson et al. 1999). In part, this is due to the fact that terrestrial DOC has chromophoric properties that absorb solar radiation and inhibit the growth of photosynthetic organisms. Consistent with previous studies (Carpenter et al. 1998, Hanson et al. 2003, Ask et al. 2009, Karlsson et al. 2009), we found that the shading effects of terrestrial DOC can reduce the biomass production and metabolism of aquatic ecosystems (Fig. 1). In addition to its effects on the underwater light environment, terrestrial

TABLE 2. Parameter values and summary statistics for the nonlinear relationship describing resilience (d⁻¹) as a function of terrestrial DOC supply (g C/m²).

| Parameter | Estimate |
|-----------------------|----------|
| <i>a</i> | 117.93 |
| <i>b</i> | -1.04 |
| <i>c</i> | 0.11 |
| <i>d</i> | -7.152 |
| <i>R</i> ² | 0.99 |

Notes: Data were fit using maximum likelihood with a scaled and shifted logistic function that had the following form: $y = a \times (\exp(b + c \times \text{supply}) / (a + \exp(b + c \times \text{load}))) + d$. Values in this table correspond with results presented in Fig. 2.

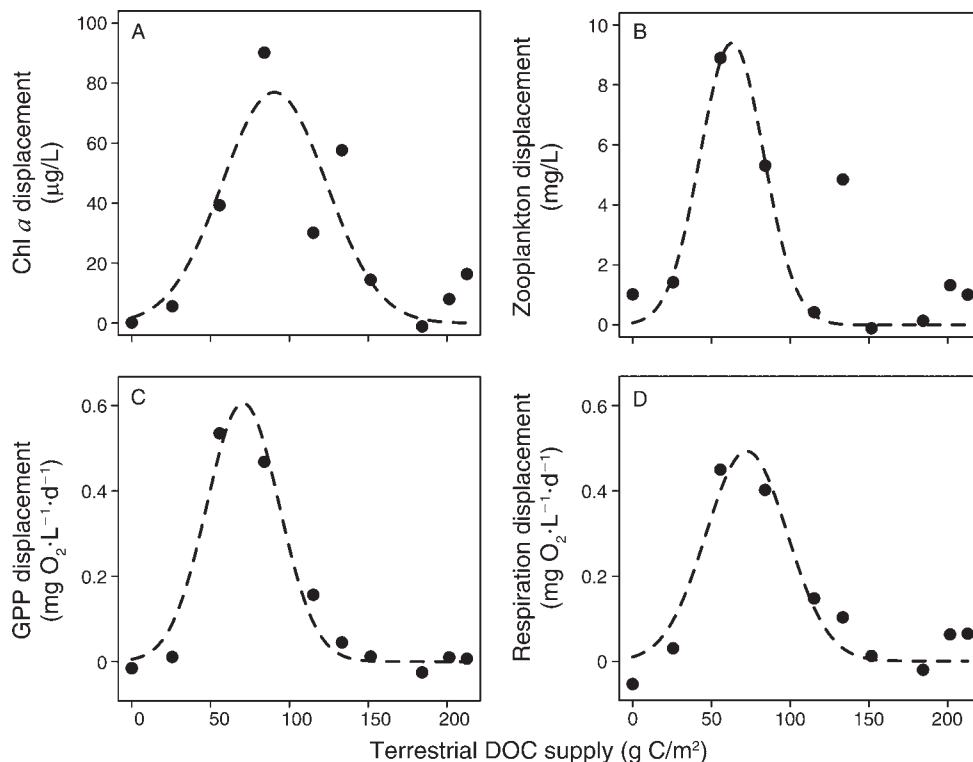


FIG. 3. Sensitivity (measured as maximum displacement) of (A) chlorophyll *a*, (B) zooplankton biomass, (C) gross primary production, and (D) ecosystem respiration in response to an inorganic nutrient perturbation along a terrestrial DOC supply gradient. Sensitivity was calculated as the largest difference between the pre-perturbation mean of the dynamic linear model (DLM) posterior probability distribution and state variable observations following the nutrient perturbation. Dashed lines are Gaussian functions fit to observed data using maximum likelihood parameter estimation. See Table 3 for the statistical model, parameter estimates, and summary statistics.

