Relationships between benthic macroinvertebrate assemblages and the physical environment in sandy SE streams, with emphasis on stream geomorphology, hydrology and watershed disturbance

by

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Sandhills, Disturbance, Macroinvertebrates, Hydrology, Geomorphology, Community Assembly

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Abstract

Biodiversity is predicted to decline over the next 100 years, and stream ecosystems are predicted to be impacted by landuse change. This predicted decline highlights the need to understand sand-bed streams and biotic response to important agents of disturbance in these understudied ecosystems when compared to gravel/cobble, upland streams. In this dissertation, I report on 4 different studies: Chapter 2) I develop and validate models for predicting discharge in sand-bed streams; in Chapter 3) I develop and validate the hydrogeomorphic reference condition; in Chapter 4) I investigate the direct and indirect effects of landuse on relevant system components with structural equation modeling within the theoretical context of the landcover cascade; and in Chapter 5) I conducted an experiment to explore the competing hypotheses that macroinvertebrate sand-bed stream assemblages are either deterministically or stochastically assembled in the face of disturbance intensity. Below I present abridged abstracts for each of the 4 studies presented in this dissertation.

Chapter 2:

Manning's equation is often used to predict discharge when stream gauging becomes logistically untenable. In this chapter, I developed and validated a regional equation that predicts discharge from easily measured channel morphology variables in sand-bed streams of the Southeastern Plains without specific estimation of Manning's n_M. The results of this study suggest that the equation developed should be favored over all other equations presented in the literature to predict in-channel discharge in sand-bed, Southeastern Plains streams.

Chapter 3:

Stream reference condition in sand-bed streams of the Sand Hills ecoregion of the Southeastern, US has not been defined. In this chapter, I develop and validate the hydrogeomorphic reference condition (HGM). The results of this study suggest that the HGM is a physico-chemical and biologically relevant reference condition. Further, the HGM stream class (reference/non-reference) can be determined from easily measured channel morphology variables. The results of this chapter should have regional implications for managing and preserving biodiversity in the SE Sand Hills, and, potentially, for sand-bed streams in general.

Chapter 4:

The landcover cascade (LCC) was developed in SE Blue Ridge streams. These systems are inherently different from lowland, sand-bed streams of the SE Sand Hills. The major question addressed in this chapter is whether the indirect effect of land cover is translated through relevant, hierarchical system components to instream biota in a similar manner as the LCC, and if direct effects of hierarchical system components are similar to those shown in the Blue Ridge. The results of this study suggest that landcover is translated differently to instream biota. Specifically, hydrologic disturbance increased when silviculture increased and decreased when restored long leaf pine decreased. Hydrology was the most important direct system component and indirect cascade to biota resulting from land use change.

Chapter 5:

How communities are assembled in the face of disturbance is an on-going question of interest to both applied and basic ecologist. Empirical data suggest 2 contrasting patterns of assembly under differing levels of disturbance intensity along a stochastic/deterministic gradient. In order to rectify this discrepancy and to explore how stream communities are assembled in the face of disturbance intensity, I conducted a randomized complete block field experiment with 5 levels of disturbance and an un-manipulated stream-bed control. The results of this study suggest that streams communities are assembled deterministically at intermediate levels of disturbance. Further, this study is the first to show the appropriate conditions for deterministic filtering are satisfied, and to show that intermediate levels of disturbance show traits that are similar to the stream-bed controls suggesting stream macroinvertebrates are deterministically assembled at intermediate disturbance.

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Chapter 1. Introduction to the dissertation. (references formatted for Ecological Applications)

Introduction

Biodiversity is predicted to decline worldwide over the next 100 years, and it has never been more important to understand the mechanisms that control biodiversity (Sala et al. 2000). Biodiversity decline is particularly relevant to stream ecosystems because anthropogenic land use change is predicted to have the largest impact on stream ecosystems (Sala et al. 2000). As a result, it is necessary to identify reference stream condition, determine the impacts of land use change, and more generally hydrologic disturbance on instream biota.

Stream ecologists have long known that watershed processes effect instream hydrology, habitat, and resultant biota (Hynes 1970). Often ecological studies in streams require a multiple-scale investigation from the watershed to the local habitat in order to explain macroinvertebrate distributions (Allan et al. 1997, Allan 2004). At the landscape scale, many studies have shown landuse to have a relationship with structuring instream biotic distributions (Walsh et al. 2005, Maloney and Weller 2011, Riseng et al. 2011, Sheldon et al. 2012). At the next level in the hierarchy, hydrology has been called the master variable in stream ecosystems (Power et al. 1995, Hart and Finelli 1999, Lake 2000, Bunn and Arthington 2002). Further, hydrologic disturbance has been suggested as an organizing force in ecological research in streams (Resh et al. 1988), and has been shown to have relationships with functional and compositional measures of instream biota (Maloney et al. 2005, Poff and Zimmerman 2010, Maloney and Weller 2011, among others). Next in the hierarchy, geomorphology has been shown to be indicative of hydrologic disturbance (Hammer 1972, Doll et al. 2002), and to have relationships with instream biota (Bravard et al. 1997, Richards et al. 1997, Burcher et al. 2007, Vandewalle et al. 2010).

Last, habitat has been conceptualized as a “habitat template”, which controls biotic assemblage structure (sensu Southwood [1977, 1988] and Townsend and Hildrew [1994]). The vast majority of the stream ecology literature investigating the link between hierarchical habitat components has focused on upland streams, and, as a result, sand-bed streams are understudied (but see, Benke et al. 1985, Maloney et al. 2005, Maloney and Feminella 2006, Maloney et al. 2008, Kosnicki et al. 2014).

Upland, gravel/cobble streams are generally characterized by high gradients and topographic relief, and consolidated sediments (Wiken et al. 2011) that require bankfull flows to initiate mobility (Doyle et al. 2007). In contrast, lowland, sand-bed streams are generally characterized by low gradients and topographic relief, and unconsolidated sediments (Wiken et al. 2011) that are subject to high bed mobility initiated at discharges as low as the annual mean (Copeland et al. 2005). In addition, these streams have different macroinvertebrate assemblages than upland streams (Feminella 2000). As a result of this sharp contrast between physical conditions of these 2 distinct stream types, it is likely that disturbance in sand-bed streams results in different geomorphic, habitat, and biotic responses than in upland streams. In this dissertation, I investigate all of these different components to elucidate how individual and synergistic effects of these different system components, with a specific interest in hydrologic disturbance, modify macroinvertebrate assemblage structure.

Aquatic macroinvertebrates are the main response organisms in 3 out of 4 data chapters, with the chapter excluding macroinvertebrates (Chapter 2) dealing with predicting the, putatively, most important physical variable affecting macroinvertebrate assemblage structure, hydrology. Aquatic macroinvertebrates have been used extensively for monitoring water quality (Barbour et al. 1996, Barbour et al. 1999, Maxted et al. 2000, among many others), and represent a taxonomic group broadly used to answer stream specific and more general ecological questions (Lepori and Malmqvist 2009, Holomuzki et al. 2010, among many others). In this dissertation, structural and functional aspects of the macroinvertebrate assemblage, with particular emphasis on the latter, are investigated for their relationship with disturbance observationally and experimentally. Specifically, I investigate 4 broad questions in sand-bed streams, and, briefly, summarize the main questions and results of each data chapter in the following paragraphs.

Chapter 2 is a hydrologic modeling study in sand-bed streams of the Southeastern Plains ecoregion, US. In this Chapter, I developed and validated models to predict stream discharge in sand-bed streams of the Southeastern Plains with easily measured channel morphology variables. First, I used leave-one-site-out cross validation to calculate goodness-of-fit metrics, specifically Akaike's Information Criteria (AIC) and Root Mean Square Error (RMSE), to choose the best performing model. Last, I validated the best performing model by utilizing USGS reference stream gauge data to calculate the AIC and RMSE and compared this model with an exhaustive list of models published in the literature. Of all models investigated, my results suggest that the model I developed is the best performing model for the Southeastern Plains, and should be used for discharge prediction when logistics constrain empirical stream gauging. I use the equation derived in this Chapter to derive important hydrologic variables in Chapter 3.

Chapter 3 is a study to determine and validate the hydrogeomorphic reference condition (HGM) in the Sand Hills sub-ecoregion of the Southeastern Plains. To these ends, data from 64 sand-bed streams were collected including a suite of relevant physico-chemical variables and macroinvertebrate assemblage data. I summarized macroinvertebrate data as mean trait values (mT; [Garnier et al. 2004]) utilizing a USGS functional trait database (Vieira et al. 2006). The HGM was developed by performing a robust regression of relevant channel morphology variables to identify the average channel morphology parameters given watershed area, similar to hydraulic geometry relationships. In this way, reference and non-reference streams were identified based on channel morphology variables. Following this, partial least squares linear discriminant analysis was used to validate reference/non-reference streams with physico-chemical and mTs independent of the data used to derive reference/non-reference stream status. The results of this study suggest that the HGM reference/non-reference stream groups are characterized by different physico-chemical and mT variables. This study has broad implications for management of stream ecosystems in the Sand Hills and will further our understanding of hydrologic, geomorphic, and macroinvertebrate functional composition relationships.

Chapter 4 is a study to investigate the relationship of land cover in Sand Hills reference streams with related system variables (e.g., hydrology) and macroinvertebrate functional composition. I used a hybrid structural equation modeling (SEM) approach that combines partial least square SEM and traditional path analysis to investigate land cover's direct and indirect effects on resultant mTs utilizing the landcover cascade (LCC; [Burcher et al. 2007]) as a theoretical basis. To these ends, *a priori* reference streams were utilized to develop and compare literature derived SEMs that represented separate system level hypotheses about how land cover is propagated through intermediate system components. The results of this study suggest that the LCC is different in sand-bed, Sand Hills streams than that observed in Blue Ridge streams where the LCC was developed. Of great importance, is the finding that long leaf pines in the watershed decreased hydrologic disturbance and resulted in a different functional composition to that in streams with pine silviculture in the watershed. This study has potential implications for how upland systems are managed to promote instream biodiversity in the Sand Hills sub-ecoregion of the Southeastern Plains, and suggest that the translation of land use to instream biota may be stream system specific.

Chapter 5 was a field experiment conducted to test the hypothesis that stream communities are deterministically assembled at intermediate disturbance intensities from the regional species pool. To these ends, I conducted a colonization field experiment in a sand-bed stream near Auburn Alabama, US, utilizing 5 levels of disturbance and a stream bed control. The results of this experiment indicate that stream communities are deterministically assembled from the regional species pool based on the traits that they possess. This study has implications for explaining how stream communities are assembled by disturbance, and, may have broader implications of how disturbance assembles communities generally. Finally, Chapter 6 presents general conclusions and future directions derived from the work presented in Chapters 2-5.

Literature Cited

Allan, J. D. 2004. Landscapes and riverscapes: the influence of land use on stream ecosystems. Annual review of ecology, evolution, and systematics **35**:257-284.

Allan, J. D., D. L. Erickson, and J. Fay. 1997. The influence of catchment land use on stream integrity across multiple spatial scales. Freshwater Biology **37**:149-161.

Barbour, M. T., J. Gerritsen, G. E. Griffith, R. Frydenborg, E. McCarron, J. S. White, and M. L. Bastian. 1996. A framework for biological criteria for Florida streams using benthic macroinvertebrates. Journal of the North American Benthological Society **15**:185-211.

Barbour, M. T., J. Gerritsen, B. D. Snyder, and J. B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates, and fish - second edition. EPA 841-B-99-002., Environmental Protection Agency; Office of Water, Washington, D.C. <https://hwbdocuments.env.nm.gov/Los%20Alamos%20National%20Labs/General/1419

5.pdf>.

Benke, A. C., R. L. Henry III, D. M. Gillespie, and R. J. Hunter. 1985. Importance of snag habitat for animal production in southeastern streams. Fisheries **10**:8-13.

Bravard, J., C. Amoros, G. Pautou, G. Bornette, M. Bournaud, M. Creuze des Chatelliers, J. Gibert, J. Peiry, J. Perrin, and H. Tachet. 1997. River incision in south-east France: morphological phenomena and ecological effects. Regulated Rivers: Research & Management **13**:75-90.

Bunn, S. E., and A. H. Arthington. 2002. Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. Environmental Management **30**:492-507.

Burcher, C. L., H. M. Valett, and E. F. Benfield. 2007. The land-cover cascade: relationships coupling land and water. Ecology **88**:228-242.

Copeland, R., P. Soar, and C. Thorne. 2005. Channel-forming discharge and hydraulic geometry width predictors in meandering sandbed rivers. In Proc., 2005 World Water and Environmental Resources Congress: Impacts of Global Change. **ASCE**:1-12.

Doll, B. A., D. E. Wise-Frederick, C. M. Buckner, S. D. Wilkerson, W. A. Harman, R. E. Smith, and J. Spooner. 2002. Hydraulic geometry relationships for urban streams throughout the piedmont of North Carolina. JAWRA Journal of the American Water Resources Association **38**:641-651.

Doyle, M. W., D. Shields, K. F. Boyd, P. B. Skidmore, and D. Dominick. 2007. Channel-forming discharge selection in river restoration design. Journal of Hydraulic Engineering **133**:831-837.

Feminella, J. W. 2000. Correspondence between stream macroinvertebrate assemblages and 4 ecoregions of the southeastern USA. Journal of the North American Benthological

Society **19**:442-461.

Garnier, E., J. Cortez, G. Billes, M.-L. Navas, C. Roumet, M. Debussche, G. Laurent, A. Blanchard, D. Aubry, A. Bellmann, C. Neill, and J.-P. Toussaint. 2004. Plant functional markers capture ecosystem properties during secondary succession. Ecology **85**:2630-2637.

Hammer, T. R. 1972. Stream channel enlargement due to urbanization. Water Resources Research **8**:1530-1540.

Hart, D. D., and C. M. Finelli. 1999. Physical-biological coupling in streams: the pervasive effects of flow on benthic organisms. Annual Review of Ecology and Systematics **30**:363-395.

Holomuzki, J. R., J. W. Feminella, and M. E. Power. 2010. Biotic interactions in freshwater benthic habitats. Journal of the North American Benthological Society **29**:220-244.

Hynes, H. B. N. 1970. The ecology of running waters. University of Toronto Press, Toronto.

Kosnicki, E., S. A. Sefick, M. H. Paller, M. S. Jarrell, B. A. Prusha, S. C. Sterrett, T. D. Tuberville, and J. W. Feminella. 2014. Defining the reference condition for wadeable streams in the Sand Hills subdivision of the Southeastern Plains Ecoregion, USA. Environmental Management **54**:494-504.

Lake, P. S. 2000. Disturbance, patchiness, and diversity in streams. Journal of the North American Benthological Society **19**:573-592.

Lepori, F., and B. Malmqvist. 2009. Deterministic control on community assembly peaks at intermediate levels of disturbance. Oikos **118**:471-479.

Maloney, K. O., and J. W. Feminella. 2006. Evaluation of single-and multi-metric benthic macroinvertebrate indicators of catchment disturbance over time at the Fort Benning

Military Installation, Georgia, USA. Ecological Indicators **6**:469-484.

Maloney, K. O., J. W. Feminella, R. M. Mitchell, S. A. Miller, P. J. Mulholland, and J. N. Houser. 2008. Landuse legacies and small streams: identifying relationships between historical land use and contemporary stream conditions. Journal of the North American Benthological Society **27**:280-294.

Maloney, K. O., P. J. Mulholland, and J. W. Feminella. 2005. Influence of catchment-scale military land use on stream physical and organic matter variables in small Southeastern Plains catchments (USA). Environmental Management **35**:677-691.

Maloney, K. O., and D. E. Weller. 2011. Anthropogenic disturbance and streams: land use and land-use change affect stream ecosystems via multiple pathways. Freshwater Biology **56**:611-626.

Maxted, J. R., M. T. Barbour, J. Gerritsen, V. Poretti, N. Primrose, A. Silvia, D. Penrose, and R. Renfrow. 2000. Assessment framework for mid-Atlantic coastal plain streams using benthic macroinvertebrates. Journal of the North American Benthological Society **19**:128-144.

Poff, N. L., and J. K. H. Zimmerman. 2010. Ecological responses to altered flow regimes: a literature review to inform the science and management of environmental flows. Freshwater Biology **55**:194-205.

Power, M. E., A. Sun, G. Parker, W. E. Dietrich, and J. T. Wootton. 1995. Hydraulic food-chain models. BioScience **45**:159-167.

Resh, V. H., A. V. Brown, A. P. Covich, M. E. Gurtz, H. W. Li, G. W. Minshall, S. R. Reice, A. L. Sheldon, J. B. Wallace, and R. C. Wissmar. 1988. The role of disturbance in stream ecology. Journal of the North American Benthological Society **7**:433-455.

Richards, C., R. Haro, L. Johnson, and G. Host. 1997. Catchment and reach scale properties as indicators of macroinvertebrate species traits. Freshwater Biology **37**:219-230.

Riseng, C. M., M. J. Wiley, R. W. Black, and M. D. Munn. 2011. Impacts of agricultural land use on biological integrity: a causal analysis. Ecological Applications **21**:3128-3146.

Sala, O. E., F. S. Chapin III, J. J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L. F. Huenneke, R. B. Jackson, A. Kinzig, R. Leemans, D. M. Lodge, H. A. Mooney, M. Oesterheld, N. L. Poff, M. T. Sykes, B. H. Walker, M. Walker, and D. H. Wall. 2000. Biodiversity - global biodiversity scenarios for the year 2100. Science **287**:1770-1774.

Sheldon, F., E. E. Peterson, E. L. Boone, S. Sippel, S. E. Bunn, and B. D. Harch. 2012. Identifying the spatial scale of land use that most strongly influences overall river ecosystem health score. Ecological Applications **22**:2188-2203.

Southwood, T. R. E. 1977. Habitat, the templet for ecological strategies? Journal of Animal Ecology **46**:337-365.

Southwood, T. R. E. 1988. Tactics, strategies and templets. Oikos **52**:3-18.

Townsend, C. R., and A. G. Hildrew. 1994. Species traits in relation to a habitat templet for river systems. Freshwater Biology **31**:265-275.

Vandewalle, M., F. De Bello, M. P. Berg, T. Bolger, S. Doledec, F. Dubs, C. K. Feld, R. Harrington, P. A. Harrison, S. Lavorel, P. Martins da Siva, M. Moretti, J. Niemela, P. Santos, T. Sattler, J. P. Sousa, M. T. Sykes, A. J. Vanbergen, and B. A. Woodcock. 2010. Functional traits as indicators of biodiversity response to land use changes across ecosystems and organisms. Biodiversity and Conservation **19**:2921-2947.

Vieira, N. M. K., N. L. M. Poff, D. M. Carlisle, S. R. Moulton II, M. L. Koski, and B. C.

Kondratieff. 2006. A database of lotic invertebrate traits for North America. U.S. Geological Survey. <http://pubs.usgs.gov/ds/ds187/>.

Walsh, C. J., A. H. Roy, J. W. Feminella, P. D. Cottingham, P. M. Groffman, and R. P. Morgan II. 2005. The urban stream syndrome: current knowledge and the search for a cure. Journal of the North American Benthological Society **24**:706-723.

Wiken, E., F. Jimenez Nava, and G. Griffith. 2011. North American terrestrial ecoregions-level III. Commission for Environmental Cooperation, Montreal, Canada. <http://www3.cec.org/islandora/en/item/10415-north-american-terrestrial-ecoregionslevel-iii-en.pdf>.

Chapter 2: Empirical estimation of stream discharge using channel geometry in low-gradient, sand-bed streams of the Southeastern Plains (Formatted for the Journal of the American Water Resources Association)

Empirical estimation of stream discharge using channel geometry in low-gradient, sand-bed streams of the Southeastern Plains

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ABSTRACT

Manning’s equation is used widely to predict stream discharge (Q) from hydraulic variables when logistics constrain empirical measurements of in-bank flow events. Uncertainty in Manning's roughness (n_M) is the major source of error in natural channels, and sand-bed streams pose difficulties because flow resistance is affected by flow-dependent bed configuration. Our study was designed to develop and validate models for estimating Q from channel geometry easily derived from cross-sectional surveys and available GIS data. A database was compiled consisting of 484 Q measurements from 75 sand-bed streams in Alabama, Georgia, South Carolina, North Carolina (Southeastern Plains) and Florida (Southern Coastal Plain), with 6 New Zealand streams included to develop statistical models to predict Q from hydraulic variables. Model error characteristics were estimated with leave-one-site-out jackknifing. Independent data of 317 Q measurements from 55 Southeastern Plains streams indicated that the model ($Q=A_cR_H^{0.6906}S^{0.1216}$; A_c=channel area, R_H=hydraulic radius, S=bed slope) best predicted Q, based on Akaike's information criterion and root mean square error. Models also were developed from smaller Q range subsets to explore if subsets increased predictive ability, but error fit statistics suggested these were not reasonable alternatives to the above equation. Thus, we recommend the above equation for predicting in-bank Q of unbraided, sandy streams of the Southeastern Plains.

KEY TERMS

Surface-water hydrology, open-channel flow, rivers/streams, channel resistance, Manning’s equation, hydraulics, discharge prediction, sand-bed, Southeastern Plains

INTRODUCTION

The Southeastern Plains is a large ecoregion of the US spanning Maryland to Louisiana with generally low-gradient streams and sandy beds (Maloney *et al.*, 2005, Wiken *et al.*, 2011)**.** Substrate in these lowland streams can be considered mobile because bed mobilization is initiated at flows as low as mean annual discharge (Copeland *et al.*, 2005). In contrast, high-gradient upland mountain streams contain predominately gravel and cobble beds, which require higher and less frequent bankfull discharges for mobilization (Doyle *et al.*, 2007). These contrasting stream types likely require strongly contrasting methods to estimate discharge empirically due to their large differences in bed mobility.

Logistical constraints often preclude empirical measurement of in-bank, instantaneous, high-flow events for developing stage-discharge relationships. The Chezy (1768 reported in Dingman (2009)) and Manning (1891) equations are frequently used to predict discharges from hydraulic variables. The Chezy equation is:

$$Q=CA_cR_H^{1/2}S_f^{1/2} \tag{1}$$

where, Q is the discharge (m^3s^{-1}), A_c is the wetted channel area (m^2), R_H is the channel hydraulic radius (m), S_f is the energy slope (m/m), and C is the reach-specific bed resistance coefficient. Manning (1891) modified Chezy's equation resulting in an empirically preferable equation (Dingman and Sharma, 1997):

$$Q=(1/n_M)A_cR_H^{2/3}S_f^{1/2} \tag{2}$$

where, n_M is Manning's roughness coefficient (in *SI* units). This equation should only be applied when flow is turbulent.

There is uncertainty in estimation of Manning's n_M, which may account for significant error in applying Manning's (1891) equation to natural channels (Dingman and Sharma, 1997; Bjerklie *et al.*, 2005; Lopez *et al.*, 2007). A major source of uncertainty in estimating n_M for natural channels is attributable to a non-rigid and highly dynamic bed (Yen, 1991), which is of particular concern in sand-bed streams because the bed can be mobilized as frequently as values approximating annual average Q (Copeland *et al.*, 2005). Three common methods for estimating n_M are: 1) selecting n_M from a table of typical values based on qualitative description of channel characteristics, 2) selecting n_M from photographs of channels displaying typical values, and 3) estimating n_M empirically using one of several equations relating hydraulic variables to n_M (Chow, 1959). Considerable work has been done relating n_M to in-stream variables in this context, with the Strickler (1923) equation being the most widely used:

$$n_M \cong 0.047 d_{50}^{1/6} \tag{3}$$

where d_{50} (m) is the 50th percentile of the bed particle size distribution (Ferguson, 2010). The Strickler equation provides an estimate of n_M that integrates bed particle size distribution (Strickler, 1923). The non-rigid bed of sand-bed streams also affects accuracy of estimating n_M with the Strickler equation (Yen, 1991).

Equation (3) above is appealing because it provides an empirical estimate of n_M, although n_M may be underestimated (Dingman 2009, Ferguson 2010). Sand-bed channels (generally characterized by $d_{50} \leq 2$ mm) pose particular problems in n_M estimation because bed forms vary with Q, which can affect n_M (Simons and Richardson, 1966; Yen, 2002). Such variation makes it problematic to apply a single value of n_M to model Q in sand-bed streams, and this variability may exacerbate known difficulties in gauging Q accurately (Isaacson and Coonrod, 2011).

Brownlie (1983) developed equations for predicting velocity and depth of flow in sand-bed streams, which can be rearranged and substituted directly for Manning's equation to predict Q from hydraulic variables (Brownlie, 1983). These equations are an improvement over estimating n_M for use in Manning's equation, but they require empirical estimates of median and geometric standard deviation of bed particle size distributions. Further, site-specific estimates of near-bed conditions and bed particle size distributions can be time- and/or cost-prohibitive, thus limiting utility of the Brownlie (1983) equations in regional assessments.

To overcome the above difficulties in estimating n_M, researchers have fit empirical regression models to hydraulic geometry and Q data without specific n_M estimation (Riggs, 1976; Bray, 1979; Dingman and Sharma, 1997; Bjerklie *et al.*, 2003, 2005; Lopez *et al.*, 2007). Dingman and Sharma (1997) and Bjerklie *et al.* (2003, 2005) fit statistical models to hydraulic variables to predict Q using a Manning-like model irrespective of bed particle composition, and accurately predicted Q at levels > 3 m^3s^{-1}. Lopez *et al.* (2007) conducted a similar analysis constraining geomorphic setting to rocky mountain streams and investigated the effect of restricting the range of data on model prediction accuracy. This restriction resulted in greatly improved accuracy when $Q > 0.1$ m^3s^{-1}, thus demonstrating that limiting the range of Q and geomorphic condition can increase Q prediction accuracy. However, none of the above studies were limited to sand-bed streams. Properties of sand-bed streams, such as variable n_M at contrasting Q, or the necessity of quantifying bed-particle size distribution, may have precluded model construction on these systems. Here we describe predictive models developed for sand-bed streams in the Southeastern Plains, USA (SE Plains). This study is the first to develop empirical Q estimation models for sand-bed streams incorporating only A_c, R_H, and S without the need to estimate bed particle size distribution, or n_M. Our primary objective was to develop and validate empirical models for estimating Q from easily measured channel geometry and GIS variables to overcome known difficulties in estimating n_M in low-gradient, sand-bed streams of the SE Plains ecoregion. The utility of such models will be to increase discharge estimation accuracy when empirical characterization of in-bank, high-flow events is unattainable or impractical.

METHODS

Data for model construction came from 75 sand-bed streams (i.e., streams with $d_{50} \leq 2$ mm or visually field verified; Fig. 1 A and B) comprising 484 Q measurements, which represented model training data. Sixty-nine of these sites were in the US coastal plains including Florida (10 sites), Alabama (21), Georgia (12), South Carolina (15), and North Carolina (11) (Fig. 2A). The coastal plains physiographic province consists of the SE Plains and Southern Coastal Plain ecoregions (Fenneman, 1917; Omernik, 1987). Florida sites were in the Southern Coastal Plain and all other sites were in the SE Plains. We included 6 sand-bed streams from New Zealand (Hicks and Mason, 1991) (Fig. 2B). Published data from New Zealand (Hicks and Mason, 1991) and Florida (Gillen, 1996) were included to increase the upper range of Q values not provided by the SE Plains data, and thus broaden model applicability. Fifty-nine of the 69 sites in the US coastal plains were unpublished data we collected from the SE Plains. Data from these 59 sites were from small watersheds (median area = 12.72 km^2, Strahler (1957) order 1-4), and were generally forested (personal observation). Model validation sites from the SE Plains, independent of the training data, consisted of 33 US Geological Survey (USGS) sites, and an additional 22 sites we collected; the total number of Q values from validation sites was 317.

The training database consisted of 3 hierarchical groups of measurements: reduced, southeastern, and full database (Table 1). We used these groups to investigate if decreasing the range of modeled data resulted in higher prediction accuracy. The *reduced database* consisted of the 344 Q measurements from 59 unpublished sites we collected, with the largest observed Q = 2.64 m^3s^{-1}. The *southeastern database* included an additional 104 Q measurements from the 10 Florida sites reported in Gillen (1996), with the largest observed Q = 85.2 m^3s^{-1}. The *full database* included an additional 36 Q measurements from 6 New Zealand sites reported in Hicks and Mason (1991), with the largest observed Q = 874 m^3s^{-1}.

For data we collected in the SE Plains, Q was estimated at cross sections perpendicular to the direction of flow using the velocity-area method (Gore, 1996) at fixed intervals across the channel. Velocity (V) was quantified with a FlowMate current meter (Hach Company, CO) and depth was measured to the nearest 0.5 cm. Wetted channel area was estimated by summing the area of the width x depth trapezoids formed by the water surface and bed, respectively, for each Q cell (A_{cell}). Q was estimated by summing $V*A_{cell}$ for the cross section. We estimated the wetted perimeter (P_w) by summing the bed segments for each Q cell from water surface to water surface. Hydraulic radius (R_H) was calculated as $R_H=A_c/P_w$. R_H, Slope (S), A_c, and Q were reported in the NZ and Florida datasets. For methods used to quantify these variables, the reader is directed to Hicks and Mason (1991) and Gillen (1996).

We assumed energy slope was equal to bed slope (S), with S estimated from high-resolution digital elevation models (DEMs, usually 10 m) or from S values reported in the National Hydrography Plus Dataset (Horizon Systems Cooperation, accessed 1 April 2012, http://www.horizon-systems.com/nhdplus/index.php) in GRASS GIS (Neteler *et al.*, 2012). Map-derived S can be used to achieve similar Q prediction accuracy to slopes measured empirically (Bjerklie *et al.*, 2003, 2005).

We constructed predictive models as follows. First, we used a simple logarithmic function based on exploratory graphical analysis of empirical Q data (model 4). Second, we fit 3 models from Bjerklie *et al.* (2003, 2005; models 5-6) and Dingman and Sharma (1997; model 7) to data from the 3 databases after transforming them with the natural logarithm (models 5-7). We fit models using ordinary least squares regression (OLS) (function lm; R Core Team, 2014) and assessed OLS assumptions with standard residual plots.

$$lnQ=K+alnA_c \tag{4}$$

$$lnQ=K+lnA_c+blnR_H+clnS \tag{5}$$

$$lnQ=K+alnA_c+blnR_H+clnS \tag{6}$$

$$lnQ=K+alnA_c+blnR_H+cln^2S \tag{7}$$

For each model investigated, we fit 2 models with K set to 0 to investigate model fit through the origin and K estimated with OLS to investigate model performance under these 2 contrasting conditions. We modified the leave-one-out jackknife method (McCuen, 2005) by leaving an entire site out at a time (“leave-one-site-out jackknifing”) to assess model predictive accuracy. Briefly, we systematically excluded all observations from one of n sites (n=75 for training data) from the database and fit the model to the remaining (n-1) sites, a step we repeated until all sites had been removed once. Exclusion of entire sites ensured that the data used to estimate goodness-of-fit statistics for the model were independent from those used to develop the model. We then used variables from the excluded site to predict Q from the model, followed by calculating several diagnostic statistics to assess model predictive accuracy (Table 2). Goodness-of-fit statistics were calculated using the antilogs of simulated and observed Q values, Q_{sim} and Q_{obs}, respectively. We used Akaike's information criteria (*AIC*), calculated following Lopez *et al.* (2007) (Table 2), to compare models fit to the same dataset, whereas we used root mean square error (*RMSE*) to compare predictive accuracy of models fit on the same and different datasets (Table 2). We used *AIC* because it selects the model explaining the most information in the empirical data out of the set of models investigated when the value is at a minimum (Burnham and Anderson, 2002). In addition, *RMSE* is useful for comparing models because it provides a measure of predictive uncertainty in the same units as the dependent variable. Both *AIC* and *RMSE* are designed to decrease as predictive accuracy increases (Helsel and Hirsch, 2002). Nonparametric bias correction was assessed to investigate the potential bias introduced by ln transformation and the effect on jackknife predictive accuracy using the method reported in Helsel and Hirsch (2002). The correction factor, i.e., $B=(\sum(Q_{obs}-Q_{sim}))/\mathrm{N}$, is multiplied by Q_{sim} resulting in a corrected estimate. We used this procedure because Dingman and Sharma (1997) used it to correct their regression models; however, they did not assess whether this correction increased prediction accuracy.