DOC can influence the structure and function of aquatic ecosystems via its effects on nutrient cycling. For example, phosphorus turnover rates during the growing season of a humic lake were more than an order of magnitude lower than the phosphorus turnover rates measured in a clearwater lake (Jones 1990). Our results demonstrate that phosphorus turnover rates decreased linearly with increasing supply of terrestrial DOC (Fig. 1H). Based on ecosystem theory, these reductions in nutrient turnover rates (NTR) that were observed in the water column should lead to a reduction in aquatic

TABLE 3. Parameter values and summary statistics describing the relationships between terrestrial DOC supply (g C/m^2) and the sensitivity (inverse of resistance) of food web and ecosystems characteristics.

| Response variable | <i>a</i> | <i>b</i> | <i>c</i> | R^2 |
|---|----------|----------|----------|-------|
| Chlorophyll <i>a</i> ($\mu\text{g/L}$) | 77.0 | 90.4 | 33.1 | 0.74 |
| Zooplankton biomass (mg/L) | 9.4 | 66.0 | 20.1 | 0.65 |
| GPP ($\text{mg O}_2\cdot\text{L}^{-1}\cdot\text{d}^{-1}$) | 19.0 | 70.6 | 23.0 | 0.96 |
| R ($\text{mg O}_2\cdot\text{L}^{-1}\cdot\text{d}^{-1}$) | 15.4 | 72.5 | 25.8 | 0.90 |

Notes: Data were fit using maximum likelihood with a Gaussian function that had the following form: $y = a \times \exp(-(\text{supply} - b)^2 / (2 \times c^2))$. Values in this table correspond with results presented in Fig. 3.

ecosystem stability (DeAngelis 1992, Cottingham and Carpenter 1994).

While humic substances appear to depress whole ecosystem metabolism, growing evidence suggests that terrestrial carbon can still be a major source of energy that fuels aquatic food webs (Carpenter et al. 2005). In

TABLE 4. ANOVA table describing the results from a full-factorial reciprocal transplant experiment designed to assess properties of terrestrial DOC on phosphorus uptake rates in aquatic ecosystems.

| Source | df | SSE | MSE | <i>F</i> | <i>P</i> |
|--|----|--------|--------|----------|----------|
| Source water | 1 | 0.9765 | 0.9765 | 604.8 | <0.001 |
| Light | 1 | 0.1000 | 0.1000 | 61.9 | <0.001 |
| Labile carbon | 1 | 1.0785 | 1.0785 | 668.0 | <0.001 |
| Pond \times light | 1 | 0.0024 | 0.0024 | 1.5 | 0.24 |
| Pond \times labile carbon | 1 | 0.4827 | 0.4827 | 298.9 | <0.001 |
| Light \times labile carbon | 1 | 0.0762 | 0.0762 | 47.2 | <0.001 |
| Pond \times light \times labile carbon | 1 | 0.0003 | 0.0003 | 0.2 | 0.66 |
| Residuals | 24 | 0.0388 | 0.0016 | | |

Notes: The three-way design manipulated source water used to fill the microcosms (low vs. high terrestrial DOC supply), light availability (incubation in ponds with low vs. high terrestrial DOC supply), and labile carbon (+ vs. - glucose). SSE is sum of squared errors, and MSE is mean-squared error. Values in this table correspond with results presented in Fig. 4.

our study system, we demonstrated that terrestrial DOC could subsidize bacterial communities and potentially higher trophic levels. First, the contribution of heterotrophic bacteria to ecosystem respiration and basal productivity (primary productivity + bacterial productivity) more than doubled along the terrestrial DOC supply gradient (Fig. 1G). Second, using the terrestrial carbon that was added to the ponds (i.e., Super Hume), we were able to isolate a diverse assemblage of aquatic bacteria that readily grew on humic substances as their sole source of carbon and energy (Lennon et al. 2013). Third, we observed that bacterial respiration (BR) significantly increased along the terrestrial DOC supply gradient, but bacterial productivity (BP) was unaffected (Lennon et al. 2013); this finding suggests that terrestrial DOC may be more important for subsidizing catabolic microbial processes than anabolic microbial processes. Last, field and laboratory observations revealed that crustacean zooplankton directly ingested colloidal fractions of the terrestrial carbon that was added to the pond. Life-table experiments demonstrated that supplementing an algal-based diet with humic substances (i.e., Super Hume) increased the fitness of a *Daphnia* clone that was isolated from a DOC-enriched pond (Lennon et al. 2013). Together, these findings suggest that terrestrial carbon had the potential to serve as a donor-controlled subsidy that could reduce aquatic consumer reliance on autochthonous resources, which according to food web theory (Huxel and McCann 1998) should affect pond stability.