In addition to the metrics summarized in Table 2, robust summaries of % error, i.e. $100*(Q_{sim}-Q_{obs})/Q_{obs}$ also were used to assess model predictive accuracy. In doing this, median instead of the mean and Rousseeuw and Croux's (1993) Qn scale estimator (*Qn*) instead of the standard deviation where used as robust measures of location and scale because of the potential outliers in USGS data (Asquith *et al.*, 2013). We used median and *Qn* of % error for both the jackknifed training data and the independent data for comparability. Summarizing % error with previously described robust statistics instead of removing outliers was done because these statistics perform similarly to the mean and standard deviation when the data do not contain outliers; however, when outliers exist, they perform better than their non-robust equivalents (Maronna *et al.*, 2006).

We calculated summary statistics of % error for 16 discrete segments of the data (bins) from $e^{-9.1}$ to $e^{7.1}$ by increasing the exponent of e by 1 (e.g., $e^{-9.1}$ m^3s^{-1}; $e^{-8.1}$ m^3s^{-1}; … ; $e^{5.1}$ m^3s^{-1}; $e^{6.1}$ m^3s^{-1}; $e^{7.1}$ m^3s^{-1}). We used binning to divide the continuous Q interval to facilitate calculation of descriptive statistics (i.e., median and *Qn* of % error). We chose the number of bins to contain the range of Q values in the full database. Last, it was important to identify the lower bound of Q prediction accuracy; therefore, we used logarithmic bins to provide increased resolution regarding error statistics in the lower range of Q examined.

We compared prediction accuracy of our models with those of other published studies (Golubtsov, 1969; Riggs, 1976; Williams, 1978; Bray, 1979; Jarrett, 1984; Meunier, 1989; Sauer, 1990; Dingman and Sharma, 1997; Bjerklie *et al.*, 2003, 2005; Lopez *et al.*, 2007). Only Manning's equation required estimation of n_M, which was estimated as 0.035 (Table 6.5, page 248 in Dingman, 2009), and used to simulate the typical application of Manning's equation in sand-bed streams. This value was within the range calculated using the method of Cowan (1956) as reported in Arcement and Schneider (1989; n_M range 0.028-0.1105), and was used as the baseline to compare values derived from Manning's equation to our models. Manning's equation using OLS to fit n_M as a model parameter, was not investigated because previous studies documented the functional relationship of Q with A_c, R_H, and S was different from that of Manning's equation (Dingman and Sharma, 1997; Bjerklie *et al.*, 2003, 2005; Lopez *et al.*, 2007; others). After fitting model (5) to the full database, we tested the R_H and S coefficients for significant differences from those of Manning's equation.

We selected model validation sites from USGS reference gauges reported in Falcone *et al.* (2010), and from our sample of independent gauged sites (i.e., field-verified sand-bed streams; Fig. 1 A and B). Only USGS Q values with quality scores of "good" or "excellent", and from sites with predominately sand substrate were included in the validation database. The quality scores represent Q values $\leq$ 5 % of actual field-measured Q (U.S. Geological Survey, National Water Information System, accessed 6 June 2013, http://waterdata.usgs.gov/nwis/help?codes_help#rated). We accessed ratings table data from the USGS website (U.S. Geological Survey, National Water Information System, accessed 6 June 2013, http://waterwatch.usgs.gov/ accessed from the gauge website "field measurements" link). R_H values were not given as part of the USGS ratings tables. All 3 models given in (5)-(7) require R_H, so we constructed a model relating A_c, wetted width (W), and mean depth (D as A_c/W) to R_H from all entries in the full database that included these variables. We fit the model to ln-transformed data to linearize and satisfy OLS assumptions. The exponential form of the resulting equation was:

$$R_{H=}(A_c^{0.24}D^{0.59})/1.79W^{0.0095};\ (R^2=0.97,\ n=418) \tag{8}$$

Equation (8) was used to predict R_H from USGS empirical hydraulic data, as D was likely different from R_H in irregular channels. We then used these data (USGS and independent gauged sites, above) to compare predictive accuracy of the best model developed in this study with those of previous studies. Error properties were assessed by applying the equation developed in this investigation to new data and calculating goodness-of-fit metrics. All analyses were conducted in the R language for statistical computing (Ihaka and Gentleman, 1996; R Core Team, 2014).

RESULTS

Examination of residual plots indicated that OLS assumptions of independence and normality of residuals were met for models developed in this paper, although plots suggested some heteroscedasticity (Fig. 3A-F). With heteroscedasticity present, OLS still provided unbiased parameter estimates (Montgomery *et al.*, 2006).

Prediction accuracy increased with K as a free parameter when model (4) was fit to all databases, compared with K fixed at 0 (*RMSE* mean decrease = 24.77 m^3s^{-1}, *AIC* mean decrease = 1047.49). In contrast, when K was set to 0 and model (5) was fit to all databases, prediction accuracy increased compared with K estimated as a free parameter (*RMSE* mean decrease = 3.87 m^3s^{-1}, *AIC* mean decrease = 337.76). For these reasons, K was allowed to vary when fitting model (4) and set to 0 when fitting model (5) for the remainder of the study.

Of the models investigated and when fit to the full database, model (5), $Q=A_cR_H^{0.6906}S^{0.1216}$, was the best model having the lowest *RMSE* and *AIC*. As a result of this, model (5) was fit to the database under investigation for comparison with model (4), which best fit the southeastern and reduced databases (Table 3). The bias corrected form of model (5), fit to the full database, had lower predictive accuracy than the uncorrected version (uncorrected *RMSE* = 9.1 m^3s^{-1} vs. 23.2 m^3s^{-1} after bias correction). The reduced and southeastern databases were best modeled with a simple logarithmic function of A_c (model 4). However, when fits were compared between models (4) and (5) fit to the reduced and southeastern database respectively, only a negligible increase in *RMSE* occurred: 0.02 m^3s^{-1} and 0.48 m^3s^{-1}. In contrast, model (5), fit to the full database, resulted in a decrease in *RMSE* of 26.4 m^3s^{-1} compared to the model (4) fit (Table 3). The R_H coefficient of model (5), fit to the full database, was not significantly different from the Manning's equation coefficient of 2/3 ($P = 0.504$). In contrast, the estimate of ~1/7 for the *S* coefficient was significantly different from the Manning's equation coefficient of 1/2 ($P <$ 2.2e-16).

When comparing fits of model (5), there was strong concordance between Q_{sim} and Q_{obs} across all 3 databases (Fig. 4A-C). A *Q* value of 0.045 m^3s^{-1} should be considered at the lower end of acceptable model application, as % error did not stabilize until the 0.045 m^3s^{-1} to 0.122 m^3s^{-1} bin (Fig. 4 D-F). The lower-end estimate of model applicability was concordant with the point of significant deviation from the 1:1 line (Fig. 4 A-C). All 3 models showed an absolute value of median % error < 24 % in the 0.045 to 0.122 m^3s^{-1} bin (range=-13 to 24 %), and all models had an absolute value of median % error < 45 % for all other bins with $Q > 0.122$ m^3s^{-1}. Model (5), when fit to the full database, consistently underestimated *Q* by < 30 % between 0.33 m^3s^{-1} to 18.17 m^3s^{-1} (Fig. 4D). At $Q > 18.17$ m^3s^{-1} there also was underestimation bias, although it was consistently < 20 % (Fig. 4D).

When model (5), fit to the full database, and models from the literature were applied to independent data during model validation, model (5) showed the highest predictive accuracy (i.e., lowest *AIC* and *RMSE)*, and a Q_{obs}~Q_{sim} slope closest to 1 (Table 4). Further, hydrologic indicators of goodness-of-fit, the Nash-Suttcliffe efficiency (0.94) and Mass Balance error (0.14) were close to 1 and 0, respectively (Table 4). The Q_{obs} - Q_{sim} relationship using the independent data showed strong concordance ($R^2 = 0.97$, Fig. 5A, Table 4). The absolute value of median error was below the jackknifed results ~ 62% of the time in the range of Q = 0.00224 m^3s^{-1} to 874 m^3s^{-1} (compare Fig. 4D and 5B). The absolute value of % errors for the validation dataset was < 26% for Q 0.00224 m^3s^{-1} to 874 m^3s^{-1}, and resulted in reduced % error when compared to the jackknifed training data results. % error for the 0.000304 m^3s^{-1} to 0.000825 m^3s^{-1} Q bin was the highest for all bins (median = 723%). The median % error for low Q (0.00224 m^3s^{-1} to 1 m^3s^{-1}) was -16.45%, medium Q (1 m^3s^{-1} to 50 m^3s^{-1}) was -19.68 %, and high Q (> 50 m^3s^{-1}) was 19.78%. This pattern suggests underestimation bias in the low and medium range, and an overestimation bias in the high range, but was within ± 20 % of Q_{obs}. Model (5) accurately predicted the independent data for $Q \geq 0.00224$ m^3s^{-1} during model validation (Fig. 5A and B), showing higher prediction accuracy with the independent data than that suggested by jackknifing with test data.

DISCUSSION AND CONCLUSIONS

The hydraulic models developed in our study should be of great utility in estimating at-a-site point discharges when compared to regional regression models or surface water hydraulic models. Regional regression models are simple to apply, but, generally, are used to estimate gross flow parameters such as flood recurrence (Riggs, 1973) or annual mean flow (Vogel *et al.*, 1999) from basin and climatic variables. Surface water hydraulic models, such as HEC-RAS (USACE, 2010), require estimation of n_M. The need to estimate n_M limits its utility because of the error associated with highly mobile sand beds.

The jackknifed and independent data model goodness-of-fit statistics indicated that model (5), when fit to the full database, had high prediction accuracy compared with all other models investigated. This result suggests that this model can be used reliably to predict in-bank discharge from hydraulic characteristics of sand-bed streams in the SE Plains ecoregion. Model (5), fit to the full database, should be preferred for application within the SE Plains ecoregion over all previously published models as it showed the highest predictive accuracy with completely independent data from sites across the expanse of this region. Percent error for training data was < 29% when 0.045 $m^3s^{-1} < Q <$ 874 m^3s^{-1}. There was no reason to favor models from the reduced databases, as % error patterns were similar among the 3 databases. *RMSE* was higher for the larger (full) database, but this result could have been produced by a larger Q range (Table 1). The validation data showed that $Q \geq$ 0.00224 m^3s^{-1} showed an absolute value of % error < 26%, suggesting model (5), fit to the full database, showed good predictive accuracy. Further, validation data resulted in % error within 20% for low, medium, and high Q ranges demonstrating the utility of model (5), fit to the full database, for predicting a wide range of Q. Further, Sauer and Meyer (1992) reported errors in empirically measured Q can range from 2-20% with a typical value of 2-3%. In addition, Pelletier (1988) reported a similar range with a median value of ~ 6%. The model presented in this paper overlaps with high end error estimates for empirically determined Q and, thus should be highly applicable to estimating Q in SE Plains streams when logistics or safety do not allow for in-bank measurement.

The equation that showed the highest predictive accuracy given *RMSE*, *AIC*, and other goodness-of-fit indicators was based on model (5) from Bjerklie *et al.* (2003). The resulting equation corresponding to the validated model (5), fit to the full database, is as follows:

$$Q = A_c R_H^{0.6906} S^{0.1216} \tag{9}$$

The above derived equation should be used for in-bank discharge prediction in unbraided, low-gradient, sand-bed streams of the SE Plains. Conservatively, we suggest that this equation only be used to predict discharge > 0.045 m^3s^{-1} even though independent data showed high prediction accuracy > 0.00224 m^3s^{-1}. We suggest this higher value because both the jackknifed and validation % errors stabilized by this point and should result in accurate Q estimates, and validation errors were within ± 20 %. Further, Equation (9) showed a higher predictive accuracy and a different empirical relationship for S than the Manning equation over the full range of Q in the sand-bed systems we examined. Even though *RMSE* increased with increasing range of modeled Q, Equation (9) should be useful for Q prediction in sand-bed streams because of increased applicability and error characteristics comparable or better than the reduced range models. Constraining the database geomorphically to sand-bed streams resulted in higher predictive accuracy of models fit in this study compared to models from unconstrained geomorphic settings.

A major advantage of using Equation (9) over typical Q-estimating methods is that it 1) does not require estimation of n_M, thus removing a major source of error, 2) does not require quantification of bed particle size distribution as in the Brownlie (1983) equation, thus making it useful for regional assessments involving quantifying Q on large geographic scales, 3) incorporates hydraulic variables (area, wetted perimeter and slope) that are easily measured in the field and/or developed from GIS-derived layers (e.g., S values), and 4) shows the highest predictive accuracy of all similar models compared from the published literature for Q estimation. In particular, Equation (9) should be highly appropriate for, and effective in, water resource management plans involving estimating Q in sand-bed streams of the SE Plains. Further, this equation has been validated with independent data spanning a large geographic range (AL, FL, GA, SC, and NC) and provides error estimates for a range of Q. This equation should increase accuracy of stream gauging and also aid in describing flow regime-ecology linkages; as a result, we recommend its use, testing, and continued refinement in these and other low-gradient sand-bed systems.

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LITERATURE CITED

Arcement, G. J., and V. Schneider, 1989. Guide for Selecting Manning's Roughness Coefficients for Natural Channels and Flood Plains. U.S. Geological Survey Water-Supply Paper 2339, 38p. http://pubs.usgs.gov/wsp/2339/report.pdf.

Asquith, W. H., G.R. Herrmann, and T.G. Cleveland, 2013. Generalized Additive Regression Models of Discharge and Mean Velocity Associated with Direct-runoff Conditions in Texas: Utility of the US Geological Survey Discharge Measurement Database. Journal of Hydrologic Engineering 18(10):1331-1348, doi:10.1061/(ASCE)HE. 1943-5584.0000635.

Bjerklie, D. M., S. L. Dingman, and C. H. Bolster, 2005. Comparison of Constitutive Flow Resistance Equations Based on the Manning and Chezy Equations Applied to Natural Rivers. Water Resources Research 41(11):1-7, doi:10.1029/2004WR003776.

Bjerklie, D. M., S. L. Dingman, C. J. Vorosmarty, C. H. Bolster, and R. G. Congalton, 2003. Evaluating the Potential for Measuring River Discharge from Space. Journal of Hydrology 278(1-4):17-38, doi:10.1016/S0022-1694(03)00129-X.

Bray, D. I., 1979. Estimating Average Velocity in Gravel-bed Rivers, (Alberta). Journal of the Hydraulics Division, American Society of Civil Engineers 105(9):1103-1123.

Brownlie, W. R., 1983. Flow depth in sand-bed channels. Journal of the Hydraulics Division, American Society of Civil Engineers 109(7):959-990, doi:10.1061/(ASCE)0733-9429(1983)109:7(959).

Burnham, K. P. and D. R. Anderson, 2002. Model Selection and Multimodel Inference: A Practical Information-theoretic Approach. Springer, New York.

Chow, V. T., 1959. Open-channel Hydraulics. McGraw-Hill, New York.

Copeland R., P. Soar, C. Thorne, 2005. Channel-forming Discharge and Hydraulic Geometery Width Predictors in Meandering Sandbed Rivers. In: *Impacts of Global Change*, Raymond Walton (Editor). American Society of Civil Engineers, Anchorage, Alaska, pp. 1-12. doi: 10.1061/40792(173)568.

Cowan, W. L., 1956. Estimating Hydraulic Roughness Coefficients. Agricultural Engineering 37(7):473-475.

Dingman, S. L., 2009. Fluvial Hydraulics. Oxford University Press, New York.

Dingman, S. L., and K. P. Sharma, 1997. Statistical Development and Validation of Discharge Equations for Natural Channels. Journal Hydrology 199(1-2):13-35, doi:10.1016/S0022-1694(96)03313-6.

Doyle, M.W., D. Shields, K.F. Boyd, P.B. Skidmore, and D. Dominick, 2007. Channel-forming discharge selection in river restoration design. Journal of Hydraulic Engineering 133(7): 831-837, doi: 10.1061/(ASCE)0733-9429(2007)133:7(831).

Falcone, J. A., D. M. Carlisle, D. M. Wolock, and M. R. Meador, 2010. GAGES: A Stream Gage Database for Evaluating Natural and Altered Flow Conditions in the Conterminous United States. Ecology 91(2):621-621, doi:10.1890/09-0889.1.

Fenneman, N.M., 1917. Physiographic Subdivision of the United States. Proceedings of the National Academy of Sciences of the United States of America 3(1):17-22, doi:10.1073/pnas.3.1.17.

Ferguson, R., 2010. Time to Abandon the Manning Equation? Earth Surface Processes and Landforms 35(15):1873-1876, doi:10.1002/esp.2091.

Gillen, D. F., 1996. Determination of Roughness Coefficients for Streams in West-central Florida. U.S. Geological Survey Open-File Report, 93p. http://pubs.usgs.gov/of/1996/0226/report.pdf.

Golubtsov, V. V., 1969. Hydraulic Resistance and Formula for Computing the Average Flow Velocity of Mountain Rivers. Journal of Soviet Hydrology 5:30-41.

Gore, J.A., 1996. Discharge Measurements and Streamflow Analysis. In: *Methods in Stream Ecology*, F.R. Hauer and G.A. Lamberti (Editors). Academic Press, San Diego, California. pp. 53-74.

Helsel, D. R., and R. M. Hirsch, 2002. Statistical Methods in Water Resources. In: *Techniques of Water-Resources Investigations of the United States Geological Survey, Book 4, Chapter A 3*, 522p. http://pubs.usgs.gov/twri/twri4a3/html/pdf_new.html.

Hicks, D. M., and P. D. Mason, 1991. Roughness characteristics of New Zealand rivers: a handbook for assigning hydraulic roughness coefficients to river reaches by the" visual comparison" approach. New Zealand Water Resources Survey, DSIR Marine and Freshwater, Wellington.

Ihaka, R. and R. Gentleman, 1996. R: A Language for Data Analysis and Graphics. Journal of Computational and Graphical Statistics 5(3): 299-314, doi: 10.1080/10618600.1996.10474713.

Isaacson, K., and J. Coonrod, 2011. USGS Streamflow Data and Modeling Sand-bed Rivers. Journal of the Hydraulics Division, American Society of Civil Engineers 137(8):847-851, doi:10.1061/(ASCE)HY.1943-7900.0000362.

Jarrett, R. D., 1984. Hydraulics of High-gradient Streams. Journal of the Hydraulics Division, American Society of Civil Engineers 110(11):1519-1539, doi:10.1061/(ASCE)0733-9429(1984)110:11(1519).

Kalin, L., S. Isik, J. E. Schoonover, and B. G. Lockaby, 2010. Predicting Water Quality in Unmonitored Watersheds Using Artificial Neural Networks. Journal of Environmental Quality 39(4):1429-1440, doi:10.2134/jeq2009.0441.

Lopez, R., J. Barragan, and M. A. Colomer, 2007. Flow Resistance Equations Without Explicit Estimation of the Resistance Coefficient for Coarse-grained Rivers. Journal of Hydrology 338(1-2):113-121, doi:10.1016/j.jhydrol.2007.02.027.

Maloney, K.O., P.J. Mulholland, and J.W. Feminella, 2005. Influence of Catchment-scale Military Land Use on Stream Physical and Organic Matter Variables in Small Southeastern Plains Catchments (USA). Environmental Management 35(5): 677-691, doi:10.1007/s00267-004-4212-6.

Manning, R., 1891. On the Flow of Water in Open Channels and Pipes. Transactions of the Institution of Civil Engineers of Ireland 20:161-207.

Maronna, R.A., R.D. Martin and V.J. Yohai, 2006. Robust Statistics Theory and Methods. John Wiley and Sons Ltd. Hoboken, NJ.

McCuen, R.H., 2005. Accuracy Assessment of Peak Discharge Models. Journal of Hydrologic Engineering 10(1):16-22, doi:10.1061/(ASCE)1084-0699(2005)10:1(16).

Meunier, M., 1989. Essai de Synthese des Connaissances en Erosion et Hydraulique Torrentielle. La Houille Blanche(5):361-376, doi:10.1051/lhb/1989040.

Montgomery, D. C., E.A. Peck, and G.G. Vining, 2006. Introduction to Linear Regression Analysis. John Wiley and Sons Ltd. Hoboken, NJ.

Neteler, M., M. H. Bowman, M. Landa, and M. Metz, 2012. GRASS GIS: A Multi-purpose Open Source GIS. Environmental Modelling & Software 31:124-130, doi:10.1016/j.envsoft.2011.11.014.

Omernik J.M., 1987. Ecoregions of the Conterminous United States. Annals of the Association of American Geographers 77(1):118-125, doi:10.1111/j.1467-8306.1987.tb00149.x.

Pelletier, P.M., 1988. Uncertainties in the Single Determination of River Discharge: a Literature Review. Canadian Journal of Civil Engineering 15(5):834-850, doi: 10.1139/l88-109.

R Core Team, 2014. R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria.

Riggs, H. C., 1973. Regional Analyses of Streamflow Characteristics. U.S. Geological Survey Techniques of Water-Resource Investigation: 04-B3, 15p. http://pubs.usgs.gov/twri/twri4b3/html/pdf.html

Riggs, H. C., 1976. A simplified slope-area method for estimating flood discharges in natural channels. Journal of Reasearch U.S. Geological Survey 4:285-291.

Rousseeuw, P. J., and C. Croux, 1993. Alternatives to the Median Absolute Deviation. Journal of the American Statistical Association 88(424):1273-1283, doi:10.1080/01621459.1993.10476408.

Sauer, V. B., 1990. written communication reported in W.F. Coon 1998, Estimation of the Roughness Coefficients for Natural Stream Channels with Vegetated Banks. U.S. Geological Survey Professional Paper 2441, 133p. http://pubs.usgs.gov/wsp/2441/report.pdf.

Sauer, V.B. and R.W. Meyer, 1992. Determination of Error in Individual Discharge Measurements. U.S. Geological Survey Open-File Report 92-144, 21p. http://pubs.usgs.gov/of/1992/ofr92-144/pdf/ofr92-144.pdf.

Simons , D.B., and E.V. Richardson, 1966. Resistance to Flow in Alluvial Channels. U.S. Geological Survey Profesional Paper 422-J, 61p. http://pubs.usgs.gov/pp/0422j/report.pdf.

Strahler, A.N., 1952. Hypsometric (Area-altitude) Analysis of Erosional Topography. Geological Society of America Bulletin 63(11):1117-1142, doi:10.1130/0016-7606(1952)63[1117:HAAOET]2.0.CO;2.

Strickler, A., 1923. Beitrage zur Frage der Geschwindigkeitsformel und der Rauhigkeitszahlen fur Stome, Kanale, und geschlossene Leitungen. Mitteilungen des eidgenschlossishen Amtes fur Wasserwirtshaft 16, Bern, Schweiz.

USACE (U.S. Army Corps of Engineers), 2010. HEC-RAS River Analysis System Hydraulic Reference Manual, Version 4.1. Institute for Water Resources, Hydrologic Engineering Center, Davis, California.

Williams, G. P., 1978. Bank-full Discharge of Rivers. Water Resources Research 14(6):1141-1154.

Wilken, E., F.J. Nava, and G. Griffith, 2011. North American Terrestrial Ecoregions – Level III. Comission for Environmental Cooperation, Montreal, Canada. ftp://ftp.epa.gov/wed/ecoregions/pubs/NA_TerrestrialEcoregionsLevel3_Final-2june11_CEC.pdf

Vogel, R. M., I. Wilson, and C. Daly, 1999. Regional Regression Models of Annual Streamflow for the United States. Journal of Irrigation and Drainage Engineering 125(3):148-157, doi:10.1061/(ASCE)0733-9437(1999)125:3(148).

Yen, B.C., 1991. Hydraulic Resistance in Open Channels. In: *Channel Flow Resistance: Centennial of Manning's Formula*, B.C. Yen (Editors). Water Resources Publications, Highlands, Colorado. pp. 1-118.

Yen, B.C., 2002. Open Channel Flow Resistance. Journal of the Hydraulics Division, American Society of Civil Engineers 128(1):20-39, doi:10.1061/(ASCE)0733-9429(2002)128:1(20).

Table 1: Range of hydraulic variables in this study. Q is discharge, Aw is watershed area, W is wetted channel width, Ac is wetted channel area, R_H is channel hydraulic radius, and S is slope.

Database	Q (m^3s^{-1})	Aw (km^2)	W(m)	Ac (m^2)	R_H (m)	S (m/m)
Reduced	0.00018-2.64	0.59-43.92	0.81-17.70	0.029-10.01	0.030-0.65	0.00008-0.04055
Southeastern	0.00018-85.23	0.59-2139	0.81-50.60	0.029-121.7	0.030-2.17	0.00008-0.04055
Full	0.00018-874	0.59-*	0.81-*	0.029-855	0.030-5.25	0.00008-0.04055
Independent	0.00053-393.6	0.039-320.5	0.84-120.7	0.039-350.5	0.031-6.75	0.00002-0.01223

***Upper range not reported in Hicks and Mason**

Table 2: Definitions of model fit statistics and fit indication. E(x) is the mean value of x, SD(x) is the standard deviation of x, k is the number of parameters fit in a given regression model, n is the number of observations. Indices have been omitted from summation operator for condensed presentation.

Model Fit Statistic	Abbreviation	Equation	Source	Better Fit
Nash-Suttcliffe Efficiency	$NSeff$	$1-[\sum (Q_{obs,i}-Q_{sim,i})^2]/[\sum (Q_{obsi}-E(Q_{obs}))^2]$	Kalin *et al.*, 2010	approaches 1
Mass Balance Error	MBe	$\sum(Q_{sim,i}-Q_{obs,i})/ \sum Q_{obs,i}$	Kalin *et al.*, 2010	approaches 0
Coefficient of Determination	R^2	$[\sum((Q_{sim,i}-E(Q_{obs}))^2]/[\sum (Q_{obs,i}-E(Q_{obs}))^2]$	Kalin *et al.*, 2010	approaches 1
Residual	e_i	$Q_{obs,i} -Q_{sim,i}$		approaches 0
Mean Residual	e_r	$E(e)$	Dingman and Sharma 1997	approaches 0
Intercept of the Q_{obs}~Q_{sim}	Int_{O-S}	β_0	Dingman and Sharma 1997	approaches 0
Slope of the Q_{obs}~Q_{sim}	S_{O-S}	β_1	Dingman and Sharma 1997	approaches 1
Standard Deviation Residuals	SDE	$SD(e)$	Dingman and Sharma 1997	approaches 0
Root Mean Square Error	$RMSE$	$[E^2(e)+SDE^2]^{0.5}$	Dingman and Sharma 1997	approaches 0
Akaike Information Criteria	AIC	$n*\ln(\sum (Q_{obs,i}-Q_{sim,i})^2/n-k-1)+2*(k+1)$	Lopez *et al.*, 2007	Decreases
Bayesian Information Criteria	BIC	$n*\ln(\sum (Q_{obs,i}-Q_{sim,i})^2/n-k-1)+k\ln(n)$	Lopez *et al.*, 2007	decreases

Table 3: Results for jackknifed model fits. Bolded equation numbers represent models of highest predictive ability based on Akaike's Information Criteria (*AIC)* and smallest root mean square error (*RMSE*). *N* is the total number of observations. Definition of model fit statistics as in Table 2. Reduced, Southeastern, and Full databases are those reported in the text (also, see Table 1).

	Reduced database		Southeastern database		Full database	
Equation	**(4)**	(5)	**(4)**	(5)	**(5)**	(4)
NSeff	0.75	0.70	0.95	0.92	0.99	0.80
MBe	-0.24	-0.17	-0.04	-0.17	-0.06	0.20
R^2	0.83	0.75	0.96	0.94	0.99	0.96
e_r	-0.06	-0.04	-0.09	-0.40	-1.06	3.19
$Int_{O\text{-}S}$	0.04	0.06	-0.28	0.05	-0.57	-2.59
$S_{O\text{-}S}$	0.62	0.60	1.08	0.81	0.97	1.35
SDE	0.17	0.19	1.85	2.30	8.99	35.31
RMSE ($\mathrm{m^3 s^{-1}}$)	0.18	0.20	1.85	2.33	9.05	35.45
AIC	-1092	-1042	524	715	2007	3247
BIC	-1087	-1037	530	721	2014	3254
N	325	325	418	418	454	454

Table 4: Validation data comparison of model (5), fit to the full database (see text), with other published models. All models are in *SI* units (*references indicate models that were reported in Lopez *et al.* (2007) in *SI* units). *N* is the total number of observations. Definition of model fit statistics as in Table 2.

Reference	Equation	*NSeff*	*MBe*	R^2	ε_r	$Int_{O\text{-}S}$	$S_{O\text{-}S}$	*SDE*	*RMSE* (m^3s^{-1})	*AIC*	*BIC*	*N*
This study	$Q = A_c R_H^{0.6906} S^{0.1216}$	0.94	0.14	0.97	0.89	-0.04	1.14	6.68	6.74	1217	1222	317
(Lopez *et al.*, 2007)	$Q = 2.93 A_c^{1.02} R_H^{0.79} S^{-0.057 \ln S}$	0.65	-0.61	0.93	-3.97	-0.30	0.44	15.96	16.44	1789	1802	317
(Bjerklie *et al.*, 2005)	$Q = 4.84 A_c^{1.10} R_H^{0.53} S^{0.33}$	0.63	0.50	0.97	3.29	-0.28	1.54	16.65	16.97	1809	1822	317
(Williams, 1978)*	$Q = 4.0 A_c^{1.21} S^{0.28}$	0.58	0.98	0.93	6.40	3.39	1.46	16.85	18.03	1844	1853	317
(Bjerklie *et al.*, 2005)	$Q = 7.14 A_c R_H^{0.67} S^{0.33}$	0.50	0.62	0.97	4.09	-0.10	1.64	19.26	19.69	1900	1909	317
(Bjerklie *et al.*, 2003)	$Q = 7.22 A_c^{1.02} R_H^{0.72} S^{0.35}$	0.37	0.60	0.98	3.94	-0.86	1.73	21.69	22.05	1975	1988	317
(Dingman and Sharma, 1997)	$Q = 1.56 A_c^{1.17} R_H^{0.40} S^{-0.0543 \ln S}$	0.23	-0.82	0.87	-5.36	0.24	0.15	23.80	24.39	2039	2052	317
(Sauer, 1990)*	$Q = 8.33 A_c R_H^{0.59} S^{0.32}$	0.20	0.92	0.97	6.05	0.75	1.81	24.13	24.87	2048	2057	317
(Riggs, 1976)*	$Q = 1.55 A_c^{1.33} S^{0.05-0.056 \ln S}$	-0.06	-1.00	0.00	-6.56	0.00	0.00	27.81	28.57	2139	2152	317
(Manning, 1891)	$Q = (1/n_M) A_c R_H^{2/3} S^{1/2}$	-0.51	1.11	0.96	7.30	-0.07	2.12	33.39	34.18	2247	2252	317
(Bray, 1979)*	$Q = 6.17 A_c R_H^{1/2} S^{0.24}$	-0.50	1.45	0.97	9.53	2.26	2.11	32.73	34.09	2248	2257	317
(Bray, 1979)*	$Q = 7.96 A_c R_H^{0.60} S^{0.29}$	-0.68	1.32	0.97	8.67	0.80	2.20	34.96	36.02	2283	2292	317
(Bray, 1979)*	$Q = 9.62 A_c R_H^{2/3} S^{0.32}$	-1.12	1.35	0.97	8.87	-0.09	2.37	39.51	40.49	2357	2366	317
(Lopez *et al.*, 2007)	$Q = 5.56 A_c^{1.03} R_H^{0.77} S^{0.27}$	-2.53	1.46	0.98	9.58	-2.21	2.80	51.39	52.28	2522	2535	317
(Lopez *et al.*, 2007)	$Q = 6.04 A_c R_H^{0.82} \mathrm{S}^{0.26}$	-3.42	1.65	0.98	10.79	-2.40	3.01	57.44	58.44	2590	2599	317
(Golubtsov, 1969)*	$Q = 4.50 A_c R_H^{2/3} S^{1/6}$	-6.02	2.54	0.97	16.67	0.18	3.52	71.74	73.65	2736	2746	317
(Jarrett, 1984)*	$Q = 3.17 A_c R_H^{0.83} S^{0.12}$	-13.35	3.09	0.97	20.27	-3.50	4.63	103.34	105.31	2963	2972	317
(Meunier, 1989)*	$Q = 1.3 A_c R_H^{0.86} S^{-0.084}$	-77.05	7.46	0.95	48.90	-5.94	9.37	240.73	245.65	3500	3509	317

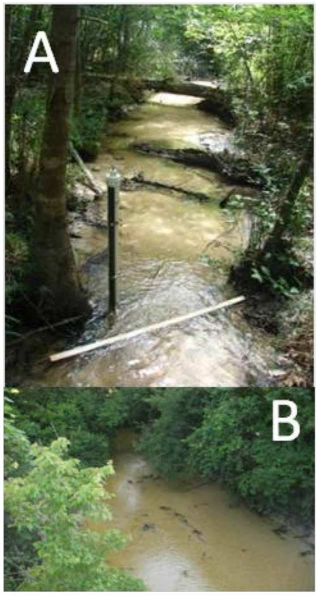

Fig. 1: Small (A) and large (B) sand-bed streams in the SE Plains typical of our study.

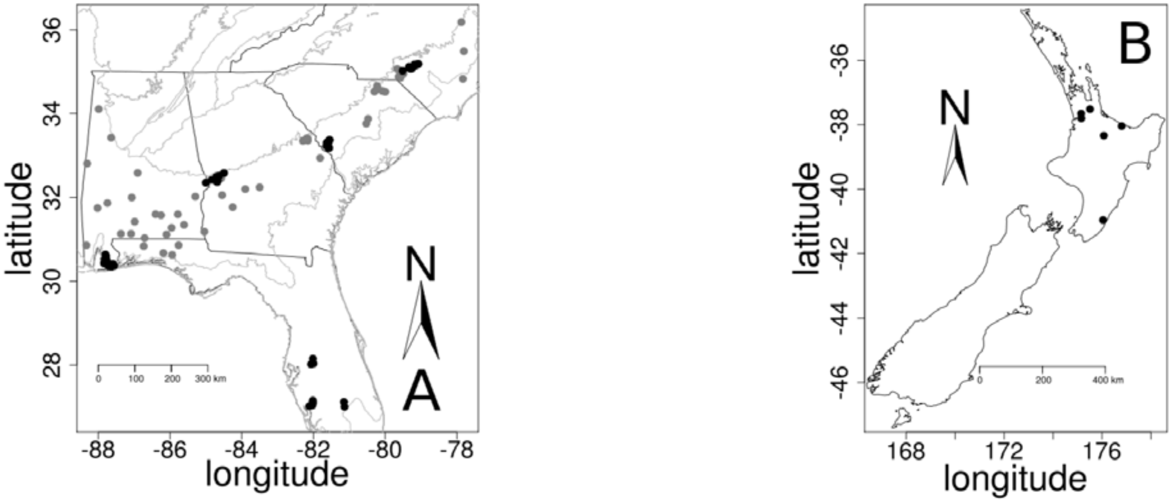

Figure 2: Training and validation sites used in this study. Southeastern, US (A) and New Zealand (B) model fit sites (●) and model validation sites (●). US Level III ecoregions are represented as lines in (A). All validation sites were in the Southeastern Plains US, below the fall line and above the Southern Coastal Plain.

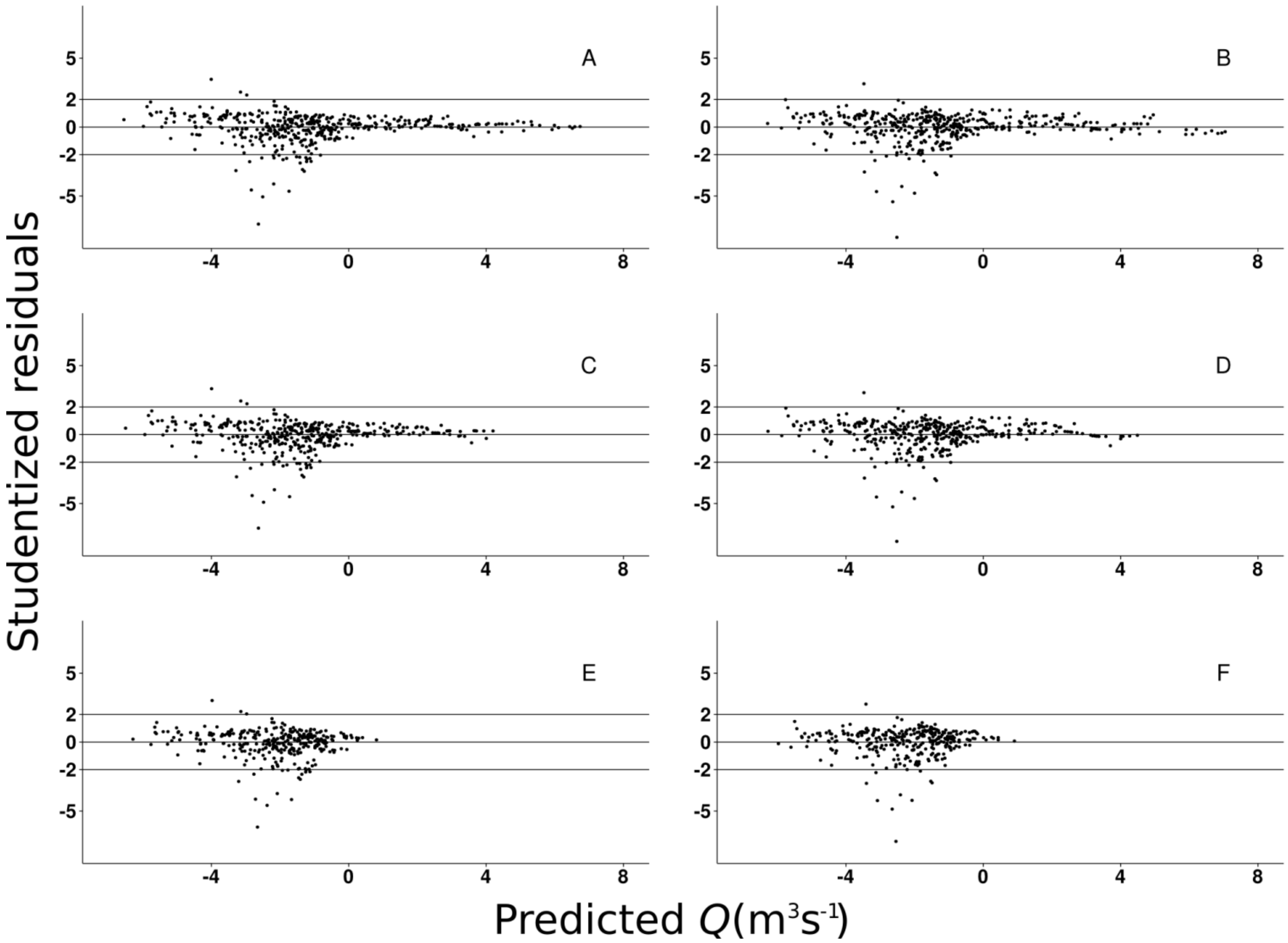

Figure 3: Predicted ln*Q* versus studentized residuals for models developed in this study. A and B are model (5) and (4) fit to the full database, C and D are model (5) and (4) fit to the southeastern database, and E and F are model (5) and (4) fit to the reduced database, respectively.

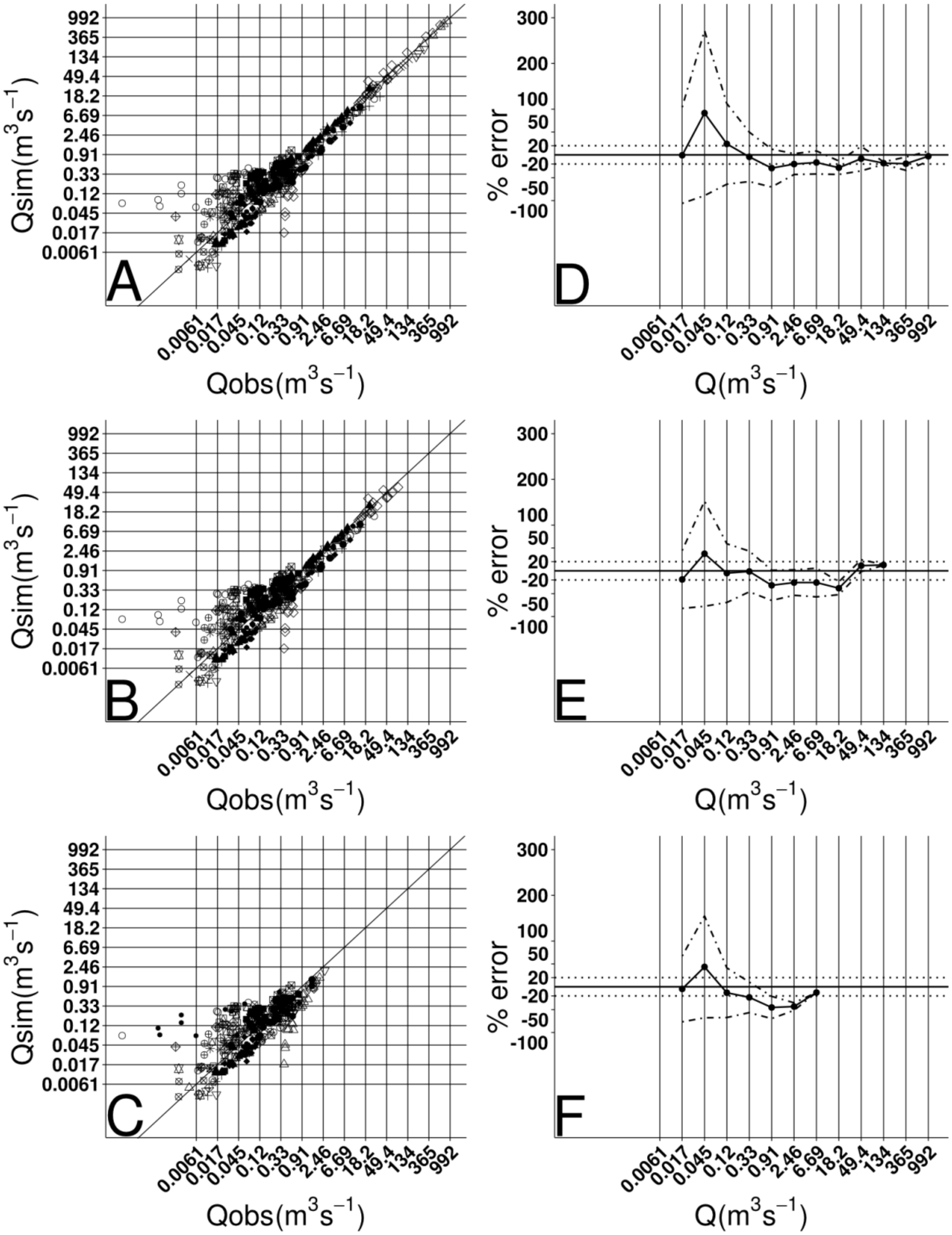

Figure 4: Model (5) fit results for all 3 databases evaluated in this study (A, full database; B, southeastern database; C, reduced database, see text) and their error characteristics (D, full database; E, southeastern database; F, reduced database). A-C) ln-transformed Q_{obs} m^3s^{-1} versus Q_{sim} m^3s^{-1} plots. Solid diagonal lines in A-C are the 1:1 lines. Solid vertical and horizontal lines in A-C and solid vertical lines in D-F represent percent error bins. Different symbols represent observations from different streams. D-F) % error plots where x-axis is Q m^3s^{-1} and y-axis is % error. Median % error (●) ± Qn (–·–) are plotted at the maximum of the bin in which they were calculated. D-F) Median % error was > 100 % for Q bins < 0.0061 m^3s^{-1} and are not presented in the graph.

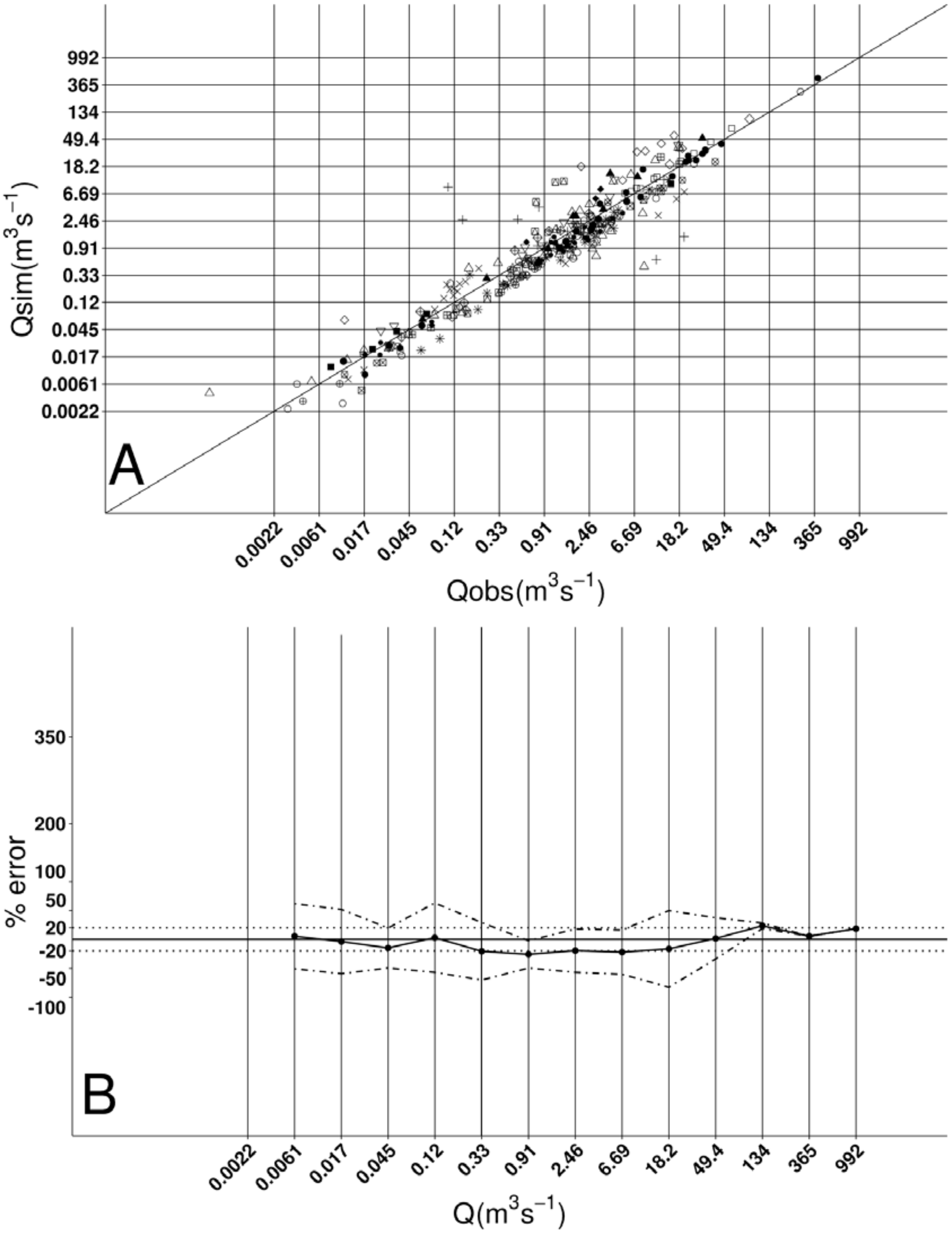

Figure 5: Results for model (5) applied to validation data. (A) and error characteristics (B). A) ln-transformed Q_{obs} m^3s^{-1} versus Q_{sim} m^3s^{-1} plots. Solid diagonal line in A is the 1:1 line. Solid vertical and horizontal lines in A and solid vertical lines in B represent percent error bins. Different symbols represent observations from different streams. B) % error plots where x-axis is Q m^3s^{-1} and y-axis is % error. Median % error (●) ± Qn (–·–) plotted at the maximum of the bin where they were calculated. B) Median % error was > 100% for Q bins < 0.0022 m^3s^{-1} and are not presented in figure.

Chapter 3: Hydrogeomorphic reference condition and its relationship with macroinvertebrate assemblages in Southeastern US Sand Hills streams (formatted for submission to Freshwater Science)

Running Head: Hydrogeomorphic reference condition

Hydrogeomorphic reference condition and its relationship with macroinvertebrate assemblages in Southeastern US Sand Hills streams

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Abstract: Defining reference conditions in streams are integral to providing benchmarks for ecological responses to anthropogenic perturbation. The hydrogeomorphic reference condition, or the channel morphologic and hydrologic condition expected when a stream is in the reference condition, in Southeastern, Sand Hills streams has not been identified. We compared differences in macroinvertebrate assemblages between hydrogeomorphically (HGM) defined reference/non-reference condition in 62 low-gradient, sand-bed streams of the Southeastern Sand Hills ecoregion, US. We identified the HGM by clustering residual deviation from robust regressions relating watershed area (*Aws*) and area (*Atob*), width (*Wtob*) and mean depth (*Dtob*) at the top of bank channel, and a multivariate residual interaction term derived from *Atob*, *Wtob*, and *Dtob*. Two distinct groups of sites were identified based on the difference between field-measured *Atob* and robust regression-derived *Atob* (*Atob* residual): streams with *Atob* residual < 0.6 m^2 and $>$ 0.6 m^2 were predicted to be HGM reference and non-reference streams, respectively. Based on 2 partial least squares linear discriminate analyses using 1) hydrologic and environmental variables, and 2) mean trait values (*mT*) of macroinvertebrates on 10 random reference and non-reference stream pairs of similar *Aws*, non-reference streams were characterized by flashier hydrographs and altered flow magnitudes, lower organic matter, coarser substrate, higher pH and specific conductivity than reference sites. Macroinvertebrate assemblages also corresponded to HGM groupings, with *mT* indicative of multivoltinism, high proportions of collector-gatherer functional feeding groups, taxa with fast current preferences, and also lower EPT richness and biotic integrity in non-reference sites. HGM is determined from field-measured channel geometry, is easy to implement, and is indicative of contemporary hydrologic disturbance resulting in contrasting macroinvertebrate assemblages in Sand Hills, sand-bed streams.

Key words: Geomorphology, hydrology, macroinvertebrates, trait-based ecology, community assembly, sand-bed, Sand Hills, coastal plains.

INTRODUCTION

Changes in hydrology associated with land use can be a major force shaping hydraulic geometry of stream channels, as streams are in dynamic equilibrium with water and sediment supplies delivered from their watersheds (Leopold 1994). Stable streams show predictable relationships between channel structure and watershed area, and deviations from predicted relationships can be used to differentiate disturbed and non-disturbed streams (Hammer 1972, Doll et al. 2002). Channel enlargement has been used to investigate altered channel geometry between contrasting rural and highly anthropogenically influenced watersheds, with hydrological changes related to shifts in watershed land use, such as increased impervious surface, being implicated as the major contributor of enlargement (Hammer 1972, Doll et al. 2002). In this context, altered hydrology results from increasing water delivery to the stream during storm events, which, in turn, alters channel geometry through incision or enlargement (Hammer 1972, Booth 1990). Hydrology also has been identified as a major influence on stream biota (Power et al. 1995, Hart and Finelli 1999, Lake 2000, Bunn and Arthington 2002); thus, changes in hydrology and channel geomorphology likely indicate altered instream habitats and biota (Bravard et al. 1997, Burcher et al. 2007).

Habitat suitability has been conceptualized as the “templet” for biotic assemblages (Southwood 1977, 1988), and has been used to investigate the match between stream habitats and benthic assemblages (Townsend and Hildrew 1994). The templet concept can be extended to assembly of whole communities, where organisms are “filtered” from the regional species pool based on interactions between species life history traits and environmental conditions (Keddy

1992). Thus, organisms at a site that “pass” an environmental filter are suited to that environment based on their traits (Keddy 1992, Poff 1997, Sokol et al. 2011). Ideally, trait values weighted by relative proportions should reflect ecosystem-level structure and function as individuals with the highest relative proportions should indicate optimal resource use efficiency (i.e., biomass-ratio hypothesis; Grime 1998, Shipley et al. 2006). Moreover, mean trait values should show strong relationships along environmental gradients where such traits are advantageous (Garnier et al. 2004, Vandewalle et al. 2010). For example, Vandewalle et al. (2010) presented a case study showing that macroinvertebrate trait values were related to principal components derived land use and hydromorphological gradients, suggesting utility in relating trait and hydrogeomorphic conditions in streams.

The Sand Hills (level IV) ecoregion of the SE Plains ecoregion in the southeastern US is characterized by streams with low gradient, sand-bed channels. In contrast to upland, gravel/cobble-bed channels, sand-bed channels are characterized by unconsolidated sediment mobilized as low as mean annual discharge (Copeland et al. 2005), and associated rapid channel adjustments to altered hydrology (Simons and Simons 1987). In addition, the biota in the SE Plains differ from upland ecoregions (Feminella 2000). As a result of such dynamic adjustments, a unique biota, and predicted increases in human population growth in this region (reviewed in Nagy et al. 2011), there is a need to characterize the state of the hydrogeomorphic reference condition in this ecoregion as a basis for biotic conservation in sandbed streams.

In this paper, we 1) described and validated simple empirical models describing the hydrogeomorphic reference condition in the Sand Hills ecoregion, 2) developed an empirical tool to classify reference and non-reference streams, and 3) evaluated the degree to which altered hydrology and geomorphology signal predictable change in structurally and functionally based benthic macroinvertebrate assemblages (Hawkins et al. 2010). Our study provides data indicating that the hydrogeomorphic reference condition reflects variation in the physical channel structure and, in turn, is a useful predictor of biotic assemblage composition and function in this altered and poorly known ecoregion.

METHODS

Study area and landscape variables

We studied 62 streams in the Sand Hills ecoregion of Georgia (Fort Benning and adjacent Nature Conservancy lands [n=11 sites] and Fort Gordon [6]), South Carolina (Savannah River Site [15], Sandhills State Forest [2], Sandhills National Wildlife Refuge [4], and Manchester State Forest [2]), and North Carolina (Sandhills Gamelands [6] and Fort Bragg [16]; Fig. 1). Sampling occurred from 2010-2012 during the low-flow period (May-Sept.). These sites were selected with exhaustive on the ground reconnaissance with the majority of sites being reference sites. Those sites that were not considered apriori reference sites were collected as test sites (sensu Kosnicki et al. 2014). These sites should represent a set of reference sites with varying degrees of deviation from this reference condition. Study watershed drainage area ranged from 0.64 to 30.29 km^2 (median=5.1 km^2) and stream order from 1 to 3, with stream channels having low-gradients, sandy substrates, and organic matter (wood and leaf litter detritus), draining mostly forested watersheds (median forest cover ~70%). We calculated site-specific watershed area (*Aws*) from 10-m National Elevation dataset digital elevation models (Gesch et al. 2002), and quantified landcover from 2006 National Landcover dataset (Wickham et al. 2013) in GRASS GIS (Neteler et al. 2011, Appendix 1). In addition, we used stream slope values from the National Hydrography Plus Dataset (Horizon Systems Cooperation, accessed 1 April 2012, http://www.horizon-systems.com/nhdplus/index.php), which was important in calculating hydraulic conditions (e.g., stream power).

Stream hydrology, channel geometry, and instream habitat variables

We used high-resolution stream stage (water level) as a measure of temporal variation in stream hydrology, estimated from Solinst Levelogger Junior® pressure transducers (Model 3001, Solinst Canada Ltd., Canada); loggers were installed at the downstream terminus of each ~150 m study reach. We adjusted stage data for ambient atmospheric pressure using Solinst Barologger Gold® pressure transducers or barometric pressure data obtained from local (within ~ 40 km) airport weather stations. We used temporary stilling wells to house loggers, constructed from schedule-40 PVC (3.81 cm ID) and perforated on the downstream side to allow water circulation. This design has been shown to produce stable water surface elevations in other SE streams (Schoonover et al. 2006). We measured stage, temperature, and barometric pressure data every 15 min for the duration of logger deployment at each reach (median duration ~1 y). Precipitation recurrence intervals were similar among sampling years (33 y [1984-2013]; range 71-88%; annual precipitation PRISM climate group data [http://www.prism.oregonstate.edu/]), thus we considered a 1-y record sufficient to capture annual and inter-annual variation during the study.

We summarized stream stage by a suite of flow metrics that were indicative of site-specific hydrologic disturbance (McMahon et al. 2003), and used the median instream temperature to characterize temperature regime. In addition, we estimated hydraulic variables from stream cross-sections (below), stage data, and standard formulas (Statzner et al. 1988). We estimated median stage discharge (Q) from a regional equation developed for SE Sand Hills streams (Sefick et al. 2015). We estimated max stage Q as a compound channel because many of the max flows entered the flood plain, which has a different Manning's *n* than the main channel, by summing estimated floodplain *Q* and instream *Q* (Chaudhry 2007), respectively, estimated with Manning's (1889) equation using Manning's *n* estimated from the methods of Acrement and Schneider (1989), and Sefick et al. (2015). Last, to estimate bed disturbance we estimated the proportion of time the median particle was in motion by quantifying frequency that critical shear stress (τ_*) was exceeded, estimated from d_{50} by linear interpolation of τ_*/sediment size data (see Berenbrock and Tranmer 2008, Appendix 1).

We quantified channel geometry following the method of Kosnicki et al. (2014). Briefly, we estimated channel cross-sectional dimension by surveying 4 to 6 equidistant channel cross sectional transects within runs of each stream reach. We established transects by staking rebar at the top of bank on both sides of the channel perpendicular to the direction of flow, corresponding to the point where water would breach the lowest bank during a storm event (Leopold 1994). We used a line level to establish the relative top of bank datum for each survey, and recorded water depths every 20 cm along each transect. We estimated top of bank area (*Atob*), top of bank mean depth (*Dtob*), and top of bank width (*Wtob*) from transect surveys at the time of macroinvertebrate sampling, and summarized these measures as a reach-specific median.

We quantified instream habitat by estimating substrate particle size (see Helms et al. 2009), amount of coarse woody debris (CWD), and benthic organic matter (BOM, organic matter material ≤ 1.6 cm diameter; Maloney et al. 2005) at each transect. Substrate size and BOM were estimated from PVC cores (7.62 cm ID, 455.8 cm^3 sample volume for substrate size; 2.5 cm ID, 49.1 cm^3 sample volume for BOM) inserted 10 cm into the substrate at midstream for substrate size or midstream and stream margin for BOM (~1 m from bank). In the laboratory, we dried substrate samples at 65°C to remove all water and combusted at 550°C for 3 hours to remove

BOM, and dry sieved for percentage by mass of representative particle sizes (phi scale -4 to 5; Lane 1947). We calculated geometric mean and SD of particle size from reach medians of specific particle size summaries (e.g., d_{50}; Bunte and Apt 2001). For BOM, we oven-dried samples to a constant mass at 65°C (typically 48 h), weighed, and ashed in a muffle furnace at 550°C for 3 h to estimate ash free dry mass. % BOM was determined as the difference between dry and ashed masses divided by total dry mass (Wallace and Grubaugh 1996). We estimated submerged CWD by measuring the length and width of each piece of CWD (wood > 2.5 cm diameter; Wallace and Benke 1984) along a 1 m^2 transect perpendicular to stream flow to obtain a transect-specific areal estimate. To estimate solar input reaching the streambed, we quantified overhead vegetative cover at each transect as % non-canopy as the mean of 4 densiometer readings (upstream, downstream, left and right bank). Last, we estimated streamwater specific conductivity (SC), pH, and, dissolved oxygen at the downstream terminus of each reach during one or more sampling dates (range=1 to 3 measurements) with samples taken in the thalweg of a representative run, and summarized the data as reach-specific medians.

Benthic Macroinvertebrate Sampling and Trait-Based Analyses

For benthic macroinvertebrates, we used 2 D-frame dip net samples (244 μm mesh) to conduct a multihabitat sampling of 1m length and 0.33m width of 3 representative microhabitats (total sampled area per reach ~2 m^2), at the downstream and upstream-most transects, respectively, resulting in a composite sample of depositional (e.g., leaf packs), CWD, root mats, and macrophyte microhabitats occurring in run habitats (see Barbour et al. 1999, Kosnicki et al. 2014). We preserved samples in 95% ethanol in the field and transported them to the laboratory. There, we elutriated samples with salt water (specific gravity ≥ 22) to separate organic from inorganic matter, and passed elutriated material through 2-mm and 250-µm stacked sieves to separate the sample into 2 size fractions. We used a 2-step processing method for macroinvertebrates samples (Feminella 1996). Coarse material retained by the 2-mm sieve was picked for ≤ 30 min to remove large and rare taxa, which was then combined with material retained by the 250-µm sieve. We combined fine material and salt water into a 1-L homogenate and subsampled by withdrawing at least two 25 mL aliquots. Subsampling continued until at least 300 organisms were recovered. We sorted all subsampled organisms sorted under a dissecting microscope at ~7x magnification (Feminella 1996, Kosnicki et al. 2014). We extrapolated the estimated individuals in the whole sample volume by multiplying counts by the number of fine subsamples needed to reach ≥ 300 organisms in 1L of homogenate. For example, if a 250mL subsample was needed to reach ≥ 300 organisms then the number of organisms in this subsample was multiplied by 4 to reach the estimated number in 1L. Next, we added large and rare taxa counts, and, last, combined the 2 samples to estimate benthic density for each reach.