Terrestrial carbon-reduced aquatic ecosystem stability

After establishing a terrestrial DOC gradient in the experimental ponds, we quantified stability by measuring the response of each system to a pulse of inorganic nutrients. Not only is this type of perturbation common in aquatic ecosystems (Nowlin et al. 2008), it is also used to test theoretical predictions regarding the stability of ecological communities. For example, pulsed resources can initiate transient dynamics that can have long lasting effects on food webs (Holt 2008). We observed an abrupt transition in aquatic ecosystem stability along the terrestrial DOC supply gradient with highly subsidized systems possessing dramatically reduced resilience to nutrient perturbation.

The results from our study are somewhat inconsistent with predictions from food web theory. Assuming that consumers can feed on autochthonous and allochthonous resources, low to moderate inputs of allochthonous resources should increase the stability of the recipient ecosystem (Huxel and McCann 1998). In simulations where ecosystems receive high levels of subsidies, however, food webs can become decoupled from locally controlled resources, which leads to the loss of species and stability (Huxel and McCann 1998).

Our results are more in line with expectations from ecosystem theory, which links stability to nutrient turnover rate (NTR). NTR is defined as the ratio of

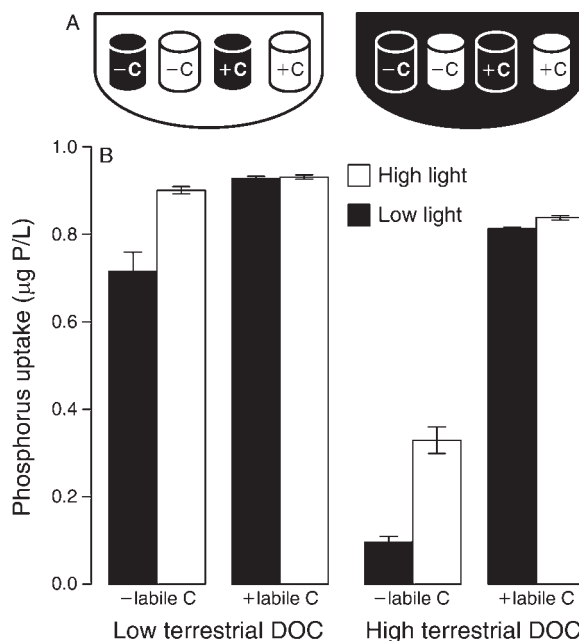


FIG. 4. We conducted a reciprocal transplant experiment to assess effects of terrestrial DOC properties on phosphorus uptake rates (mean \pm SD) in the pond systems. (A) The full factorial experiment ($n = 4$) involved filling 32 microcosms (cylinders) with source water from a pond with low terrestrial DOC (white) or a pond with high terrestrial DOC (black). We manipulated the effects of DOC-mediated light attenuation by incubating microcosms in different ponds at 0.5 m depth. Last, we assessed the effect of DOC quality by adding labile C (glucose) to half of the microcosms (+C) relative to controls (-C). Phosphorus uptake rates for the reciprocal transplant experiment are shown in panel (B). See Table 4 for ANOVA and summary statistics.

nutrient flow to nutrient standing stock and is proportional to other metrics of resilience, namely the dominant eigenvalue (λ_{\max}) from the linear model of a given system (Cottingham and Carpenter 1994). An ecosystem with a high NTR has the ability to quickly process a nutrient pulse and return to pre-perturbation conditions (Odum and Pinkerton 1955, O'Neill 1976, DeAngelis 1980). We observed that NTR in the water column decreased linearly along a terrestrial DOC gradient (Fig. 1H) possibly due to the fact that humic substances adsorb mineral nutrients (de Haan et al. 1990), inhibit enzymatic processes (Hättenschwiler and Vitousek 2000), and reduce microbial activity via light attenuating properties and biochemical recalcitrance (Wetzel 1995, Lischke et al. 2014).

Mechanistic insight into diminished resilience

We hypothesized that the nonlinear subsidy-stability relationship (Fig. 2) reflects the fact that terrestrial DOC simultaneously acts as a resource subsidy and an ecosystem regulator (Williamson et al. 1999). As a resource subsidy, terrestrial DOC can decouple producer-decomposer interactions and promote phosphorus

uptake by heterotrophic bacteria (Grover 2000), a compensatory mechanism that should enhance ecosystem stability in response to a nutrient pulse. Terrestrial DOC is also an ecosystem regulator, which may reduce aquatic stability in response to nutrient perturbations via two non-mutually exclusive ways. First, the shading properties of terrestrial DOC should directly reduce phosphorus uptake by light-limited primary producers (Fig. 1D). Second, if there is strong producer–decomposer coupling, terrestrial DOC should indirectly reduce phosphorus uptake by heterotrophic bacteria that rely on the labile carbon that is released by primary producers (Cole et al. 1988).