We identified macroinvertebrates to the lowest practical taxonomic level (usually genus or species) using available keys (Brigham et al. 1982, Kowalyk 1985, Epler 2001, Merritt et al. 2008, Thorp and Covich 2009, Epler 2010). We resolved ambiguous taxa with consensus of 3 identifiers following Cuffney et al. (2007). We used Ephemeroptera, Plecoptera, and Trichoptera (EPT) richness, the Florida Stream Condition Index (SCI), Georgia Stream Condition Index (GASCI), and the Georgia Biotic Index (GABI) as a tolerance and compositional summary of macroinvertebrate assemblages. EPT richness is a well know metric predicted to decrease with increasing disturbance (Barbour et al. 1999), whereas SCI is predicted to decrease with disturbance and is the successor to the Florida Index (Fore et al. 2007). The Florida Index, indicative of disturbance in Sand Hill streams (Maloney and Feminella 2006), was highly correlated to the SCI (0.69; [Fore et al. 2007]); thus, we considered it useful in indicating potential disturbance in our study. We included the GABI using tolerance values from Georgia Environmental Protection Division (GAEPD). (http://epd.georgia.gov/sites/epd.georgia.gov/files/related_files/site_page/GAEPD_Taxa_withFFG_Habit_Tol_Val_2012_sorted.xls) and GASCI, calibrated for the Sand Hills, in our study because Maloney and Feminella (2006) found these indexes also responded to disturbance. We predicted GABI and GASCI to increase and decrease with increasing disturbance, respectively.

In addition to the above compositional and tolerance metrics, we used a trait-based approach to assess habitat-assemblage matches, which integrated individualized measures of trait states weighted by relative abundance to produce a community-aggregated mean trait (*mT*, Garnier et al. 2004). we used trait values recorded from the USGS database http://pubs.usgs.gov/ds/ds187/ (Vieira et al. 2006), coded so that states of a given trait summed to 1 at the genus level, similar to fuzzy coding (Chevenet et al. 1994). For example, values for the 3 trait states of voltinism (i.e., univoltine, multivotine, semivoltine) were summed across all taxa in a genus, substituting family values when genus values were unavailable, and then divided by the total value of all voltinism trait states. This step resulted in all trait states summing to 1 thus representing the proportion of each trait state the taxon (usually genus) possessed. We calculated mean trait values (*mT*) as, $mT = \sum_{i=1}^{n} p_i * trait_i$, where p_i was taxa relative abundance and $trait_i$ was the value of a given trait (Garnier et al. 2004) (functcomp, FD package [Laliberte and Shipley 2011]). We used the majority of traits contained in the database because validation/prediction of HGM reference condition was a study objective (Appendix 1). We predicted that *mT* would be a useful means of relating macroinvertebrate assemblage trait structure to instream hydrogeomorphic conditions because aggregate traits integrate biotic organization over contrasting abiotic constraints (Vandewalle et al. 2010).

Clustering and Classification of Reference and Non-Reference Sites

Robust regressions.– We hypothesized that hydrogeomorphically contrasting stream types would conservatively represent at least 2 statistical populations (i.e., reference and non-reference streams), based on the assumptions that 1) predictable relationships exist between channel geometry and *Aws* in stable channels (Leopold 1994), and 2) streams deviating in channel geometry measures from the expected (reference) value, given *Aws*, defined a non-reference population. To quantify deviation from this expectation, we used robust regression models relating *Aws* (x) and 3 geomorphic variables (y = *Atob*, *Dtob* and, *Wtob*), fit with iteratively re-weighted least squares regression (rlm, MASS package, Venables and Ripley 1994), and then tested for significance with a Wald test (robftest, sfsmisc package, Maechler 2012). We then calculated the centroid distance from 0 (Cd_o), the distance from the channel geometry reference condition in multivariate space, to describe variability in the channel adjustments related to more than one of the channel geometry variables, as:

$$Cd_0 = \sqrt{\left\{\left[0 - \left({}^{A_e}\!/_{3}\right)\right]^2\right\} + \left\{\left[0 - \left({}^{D_e}\!/_{3}\right)\right]^2\right\} + \left\{\left[0 - \left({}^{W_e}\!/_{3}\right)\right]^2\right\}} \qquad (1)$$

where A_e = *Atob residuals*, D_e = *Dtob residuals*, and W_e = *Wtob residuals*. We used these 3 groups of residuals and Cd_0, rather than a single measure of channel geometry, to ensure that multivariate differences in channels were accounted for in stream clustering (below) and, thus, adjustments of channels to changes in hydrology were not constrained by a single geometry measure. For example, channels having similar *Atob* could be incised having large values for *Dtob* and *Atob,* but not *Wtob*, or channels could be excessively wide and not deep having large *Wtob* and *Atob*, but a small *Dtob*. Because there was no guidance in the literature for these understudied Sand Hills streams we chose to include all three geomorphic variables in cluster analyses.

Classification of reference and non-reference streams.–We standardized channel geometry residuals and Cd_0 data (mean=0, SD=1) to remove the influence of scale, and clustered sites with Partitioning Around Medoids (pam, cluster package, Maechler et al. 2012), a robust alternative to kmeans (Borcard et al. 2011), with the number of groups determined objectively by maximizing mean silhouette width between clusters from 2 to N-1 clusters (Borcard et al. 2011). The silhouette width for an observation is defined as the ratio of the distance to the nearest non-member cluster minus the distance to the member cluster divided by the maximum of these 2 distances; when the mean value was maximum the result was "tight" groups relative to the other groups in the dataset. For example, under conditions of maxed silhouette width, streams assigned to a cluster are the most similar to one another while being the most dissimilar to streams in other clusters (Rousseeuw 1987, see also Kosnicki et al. 2014). We used principal components analysis (PCA) to visualize stream clusters based on standardized data. We then used a classification tree to determine which unstandardized geomorphic variable(s) best classified streams as reference and non-reference (rpart, rpart package [Therneau et al. 2010]). In this way, we developed a method to classify other Sand Hills streams not investigated in our study.

Validation of Reference and Non-reference Classification

To validate reference/non-reference stream classifications, we paired reference and non-reference streams determined from the cluster analysis with those of similar *Aws* values. This procedure generated 10 reference and non-reference pairs (20 streams) for validation analyses. We used Partial Least Squares (PLS) regression for evaluating which *mT* values and environmental variables best discriminated between reference and non-reference streams (Turkmen and Billor 2013). PLS is useful when collinearity is high or when there are more predictor variables than sites, which often occurs in ecological studies, thus precluding use of standard statistical techniques (e.g., multiple linear regression) when investigating relationships (Carrascal et al. 2009).

PLS is similar to PCA as they both reduce data dimensionality by projecting linear combinations of the original variables onto fewer derived variables (i.e., PCA components or PLS latent variables, LV). PCA projects the X (e.g., environmental) matrix onto orthogonal components in the direction of maximum variance in X (Borcard et al. 2011). PCA loadings are weights that describe the association between the original data and the ordered scores along the derived component (Wold et al. 1987). In contrast, PLS projects the X matrix onto a series of LVs with the condition that the weight maximizes the covariance between Y and X (Abdi 2007). Thus, PLS loadings are weights describing the association of the original data to the ordered scores on the LVs that maximally predict Y. In contrast to PCA, there are cross-validation methods that can objectively determine the number of important LVs that maximally predict Y (Boulesteix and Strimmer 2007), and variable-selection methods to identify important variables predicting Y (Chong and Jun 2005).

Partial least squares regression modeling.–We used PLS modeling (specifically PLS followed by linear discriminant analysis [PLS-LDA]) to determine which predictor variables best discriminated between reference and non-reference streams. This procedure maximizes the environmental (or trait) variables ability to predict group membership. Use of retained LVs in an LDA to predict group membership has been shown to increase classification accuracy over PLS alone (Boulesteix 2004, Boulesteix and Strimmer 2007, Turkmen and Billor 2013). We fit PLS models with de Jong’s (1993) SIMPLS algorithm (plsr, pls package, Mevik and Wehrens 2007).

Our PLS-LDA modeling procedure was as follows. First, we chose the number of LVs to minimize root mean square error of prediction based on leave one out cross-validation (LOOCV). Second, we fit the model with the number of LVs identified by LOOCV. We then calculated variable importance in the projection (vip) scores (get.biom, BioMark package, Wehrens et al. 2013). Third, we removed variables with values <1 as a variable selection step (see Sonesten 2003). We repeated steps 1 and 2 on the dataset derived from the variable selection step (step 3), and the retained LV scores were used in LDA (lda, MASS package, Venables and Ripley 1994) to discriminate reference and non-reference streams (Turkmen and Billor 2013).

PLS misclassification error and significance. –We were interested in model discrimination accuracy because we wanted to know how well geomorphic groups were predicted by environmental variables and *mT*; thus, we assessed the number of streams misclassified (NMC) by the models for likely predictive ability with LOOCV (Turkmen and Billor 2013). PLS does not have a test for overall significance analogous to the *F* test of regression, so we assessed overall model significance by permutation (Szymanska et al. 2012). Here, we randomly assigned group labels 10,000 times to determine how often NMC of permuted models was ≤ NMC from the original model. We divided NMC from the permutation procedure ≤ NMC by 10,000, and thus assigned a probability for accessing model overall significance in discriminating reference and non-reference streams.

To test for differences using traditional assessment metrics we used *t tests* with equal variance (i.e., parametric assumptions were satisfied) to test predictions of EPT, SCI, GASCI, and GABI between reference and non-reference stream pairs, but we adjusted resulting *P* values for multiple testing with the false discovery rate (Benjamini and Hochberg 1995) because the Bonferroni correction was considered too conservative (Doledec et al. 2006). The adjusted *P* values were compared against alpha=0.05. All statistical analyses were done in the R language (v. 3.0.1; R Core Team 2013; Ihaka and Gentleman 1996).

RESULTS

Hydrogeomorphic Reference Condition and Site Classification

Regression analysis showed that *Aws* was significantly related to *Atob* ($n = 62$, $F = 75.69$, $P < 0.0001$; equation: $Atob = 0.7381+0.0839*Aws$, Fig. 2A), *Dtob* ($n = 62$, $F = 25.21$, $P < 0.0001$; $Dtob = 0.3472+0.0121*Aws$, Fig. 2B) and *Wtob* ($n = 62$, $F = 43.14$, $P < 0.0001$; $Wtob = 2.5159+0.0954*Aws$, Fig. 2C). Two stream groups displaying contrasting hydrogeomorphic conditions were identified from the cluster analysis (Fig. 3), which formed the basis for the reference and non-reference classification. PC1 was highly related (81% of variance) to *Atob* and Cd_0, underscoring the importance of *Atob* in separating stream groups (Fig. 3). Cd_0 also highly loaded on PC1, although this likely was because of Cd_0 ‘s strong relationship with *Atob* (r=0.92; $P<0.001$), and its relationship to *Wtob* and *Dtob* to a lesser extent (r=0.80 and 0.62; $P<0.001$, respectively). Of the geomorphic variables investigated, classification tree analysis indicated that a residual (unexplained variation) value of 0.6 m^2 *Atob* from the *Atob* regression equation best classified reference and non-reference streams with an error rate of <2% (1 of 62 sites). Streams with *Atob* residuals >0.6 m^2 were considered non-reference streams, whereas sites with *Atob* residuals <0.6 m^2 were classified as reference streams.

Validation of Reference and Non-Reference Classifications

PLS-LDA revealed that environmental variables (Env_{PLS}) and macroinvertebrate traits ($Trait_{PLS}$) each significantly discriminated the 2 stream groups (permutation values $P = 0.02$ and < 0.001, respectively). All variables retained in the final model had vip ≥ 1 in the variable-selection step (grey-highlighted variables in Appendix 1), indicating their importance in discriminating reference and non-reference streams.

Env_{PLS} identified 1 LV describing putative hydrologic disturbance variables loading in the non-reference direction (right side of Fig. 4); only organic matter variables (OM and CWD) loaded in the reference direction (left side of Fig. 4, explained variance in X and Y, 35 and 68%, respectively). Streamwater specific conductance and pH also were important in discriminating the 2 stream groups (highest loadings), being higher in non-reference streams (Fig. 4). Hydrologic measures of high-magnitude stage duration (i.e., maximums above specified quartiles 75th and 95th) and low-magnitude stage duration (i.e., maximums below specified quartiles 10th and 25th) were higher in non-reference streams (Fig. 4). Stream flashiness, as falling limb differences in event hydrographs, also was higher in non-reference streams. Last, substrate size and variation (as Sed Mean and Sed SD, respectively) and % developed land in the watershed were higher in non-reference streams (Fig. 4). NMC for Env_{PLS} was 10% (i.e., 90% correct classification).

Compared to non-reference sites, EPT richness SCI, and GASCI were significantly higher ($t = 2.74$; $P = 0.0154$, $t = 2.76$; $P = 0.0154$, $t = 2.15$; $P = 0.0315$, respectively) and GABI trended lower ($t = -1.30$; $P = 0.106$) in reference than non-reference sites (Fig. 5).

For macroinvertebrates, $\text{Trait}_{\text{PLS}}$ identified 6 LVs explaining ~ 90% of variance in X and Y. LDA standardized coefficients derived from $\text{Trait}_{\text{PLS}}$ scores predicting group membership showed that LDA coefficient for LV 1 was ~2x higher than LV 2 and 3 (Table 1). Given the paramount importance of LV 1 in discriminating sites (36 and 49% of X and Y variance, respectively), interpretation was centered on this LV, and data visualization presented as a biplot of LVs 1 and 2 (Fig. 6) and as PLS loadings for LV 1 (Fig 7). Several traits loaded highly on LV 1 in the non-reference direction (right side of Figs. 6 and 7), and included taxa 1) in the collector-gatherer functional feeding group, 2) with multiple generations per year (multivoltine), 3) preference for fast current, and 3) bluff (bricklike) body shapes. In contrast, traits loading highly on LV 1 in the reference direction (left side of Fig. 6 and 7) included taxa 1) with dorsoventrally flattened bodies, 2) in the shredder functional feeding group, and with 3) a moderate adult life span, 4) hemimetabolous development (only 2 aquatic stages), 5) one generation per year (univoltine), 6) fast seasonal cycles, and 7) habitat preferences for the stream bed (Fig. 6).NMC for $\text{Trait}_{\text{PLS}}$ was 15% (i.e., 85% correct classification).

DISCUSSION

Results of our study provide evidence supporting the existence of a hydrogeomorphic reference condition for Sand Hills streams of the SE Plains ecoregion, based on a multivariate cluster analysis of commonly used and easily measured channel geometry variables. Further, we have developed a useful empirical tool to classify Sand Hills streams not investigated in this study as reference or non-reference by measuring top of bank area (*Atob*) and comparing with expected *Atob* from the robust regression developed in this study (see Appendix 1). PLS results suggest a strong linkage between watershed land use, altered hydrology and geomorphology, and instream habitats and associated biotic assemblages, which parallels models describing landscape-instream linkages developed for stony upland streams (sensu Burcher et al. [2007]). In addition, a broad suite of traditional macroinvertebrate assessment metrics and traits and habitat conditions showed predictable differences between reference and non-reference streams related to channel enlargement, primarily as increases in *Atob*. Last, the reference condition criterion that we developed differed from 3 typical ways others have defined the reference condition: 1) landscape level assessment (Carlisle and Meador 2007), 2) best professional judgment (Stoddard et al. 2006), or 3) screening abiotic variables (Whittier et al. 2007, but see Kosnicki et al. [2014] for a discussion selecting reference streams in the Sand Hills). The main advantage of our reference condition criteria is the relative ease of implementation, and the link between contemporary channel morphology and hydrologic disturbance known to affect biota (Bunn and Arthington 2002).

Geomorphic Classification and Discrimination of Streams

Our results showed that differences in morphology of non-reference stream channel appeared mostly related to increases in *Atob*. Related changes in *Wtob* and *Dtob* driving changes in *Atob* showed that non-reference channels were both wider and/or deeper, and not simply incised. We acknowledge that our data cannot identify causal relationships between hydrology and channel morphology; however, channel enlargement was an indicator of contemporary hydrologic disturbance, and we infer that changes in hydrology likely caused the changes to channel morphology we observed in the study streams. Thus, an *Atob* residual threshold of 0.6 m^2 appears useful in classifying other Sand Hills ecoregion streams as reference or non-reference in a hydrogeomorphic context. Our results also suggest that this method should identify streams with contemporary hydrologic disturbance because contemporary channel enlargement was related to hydrologic disturbance. As a result, this tool should be useful to managers and other researchers as a relatively rapid means to assess the status of other streams in the Sand Hills ecoregion. An example of how the *Atob* residual criterion could be used to classify other Sand Hills streams is found in Appendix 2. We lack empirical data suggesting the efficacy of this approach outside of the SE Sand Hills, although we hypothesize that this method should be applicable to other ecoregions containing low-gradient, sand-bed streams. When applying this method to new sites, thorough stream reconnaissance should be undertaken to ensure that channel morphology is consistent upstream and downstream of the study reach.

Environmental and Macroinvertebrate Contrasts Between Stream Groups

Hydrology and other environmental variables. – Results of the Env_{PLS} analysis showed that 50% of the best reference/non-reference discriminating variables retained were those describing flow regimes, specifically variables reflecting hydrologic disturbance. This result highlights the high importance of differences in hydrology between reference and non-reference streams, thus further underscoring use of “hydrogeomorphic” in characterizing the reference condition. Percent of developed land was higher in non-reference streams and has been indicated in driving changes in hydrology (Burcher et al. 2007). Further, landuse legacy effects have been implicated in accounting for variation in contemporary instream habitat and macroinvertebrate assemblages on one of the bases included in this study (i.e., Fort Benning, Maloney et al. 2008).

Our study was not designed to investigate landuse legacy specifically; however, we cannot rule out potential historical landuse effects on instream variables. This pattern may explain why developed land, while important, was not more highly loaded in the LDA analysis. Increases in SC and pH, higher in non-reference streams, also have been shown to indicate watershed disturbance in coastal plains streams (Houser et al. 2006, Zampella et al. 2006). Zampella et al. (2006) found high correlations between both pH and SC and nitrogen species, thus higher pH and SC in our non-reference streams could signal increased nutrient levels from runoff from developed or non-natural areas. Generally, streamwater pH and SC were low in our Sand Hills streams, and our results suggest these relatively easy in-stream measures could be useful in preliminary screening of reference and non-reference stream, with high values signaling potential impacts. Benthic organic matter and coarse woody debris were both lower and sediment mean diameter and standard deviation were larger in non-reference (vs. reference), respectively. Likewise, these conditions also were indicative of hydrologic disturbance (Maloney et al. 2005). As hydrologic disturbance increases, fine sediments are exported from the system and bed material coarsening occurs (Helms et al. 2009). Taken together, our results suggest that hydrologic disturbance and its effects on instream habitat conditions are greater in non-reference, Sand Hill streams, and that contemporary hydrologic disturbance appears to have resulted in enlarged channels observed in this study.

Stream channels in our study displayed 2 distinctly contrasting morphological characters (related to *Atob*) with reference channels showing smaller substrate size and *Atob.* This finding contrasts with observations of Piedmont streams reported in Sweeney et al. (2004) where streams in more undisturbed watersheds were wider and showed larger substrates. We surmise that ecoregional differences in landscape setting (e.g., sandy-based geology, relief, etc.), watershed size differences, and variation in vegetation type and capacity to resist shearing forces during storm events likely result in the differences between the 2 studies. An important parallel between our study and Sweeney et al. (2004) was that water velocity was lower in more undisturbed watersheds (reference). We suggest that in our non-reference Sand Hills streams increased hydrologic disturbance exported the less consolidated sandy sediments increasing *Atob*, coarsening the bed material, and resulting in lower abundance of BOM and CWD, mechanisms that are consistent with established ideas about increases in hydrologic forcings in larger channels (Hammer 1972, Leopold 1994, Doll 2002).

Biotic assemblage contrasts in reference and non-reference streams. – Patterns in macroinvertebrate assemblage-level trait data (*mT*) and traditional metrics suggest the presence of 2 distinct benthic assemblages in reference and non-reference streams. Patterns in trait data (*mT*) support the assertion that benthic assembly occurs through filtering from the regional species pool, based on traits that best suit their occurrence in these contrasting stream types (sensu Keddy 1992, Poff 1997). In particular, the highest loaded trait in the non-reference direction, collector-gatherer feeding group *mT* values, also has been shown to be resistant to hydrologic disturbance from fire in southwestern, US streams (Vieira et al. 2004), and *mT* values also were shown to increase along an increasing human impact landuse/hydromorphologic gradient (Vandewalle et al. 2010). Further, Burcher et al. (2007) found an increase in collector-gatherers in response to an urban landcover cascade related to channel morphology change and decreased percent bed substrate fines, a finding similar to our study. In a study of Florida coastal plain streams, Barbour et al. (1996), suggested that this generalist feeding group should be calibrated for specific stressors as there is not a consistent expectation for collector-gatherer responses to disturbance in the literature (reviewed in Statzner and Beche 2010). Our study suggests that gathering-collectors, multivoltine taxa, and those with preference for fast current velocity, were all favored in hydrologically disturbed habitats (Townsend and Hildrew 1994, Townsend et al. 1997, Carlisle et al. 2010, Vandewalle et al. 2010), and were strongly associated with non-reference streams. For reference streams, our result showing univoltine trait occurrence was consistent with Richards et al. (1997), where univoltine taxa decreased with increasing bankfull area (similar to *Atob* for stable streams). This result is consistent with the predictions of the habitat templet theory (Townsend and Hildrew 1994) for stream ecosystems because habitats that are temporally less variable, such as in reference streams, should select traits that are related to habitat stability (Townsend and Hildrew 1994, Townsend et al. 1997). In contrast, multivoltinism and velocity preferences likely confer resilience (sensu Southwood 1977, Townsend and Hildrew 1994, Carlisle et al. 2010) to repeated temporal hydrologic disturbance allowing for organisms persistence in non-reference streams

In our study, taxa with traits showing fast current preferences were related to non-reference streams, which experienced higher duration maximum flows than reference streams as shown in the $\mathrm{Env}_{\mathrm{PLS}}$. These patterns associated with voltinism and velocity preferences are consistent with the premise that assemblages in non-reference streams are subject to higher hydrologic disturbance (vs. reference streams). These results underscore the paramount importance of the abiotic environment in structuring biotic assemblages in Sand Hills streams. Similarly, organic matter abundance was lower in non-reference streams, likely resulting from increased high-flow events, as flashiness or large magnitude flows, eroding instream benthic organic matter and CWD (Cuffney and Wallace 1989, Maloney et al. 2005). In turn, decreased organic matter was likely indicative of lower coarse particulate organic matter resources for shredders in non-reference streams, thus reducing their *mT* values relative to reference sites.

There were traits that differed between reference and non-reference streams for which we do not have mechanistic explanations, such as the prevalence of dorsoventrally flattened organisms and hemimetabolous-based life stages in reference streams. For instance, the shredder *Leuctra* occurred in high abundances in reference streams and is semivoltine/univoltine (0.2 and 0.8 trait affinities), dorsoventrally flattened, and has 2 aquatic life stages. We suggest that the shredder trait and voltinism co-determines occurrence of this taxon in reference streams because of observed higher food resources (e.g., CPOM) and voltinism's relationship with hydrologic disturbance, whereas dorsoventrally flattened and 2 aquatic life stages traits are phylogenetically constrained and thus may not be filtered based on the environment (Poff et al. 2006).

Last, the presence of strongly contrasting assemblage richness (as EPT) and Biotic Integrity (as SCI and GASCI) between non-reference and reference streams, also suggested that disturbance-insensitive taxa were more likely to occur in non-reference than reference sites. EPT richness often is used as a measure of ecological integrity and thus is predicted to decrease in response to perturbation (Barbour et al. 1996, Maxted et al. 2000). Reference streams have a significantly higher number of EPT taxa, which is consistent with higher observed hydrologic disturbance in these streams. While not significant at the 0.05 level, GABI responded in the predicted direction and SCI and GASCI were significantly higher in reference streams and suggested that non-reference streams have lower biotic integrity than reference streams. Taken together, macroinvertebrate trait and assemblage-based responses suggest that the hydrogeomorphic reference approach identifies a biologically relevant reference condition in Sand Hill streams.

CONCLUSIONS

The hydrogeomorphic reference condition approach to define a biologically relevant reference condition appears useful in determining the likelihood that a stream is in the reference condition. A simple empirical measure of *Atob* and its deviation (*Atob residual* given *Aws*) can be used as a basis for evaluating the non-reference condition using the *Atob residual* threshold of 0.6 m^2. Such field-based empirical approachs appear useful in providing expectations for instream environmental and macroinvertebrate trait conditions as well as other key response variables for use in assessment. We suggest that the hydrogeomorphic approach be expanded and evaluated in the Sand Hills and other Atlantic Coastal Plain ecoregions where low-gradient, sand-bed streams predominate.

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LITERATURE CITED

Abdi, H. 2007. Partial least squares regression pls-regression. Pages 740-744 *in* N. J. Salkind (editor). Encyclopedia of measurement and statistics. Sage, Thousand Oaks, California.

Arcement, G. J., and V. Schneider. 1989. Guide for selecting manning's roughness coefficients for natural channels and flood plains. Water-Supply Paper 2339. U.S. Geological Survey, Denver, Colorado.

Barbour, M. T., J. Gerritsen, G. E. Griffith, R. Frydenborg, E. McCarron, J. S. White, and M. L. Bastian. 1996. A framework for biological criteria for florida streams using benthic macroinvertebrates. Journal of the North American Benthological Society 15:185-211.

Barbour, M. T., J. Gerritsen, B. D. Snyder, and J. B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: Periphyton, benthic macroinvertebrates, and fish - second edition. EPA 841-b-99-002. Environmental Protection Agency; Office of Water, Washington, D.C.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society. Series B (Methodological) 57:289-300.

Berenbrock, C., and A. W. Tranmer. 2008. Simulation of flow, sediment transport, and sediment mobility of the Lower Coeur D'alene River, Idaho. Scientific Investigations Report 2008-5093. U.S. Geological Survey, Reston, Virginia.

Booth, D. B. 1990. Stream-channel incision following drainage-basin urbanization. Water Resources Bullitin 26:407-417.

Borcard, D., F. Gillet, and P. Legendre. 2011. Numerical ecology with r. Springer, New York.

Boulesteix, A.-L. 2004. PLS dimension reduction for classification with microarray data. Statistical Applications in Genetics and Molecular Biology 3:1-32.

Boulesteix, A.-L., and K. Strimmer. 2007. Partial least squares: a versatile tool for the analysis of high-dimensional genomic data. Briefings in Bioinformatics 8:32-44.

Bravard, J., C. Amoros, G. Pautou, G. Bornette, M. Bournaud, M. Creuze des Chatelliers, J. Gibert, J. Peiry, J. Perrin, and H. Tachet. 1997. River incision in south-east France: Morphological phenomena and ecological effects. Regulated Rivers: Research & Management 13:75-90.

Brigham, A. R., W. U. Brigham, and A. Gnilka. 1982. Aquatic insects and oligochaetes of north and South Carolina. Midwest Aquatic Enterprises, Mahomet, Illinois.

Bunn, S. E., and A. H. Arthington. 2002. Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. Environmental Management 30:492-507.

Bunte, K., and S. R. Abt. 2001. Sampling surface and subsurface particle-size distributions in wadable gravel- and cobble-bed streams for analyses in sediment transport, hydraulics, and streambed monitoring. General Technical Report RMRS-GTR-74. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fort Collins, Colorado.

Burcher, C. L., H. M. Valett, and E. F. Benfield. 2007. The land-cover cascade: relationships coupling land and water. Ecology 88:228-242.

Carlisle, D. M., J. Falcone, D. M. Wolock, M. R. Meador, and R. H. Norris. 2010. Predicting the natural flow regime: Models for assessing hydrological alteration in streams. River Research and Applications 26:118-136.

Carlisle, D. M., and M. R. Meador. 2007. A biological assessment of streams in the Eastern United States using a predictive model for macroinvertebrate assemblages. Journal of the American Water Resources Association 43:1194-1207.

Carrascal, L. M., I. Galvan, and O. Gordo. 2009. Partial least squares regression as an alternative to current regression methods used in ecology. Oikos 118:681-690.

Chaudhry, M. H. 2007. Open-channel flow. 2nd edition. Springer Science+Business Media, New York.

Chevenet, F., S. Doledec, and D. Chessel. 1994. A fuzzy coding approach for the analysis of long-term ecological data. Freshwater Biology 31:295-309.

Chong, I. G., and C. H. Jun. 2005. Performance of some variable selection methods when multicollinearity is present. Chemometrics and Intelligent Laboratory Systems 78:103-112.

Copeland, R., P. Soar, and C. Thorne 2005. Channel-forming discharge and hydraulic geometry width predictors in meandering sandbed rivers. Proceedings, 2005 World Water and Environmental Resources Congress: Impacts of Global Change. ASCE Reston, VA.

Cuffney, T. F., M. D. Bilger, and A. M. Haigler. 2007. Ambiguous taxa: effects on the characterization and interpretation of invertebrate assemblages. Journal of the North American Benthological Society 26:286-307.

Cuffney, T. F., and J. B. Wallace. 1989. Discharge-export relationships in headwater streams: the influence of invertebrate manipulations and drought. Journal of the North American Benthological Society 8:331-341.

de Jong, S. 1993. Simpls: an alternative approach to partial least squares regression. Chemometrics and Intelligent Laboratory Systems 18:251-263.

Doledec, S., N. Phillips, M. Scarsbrook, R. H. Riley, and C. R. Townsend. 2006. Comparison of structural and functional approaches to determining landuse effects on grassland stream invertebrate communities. Journal of the North American Benthological Society 25:44-60.

Doll, B. A., D. E. Wise-Frederick, C. M. Buckner, S. D. Wilkerson, W. A. Harman, R. E. Smith, and J. Spooner. 2002. Hydraulic geometry relationships for urban streams throughout the piedmont of North Carolina. Journal of the American Water Resources Association 38:641-651.

Epler, J. 2010. The water beetles of Florida, an identification manual for the families Chrysomelidae, Curculionidae, Dryopidae, Elmidae, Gyrinidae, Haliplidae, Helophoridae, Hydraenidae, Hydrochidae, Hydrophilidae, Noteridae, Psephenidae, Ptilodactylidae and Scirtidae. Florida Department of Environmental Protection, Tallahassee, Florida. (Avaliable from: http://www.floridadep.org/labs/cgi-bin/sbio/keys.asp).

Epler, J. H. 2001. Identification manual for the larval Chironomidae (Diptera) of North and South Carolina. A guide to the taxonomy of the midges of the southeastern United States including Florida. Special Publication SJ2001-SP13, North Carolina Department of Environmental and Natural Resources, Raleigh, NC, and St. Johns River Water Managment district, Palatka, FL. (Avaliable from: http://www.floridadep.org/labs/cgi-bin/sbio/keys.asp).

Feminella, J. W. 1996. Comparison of benthic macroinvertebrate assemblages in small streams along a gradient of flow permanence. Journal of the North American Benthological Society 15:651-669.

Feminella, J. W. 2000. Correspondence between stream macroinvertebrate assemblages and 4 ecoregions of the southeastern USA. Journal of the North American Benthological Society 19:442-461.

Fore, L. S., R. Frydenborg, D. Miller, T. Frick, D. Whiting, J. Espy, and L. Wolfe. 2007. Development and testing of biomonitoring tools for macroinvertebrates in Florida streams (stream condition index and biorecon). Florida Department of Environmental Protection, Tallahassee, FL, USA. (Avaliable from: http://www.dep.state.fl.us/water/bioassess/pubs.htm).

Garnier, E., J. Cortez, G. Billes, M.-L. Navas, C. Roumet, M. Debussche, G. Laurent, A. Blanchard, D. Aubry, A. Bellmann, C. Neill, and J.-P. Toussaint. 2004. Plant functional markers capture ecosystem properties during secondary succession. Ecology 85:2630-2637.

Gesch, D., M. Oimoen, S. Greenlee, C. Nelson, M. Steuck, and D. Tyler. 2002. The national elevation dataset. Photogrammetric Engineering and Remote Sensing 68:5-11.

Grime, J. P. 1998. Benefits of plant diversity to ecosystems: immediate, filter and founder effects. Journal of Ecology 86:902-910.

Hammer, T. R. 1972. Stream channel enlargement due to urbanization. Water Resources Research 8:1530-1540.

Hart, D. D., and C. M. Finelli. 1999. Physical-biological coupling in streams: the pervasive effects of flow on benthic organisms. Annual Review of Ecology and Systematics 30:363-395.

Hawkins, C. P., J. R. Olson, and R. A. Hill. 2010. The reference condition: predicting benchmarks for ecological and water-quality assessments. Journal of the North American Benthological Society 29:312-343.

Helms, B. S., J. E. Schoonover, and J. W. Feminella. 2009. Assessing influences of hydrology, physicochemistry, and habitat on stream fish assemblages across a changing landscape. Journal of the American Water Resources Association 45:157-169.

Houser, J. N., P. J. Mulholland, and K. O. Maloney. 2006. Upland disturbance affects headwater stream nutrients and suspended sediments during baseflow and stormflow. Journal of Environmental Quality 35:352-365.

Ihaka, R., and R. Gentleman. 1996. R: A language for data analysis and graphics. Journal of Computational and Graphical Statistics 5:299-314.

Keddy, P. A. 1992. Assembly and response rules: Two goals for predictive community ecology. Journal of Vegetation Science 3:157-164.

Kosnicki, E., S. A. Sefick, M. H. Paller, M. S. Jarrell, B. A. Prusha, S. C. Sterrett, T. D. Tuberville, and J. W. Feminella. 2014. Defining the reference condition for wadeable streams in the Sand Hills subdivision of the Southeastern Plains ecoregion, USA. Environmental Management 54:494-504.

Kowalyk, H. E. 1985. The larval cephalic setae in the Tanypodinae (Diptera: Chironomidae) and their importance in generic determinations. Canadian Entomologist 117:67-106.

Lake, P. S. 2000. Disturbance, patchiness, and diversity in streams. Journal of the North American Benthological Society 19:573-592.

Laliberte, E., and B. Shipley. 2011. Fd: Measuring functional diversity (fd) from multiple traits, and other tools for functional ecology. R package version 1.0-11. (Avaliable from: https://www.r-project.org/)

Lane, E. W. 1947. Report of the subcommittee on sediment terminology. Transactions, American Geophysical Union 28:936-938.

Leopold, L. B. 1994. A view of the river. Harvard Univiversity Press, Cambridge, Massachuessets.

Maechler, M. 2012. Sfsmisc: Utilities from seminar fuer statistik eth zurich. R package version:1.0-20. (Avaliable from: https://www.r-project.org/)

Maechler, M., P. Rousseeuw, A. Struyf, M. Hubert, and K. Hornik. 2012. Cluster: Cluster analysis basics and extensions. R package version 1. (Avaliable from: https://www.r-project.org/)

Maloney, K. O., and J. W. Feminella. 2006. Evaluation of single-and multi-metric benthic macroinvertebrate indicators of catchment disturbance over time at the Fort Benning Military Installation, Georgia, USA. Ecological Indicators 6:469-484.

Maloney, K. O., J. W. Feminella, R. M. Mitchell, S. A. Miller, P. J. Mulholland, and J. N. Houser. 2008. Landuse legacies and small streams: identifying relationships between historical land use and contemporary stream conditions. Journal of the North American Benthological Society 27:280-294.

Maloney, K. O., P. J. Mulholland, and J. W. Feminella. 2005. Influence of catchment-scale military land use on stream physical and organic matter variables in small southeastern plains catchments (USA). Environmental Management 35:677-691.

Manning, R. 1889. On the flow of water in open channels and pipes. Transactions of the Institution of Civil Engineers of Ireland 20:161-195.

Maxted, J. R., M. T. Barbour, J. Gerritsen, V. Poretti, N. Primrose, A. Silvia, D. Penrose, and R. Renfrow. 2000. Assessment framework for mid-atlantic coastal plain streams using benthic macroinvertebrates. Journal of the North American Benthological Society 19:128-144.

McMahon, G., J. D. Bales, J. F. Coles, E. M. P. Giddings, and H. Zappia. 2003. Use of stage data to characterize hydrologic conditions in an urbanizing environment. Journal of the American Water Resources Association 39:1529-1546.

Merritt, R. W., K. W. Cummins, and M. B. Berg. 2008. An introduction to the aquatic insects of north america. 4th edition. Kendall Hunt Publishing, Dubuque, Iowa.

Mevik, B., and R. Wehrens. 2007. The pls package: principal component and partial least squares regression in r. Journal of Statistical Software 18:1-24. (Avaliable from: https://www.r-project.org/)

Nagy, R. C., B. G. Lockaby, B. Helms, L. Kalin, and D. Stoeckel. 2011. Water resources and land use and cover in a humid region: the southeastern United States. Journal of Environmental Quality 40:867-878.

Neteler, M., M. H. Bowman, M. Landa, and M. Metz. 2011. Grass GIS: a multi-purpose open source GIS. Environmental Modelling & Software 31:124-130.

Poff, N. L. 1997. Landscape filters and species traits: towards mechanistic understanding and prediction in stream ecology. Journal of the North American Benthological Society 16:391-409.

Poff, N. L., J. D. Olden, N. K. M. Vieira, D. S. Finn, M. P. Simmons, and B. C. Kondratieff. 2006. Functional trait niches of North American lotic insects: traits-based ecological applications in light of phylogenetic relationships. Journal of the North American Benthological Society 25:730-755.

Power, M. E., A. Sun, G. Parker, W. E. Dietrich, and J. T. Wootton. 1995. Hydraulic food-chain models. BioScience 45:159-167.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. (Avaliable from: https://www.r-project.org/)

Richards, C., R. Haro, L. Johnson, and G. Host. 1997. Catchment and reach scale properties as indicators of macroinvertebrate species traits. Freshwater Biology 37:219-230.

Rousseeuw, P. J. 1987. Silhouettes: a graphical aid to the interpretation and validation of cluster analysis. Journal of Computational and Applied Mathematics 20:53-65.

Schoonover, J. E., B. G. Lockaby, and B. S. Helms. 2006. Impacts of land cover on stream hydrology in the West Georgia Piedmont, USA. Journal of Environmental Quality 35:2123-2131.

Sefick, S. A., L. Kalin, E. Kosnicki, B. P. Schneid, M. S. Jarrell, C. J. Anderson, M. H. Paller, and J. W. Feminella. 2015. Emprical estimation of stream discharge using channel geometry in low-gradient, sand-bed streams of the southeastern plains. Journal of the American Water Resources Association 51:1060-1071.

Shipley, B., D. Vile, and E. Garnier. 2006. From plant traits to plant communities: a statistical mechanistic approach to biodiversity. Science 314:812-814.

Simons, D. B., and R. K. Simons 1987. Differences between gravel and sand-bed rivers. Pages 3-11 *in* J. C. Thorne and R. D. Hey (editors). Sediment transfer in gravel-bed rivers. John Wiley & Sons, New York.

Sokol, E. R., E. F. Benfield, L. K. Belden, and M. H. Valett. 2011. The assembly of ecological communities inferred from taxonomic and functional composition. American Naturalist 177:630-644.

Sonesten, L. 2003. Catchment area composition and water chemistry heavily affects mercury levels in perch (*Perca fluviatilis* l.) in circumneutral lakes. Water, Air, and Soil Pollution 144:117-139.

Southwood, T. R. E. 1988. Tactics, strategies and templets. Oikos 52:3-18.

Southwood, T. R. E. 1977. Habitat, the templet for ecological strategies? Journal of Animal Ecology 46:337-365.

Statzner, B., and L. A. Beche. 2010. Can biological invertebrate traits resolve effects of multiple stressors on running water ecosystems? Freshwater Biology 55:80-119.

Statzner, B., J. A. Gore, and V. H. Resh. 1988. Hydraulic stream ecology: observed patterns and potential applications. Journal of the North American Benthological Society 7:307-360.

Stoddard, J. L., D. P. Larsen, C. P. Hawkins, R. K. Johnson, and R. H. Norris. 2006. Setting expectations for the ecological condition of streams: the concept of reference condition. Ecological Applications 16:1267-1276.

Szymanska, E., E. Saccenti, A. K. Smilde, and J. A. Westerhuis. 2012. Double-check: validation of diagnostic statistics for pls-da models in metabolomics studies. Metabolomics 8:3-16.

Sweeney, B. W., T. L. Bott, J. K. Jackson, L. A. Kaplan, J. D. Newbold, L. J. Standley, W. C. Hession, and R. J. Horwitz. 2004. Riparian deforestation, stream narrowing, and loss of stream ecosystem services. Proceedings of the National Academy of Sciences of the United States of America 101:14132-14137.

Therneau, T. M., B. Atkinson, and B. Ripley. 2010. Rpart: Recursive partitioning. R package version 3. (Avaliable from: https://www.r-project.org/)

Thorp, J. H., and A. P. Covich (editors). 2009. Ecology and classification of North American freshwater invertebrates. 3rd edition. Academic Press, Burlington, MA.

Townsend, C. R., and A. G. Hildrew. 1994. Species traits in relation to a habitat templet for river systems. Freshwater Biology 31:265-275.

Townsend, C. R., M. R. Scarsbrook, and S. Doledec. 1997. Quantifying disturbance in streams: alternative measures of disturbance in relation to macroinvertebrate species traits and species richness. Journal of the North American Benthological Society 16:531-544.

Turkmen, A., and N. Billor. 2013. Partial least squares classification for high dimensional data using the pcout algorithm. Computational Statistics 28:771-778.

Vandewalle, M., F. De Bello, M. P. Berg, T. Bolger, S. Doledec, F. Dubs, C. K. Feld, R. Harrington, P. A. Harrison, S. Lavorel, P. Martins da Siva, M. Moretti, J. Niemela, P. Santos, T. Sattler, J. P. Sousa, M. T. Sykes, A. J. Vanbergen, and B. A. Woodcock. 2010. Functional traits as indicators of biodiversity response to land use changes across ecosystems and organisms. Biodiversity and Conservation 19:2921-2947.

Venables, W. N., and B. D. Ripley. 1994. Modern applied statistics with s-plus. 4th edition. Springer, New York.

Vieira, N. K. M., W. H. Clements, L. S. Guevara, and B. F. Jacobs. 2004. Resistance and resilience of stream insect communities to repeated hydrologic disturbances after a wildfire. Freshwater Biology 49:1243-1259.

Vieira, N. M. K., N. L. M. Poff, D. M. Carlisle, S. R. Moulton II, M. L. Koski, and B. C. Kondratieff. 2006. A database of lotic invertebrate traits for north america. Data Series 187. U.S. Geological Survey. (Avaliable from: http://pubs.usgs.gov/ds/ds187/)

Wallace, J., and J. Grubaugh 1996. Transport and storage of FPOM. Pages 191-215 *in* F. R. Hauer and G. A. Lamberti (editors). Methods in stream ecology. Academic Press, San Diego, California.

Wallace, J. B., and A. C. Benke. 1984. Quantification of wood habitat in subtropical coastal plain streams. Canadian Journal of Fisheries and Aquatic Sciences 41:1643-1652.

Wehrens, R., P. Franceschi, M. R. Wehrens, and M. Suggests. 2013. Package biomark. (Avaliable from: https://www.r-project.org/)

Whittier, T. R., J. L. Stoddard, D. P. Larsen, and A. T. Herlihy. 2007. Selecting reference sites for stream biological assessments: best professional judgment or objective criteria. Journal of the North American Benthological Society 26:349-360.

Wickham, J. D., S. V. Stehman, L. Gass, J. Dewitz, J. A. Fry, and T. G. Wade. 2013. Accuracy assessment of nlcd 2006 land cover and impervious surface. Remote Sensing of Environment 130:294-304.

Wold, S., K. Esbensen, and P. Geladi. 1987. Principal component analysis. Chemometrics and Intelligent Laboratory Systems 2:37-52.

Zampella, R. A., J. F. Bunnell, K. J. Laidig, and N. A. Procopio. 2006. Using multiple indicators to evaluate the ecological integrity of a coastal plain stream system. Ecological Indicators 6:644-663.

Appendix 1. Definitions of environmental and stream benthic macroinvertebrate variables used in Partial Least Squares (PLS) regression modeling. Variables highlighted grey were those retained in PLS modeling after the variable importance in the projection (vip), variable selection step (see text). ENV=Environmental; MACRO=Macroinvertebrate; GIS LULC = Land use/Land Cover from GIS data.

Variable Category	Variable Class	Variable Condition	Variable Code	Variable Definition
ENV	***LANDSCAPE***		Elevation	Stream Elevation
			Slope	GIS stream slope
			Devel	GIS LULC Low+Medium+High Development
			Forest	GIS LULC mixed+evergreen+ deciduous forest+woody wetlands
	HYDROLOGY (STAGE)	**Duration of Low Stage**	MAX_q10	Maximum duration below 10th quartile
			MAX_q25	Maximum duration below 25th quartile
			MAX_q5	Maximum duration below 5th quartile
			MED._q10	Median duration below 10th quartile
			MED._q25	Median duration below 25th quartile
			MED._q5	Median duration below 5th quartile
		Duration of High Stage	MAX_q75	Maximum duration above 75th quartile
			MAX_q90	Maximum duration above 90th quartile
			MAX_q95	Maximum duration above 95th quartile
			MED._q75	Median duration above 75th quartile
			MED._q90	Median duration above 90th quartile
			MED._q95	Median duration above 95th quartile
		Frequency of Stage Change (Flashiness)	rising 15.24 cm	Number of hours stage rises at least 15.24 cm
			rising 21.336 cm	Number of hours stage rises at least 21.336 cm
			rising 27.432 cm	Number of hours stage rises at least 27.432 m
			rising 3.048 cm	Number of hours stage rises at least 3.048 cm
			rising 9.144 cm	Number of hours stage rises at least 9.144 cm
			falling 15.24 cm	Number of hours stage falls at least 15.24 cm
			falling 21.336 cm	Number of hours stage falls at least 21.336cm

			falling 27.432 cm	Number of hours stage falls at least 27.432cm
			falling 3.048 cm	Number of hours stage falls at least 3.048 cm
			falling 9.144 cm	Number of hours stage falls at least 9.144 cm
			CV	Coefficient of Variation for the Period of Record of stage
	HYDROLOGY (Discharge)		max_Q	Maximum Discharge
			median_Q	Median Discharge
	HYDRAULICS		max_power	Maximum Stream Power
			median_power	Median Stream Power
			max_Fr	Maximum Froude Number
			median_Fr	Median Froude number
			max_t	Maximum Tractive Force
			median_t	Median Tractive Force
			prop_sed_move	Proportion of time median particle entrained
			max_U_shear	Maximum Shear Velocity
			median_U_shear	Median Shear Velocity
	INSTREAM HABITAT	**Organic Matter**	CWD	Coarse woody debris (wood > 0.25 m diam.)
			OM	Benthic organic matter deposited in channel
		Stream Chemistry	pH	pH
			SC	Specific Conductance
			DO %	Dissolved oxygen %
			Temperature	Water Temperature
		Insolation	prop_NC	Proportion stream canopy
		Bed Sediment	Sed Mean	Mean diameter substrate particles in stream bed
			SED SD	Substrate size Standard Deviation
MACRO		**Reproductive Preference**	ovi1	oviposition (egg laying) on algal mats
			ovi2	oviposition (egg laying) on bank soil
			ovi3	oviposition (egg laying) on bed substrate
			ovi4	oviposition (egg laying) on floating debris
			ovi5	oviposition (egg laying) on moss/ submerged macrophytes

		ovi6	oviposition (egg laying) on wet wood
		ovi7	oviposition (egg laying) on/under stones
		ovi8	oviposition (egg laying) on overhanging substrate dry
		eggc1	Laying cemented eggs
		eggc2	Laying Non-cemented eggs
HABITAT	**Waterbody Preference**	wb1	Lentic
		wb2	Warm spring
		Cold Spring	Cold spring
		Headwater	Headwater
		wb5	2nd to 4th order
		wb6	River
		wb7	Temporary habitat
	Current Preference	vel1	Quiet current
		Slow Current	Slow current
		vel3	Fast laminar current
		Fast Current	Fast turbulent current
	Current Adaptation	mflo1	Adaptations to flow
		mflo2	No flow adaptations
	Microhabitat Preference	mh1	Sand
		mh2	Silt
		mh3	Gravel
		mh4	Rocks
		mh5	Boulder
		mh6	Large woody debris
		Detritus Micro. Hab.	Detritus
		mh8	Phytoplankton
		Algae	Algae
		mh10	Pelagic
	Lateral Preference	lat1	Lotic margin
		lat2	Lentic shore

		lat3	Pools
		lat4	Riffle
		lat5	Hyporheic (subsurface)
	Vertical Preferences	vrt1	Water surface
		Macrophytes	Aquatic plants
		vrt3	Pelagic
		Bed Hab. Pref.	Stream bed
		vrt5	Hyporheic (subsurface)
PHYSIOLOGICAL	**Oxygen Tolerance**	oxy1	Normal
		oxy2	Low
	Chemical Tolerance	pH1	Acidic (< 6.0)
		pH2	Circumneutral (~7.0)
		pH3	Alkaline (> 8.0)
		sal1	Fresh water
		sal2	Brackish
		sal3	Salt water
	Thermal Preference	thrm1	Cold water
		thrm2	No temperature preference
		thrm3	Warm water
	Turbidity Preference	trb1	Low turbidity
		trb2	No turbidity preference
		trb3	High turbidity
ECOLOGY	**Functional Feeding Groups**	ffg1	Collector-filterer
		Collector Gatherer	Collector-gatherer
		ffg3	Parasite
		ffg4	Predator
		ffg5	Scraper/grazer
		Shred.	Shredder
	Habit	hab1	Burrower
		hab2	Climber
		hab3	Clinger

		hab4	Sprawler
		hab5	Swimmer
LIFE HISTORY		2 aqu. stages	egg and nymph
		ast3	egg, larvae, and pupae
		ast4	egg, larvae, pupae, and adult
	Voltinism	vlt1	Semivoltine (< 1 generation per year)
		Uni Volt.	Univoltine (1 generation per year)
		Multi Volt.	Multivoltine (> 1 generation per year)
	Development Speed	dsp1	Slow seasonal cycle
		Fast Seasonal	Fast seasonal cycle
		dsp3	Non-seasonal
	Adult Life Span	Short Adult Life.	Hours
		Moderate Adult Life.	Weeks
		Long Adult Life.	Months
	Fecundity	fec1	< 100 eggs
		fec2	100-1000
		fec3	1000-10,000
		Diapause	Diapause (resting stage)
		No Diapause	No Diapause (resting stage)
MOBILITY	**Drift**	drf1	Weak drifter
		drf2	Passive/occasional drifter
		Strong Drift	active/frequent drifter
	Larval Dispersal	lds1	Dispersal distance < 1 m
		lds2	Dispersal distance 1-10 m
		lds3	Dispersal distance 11-100 m
	Adult Dispersal	dis1	Dispersal distance 10 m
		dis2	Dispersal distance 1 km
		dis3	Dispersal distance 10 km
		dis4	Dispersal distance 100 km
		ext1	Ability to temporarily exit water

		ext2	Inbility to temporarily exit water
MORPHOLOGY	**Larval size**	siz1	length < 9 mm
		siz2	length 9-16 mm
		siz3	length > 16 mm
	Body shape	Bluff	Bluff (bricklike)
		shp2	Round (humped)
		shp3	Tubular
		shp4	Streamlined/fusiform
		D. Flat.	Dorsoventrally flattened
	Sclerotization (body armor)	arm1	Soft (unhardened) body
		arm2	Partially sclerotized (hardened)
		Hard Shelled	Hard Shelled
		arm4	All sclerotized (completely hardened)
	Respiration mode	rsp1	Cutaneous respiration (through cuticle)
		rsp2	Spiracular gills (water breather)
		rsp3	Hemolymph with hemoglobin
		rsp4	Tracheal gills (water breather)
		rsp5	Atmospheric respiration (air breather)
		rsp6	Plastron (water breather)
		rsp7	Temporary air store (air breather)

Appendix 2. Example application showing empirical determination of hydrogeomorphic (HGM) condition for a stream channel on Fort Benning, GA.

Watershed area (*Aws*, km^2) is estimated using GPS (as UTM coordinates at the downstream terminus of the sample reach) and GIS algorithms (e.g., r.stream.basins in GRASS GIS 6.4). Observed area at top of bank ($Atob_o$, m^2) is measured in the field (see text for explanation). Predicted area at top of bank ($Atob_p$, m^2) is estimated using the robust regression equation $Atob_p$=0.7381+ 0.0839**Aws*, which, when subtracted from $Atob_o$ yields *Atob residual* for the site. If *Atob residual* is < 0.6 m^2 then the site is considered reference; if this value is > 0.6 m^2 then the site is considered hydrogeomorphically disturbed. Here, estimated *Atob residual* was < 0.6 m^2, resulting in a site classification of reference. Instream environmental and biological variables with the highest discrimination potential (determined from PLS-LDA analyses, above) can be used set expectations for conditions likely to occur based on HGM classification state.

Variable	**Value**
UTM Coordinate (Zone 16)	3588705 N 0710570 E
Aws (from GIS)	0.636 km^2
$Atob_p$ = 0.7381+ 0.0839**Aws*	0.792 m^2
$Atob_o$	0.668 m^2
Atob residual = $Atob_o$ - $Atob_p$	-0.124 m^2
Atob residual < 0.6 m^2 – REFERENCE	YES
Atob residual > 0.6 m^2 - NON-REFERENCE	NO

Fig. 1. Map of SE United States showing study sites (●) in GA, SC, and NC. Sites were in the Sand Hills ecoregion below the Piedmont and above the Coastal Plain (light grey lines).

Fig. 2. Iteratively reweighted least squares regressions of watershed area (*Aws*) and A) the top of bank area (*Atob*), B) the top of bank mean depth (*Dtob*), and C) the top of bank width (*Wtob*) for the 62 study sites. Colors and shapes are indicative of geomorphic group based on the clustering analysis and iteratively re-weighted least squares (see text). ● and ● are reference sites as indicated by Partitioning Around Mediods clustering. ● are reference sites that were randomly paired with similar *Aws* to non-reference sites (▲) for site comparisons using macroinvertebrate and environmental data.

Fig. 3. Principal components cluster biplot. Reference and non-reference sites are represented by ● and ▲, respectively. A_e = *Atob*, W_e = *Wtob*, and D_e *Dtob* (defined in Fig. 2) residuals from robust regression (see text), respectively. Centroid distance is Cd_0 (see text). The variance explained by PC1 and PC2 was 81.1 and 13.4%, respectively.

Fig. 4. Partial Least Squares (PLS) loadings of original environmental variables onto PLS latent variable (LV) 1. Variables are ordered by decreasing absolute magnitude of their loading on LV 1. Reference stream scores were negative values whereas non-reference stream scores were positive. The LDA standardized coefficient derived from the $\mathrm{Env}_{\mathrm{PLS}}$ scores was 7.52. Variables defined in Appendix 1.

Fig. 5. Boxplots comparing Ephemeroptera, Plecoptera and Trichoptera richness (EPT) (A), Florida Stream Condition Index (SCI) (B), Georgia Stream Condition Index (GASCI) (C), and Georgia Biotic Index (GABI) (D) between reference and non-reference streams, as defined by contrasting site hydrogeomorphology (see text). Difference between groups for EPT, SCI, and GASCI were significant (∗) at *P*≤0.0315.

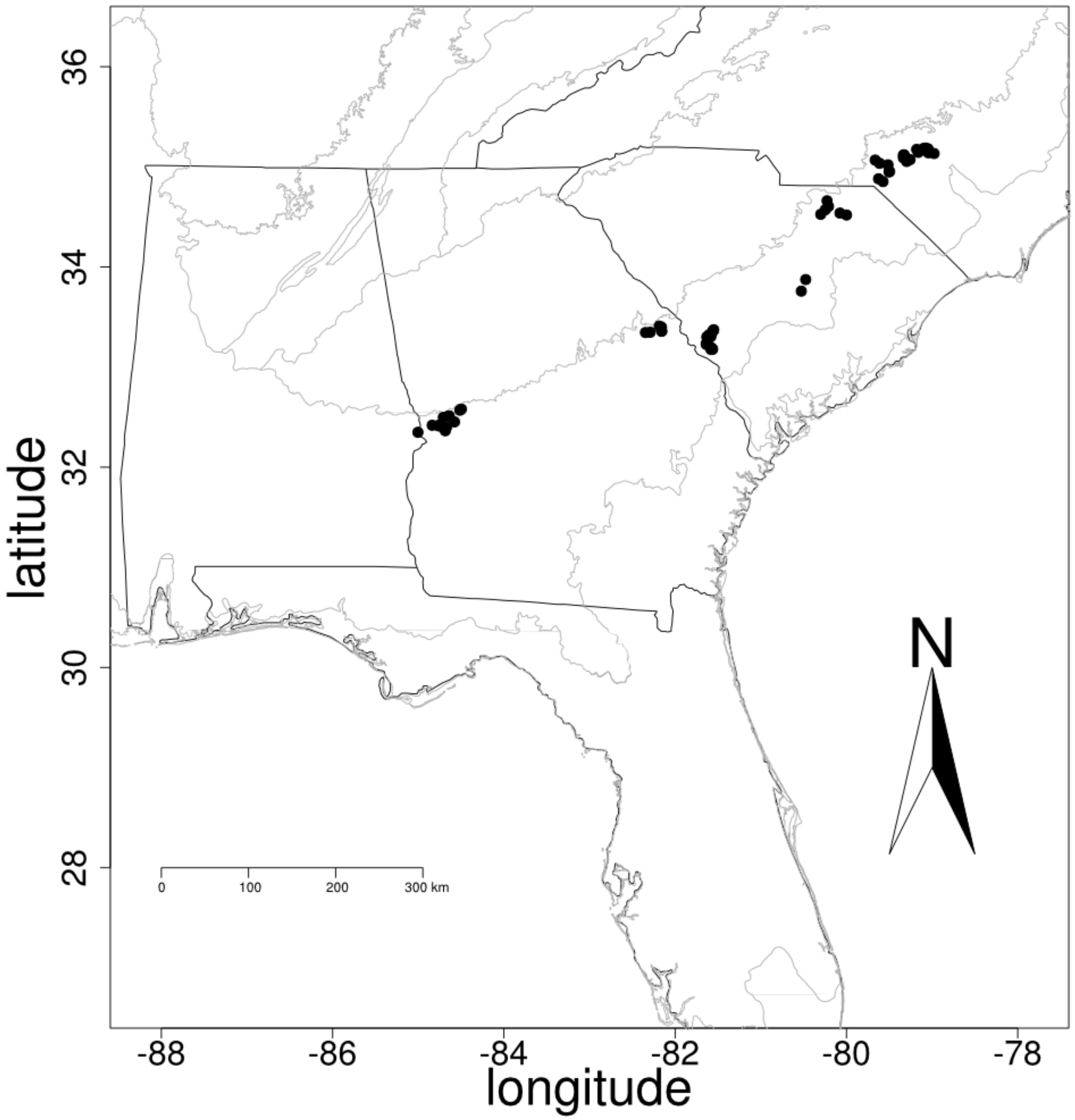
Fig. 6. Partial Least Squares (PLS) score and loadings biplot displaying PLS latent variable 1 and 2 for the macroinvertebrate assemblage trait data from the study sites. ● are reference sites and ▲ are non-reference sites as indicated by Partitioning Around Mediods clustering. Variables furthest from the origin exerted the greatest influence on the resulting site scores (see Fig. 6 for variable loading plots). Variance explained for X and Y matrices are X=35.97 and Y=49.30% and X=14.84 and Y=21.04%, x and y axes respectively. Traits important based on the variable selection step that were not highly loaded were removed to simplify figure. **DH** is Detritus Micro. Hab. and **FS** is Fast Seasonal; all Variables defined in Appendix 1.