To tease apart the complex effects of terrestrial DOC on nutrient processing, we performed a full-factorial reciprocal transplant experiment crossed with a labile carbon amendment. The strong response of phosphorus uptake rates to light and labile carbon suggests that the reduced uptake of phosphate was not due to terrestrial DOC-mediated immobilization (Jackson and Hecky 1980) or inhibition of microbial enzyme activity (Hättenschwiler and Vitousek 2000). Instead, our findings suggest that the role of terrestrial DOC as a resource subsidy is limited by its chemical recalcitrance, while the ecosystem regulating effects of terrestrial DOC (i.e., direct and indirect effects of shading) are primarily responsible for destabilizing aquatic ecosystem functioning.

Terrestrial carbon altered the flow of nutrients to higher trophic levels

Our results demonstrate that terrestrial DOC inputs may determine how higher trophic levels and ecosystem processes respond to nutrient perturbations. The sensitivity of algal biomass, zooplankton biomass, gross primary productivity, and ecosystem respiration exhibited a hump-shaped relationship along the terrestrial DOC gradient (Fig. 3). As discussed in the previous section, in ponds receiving high terrestrial DOC supply, the light attenuating properties and recalcitrance of terrestrial carbon diminished the uptake of phosphorus by the phototrophic and heterotrophic microorganisms at the base of the food web. Our results suggest that these two properties limited the flow of nutrient pulses to higher trophic levels. In addition, we observed reduced sensitivity of food web and ecosystem processes to the nutrient pulse in ponds that received low DOC supply. This result was somewhat surprising since phosphate concentrations were rapidly depleted in low-DOC ponds following the nutrient pulse (Fig. 2). We hypothesize that benthic communities were responsible in part for sequestering inorganic nutrients from the pulse. In the systems with low DOC supply, light penetrated to the bottom of the ponds (Fig. 1D), which would have allowed for phosphorus uptake by benthic phototrophic microorganisms (i.e., periphyton). Our hypothesis is consistent with expectations that terrestrial DOC supply may

regulate the importance of benthic–pelagic coupling with consequences for whole-ecosystem nutrient dynamics (Vadeboncoeur et al. 2002, Jäger and Diehl 2014). Finally, intermediate rates of DOC supply elicited the most change in planktonic food web and ecosystem processes. Under these conditions, terrestrial DOC concentrations were apparently below the shading threshold of planktonic primary producers, while the sensitivity of zooplankton and ecosystem respiration may have been enhanced owing to synergistic interactions between allochthonous and autochthonous resource supply.

Implications of browning: thresholds of ecosystem stability

The flux of material and energy across habitat boundaries is critical for understanding various ecological processes, including the response of an ecosystem to environmental perturbations. As such, global-change-mediated alterations to subsidy supply are likely to impact the stability of important ecosystem functions (Greig et al. 2012). We documented a threshold response of aquatic ecosystems to increased inputs of terrestrial carbon that are associated with the relatively recent, but widespread browning phenomenon (Roulet and Moore 2006, Monteith et al. 2007). Our findings suggest that small changes in terrestrial DOC supply have the potential to dramatically alter the stability of aquatic ecosystems in response to nutrient inputs. Our findings suggest that aquatic ecosystems with low to intermediate DOC concentrations may be most sensitive to ongoing changes in terrestrial DOC supply and should perhaps be targeted for management (Stanley et al. 2012). For example, approximately half of the world's lakes have DOC concentrations below our observed stability threshold (Sobek et al. 2007). Given that terrestrial DOC concentrations in some aquatic ecosystems are increasing by 1.6–6.25% per year (Findlay 2005, Monteith et al. 2007), we estimate that over the next decade 18–52% of lakes that possess DOC concentrations below the stability threshold are at risk of a precipitous drop in ecosystem stability that would fundamentally alter how aquatic habitats process nutrient inputs. Together, our results indicate that chronic changes in the terrestrial environment may diminish the ability of aquatic ecosystems to respond to common perturbations, such as episodic nutrient influxes, and reduce the capacity for natural ecosystems to provide key nutrient recycling and removal services.

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SUPPLEMENTAL MATERIAL

Ecological Archives

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