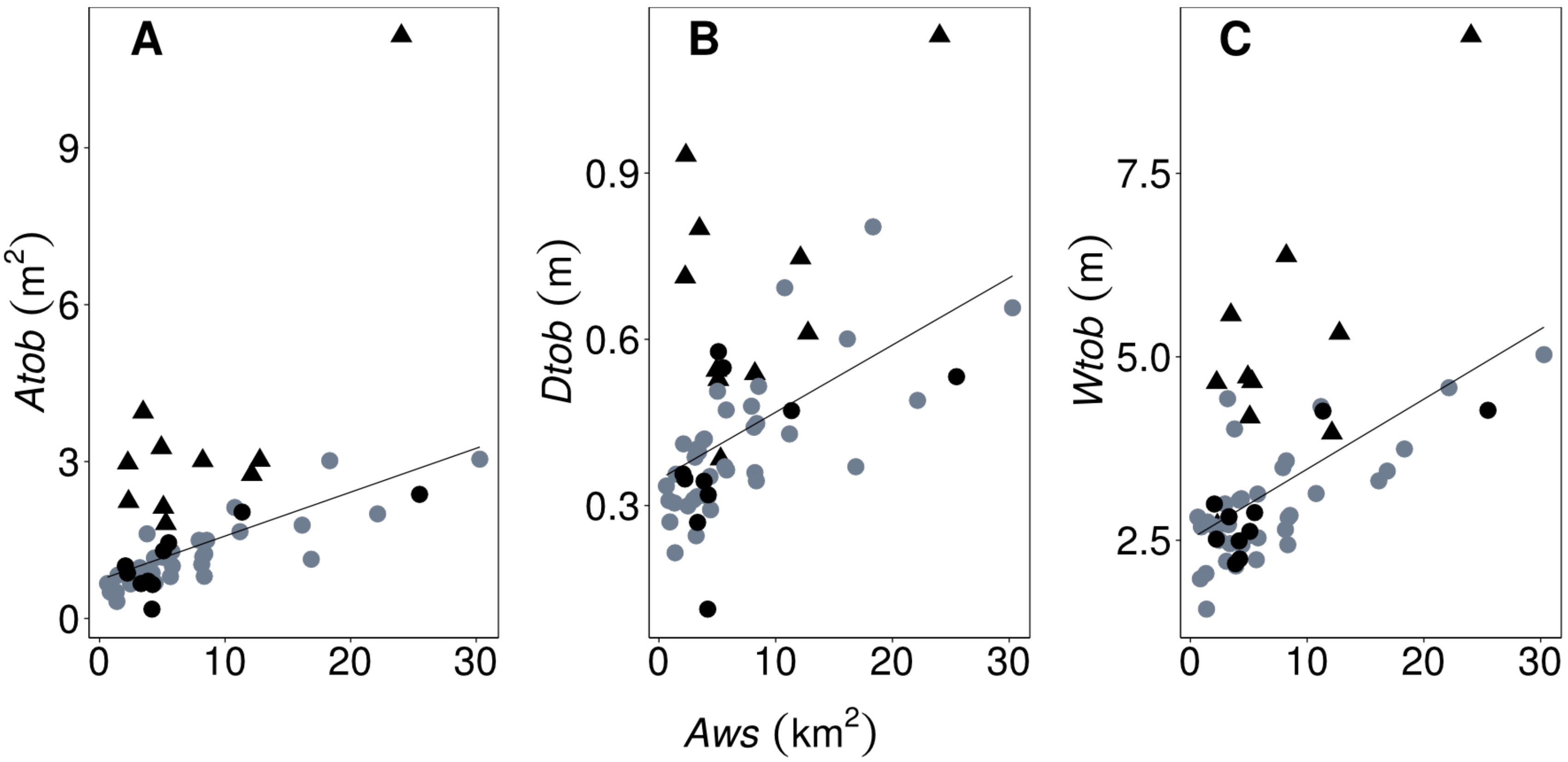
Fig. 7. Partial Least Squares (PLS) loadings of macroinvertebrate mean trait variables onto PLS latent variable (LV) 1. Variables are ordered by the absolute magnitude of their loading on LV 1. Reference stream scores were negative values whereas non-reference stream scores were

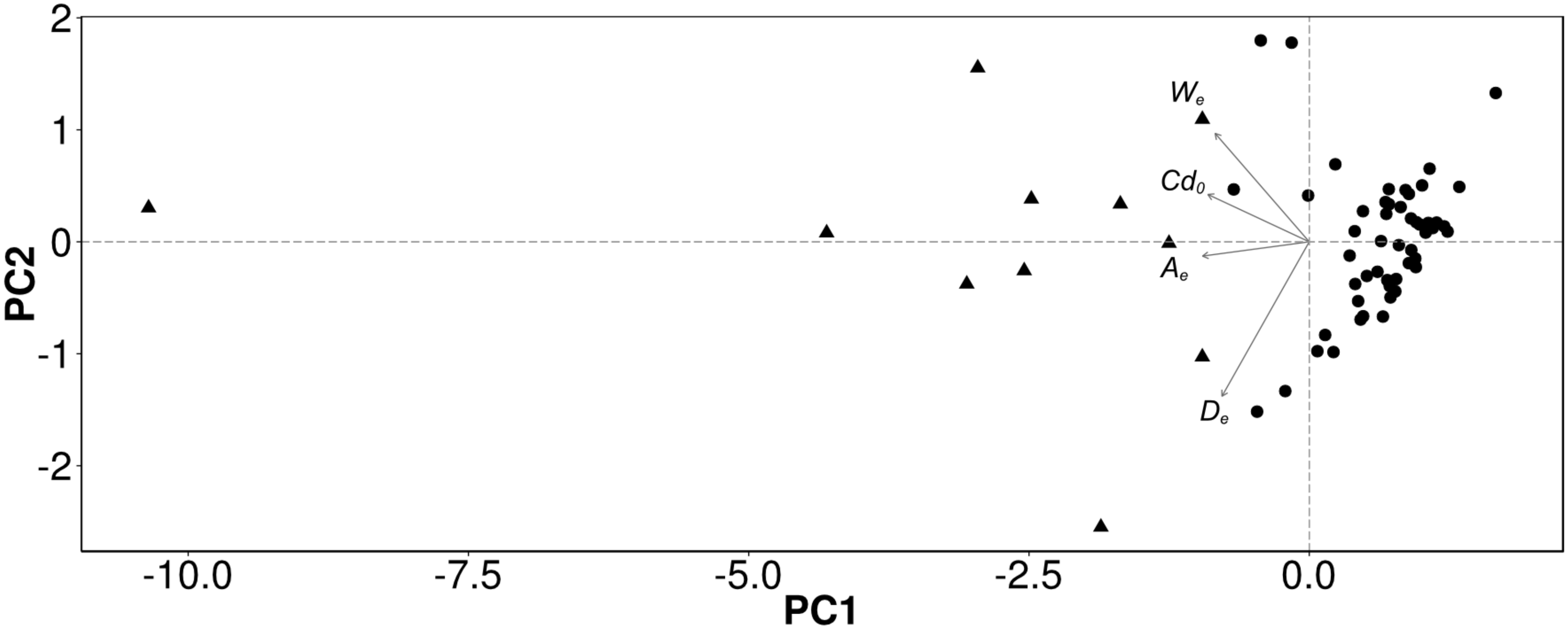
positive. Loadings ordered in terms of decreasing loading magnitude. Variables defined in Appendix 1.

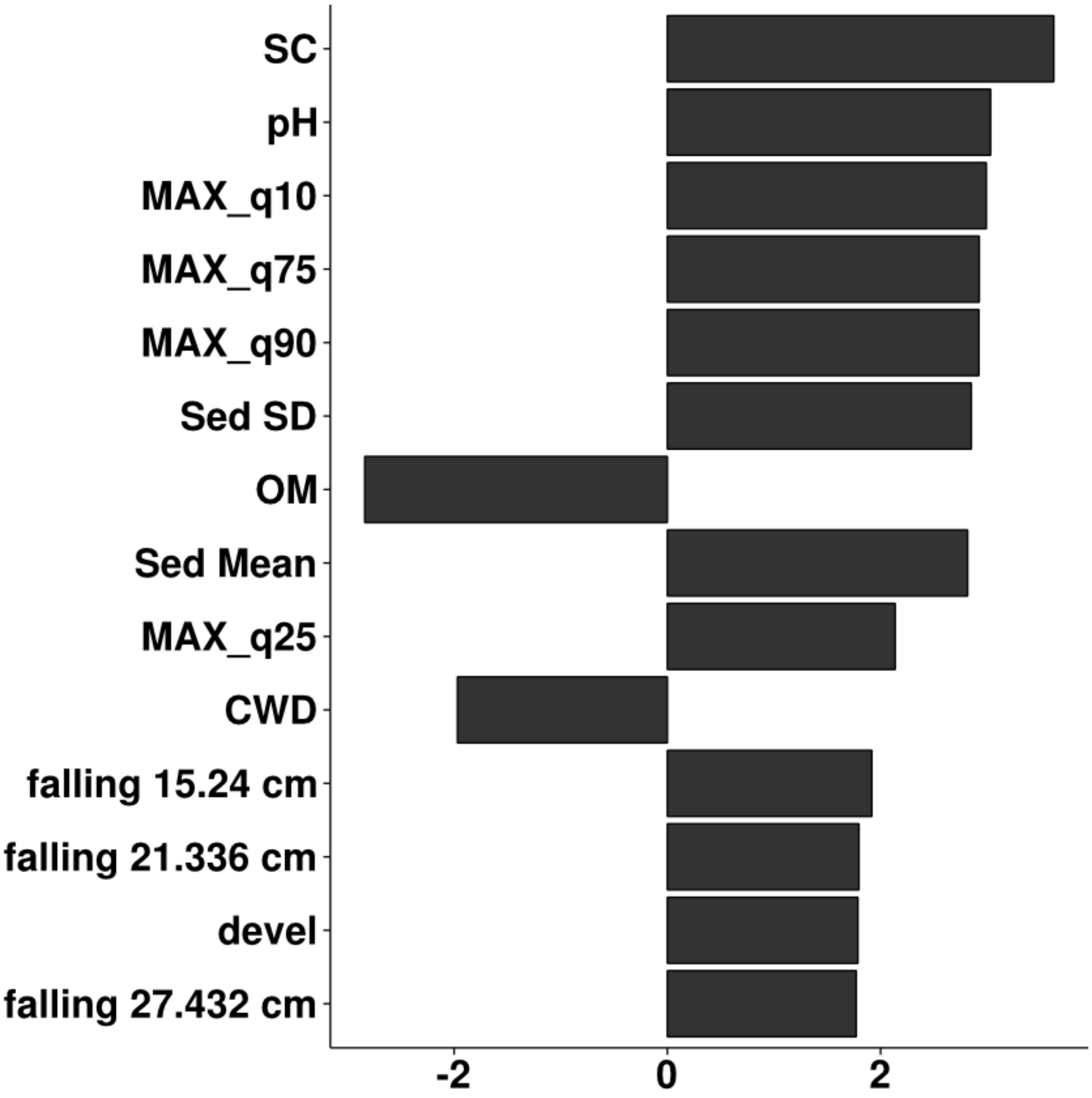
Table 1: Standardized linear discriminant analysis (LDA) coefficients utilizing partial least squares (PLS) latent variables (LV 1-6 from macroinvertebrate traits, $\text{trait}_{\text{PLS}}$), used to predict reference/non-reference streams based on mean macroinvertebrate trait values (see text).

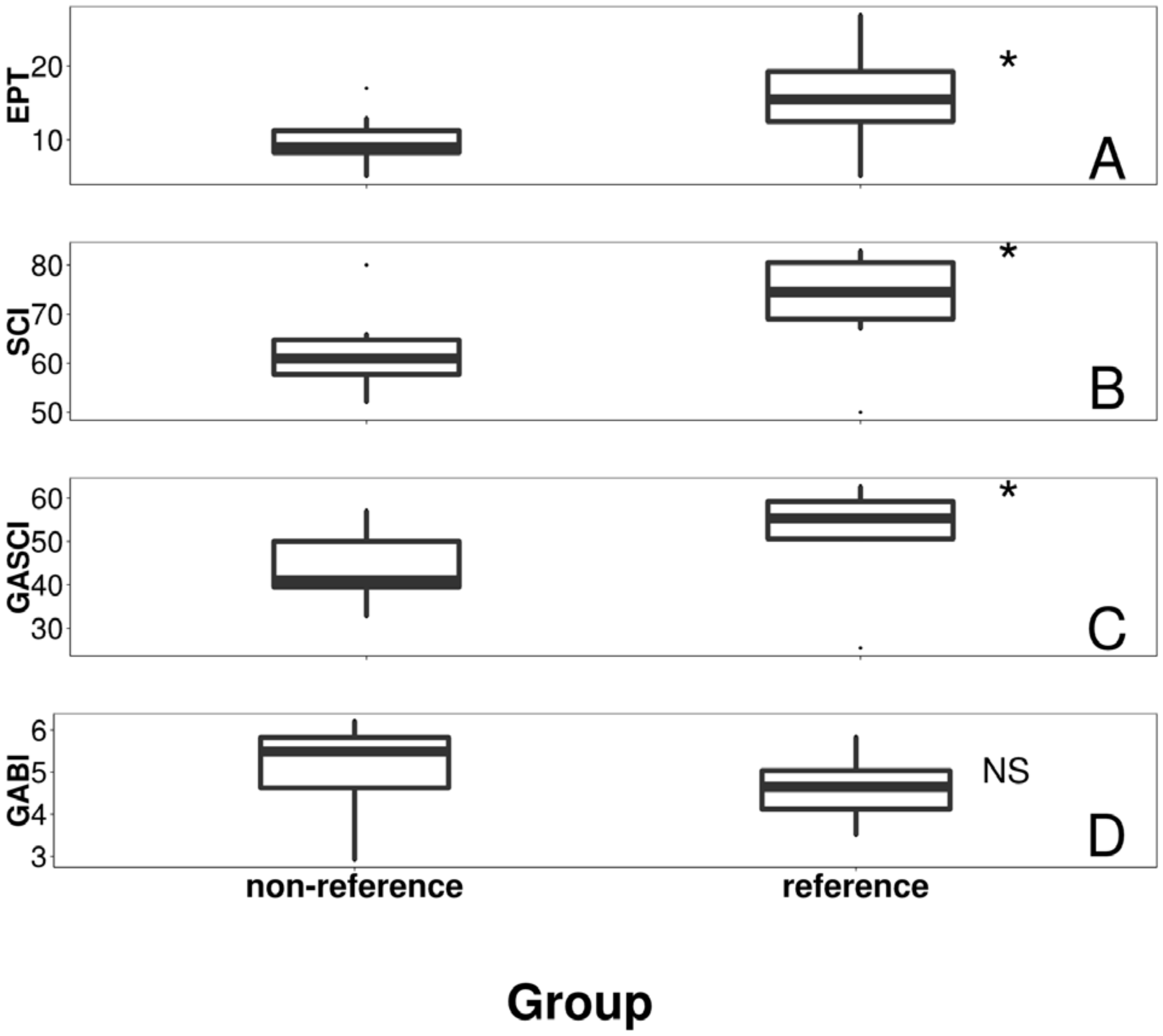
Latent Variable	Standardized LDA Coefficient
LV 1	9.9
LV 2	5.5
LV 3	6
LV 4	2.1
LV 5	2.5
LV 6	1.9

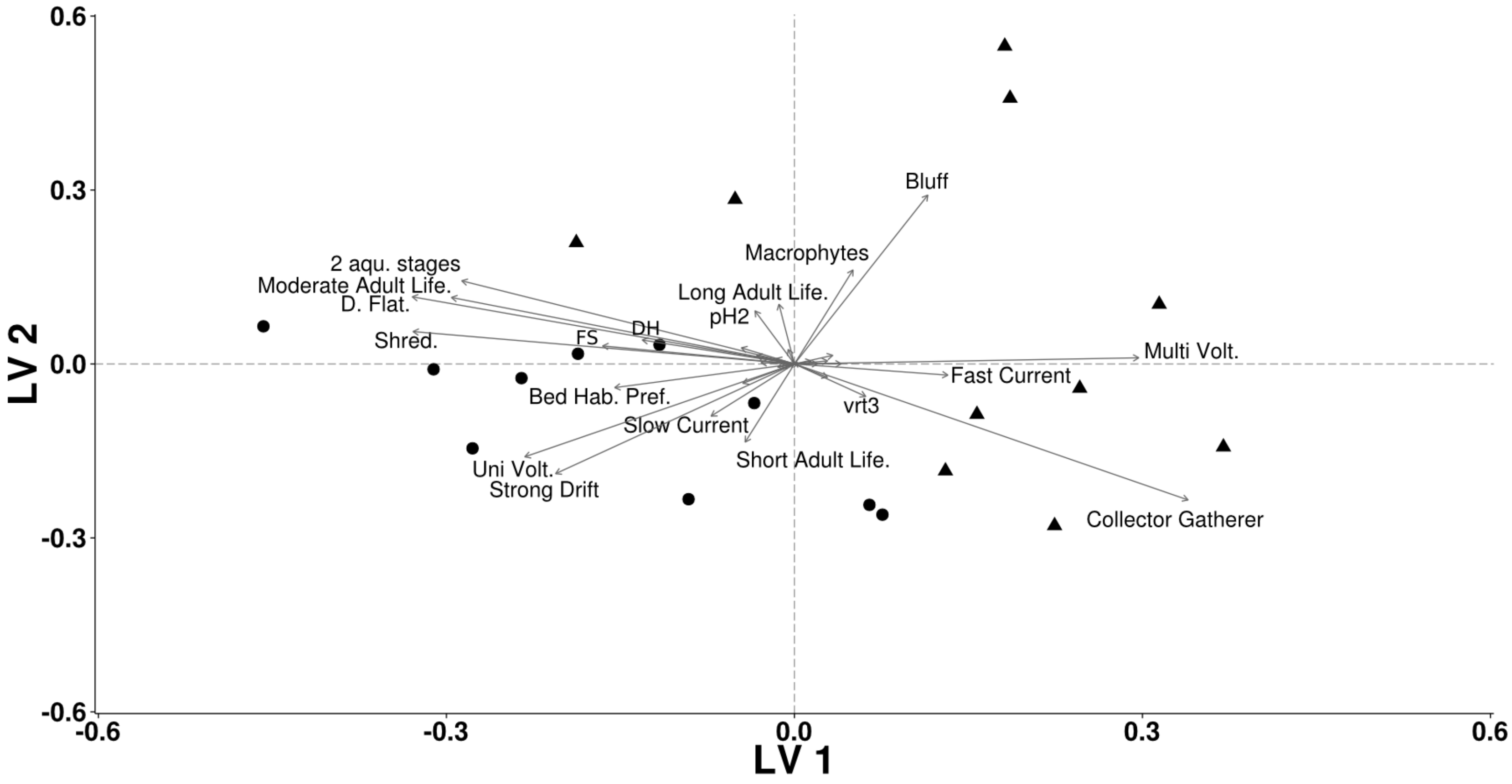

36
34
32
30
28
latitude
-88
-86
-84
-82
-80
-78
longitude
0
100
200
300 km
N


A
B
C
Atob (m2)
Dtob (m)
Wtob (m)
Aws (km2)
0
3
6
9
0.3
0.6
0.9
2.5
5.0
7.5
0
10
20
30

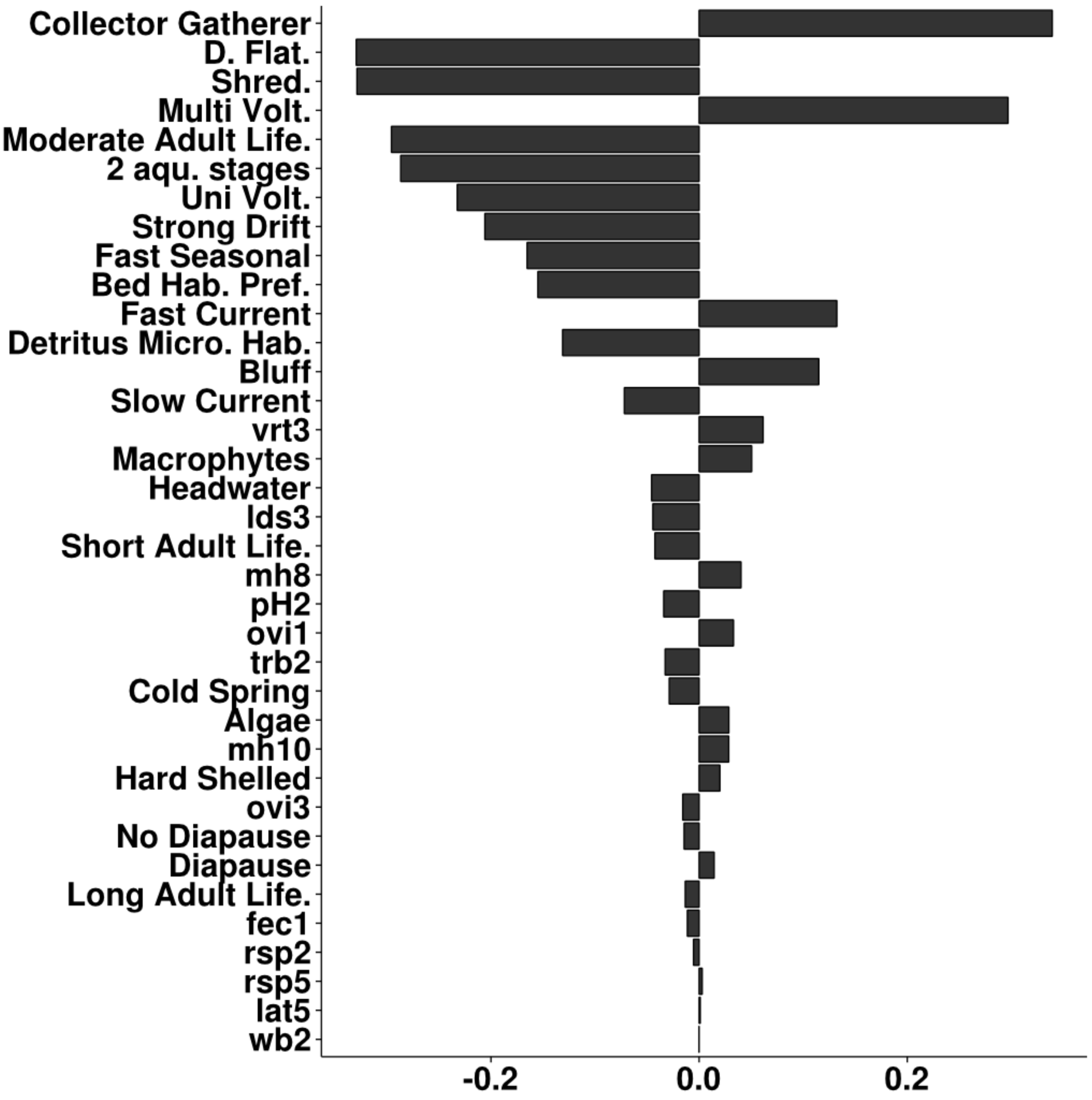

2
1
0
-1
-2
PC2
-10.0
-7.5
-5.0
-2.5
0.0
PC1
W_e
Cd_0
A_e
D_e


SC
pH
MAX_q10
MAX_q75
MAX_q90
Sed SD
OM
Sed Mean
MAX_q25
CWD
falling 15.24 cm
falling 21.336 cm
devel
falling 27.432 cm
-2
0
2


EPT
20
10
*
A
SCI
80
70
60
50
*
B
GASCI
60
50
40
30
*
C
GABI
6
5
4
3
NS
D
non-reference
reference
Group



LV 2
LV 1
0.6
0.3
0.0
-0.3
-0.6
-0.6
-0.3
0.0
0.3
0.6
Bluff
Macrophytes
Long Adult Life.
pH2
2 aqu. stages
Moderate Adult Life.
D. Flat.
Shred.
FS
DH
Multi Volt.
Fast Current
vrt3
Bed Hab. Pref.
Slow Current
Short Adult Life.
Uni Volt.
Strong Drift
Collector Gatherer


Collector Gatherer
D. Flat.
Shred.
Multi Volt.
Moderate Adult Life.
2 aqu. stages
Uni Volt.
Strong Drift
Fast Seasonal
Bed Hab. Pref.
Fast Current
Detritus Micro. Hab.
Bluff
Slow Current
vrt3
Macrophytes
Headwater
lds3
Short Adult Life.
mh8
pH2
ovi1
trb2
Cold Spring
Algae
mh10
Hard Shelled
ovi3
No Diapause
Diapause
Long Adult Life.
fec1
rsp2
rsp5
lat5
wb2
-0.2
0.0
0.2

Chapter 4: Is the land-cover cascade system-specific? A case study in sand-bed stream of the US Southeastern Plains ecoregion. (Formatted for submission to Ecological Applications)

Running Head: LCC in Southeastern Plains streams

Is the land-cover cascade system-specific? A case study in sand-bed stream of the US Southeastern Plains ecoregion.

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Abstract. How land use/land-cover (LC) directly and indirectly affect intermediate system components is important to understand stream macroinvertebrate functional composition (FC) in sand-bed streams. The Land-Cover Cascade (LCC) that was developed and tested in the SE Blue Ridge (EPA level III ecoregion) operationalized how LC is translated to stream biota, but has not tested elsewhere. Because of fundamental differences in the physical variables in Blue Ridge and Southeastern Plains (SEP) streams, we conducted a study to investigate if the LCC is an appropriate model for how LC affects biota in sand-bed streams of the SEP. To these ends, we collected LC, hydrologic, geomorphic, habitat, and aquatic macroinvertebrate data summarized as functional composition (FC) in 46 US SEP reference streams in GA, SC, and NC. We compared 5 structural equation models using an information theoretic approach: the LCC, LCC with additional hydrologic direct effects, hydrology as a master variable model (HMV) that we developed from the literature, and 2 models to ensure parameter parsimony of the HMV. In order to investigate whether regional background variability affected FC, we developed a natural gradients model and assessed the variance explained in FC. The results of this study suggest that the HMV best explained the effects of LC on FC, and was not confounded by regional environmental variability. The HMV showed, in contrast to the results of the original LCC study, that hydrology was the most important system variable directly effecting FC. Indirectly, LC as increases in pine silviculture and decreases in longleaf pine in the watershed increased hydrologic disturbance and mainly resulted in increased Collector-filterer and decreased shredder functional feeding groups.

Key words: Land-cover Cascade; Southeastern Plains Streams; sand-bed; Longleaf Pine; Functional Composition; Hydrology; Geomorphology.

Introduction

Stream ecologists have long known that watershed processes are intimately linked to instream biotia (Hynes 1970), and often use a multi-spatial scale approach from the entire watershed to the local habitat to study assemblage responses (reviewed in Allan 2004). First, the relationship of separate system components from watershed land use/land-cover (LC) to habitat with various groups of aquatic assemblages has been extensively investigated and continues to be an active area of research (Walsh et al. 2005, Maloney and Weller 2011, Riseng et al. 2011, Sheldon et al. 2012, Liu et al. 2015, Marion et al. 2015, others). Second, hydrologic disturbance has been suggested as a central organizing force in stream ecosystems (Resh et al. 1988, Lake 2000, Walsh et al. 2005), has been extensively investigated in stream ecosystems (Maloney et al. 2005, Poff and Zimmerman 2010, Maloney and Weller 2011, others), and has been called the even been called the master variable (Bunn and Arthington 2002). Third, instream geomorphology has been shown to have affect biotia (Bravard et al. 1997, Richards et al. 1997, Vandewalle et al. 2010). Last, habitat has been conceptualized as a template that filters biotic assemblage structure (sensu Southwood 1977, 1988, Townsend and Hildrew 1994, Poff et al. 2006).

Translation of the above system components from watershed LC effects to instream biota has been operationalized as the Land-cover Cascade (LCC) by Burcher et al. (2007). The LCC provides a conceptual framework for describing how watershed LC affects different hierarchical system components that ultimately alter structural and functional aspects of biotic assemblages. In the strict sense, the LCC hypothesizes that changes to watershed LC will have an effect on hydrology (e.g., higher magnitude flows), which, in turn, affects channel morphology because stream channels are in dynamic equilibrium with water and sediment supplied by the watershed (Leopold 1994). As a result of channel changes, erosional (e.g., stream bed sediment) and depositional habitat (e.g., particulate organic matter standing stocks) variables change, and ultimately result in altered biotic assemblage structure and function (see Burcher et al. [2007] Fig. 2).

The LCC was developed in SE US Blue Ridge ecoregion (EPA level III) streams (Burcher et al. 2007) where the terrain is, typically, mountainous and results in streams that are characterized by high gradients and consolidated, bedrock/cobble sediments, and the dominant watershed vegetation is broadleaf deciduous trees (Wiken et al. 2011). In these systems, bankfull flows are required to mobilize substrate that result in equilibrium channel morphology (Doyle et al. 2007). In contrast, the SE US Southeastern Plains ecoregion (SEP; EPA level III) is characterized by terrain that is moderate to flat and results in streams that are generally characterized by moderate to low gradients and unconsolidated, sandy sediments, and the dominant, natural watershed vegetation was longleaf pine (Wiken et al. 2011). In sharp contrast to Blue Ridge channel-forming flows that happen on average every 2 years (Leopold 1994), substrate mobility is initiated many times per year and resulting channel morphology changes often in SEP streams (Copeland et al. 2005). Further, distinct physicochemical conditions and macroinvertebrate assemblages occur between Blue Ridge and SEP streams (Feminella 2000). Thus, fundamental differences in Blue Ridge and SEP streams could modify the degree to which LCC operates in sand-bed, SEP streams.

We investigated the LCC in SEP streams result in similar system responses as that in Blue Ridge streams. We conducted a study in the SEP, developed literature-derived, competing structural equation models (SEM), and compared the models with an information theoretic approach. In contrast to path analysis (Burcher et al. 2007), SEM represents an advance because system components are modeled as multivariate responses allowing for a more realistic explanation of variance in ecosystem components. In this paper, we utilize the LCC as the literature derived NULL model, and develop and compare multiple competing hypotheses, posed as path diagrams, of how LC and other system components directly and indirectly effect aquatic macroinvertebrate functional composition (FC).

METHODS

Study sites

We studied 46 SEP reference streams in GA (Fort Benning [n=7] and adjacent Nature Conservancy Lands [3] and Fort Gordon [2]), SC (Savannah River Site [10], Manchester State Forest [2], Sand Hills State Forest [1], and Sandhills National Wildlife Refuge [4]), and NC (Fort Bragg [12] and Sandhills Gamelands [5]). These streams were selected with on-the-ground reconnaissance and were considered a priori reference sites (Fig. 1). Briefly, sites were low-gradient, unbraided, sand-bed streams draining mostly forested watersheds (mean forest cover = 78% and range=38-97%; mean urban and developed=4% and range=0-12%), and contained appreciable amounts of course woody debris and deposited organic matter. Instream habitat and benthic macroinvertebrate sampling occurred at these sites during the summer low flow period of 2010-2012.

Land use/land-cover

We quantified land use/land-cover as percent of watershed area developed from national elevation dataset digital elevation models (Gesch et al. 2002) and U.S. Geological Survey National GAP Analysis Program (GAP) Analysis Program vegetation and LC data (http://gapanalysis.usgs.gov/gaplandcover/data/download/) with GRASS GIS (Neteler et al. 2012). We used macrogroup vegetation classification level, an ecologically-derived classification described in Faber-Langendoen (2014), because it provides increased resolution when compared to the National Landcover Dataset (NLCD) (Wardlow and Egbert 2003). For example, the NLCD evergreen forest category, an important vegetation type in the SEP, can be further divided into longleaf pine or loblolly pine from the GAP data. Land use/land-cover encountered in our study watersheds are summarized in Table 1 (see Appendix A for extended descriptions). Geology and mean annual precipitation at each sample location was summarized from USGS (http://mrdata.usgs.gov/geology/state/) and PRISM climate group data (http://www.prism.oregonstate.edu/), respectively.

Hydrology and Geomorphology Variables

To quantify hydrology, we instrumented each site with Solinst Levelogger Junior® pressure transducers (Model 3001, Solinst Canada Ltd., Canada) in semi-permanent stilling wells to measure stage (and temperature) every 15 min. We adjusted stage for ambient barometric pressure with local barometric pressure (Solinst Barologger Gold® pressure transducers or airport weather stations within ~ 40 km). Stage was summarized for the period of record (median~1.4 y) with measures indicative of disturbance (TABLE 2; McMahon et al. 2003). Temperature was summarized as the median temperature during the sampling period. We quantified channel geomorphology following Sefick et al. (Sefick CH2, see also Kosnicki et al. [2014]) at the top of bank. Briefly, we surveyed 4 to 6 equidistant channel cross sections in runs perpendicular to the direction of flow by recording top of bank and water depths every 20 cm along each transect. We calculated reach specific medians of top of bank area (Atob), depth (Dtob), and width (Wtob) from these surveys.

Instream Habitat Sampling

We quantified habitat following methods in Sefick et al. (Sefick CH2). Briefly, we quantified coarse woody debris as percent of the channel (CWD > 2.5 cm diameter; Wallace and Benke 1984), benthic organic matter (BOM ≤1.6 cm diameter; Maloney et al. 2005), and substrate particle size at each transect (similar to Helms 2009a). To obtain percent CWD in the channel, length and width of each piece of CWD >2.5 cm in diameter was measured along a 1 m transect perpendicular to the flow and summarized as reach-specific median. Substrate size and BOM were estimated from PVC cores (7.62 cm ID diam., 455.8 cm^3 sample volume for substrate size; 2.5 cm ID, 49.1 cm^3 sample volume for BOM) inserted to a depth of 10 cm near the center of the channel directly above each transect (substrate size) or at 2 locations (midstream and stream margin for BOM) along each transect. We oven dried and combusted substrate samples to remove BOM (below), and then dry-sieved for representative particle sizes (i.e., d_5, d_{50}, d_{95}, etc., phi scale -4-5, Lane [1947]). We calculated geometric mean and standard deviation of particle size distribution (Bunte and Abt 2001). We oven-dried BOM samples at 65°C (typically 48 h), weighed, and ashed in a muffle furnace at 550°C for 3 h to determine ash-free dry mass (AFDM). Samples were cooled in a desiccator and reweighed; % BOM was determined as the difference between dry and ashed masses divided by total dry mass (Wallace and Grubaugh 1996).

Benthic Macroinvertebrate Sampling

We quantified macroinvertebrate assemblages after Sefick et al. (Sefick CH2, see also Kosnicki et al. [2014]). Briefly, we took 1 1 m by 0.33 m composite sample of 3 available depositional organic matter (e.g., leaf packs), CWD, root mats, and macrophyte microhabitats (generally in run habitats) directly above and below the first and last channel morphology survey

(244 µm D-frame net; total area sampled ~ 2m^2 per site) (see Barbour et al. 1999, Kosnicki et al. 2014). Samples were preserved in the field with 95% ethanol and stored ≤ 0 ºC until sorted in the laboratory. In the laboratory, we used a two-stage sorting procedure (Feminella 1996). We washed the sample with running water through 2 mm and 250 µm stacked sieves to separate coarse and fine material, respectively. The coarse material was picked for ≤ 30 min to remove all large and rare taxa. After time had elapsed, the coarse material was washed a second time through the sieve stack to ensure any small organisms in the coarse part of the sample were washed onto the 250 µm sieve. All retained fine material was mixed with salt water (specific gravity ≥ 22) to a 1L homogenate, and ~50 mL subsample was removed and sorted with a dissecting scope at ~7x magnification. Subsampling continued until at least 300 organisms were recovered (Vinson and Hawkins 1996). We identified macroinvertebrates to the lowest practical taxonomic level (typically genus; including Chironomidae) with available literature (Brigham et al. 1982, Kowalyk 1985, Epler 1996, 2001, 2010, Merritt et al. 2008, Thorp and Covich 2009). To estimate abundance, we corrected the raw fine sample counts by dividing by the proportion of homogenate subsampled, added large and rare taxa, and arrived at an estimate for macroinvertebrate abundance (no. organisms/m^2). We resolved ambiguous taxa with the consensus of 3 independent identifiers, and proportionally distributed ambiguous parents among children taxa (Cuffney et al. 2007). Because the focus of our study was on functional aspects of the macroinvertebrate assemblage, we collapsed identifications at lower taxonomic levels (e.g., species to genus) in order to match functional trait database provided by Viera et al. (2006). Because there were multiple enteries for each taxon in the trait database, we calculated trait affinities (i.e., most likely generic trait) by fuzzy coding functional trait data to sum to 1 across all trait modalities (i.e., sum of semivoltine, univoltine, multivoltine is 1) for a given trait within

a taxa, usually at the genus level (Chevenet et al. 1994). For example, the genus Leuctra has trait affinities 0.2 for multivoltinism and 0.8 for univoltinism. We then calculated mean trait values (mT) by multiplying macroinvertebrate relative abundance by trait affinities and summing these values for a site (Garnier et al. 2004, Vandewalle et al. 2010). As a result, we developed an abundance-weighted trait value that should be useful to identify those traits that allow the assemblage to persist with encountered environmental constraints (sensu species sorting Leibold et al. 2004, Shipley et al. 2006).

Statistical analysis

We used structural equation modeling (SEM) to investigation of stream system-level multivariate hypotheses. With this method, latent variables (LV), such as LC, are constructed from measured indicator variables (IV), such as Southeastern North American Ruderal Forest & Plantation, Longleaf Pine & Sand Pine Woodland, etc., and relationships among these constructs can be investigated (Shipley 2002). LV and path coefficient estimation was conducted with partial least squares SEM (PLS-PM) followed by covariance based SEM modeling on the LV scores derived from PLS-PM (hereafter PLS-SEM; see Grace and Jutila 1999). This 2-stage method combines the strengths of PLS-PM and SEM. PLS-PM has no distributional assumptions, is minimally affected by multi-collinearity, and does not require large sample size (Grace and Jutila 1999); however, PLS-PM cannot assess model fit to the data as the methodology is concerned primarily with prediction. For this reason we conducted SEM of the LVs to use the model to data fit statistics important in assessing SEM. We bootstrapped SEM diagnostic statistics 10,000 times from the LV scores after Bollen and Stine (1992) to provide χ^2, and calculated all other fit statistics were from this value. Path coefficient standard errors were estimated with 10,000 normal bootstrap draws from the LV scores to access parameter significance (Ievers-Landis et al. 2011).

We compared models using information theoretic criteria to determine which model best explained the data (Burnham and Anderson 2002). We used the consistent Akaike's Information criteria (*AICc*) to account for small sample size (Anderson and Burnham 2002). Of particular interest were the *AICc* weights (*AICcWt*), which could be interpreted as the probability that a model was the true model out of the set of competing models (Burnham and Anderson 2002). Additionally, we compared models goodness-of-fit with χ^2 (and the related *P-value*), comparative fit index (*CFI*), and root mean square error of approximation (*RMSEA*).

Latent Variable Construction

We used LC, hydrology (Hyd), geomorphology (Geo), habitat (Hab), and functional composition (FC) as LVs estimated by PLS-PM because they were identified as important LCC variables (Burcher et al. 2007). To ensure unidimensionality, or the property that IVs measured the same underlying construct of LVs we reversed the sign (i.e., multiplied by -1) of those variables that were negatively loaded on a LV, and calculated Dillon's Rho (Hair et al. 2012). To guard against overfitting of models and because there was no a priori justification in the literature for which variables to use in our reference streams, we reduced IV for LC and Hyd with a variable-selection step by bootstrapping the eigenvectors 1000 times to determine if the bootstrap 95% confidence intervals (CI) of each eigenvector on principal component 1 contained 0. If the CI contained 0 we considered the variable not to be important on that principal component (PC) and removed before LV estimation with PLS-PM (Jackson 1993, Peres-Neto et al. 2003) (TABLE 1 and 2). We used only PC1 in this variable selection step because it explained most of the variation in LC and Hyd IVs prior to the variable selection step (i.e., 61 and 87%, respectively. The IV for Geo were Atob, Dtob, and Wtob, and these variables are the typical variables used for characterizing geomorphology at a regional scale (e.g., regional curves; Leopold 1994). These variables can predict hydrologic disturbance and associated macroinvertebrate FC (Sefick CH2). IVs for Hab were organic matter (OM; CWD and BOM) and summaries of sediment size characteristics (geometric mean and standard deviation) that were shown to be important in many significant LCCs (Burcher et al. 2007). The IVs for FC were functional feeding groups found to respond to the LCC (Burcher et al. 2007) and multivoltinism, which was predicted to increase with increasing hydrologic disturbance (i.e., temporal variability) (Townsend and Hildrew 1994), and has been shown to be related to hydrologic disturbance in the study streams (Sefick CH2).

SEM construction and competing hypotheses

We used the LCC model as the theoretical basis for model construction and comparison in this study (Burcher et al. 2007). The LCC model and competing models represent specific hypotheses about how effects of LC propagate through important system components to macroinvertebrate assemblages in SEP streams (FIG. 2A-F). The LCC model we investigated represented a generalized model that Burcher et al. (2007) found to be important in explaining variation in macroinvertebrate assemblages in Blue Ridge streams (FIG. 2A). There were no direct effects of Hyd on Hab or FC in the significant models reported in Burcher et al. (2007), although Hyd regime can have direct effects on macroinvertebrate assemblages (Lake 2000, Bunn and Arthington 2002), or as changes to hydraulic habitat (Doledec et al. 2006). Hyd also may affect Hab in streams (Maloney et al. 2005, Burcher et al. 2007, Helms et al. 2009b). Thus, one competing hypothesis we tested was that LCC operated, but with the addition of direct effects of Hyd on Hab and FC (FIG. 2B). Another system level hypothesis we investigated was that Hyd acts as an instream master variable by affecting all system components lower in the hierarchy (e.g., Hab or FC). There was literature support to suggest that changes in Hyd change Geo (Hammer 1972, Doll et al. 2002), and changes in Geo can affect FC (Richards et al. 1997, Burcher et al. 2007, Vandewalle et al. 2010). Hab has been conceptualized as a major templet related to macroinvertebrate assemblage composition (Townsend and Hildrew 1994, Townsend et al. 1997) and can respond to Hyd (Maloney et al. 2005, Helms et al. 2009b), so we developed the hydrology as a master variable model (HMV) as a competing hypothesis in SEP streams (FIG. 2C). We included 2 modified HMV models to address parameter parsimony by removing the direct effect of Geo and Hab on FC (FIG. 2D and Fig. 2E, respectively). We compared the above 5 models with Information theoretic approaches (*Statistical analysis* above). Last, to ensure that natural watershed attributes were not driving FC changes, we developed a natural model that included the main natural gradients that might account for changes in FC, including watershed area, instream temperature, geology, and average annual precipitation (FIG. 2F), and accounted explicitly for spatial position with latitude and longitude of the reach terminus. We could not compare this model directly to the other system hypotheses with information theoretic techniques, but was accessed for significance and goodness-of-fit. For all statistical tests an α of 0.05 was used.

RESULTS

Model Comparison

Model comparisons indicated that the HMV model best represented the data (*AICcWt*=0.81). The next highest competing model, the HMV without the direct effect of geomorphology, had considerably less support (*AICcWt*=0.16). Further, standard SEM goodness-of-fit measures indicated that HMV, highest *CFI* (1), lowest *RMSEA* (0), and lowest χ^2 (3.58; P = 0.52), thus fit the data better than other competing models (TABLE 3). Further, the HVM model was not confounded by natural gradients as the natural model showed low *CFI*, high *RMSEA*, and high χ^2 (TABLE 3). As a result of these data, only the HMV model will be further discussed.

Latent Variables

The 5 latent variables represented gradients describing the following: 1) LC was mainly composed of decreasing pine silviculture and increasing native longleaf pine; 2) Hyd was composed of increasing hydrologic disturbance as longer duration low and high flows; 3) Geo consisted of increasing channel enlargement; 4) Hab was composed of decreasing sediment size and sorting and increasing OM; and 5) FC was composed of decreasing shredders and increasing collector-filter functional feeding groups, and secondarily, scraper/grazer, collector-gatherer functional feeding groups, and multivoltine life cycles (TABLE 4). Dillon's rho for LVs were > 0.6 (0.67-0.95) indicating that IVs reliably measured the same LV (Hair et al. 2012).

HMV Structural Equation Model

All direct effect model parameters were significant ($P \leq 0.05$). Further, the explained variance was > 50% for the Hyd and FC-LVs, supporting the use of this model in SEP streams (FIG. 3). While not as high as Hyd and FC, > 30% of the variation in Hab and Geo also were explained by the model (FIG. 3). The HMV model showed that increases in longleaf pine had direct effects of decreasing hydrologic disturbance. Further, increasing hydrologic disturbance directly increased sediment particle size and decreased OM, increased channel enlargement, and decreased mT shredders and increased mT collector-filterers, scraper/grazer, collector-gatherer, and multivoltine. Increasing channel enlargement (Geo) and sediment size and decreasing organic matter (Hab) also showed the same direct effects. However, Hyd showed the largest direct effect on FC (FIG. 3). Total and indirect effects of LC on FC were calculated and tested for significance because investigation of LC's effect on stream system components was an objective of this study. The total effect of LC on FC was highly significant -0.484 ($P \leq 0.001$). LC had a non-significant indirect effect through Hyd and Hab on FC, LC decreased FC through Hyd and Geo (-0.181; P=0.010), and the highest indirect effect of decreased FC through Hyd (-0.382; P=0.004).

DISCUSSION

The results of this study suggested that a different translation of landcover (LC) changes to functional composition (FC) was operating in SE Plains (SEP) (i.e., HMV model) reference streams in contrast to that predicted by the landcover cascade (LCC). Inferences from the HMV in relation to FC were not confounded by natural factors because the natural model did not significantly explain variability in FC. This result suggested functional traits are less structured by variation in spatial position, climate, geology, or system size, but more strongly structured by local habitat constraints sorting organisms from the regional species pool based on traits they possess (i.e., HMV model; [Southwood 1977, Townsend and Hildrew 1994, Poff 1997, Leibold et al. 2004]). This has not been demonstrated until now for SEP streams, but results from other studies from other regions also show this pattern (Burcher et al. 2007, Heino et al. 2007, Sokol et al. 2011, Schmera et al. 2013, and others). Because of its fit characteristics, we will limit our following discussion to the HMV model.

Direct effects

Land use/land-cover.–Because these streams were mostly forested, reference streams, the major LC gradient represented different forestry practices in the watersheds. This result is in contrast to Burcher et al. who found that both agriculture and low levels of urbanization to be important in developed LCCs (Burcher et al. 2007). In contrast, our systems we found agriculture and urban to explain enough variation to be included in our latent variable construction, but had relatively small loadings (0.26 and 0.51 respectively) when compared to forestry practices. As a result, we will restrict our discussion to the most important variables found in our study. Our results show decreases in managed pine plantations and increases in longleaf pine were linked to less hydrologically disturbed streams and biota. Hydrologic disturbance was composed of higher duration low and high magnitude flows. This has management implications for the SEP ecoregion and the coastal plains in general as this area was historically dominated by longleaf pine forests (Noss et al. 2015). In addition, Noss et al. (2015) argue the coastal plain should be considered a biodiversity hotspot, which further highlights the importance of this study in understanding how forest management activities effect instream processes in SEP streams. The results of this study suggest that forest management has a strong direct effect on changing in-stream hydrology in SEP streams. Further, loblolly pine silviculture plantations were positively correlated with blackwater stream land-cover on the LC LV. We do not have any data to explain this association, but speculate that site factors such as topographic relief, soil factors, etc. conducive to silviculture occur in the uplands of areas where lowlands are conducive to blackwater stream occurrence. This secondary gradient highlights the importance of silviculture in the watersheds that support blackwater streams as they are important and unique ecosystems (Benke et al. 1984) that might be disproportionately impacted by silviculture in the SEP. A possible mechanism to explain change in hydrologic disturbance related to silviculture practices is the difference in understory vegetation, mostly dominated by warm season grasses, associated with longleaf pine management (Noss et. al 2015). These grasses would likely result in increased hydraulic roughness that would increase the travel time of water from the watershed to the stream and result in decreased instream hydrologic disturbance. This is in sharp contrast to managed loblolly pine plantations where low/no understory vegetation and compacted soils would result in decreased water travel time to the stream, and, as a result, increase hydrologic disturbance.

Hydrology.–Hydrology has been called the master variable in stream ecosystems (Power et al. 1995, Hart and Finelli 1999, Lake 2000, Bunn and Arthington 2002). Our results support this assertion because hydrology had high direct effects on all system variables lower in the cascade. Burcher (2007) found that geomorphology (Geo) was important in all 7 macroinvertebrate LCCs investigated. They reasoned that because of potential difficulties in quantifying hydrologic events they might have missed this signal and state "geomorphic entities should be influenced by hydrology and future research should focus on hydrologic features manifested during stormflow to capture this relationship" (p. 239 Burcher et al. 2007). In this study, we quantified and summarized hydrologic data over a long temporal extent and at a high temporal resolution, capturing multiple storm events, and showing the primary importance of hydrology in structuring Geo, Hab, and FC. We speculate that if hydrology data was quantified in a similar manner Burcher et al. (2007) would have found a similar importance of hydrology in Blue Ridge LCCs. While we do not have estimates of channel forming discharges for the SEP (sensu Leopold 1994), Copeland et al. (2005) have found that discharges as low as mean annual discharge could be considered channel forming flows. In this study, it is concordant with our knowledge of channel morphology/hydrologic relationships that the higher duration maximum flows eroded highly mobile sand-beds/banks resulting in enlarged channels (sensu Hammer 1972). In addition, these higher duration flows associated with the Hyd LV had an effect of coarsening substrate and decreasing OM. We speculate the high direct effect (highest of all direct effects) of Hyd on FC might have been due to an important habitat variable not being measured increasing the direct effect of Hyd, such as coarse particulate organic matter (CPOM) an important food resource for shredders. If this was the case, CPOM was likely susceptible to being washed out by high duration flow events and could have inflated the Hyd direct effect (Cuffney and Wallace 1989, Maloney et al. 2005). However, it is equally likely that aquatic macroinvertebrate assemblages are directly responding to hydrologic disturbance (Doledec et al. 2006). Future studies should attempt to gather appropriate data to clarify these different possible explanations.

Geomorphology.–Channel enlargement had the second highest direct effect (after Hyd) on FC. Geomorphology could have a direct effect on FC by being a potential barrier to emergence of adult insects. Surprisingly, there have been no direct investigations of aquatic insect emergence related to changes in channel morphology; however, it has been shown that increases in bank height act as a dispersal barrier for crayfish (Helms et al. 2013), and it has been shown that bank height and bank erosion potential were related to changes in macroinvertebrate assemblage structure (Simpson et al. 2014). Burcher et al. (2007) found that Geo was implicated in all macroinvertebrate LCCs and suggested that Geo should be affected by hydrology and that would in turn affect aquatic insects and fishes. However, the primacy of Geo as the most influential on LCCs was most likely due to inadequate quantification of long-term discharge records, a stated deficiency in the original paper (Burcher et al. 2007), and, as stated above, we quantified these data, and found that increased hydrologic disturbance enlarged stream channels.

Habitat.–Direct effects of habitat on FC were the lowest of all LVs. This was likely due to the habitat variables being indicators of hydrologic disturbance, and not those that directly affect FC. As stated earlier, we believe that if CPOM was quantified there might have been a higher direct effect of Hab. This highlights the need for further research that directly attempt to uncover relationships of Hab and FC. However, this result may represent the difficulty in quantifying habitat parameters that are influential at the scale of macroinvertebrates.

Functional composition.–Functional composition primarily (i.e., highest loadings) was related to a gradient of decreasing shredders and increasing filtering organisms. Filtering organisms may have responded to higher mean particle size because larger particles have relativly greater stability that might allow for net attachment when compared to smaller, relatively more unstable, sand substrate, which may explain the small, but significant direct effect of Hab. There was a secondary gradient related to increasing Scraper/grazer and Multivoltine organisms. Data from an experiment in sand-bed streams investigating bed movement disturbance showed Collector-filterer, and to a lesser extent scraper/grazer, and Collector-gatherer functional feeding groups related to higher bed disturbance, and shredder functional feeding groups were related to "natural" streambed disturbance in a sand-bed stream (Sefick et al. unpublished experimental data [CH4.]). This suggests that hydrologic disturbance results in bed movement and filters the functional feeding groups from the regional species pool, and may be a peculiarity of sand-bed streams for which we do not have a satisfying explanation from the data we collected. In addition, Multivoltine organisms increased with hydrologic disturbance and increases in channel dimensions (mostly related to Atob). These findings were consistent with the habitat templet predictions (Townsend and Hildrew 1994) and empirical studies (Sefick et al. unpublished MS [CH.2], Richards et al. (1997)). All of these findings suggested that there was a different FC mainly related to hydrologic disturbance in the SEP.

Total and indirect effects

One of our major objectives was to investigate the relationship of land use/land-cover change with FC response. Thus, we investigated total and indirect effects of LC on FC. Our results suggest that the main land use gradient in SEP reference streams in our study was a long leaf pine forest to heavily managed pine plantation gradient. We speculate that this was due to increased roughness in the understory of long leaf pine forests that were in stark contrast to those of managed pine plantations. Of the total effect, LC through hydrology had the largest effect on FC. This reinforces hydrology's importance to stream ecosystems, and is a modification for the SEP of Burcher et al.'s (2007) LCCs developed in Blue Ridge streams. Further, this finding has potential important implications for managing upland watersheds in the SEP and shows that watershed LC has an important effect on FC indirectly through hydrology.

Conclusion

The results of this study suggest that a modified LCC is operating in sand-bed, SEP streams with hydrologic disturbance being the primary instream variable structuring FC. In addition, restoring the native longleaf pine ecosystem to upland systems not only have positive effects on upland biodiversity, but have impacts on stream ecosystems as well. The results of this study should help further justify longleaf pine restoration activities because of the effect they have on stream ecosystems, and aid in management decisions in the SEP. Further, this study can be used to help set FC expectations for reference streams in the SEP under different upland management regimes. In addition, this study suggests that the translation of landcover to instream biota is not a simple cascade model and might be different depending on system context, and suggest further research on the generality of LCC on other ecosystems, such as lake or estuaries.

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LITERATURE CITED

Allan, J. D. 2004. Landscapes and riverscapes: the influence of land use on stream ecosystems. Annual review of ecology, evolution, and systematics 35:257-284.

Anderson, D. R., and K. P. Burnham. 2002. Avoiding pitfalls when using information-theoretic methods. Journal of Wildlife Management 66:912-918.

Barbour, M. T., J. Gerritsen, B. D. Snyder, and J. B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates, and fish - second edition. EPA 841-B-99-002., Environmental Protection Agency; Office of Water, Washington, D.C.

Benke, A. C., T. C. Van Arsdall Jr, D. M. Gillespie, and F. K. Parrish. 1984. Invertebrate productivity in a subtropical blackwater river: the importance of habitat and life history. Ecological Monographs:25-63.

Bollen, K. A., and R. A. Stine. 1992. Bootstrapping goodness-of-fit measures in structural equation models. Sociological Methods & Research 21:205-229.

Bravard, J., C. Amoros, G. Pautou, G. Bornette, M. Bournaud, M. Creuze des Chatelliers, J. Gibert, J. Peiry, J. Perrin, and H. Tachet. 1997. River incision in south-east France: morphological phenomena and ecological effects. Regulated Rivers: Research & Management 13:75-90.

Brigham, A. R., W. U. Brigham, and A. Gnilka. 1982. Aquatic insects and oligochaetes of North and South Carolina. Midwest Aquatic Enterprises, Illinois.

Bunn, S. E., and A. H. Arthington. 2002. Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. Environmental Management 30:492-507.

Bunte, K., and S. R. Abt. 2001. Sampling surface and subsurface particle-size distributions in wadable gravel- and cobble-bed streams for analyses in sediment transport, hydraulics, and streambed monitoring. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fort Collins, Colorado.

Burcher, C. L., H. M. Valett, and E. F. Benfield. 2007. The land-cover cascade: relationships coupling land and water. Ecology 88:228-242.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. Springer.

Chevenet, F., S. Doledec, and D. Chessel. 1994. A fuzzy coding approach for the analysis of long-term ecological data. Freshwater Biology 31:295-309.

Copeland, R., P. Soar, and C. Thorne. 2005. Channel-forming discharge and hydraulic geometry width predictors in meandering sandbed rivers. In Proc., 2005 World Water and Environmental Resources Congress: Impacts of Global Change. ASCE:1-12.

Cuffney, T. F., M. D. Bilger, and A. M. Haigler. 2007. Ambiguous taxa: effects on the characterization and interpretation of invertebrate assemblages. Journal of the North American Benthological Society 26:286-307.

Doledec, S., N. Lamouroux, U. Fuchs, and S. Merigoux. 2006. Modelling the hydraulic preferences of benthic macroinvertebrates in small European streams. Freshwater Biology 52:145-164.

Doll, B. A., D. E. Wise-Frederick, C. M. Buckner, S. D. Wilkerson, W. A. Harman, R. E. Smith, and J. Spooner. 2002. Hydraulic geometry relationships for urban streams throughout the piedmont of North Carolina. JAWRA Journal of the American Water Resources Association 38:641-651.

Doyle, M. W., D. Shields, K. F. Boyd, P. B. Skidmore, and D. Dominick. 2007. Channel-forming discharge selection in river restoration design. Journal of Hydraulic Engineering 133:831-837.

Epler, J. 2010. The water beetles of Florida, an identification manual for the families Chrysomelidae. Curculionidae, Dryopidae, Elmidae, Gyrinidae, Haliplidae, Helophoridae, Hydraenidae, Hydrochidae, Hydrophilidae, Noteridae, Psephenidae, Ptilodactylidae and Scirtidae. Florida Department of Environmental Protection, Tallahassee, Florida. (Avaliable from: http://www.floridadep.org/labs/cgi-bin/sbio/keys.asp).

Epler, J. H. 1996. Identification Manual for the Water Beetles of Florida:(Coleoptera: Dryopidae, Dytiscidae, Elmidae, Gyrinidae, Haliplidae, Hydraenidae, Hydrophilidae, Noteridae, Psephenidae, Ptilodactylidae, Scirtidae). State of Florida, Department of Environmental Protection, Division of Water Facilities. (Avaliable from: http://www.floridadep.org/labs/cgi-bin/sbio/keys.asp).

Epler, J. H. 2001. Identification Manual for the larval Chironomidae (Diptera) of North and South Carolina. A guide to the taxonomy of the midges of the southeastern

United States including Florida. Special Publication SJ2001-SP13, North Carolina Department of Environmental and Natural Resources, Raleigh, NC, and St. Johns River Water Managment district, Palatka, FL. (Avaliable from: http://www.floridadep.org/labs/cgi-bin/sbio/keys.asp).

Faber-Langendoen, D., T. Keeler-Wolf, D. Meidinger, D. Tart, B. Hoagland, C. Josse, G. Navarro, S. Ponomarenko, J.-P. Saucier, and A. Weakley. 2014. EcoVeg: a new approach to vegetation description and classification. Ecological Monographs 84:533-561.

Feminella, J. W. 1996. Comparison of benthic macroinvertebrate assemblages in small streams along a gradient of flow permanence. Journal of the North American Benthological Society 15:651-669.

Feminella, J. W. 2000. Correspondence between stream macroinvertebrate assemblages and 4 ecoregions of the southeastern USA. Journal of the North American Benthological Society 19:442-461.

Garnier, E., J. Cortez, G. Billes, M.-L. Navas, C. Roumet, M. Debussche, G. Laurent, A. Blanchard, D. Aubry, A. Bellmann, C. Neill, and J.-P. Toussaint. 2004. Plant functional markers capture ecosystem properties during secondary succession. Ecology 85:2630-2637.

Gesch, D., M. Oimoen, S. Greenlee, C. Nelson, M. Steuck, and D. Tyler. 2002. The national elevation dataset. Photogrammetric Engineering and Remote Sensing 68:5-11.

Grace, J. B., and H. Jutila. 1999. The relationship between species density and community biomass in grazed and ungrazed coastal meadows. Oikos 85:398-408.

Hair, J. F., M. Sarstedt, C. M. Ringle, and J. A. Mena. 2012. An assessment of the use of partial least squares structural equation modeling in marketing research. Journal of the Academy of Marketing Science 40:414-433.

Hammer, T. R. 1972. Stream channel enlargement due to urbanization. Water Resources Research 8:1530-1540.

Hart, D. D., and C. M. Finelli. 1999. Physical-biological coupling in streams: the pervasive effects of flow on benthic organisms. Annual Review of Ecology and Systematics 30:363-395.

Heino, J., H. Mykra, J. Kotanen, and T. Muotka. 2007. Ecological filters and variability in stream macroinvertebrate communities: do taxonomic and functional structure follow the same path? Ecography 30:217-230.

Helms, B., W. Budnick, P. Pecora, J. Skipper, E. Kosnicki, J. Feminella, and J. Stoeckel. 2013. The influence of soil type, congeneric cues, and floodplain connectivity on the local distribution of the devil crayfish (Cambarus diogenes Girard). Freshwater Science 32:1333-1344.

Helms, B. S., J. E. Schoonover, and J. W. Feminella. 2009a. Assessing Influences of Hydrology, Physicochemistry, and Habitat on Stream Fish Assemblages Across a Changing Landscape1. JAWRA Journal of the American Water Resources Association 45:157-169.

Helms, B. S., J. E. Schoonover, and J. W. Feminella. 2009b. Seasonal variability of landuse impacts on macroinvertebrate assemblages in streams of western Georgia, USA. Journal of the North American Benthological Society 28:991-1006.

Hynes, H. B. N. 1970. The ecology of running waters. University of Toronto Press, Toronto.

Ievers-Landis, C.E., C.J. Burant, and R. Hanzen. 2011. The concept of bootstrapping of structural equation models with smaller samples: and illustration using mealtime rituals in diabetes management. Journal of Developmental & Behavioral Pediatrics 32:619-626.

Jackson, D. A. 1993. Stopping rules in principal components analysis: a comparison of heuristical and statistical approaches. Ecology:2204-2214.

Kosnicki, E., S. A. Sefick, M. H. Paller, M. S. Jarrell, B. A. Prusha, S. C. Sterrett, T. D. Tuberville, and J. W. Feminella. 2014. Defining the reference condition for wadeable streams in the Sand Hills subdivision of the Southeastern Plains Ecoregion, USA. Environmental Management 54:494-504.

Kowalyk, H. E. 1985. The larval cephalic setae in the Tanypodinae (Diptera: Chironomidae) and their importance in generic determinations. The Canadian Entomologist 117:67-106.

Lake, P. S. 2000. Disturbance, patchiness, and diversity in streams. Journal of the North American Benthological Society 19:573-592.

Lane, E. W. 1947. Report of the Subcommittee on Sediment Terminology. Transactions American Geophysical Union 28:936-938.

Leibold, M. A., M. Holyoak, N. Mouquet, P. Amarasekare, J. M. Chase, M. F. Hoopes, R. D. Holt, J. B. Shurin, R. Law, D. Tilman, M. Loreau, and A. Gonzalez. 2004. The metacommunity concept: a framework for multi-scale community ecology. Ecology letters 7:601-613.

Leopold, L. B. 1994. A View of the River. Harvard Univ Press, Cambridge, Massachuessets.

Liu, S., G. Xie, L. Wang, K. Cottenie, D. Liu, and B. Wang. 2015. Different roles of environmental variables and spatial factors in structuring stream benthic diatom and macroinvertebrate in Yangtze River Delta, China. Ecological Indicators.

Maloney, K. O., P. J. Mulholland, and J. W. Feminella. 2005. Influence of catchment-scale military land use on stream physical and organic matter variables in small Southeastern Plains catchments (USA). Environmental Management 35:677-691.

Maloney, K. O., and D. E. Weller. 2011. Anthropogenic disturbance and streams: land use and land-use change affect stream ecosystems via multiple pathways. Freshwater Biology 56:611-626.

Marion, C. A., M. C. Scott, and K. M. Kubach. 2015. Multiscale Environmental Influences on Fish Assemblage Structure of South Atlantic Coastal Plain Streams. Transactions of the American Fisheries Society 144:1040-1057.

McMahon, G., J. D. Bales, J. F. Coles, E. M. P. Giddings, and H. Zappia. 2003. Use of stage data to characterize hydrologic conditions in an urbanizing environment. JAWRA Journal of the American Water Resources Association 39:1529-1546.

Merritt, R. W., K. W. Cummins, and M. B. Berg. 2008. An Introduction to the Aquatic Insects of North America. 4th edition. Kendall Hunt Publishing, Dubuque, Iowa.

Neteler, M., M. H. Bowman, M. Landa, and M. Metz. 2012. GRASS GIS: a multi-purpose Open Source GIS. Environmental Modelling & Software 31:124-130.

Noss, R. F., W. J. Platt, B. A. Sorrie, A. S. Weakley, D. B. Means, J. Costanza, and R. K. Peet. 2015. How global biodiversity hotspots may go unrecognized: lessons from the North American Coastal Plain. Diversity and Distributions 21:236-244.

Peres-Neto, P. R., D. A. Jackson, and K. M. Somers. 2003. Giving meaningful interpretation to ordination axes: assessing loading significance in principal component analysis. Ecology 84:2347-2363.

Poff, N. L. 1997. Landscape filters and species traits: Towards mechanistic understanding and prediction in stream ecology. Journal of the North American Benthological Society 16:391-409.

Poff, N. L., J. D. Olden, N. K. M. Vieira, D. S. Finn, M. P. Simmons, and B. C. Kondratieff. 2006. Functional trait niches of North American lotic insects: traits-based ecological applications in light of phylogenetic relationships. Journal of the North American Benthological Society 25:730-755.

Poff, N. L., and J. K. H. Zimmerman. 2010. Ecological responses to altered flow regimes: a literature review to inform the science and management of environmental flows. Freshwater Biology 55:194-205.

Power, M. E., A. Sun, G. Parker, W. E. Dietrich, and J. T. Wootton. 1995. Hydraulic food-chain models. BioScience 45:159-167.

Resh, V. H., A. V. Brown, A. P. Covich, M. E. Gurtz, H. W. Li, G. W. Minshall, S. R. Reice, A. L. Sheldon, J. B. Wallace, and R. C. Wissmar. 1988. The role of disturbance in stream ecology. Journal of the North American Benthological Society 7:433-455.

Richards, C., R. Haro, L. Johnson, and G. Host. 1997. Catchment and reach scale properties as indicators of macroinvertebrate species traits. Freshwater Biology 37:219-230.

Riseng, C. M., M. J. Wiley, R. W. Black, and M. D. Munn. 2011. Impacts of agricultural land use on biological integrity: a causal analysis. Ecological Applications 21:3128-3146.

Schmera, D., T. Eros, and J. Heino. 2013. Habitat filtering determines spatial variation of macroinvertebrate community traits in northern headwater streams. Community Ecology 14:77-88.

Sheldon, F., E. E. Peterson, E. L. Boone, S. Sippel, S. E. Bunn, and B. D. Harch. 2012. Identifying the spatial scale of land use that most strongly influences overall river ecosystem health score. Ecological Applications 22:2188-2203.

Shipley, B. 2002. Cause and correlation in biology: a user's guide to path analysis, structural equations and causal inference. Cambridge Univ Press.

Shipley, B., D. Vile, and E. Garnier. 2006. From plant traits to plant communities: a statistical mechanistic approach to biodiversity. Science 314:812-814.

Simpson, A., I. Turner, E. Brantley, and B. Helms. 2014. Bank erosion hazard index as an indicator of near-bank aquatic habitat and community structure in a southeastern Piedmont stream. Ecological Indicators 43:19-28.

Sokol, E. R., E. F. Benfield, L. K. Belden, and M. H. Valett. 2011. The assembly of ecological communities inferred from taxonomic and functional composition. American Naturalist 177:630-644.

Southwood, T. R. E. 1977. Habitat, the templet for ecological strategies? Journal of Animal Ecology 46:337-365.

Southwood, T. R. E. 1988. Tactics, strategies and templets. Oikos 52:3-18.

Thorp, J. H., and A. P. Covich. 2009. Ecology and classification of North American freshwater invertebrates. 3rd edition. Academic Press, Burlington, MA.

Townsend, C. R., S. Doledec, and M. R. Scarsbrook. 1997. Species traits in relation to temporal and spatial heterogeneity in streams: a test of habitat templet theory. Freshwater Biology 37:367-387.

Townsend, C. R., and A. G. Hildrew. 1994. Species traits in relation to a habitat templet for river systems. Freshwater Biology 31:265-275.

Vandewalle, M., F. De Bello, M. P. Berg, T. Bolger, S. Doledec, F. Dubs, C. K. Feld, R. Harrington, P. A. Harrison, S. Lavorel, P. Martins da Siva, M. Moretti, J. Niemela, P. Santos, T. Sattler, J. P. Sousa, M. T. Sykes, A. J. Vanbergen, and B. A. Woodcock. 2010. Functional traits as indicators of biodiversity response to land use changes across ecosystems and organisms. Biodiversity and Conservation 19:2921-2947.

Vieira, N. M. K., N. L. M. Poff, D. M. Carlisle, S. R. Moulton II, M. L. Koski, and B. C. Kondratieff. 2006. A database of lotic invertebrate traits for North America. U.S. Geological Survey.

Vinson, M. R., and C. P. Hawkins. 1996. Effects of sampling area and subsampling procedure on comparisons of taxa richness among streams. Journal of the North American Benthological Society:392-399.

Wallace, J., and J. Grubaugh. 1996. Transport and storage of FPOM. Pages 191-215 *in* F. R. Hauer and G. A. Lamberti, editors. Methods in stream ecology. Academic Press, San Diego, California.

Wallace, J. B., and A. C. Benke. 1984. Quantification of wood habitat in subtropical coastal plain streams. Canadian Journal of Fisheries and Aquatic Sciences 41:1643-1652.

Walsh, C. J., A. H. Roy, J. W. Feminella, P. D. Cottingham, P. M. Groffman, and R. P. Morgan II. 2005. The urban stream syndrome: current knowledge and the search for a cure. Journal of the North American Benthological Society 24:706-723.

Wardlow, B. D., and S. L. Egbert. 2003. A state-level comparative analysis of the GAP and NLCD land-cover data sets. Photogrammetric Engineering & Remote Sensing 69:1387-1397.

Wiken, E., F. Jimenez Nava, and G. Griffith. 2011. North American terrestrial ecoregions-level III. Commission for Environmental Cooperation, Montreal, Canada.

TABLE 1. Land use/land-cover variables derived from USGS GAP data (v.2). Grey highlighted variables were retained after PCA variable selection step (see methods).

Macrogroup	Abbreviated Description	Status (Natural/Disturbed)	Ecological System
Atlantic & Gulf Coastal Plain Bog & Fen		Natural	Atlantic Coastal Plain Peatland Pocosin
Barren	Barren Land	Disturbed	Undifferentiated Barren Land
			Unconsolidated Shore
Central Mesophytic Hardwood Forest	Upland forest; generally deciduous		Atlantic Coastal Plain Mesic Hardwood and Mixed Forest
Developed & Urban	Developed high, medium, low intensity and open space	Disturbed	Developed High Intensity
			Developed Low Intensity
			Developed Medium Intensity
			Developed Open Space
Herbaceous Agricultural Vegetation	Agricultural vegetation – row crops (e.g., soybeans) and grazing land	Disturbed	Cultivated Cropland
			Pasture Hay
Longleaf Pine & Sand Pine Woodland	Southeastern Plains native forest	Natural	Atlantic Coastal Plain Fall line Sandhills Longleaf Pine Woodland- Open Understory Modifier
			Atlantic Coastal Plain Upland Longleaf Pine Woodland
			East Gulf Coastal Plain Interior Upland Longleaf Pine Woodland- Open Understory Modifier
			Atlantic Coastal Plain Fall line Sandhills Longleaf Pine Woodland- Scrub Shrub Understory Modifier
			West Gulf Coastal Plain Upland Longleaf Pine Forest and Woodland
Open Water	Stream, river, ponds, and lakes	Natural	Open Water- Fresh
Quarries, Mines, Gravel Pits and Oil Wells	Mining activities	Disturbed	Quarries, Mines, Gravel Pits, and Oil Wells
Recently Disturbed or Modified	Generally dominated by herbaceous, primary, herbaceous succession species, shrubs, or stunted trees associated with a recent disturbance (e.g., tree harvesting)	Disturbed	Disturbed Successional- Grass Forb Regeneration
			Disturbed Successional- Shrub Regeneration
			Harvested Forest- Grass Forb

			Regeneration Harvested Forest- Shrub Regeneration
Southeastern North American Ruderal Forest & Plantation	Land converted from Longleaf Pine Stands to Loblolly Pine by disturbance or Evergreen and Deciduous Silviculture	Disturbed	Atlantic Coastal Plain Fall Line Sandhills Longleaf Pine Woodland- Loblolly Modifier Deciduous Plantations East Gulf Coastal Plain Interior Upland Longleaf Pine Woodland- Loblolly Modifier East Gulf Coastal Plain Interior Upland Longleaf Pine Woodland- Offsite Hardwood Modifier Evergreen Plantations or Managed Pine
Southern Coastal Plain Basin Swamp	Swamps characteristic of the upper Coastal Plain generally dominated by tupelo and blackgum	Natural	Atlantic Coastal Plain Nonriverine Swamp and Wet Hardwood Forest- Taxodium Nyssa Modifier Southern Coastal Plain Nonriverine Basin Swamp
Southern Coastal Plain Evergreen Hardwood & Conifer Swamp	Generally the same as Southern Coastal Plain Basin Swamp, but can have some pine species	Natural	Southern Coastal Plain Hydric Hammock Atlantic Coastal Plain Streamhead Seepage Swamp, Pocosin, and Baygall
Southern Floodplain Hardwood Forest	Associated with small brown/blackwater streams initiated in the Coastal Plains	Natural	Atlantic Coastal Plain Blackwater Stream Floodplain Forest- Forest Modifier Atlantic Coastal Plain Small Blackwater River Floodplain Forest East Gulf Coastal Plain Small Stream and River Floodplain Forest
Southern Mixed Deciduous-Evergreen Broadleaf Forest	Upland mixed forest	Natural	Southern Coastal Plain Dry Upland Hardwood Forest
Southern-Central Oak-Hardwood & Pine Forest	Generally dry upland forest of either natural vegetation or indicative of longleaf pine forest conversion to hardwoods	Natural/Disturbed	Atlantic Coastal Plain Dry and Dry Mesic Oak Forest Atlantic Coastal Plain Fall line Sandhills Longleaf Pine Woodland- Offsite.Hardwood Modifier
Wet Longleaf Pine & Southern	Small forested wetlands	Natural	Southern Coastal Plain Nonriverine Cypress Dome

TABLE 2. Variables calculated to summarize stage. Grey highlighted variables were retained after PCA variable selection step (see methods).

Stage Summary Category	**Variable**	**Description**
Duration of Low Stage	MAX_q10	Maximum duration below 10th quartile
	MAX_q25	Maximum duration below 25th quartile
	MAX_q5	Maximum duration below 5th quartile
	MED._q10	Median duration below 10th quartile
	MED._q25	Median duration below 25th quartile
	MED._q5	Median duration below 5th quartile
Duration of High Stage	MAX_q75	Maximum duration above 75th quartile
	MAX_q90	Maximum duration above 90th quartile
	MAX_q95	Maximum duration above 95th quartile
	MED._q75	Median duration above 75th quartile
	MED._q90	Median duration above 90th quartile
	MED._q95	Median duration above 95th quartile
Frequency of Stage Change (Flashiness)	rising 15.24 cm	Number of hours stage rises by at least 15.24 cm
	rising 21.336 cm	Number of hours stage rises by at least 21.336 cm
	rising 27.432 cm	Number of hours stage rises by at least 27.432 m
	rising 3.048 cm	Number of hours stage rises by at least 3.048 cm
	rising 9.144 cm	Number of hours stage rises by at least 9.144 cm
	falling 15.24 cm	Number of hours stage falls by at least 15.24 cm
	falling 21.336 cm	Number of hours stage falls by at least 21.336cm
	falling 27.432 cm	Number of hours stage falls by at least 27.432cm
	falling 3.048 cm	Number of hours stage falls by at least 3.048 cm
	falling 9.144 cm	Number of hours stage falls by at least 9.144 cm

Notes: Coefficient of Variation was not included in the
hydrology summary because McMahon (2003)
found that it was not representative of hydrologic disturbance.
The Principal Components variable (Hyd1)
was not included because PLS was used to investigate
multivariate hydrologic influences on other
System variables

TABLE 3. Model Fit Comparisons. HMV is the hydrology as a master variable model, Geo is channel morphology, Hab is habitat, **LCC** is the land-cover cascade, and DF is degrees of freedom (see text for other abbreviations).

Models	**Df**	**χ2**	***P***	***CFI***	***RMSEA***	***AICc***	***AICcWt***
HMV **(C)**	4	3.58	0.52	1.00	0.00	561.87	0.81
HMV no Geo **(D)**	4	4.51	0.38	1.00	0.05	565.08	0.16
HMV no Geo or Hab **(E)**	3	4.50	0.27	0.99	0.10	569.61	0.02
LCC with Hyd direct effects **(B)**	4	11.20	0.06	0.93	0.20	571.42	0.01
LCC **(A)**	6	25.90	0.00	0.76	0.27	602.56	0.00
Natural Model **(F)**	6	20.29	0.04	0.87	0.23	[redacted]	[redacted]

Note: *AICcWt* is the *AICc* weight. *CFI* and *RMSEA* indicate a good model fit with values approaching 1 and 0, respectively. Bold letters represent models in FIG. 2

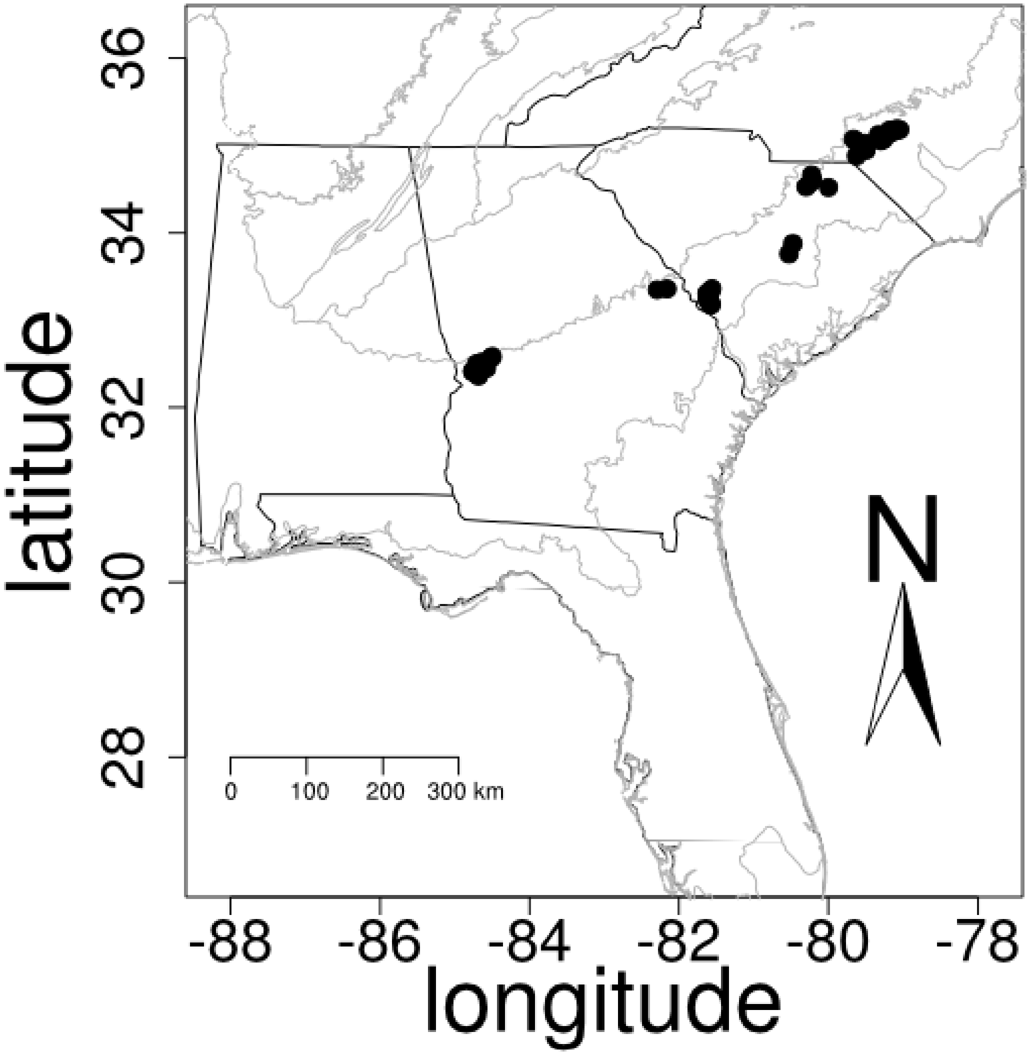
TABLE 4. Latent Variable Loadings Table. Values highlighted in grey are loadings that are greater than 0.4 and if the IV had Negative relationship with the LV and had to be multiplied by -1. Please see text for LV abbreviations, TABLES 1 and 2 for LC and Hyd indicator abbreviations, respectively, and Atob, Wtob, and Dtob are area, width, and depth at the top of bank, respectively.

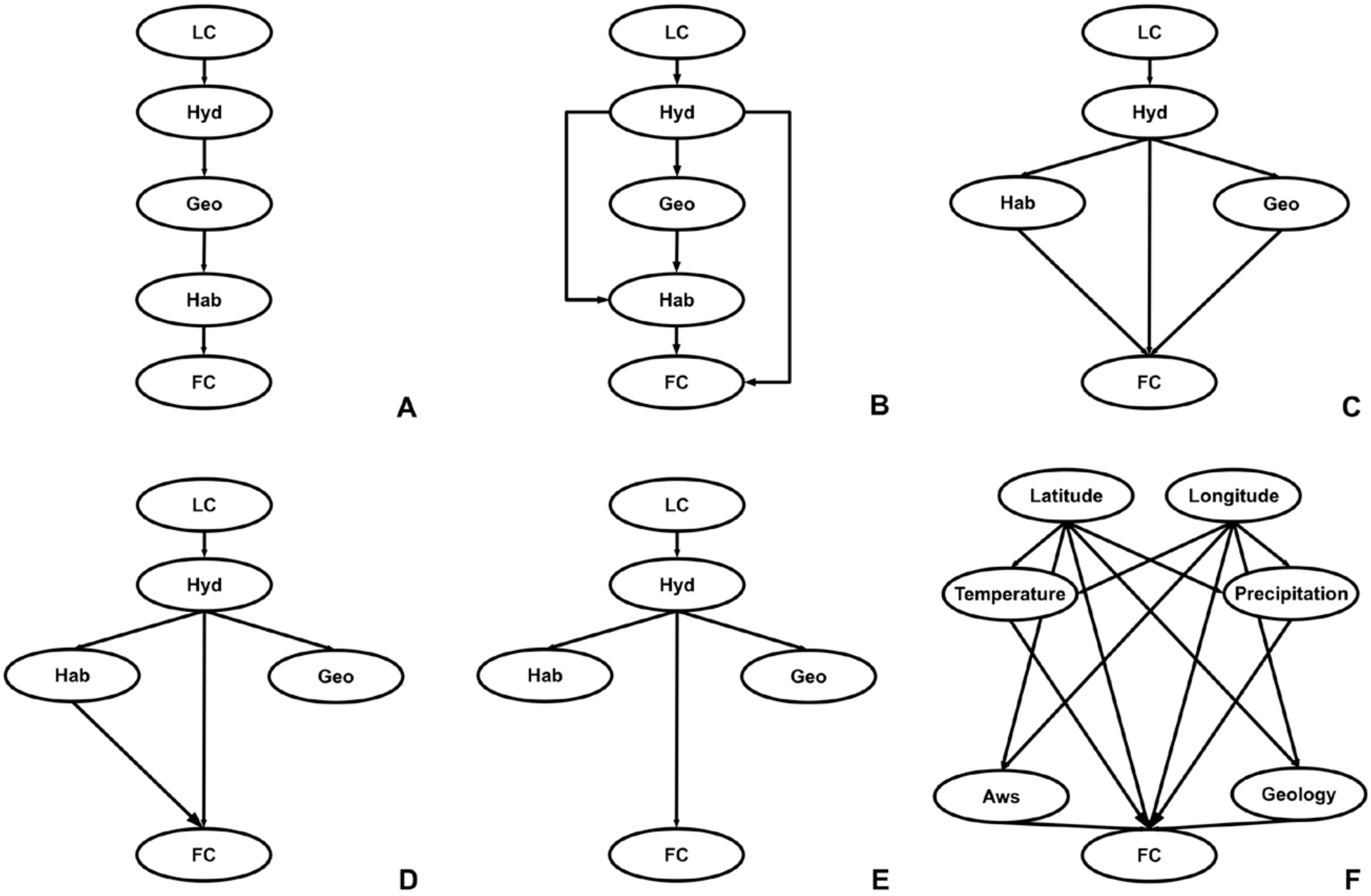
LV	Indicator Variable	Loading	Relationship to Latent Variable
LC	Southeastern North American Ruderal Forest & Plantation	0.913422122	Negative
	Longleaf Pine & Sand Pine Woodland	0.704708567	
	Southern Floodplain Hardwood Forest	0.665317484	Negative
	Developed & Urban	0.506882702	
	Herbaceous Agricultural Vegetation	0.259806829	
Hyd	MAX_q10	0.924042198	
	MAX_q5	0.862943563	
	MAX_q75	0.802573189	
	MAX_q25	0.770793154	
	MAX_q95	0.745167943	
	MAX_q90	0.686141637	
	MED._q10	0.5997491	
	MED._q5	0.597814978	
	MED._q25	0.371391499	
	rising 3_048 cm	0.200454072	
	falling 21_336 cm	0.081900298	
Hab	Sediment geometric mean	0.91426283	Negative
	Sediment standard deviation	0.837681809	Negative
	OM	0.40098463	
	submerged_cwd_per_measured_median2	0.298147046	
Geo	Atob	0.994316428	
	Wtob	0.899465483	
	Dtob	0.881092777	
FC	Collector-filterer	0.81917475	
	Shredder	0.682435904	Negative
	Scraper/grazer	0.457010058	
	Multivoltine	0.402867674	
	Collector-gatherer	0.037165127	

FIG. 1. Forty six reference streams (●) in the Southeastern Plains used to build Structural Equation Models. Grey lines are EPA level III ecoregions.

FIG. 2. Structural Equation Models that were investigated in this study. The models are as follows: **A**) the land-cover cascade, **B**) the land-cover cascade with direct effects of hydrology, **C**) hydrology as a master variable, **D**) hydrology as a master variable with no direct effect of geomorphology, **E**) hydrology as a master variable with no direct effect of geomorphology or habitat, and **F**) natural background model.

FIG. 3. Parameterized path diagram of the hydrology as a master variable model. Values along paths are path parameters and R^2 inside of each latent variable is the variance explained by the model (see text for latent variable definitions).


36
34
32
30
28
latitude
-88
-86
-84
-82
-80
-78
longitude
N
0
100
200
300 km


LC
Hyd
Geo
Hab
FC
A
LC
Hyd
Geo
Hab
FC
B
LC
Hyd
Hab
Geo
FC
C
LC
Hyd
Hab
Geo
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Hab
Geo
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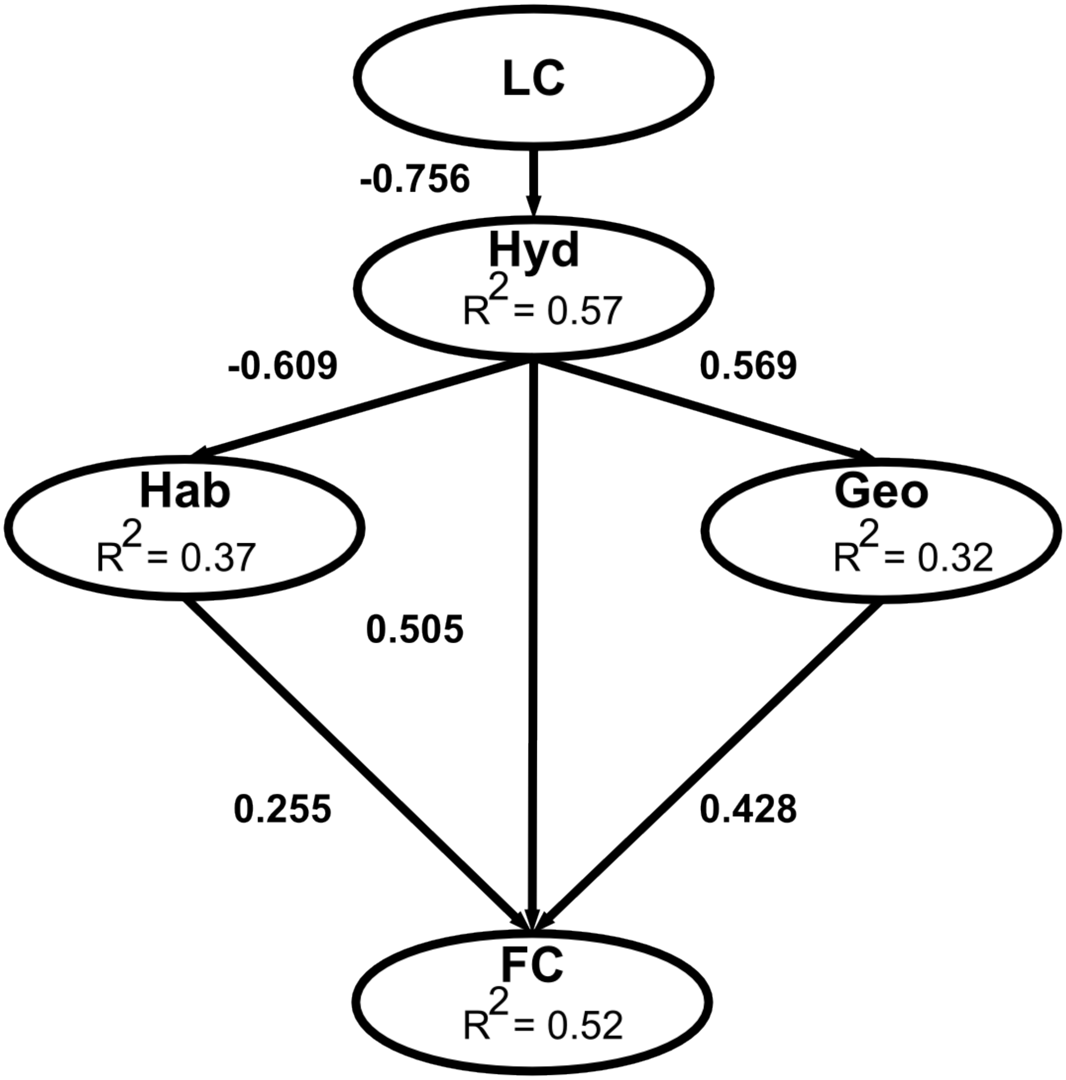
E
Latitude
Longitude
Temperature
Precipitation
Aws
Geology
FC
F


LC
-0.756
Hyd
R^2 = 0.57
-0.609
0.569
Hab
R^2 = 0.37
Geo
R^2 = 0.32
0.505
0.255
0.428
FC
R^2 = 0.52

Appendix A. Macrogroups and associated Ecological System and description for USGS GAP (v.2) data. The description presented is from metadata associated with USGS GAP data and represents the source data for the "Abbreviated Description" in Table 1. Grey highlighted variables were retained after PCA variable selection step (see methods).

Macrogroup	Ecological System	Description
Atlantic & Gulf Coastal Plain Bog & Fen	Atlantic Coastal Plain Peatland Pocosin	This system includes wetlands of organic soils on the outer terraces of the coastal plain from southeastern Virginia to the Carolinas. Occurring on broad flats or gentle basins, the vegetation is predominantly dense shrubland and very shrubby open woodlands. A characteristic suite of primarily evergreen shrubs, greenbriars, and pond pine dominates. These shrubs include inkberry, fetterbush, staggerbush, littleleaf titi, big gallberry, and honeycups, along with laurel greenbrier. Pond pine is the characteristic tree, along with loblolly-bay, sweetbay, and swamp bay. Herbs are scarce and largely limited to small open patches. Under pre-European settlement fire regimes, stands of switch cane (canebrakes) would have been more common and extensive. Soil saturation, sheet flow, and peat depth create a distinct zonation, with the highest stature woody vegetation on the edges and lowest in the center. Catastrophic fires are important in this system, naturally occurring at moderate frequency. Fires generally kill all above-ground vegetation in large patches, which recovers rapidly in most of the burned areas, primarily by sprouting.
Barren	Undifferentiated Barren Land	Barren areas of bedrock, desert pavement, scarps, talus, slides, volcanic material, glacial debris, sand dunes, strip mines, gravel pits and other accumulations of earthen material. Generally, vegetation accounts for less than 15% of total cover.
	Unconsolidated Shore	Unconsolidated material such as silt, sand, or gravel that is subject to inundation and redistribution due to the action of water. Characterized by substrates lacking vegetation except for pioneering plants that become established during brief periods when growing conditions are favorable. Erosion and deposition by waves and currents produce a number of landforms representing this class.
Central Mesophytic Hardwood Forest	Atlantic Coastal Plain Mesic Hardwood and Mixed Forest	This upland system of the Atlantic Coastal Plain ranges from southern New Jersey south to Georgia in a variety of moist but non-wetland sites that are naturally sheltered from frequent fire. Such sites include lower slopes and bluffs along streams and rivers in dissected terrain, mesic flats between drier pine-dominated uplands and floodplains, and local raised areas within bottomland terraces or wet flats. Soils are variable in both texture and pH. The vegetation consists of forests dominated by trees that include a significant component of mesophytic deciduous hardwood species, such as beech or southern sugar maple. Upland and bottomland oaks at the mid range of moisture tolerance are usually also present, particularly white oak, but sometimes also southern red oak, cherrybark oak, or Shumard oak. Loblolly pine is sometimes present, but it is unclear if it is a natural component or has entered only as a result of past cutting. Understories are usually well-

		developed. Shrub and herb layers may be sparse or moderately dense.
Developed & Urban	Developed High Intensity	Includes highly developed areas where people reside or work in high numbers. Examples include apartment complexes, row houses and commercial/industrial. Impervious surfaces account for 80 to100 percent of the total cover.
	Developed Low Intensity	Includes areas with a mixture of constructed materials and vegetation. Impervious surfaces account for 20-49 percent of total cover. These areas most commonly include single-family housing units.
	Developed Medium Intensity	Includes areas with a mixture of constructed materials and vegetation. Impervious surfaces account for 50-79 percent of the total cover. These areas most commonly include single-family housing units.
	Developed Open Space	Includes areas with a mixture of some constructed materials, but mostly vegetation in the form of lawn grasses. Impervious surfaces account for less than 20 percent of total cover. These areas most commonly include large-lot single-family housing units, parks, golf courses, and vegetation planted in developed settings for recreation, erosion control, or aesthetic purposes.
Herbaceous Agricultural Vegetation	Cultivated Cropland	Areas used for the production of annual crops, such as corn, soybeans, vegetables, tobacco, and cotton, and also perennial woody crops such as orchards and vineyards. Crop vegetation accounts for greater than 20 percent of total vegetation. This class also includes all land being actively tilled.
	Pasture Hay	Areas of grasses, legumes, or grass-legume mixtures planted for livestock grazing or the production of seed or hay crops, typically on a perennial cycle. Pasture/hay vegetation accounts for greater than 20 percent of total vegetation.
Longleaf Pine & Sand Pine Woodland	Atlantic Coastal Plain Fall line Sandhills Longleaf Pine Woodland- Open Understory Modifier	This system occurs in the Fall-Line Sandhills region of from central North Carolina extending into central Georgia. It is the predominant system in its range, covering most of the natural landscape of the region. It occurs on upland sites ranging from gently rolling, broad ridgetops to steeper side slopes, as well as locally in mesic swales and terraces. Most soils are well- to excessively drained. The vegetation is naturally dominated by longleaf pine (Pinus palustris). Most associations have an understory of scrub oaks. The herb layer is generally well-developed and dominated by grasses. Wiregrass (Aristida stricta in the north, Aristida beyrichiana in the south) dominates in most of the range, but other grasses dominate where it is absent. Forbs, including many legumes, are also abundant. Frequent, low-intensity fire is the dominant natural ecological force._x000D_ _x000D_ Variation: Open Understory - Grasses, widely scattered shrubs, or bare sand and soil dominate the understory. The open aspect usually maintained through frequent fire or other management._x000D_ _x000D_ Other Variation(s): Loblolly, Offsite Hardwood, Scrub/Shrub Understory.
	Atlantic Coastal Plain Upland Longleaf Pine Woodland	This system of upland longleaf pine -dominated vegetation ranges from southern Virginia (beginning approximately at the James River) to northeastern Florida, and was perhaps the most extensive system in that area of the outer coastal plain. It is characterized by

		longleaf pine in an upland setting with natural exposure to frequent fire. A few occurrences just north of the range of longleaf pine are dominated by old-growth loblolly pine. It occurs on a variety of well- to excessively drained soils, and on the higher parts of upland-wetland mosaics. Most areas have an understory of scrub oaks. The herb layer is generally well-developed and dominated by three-awn and other grasses. Frequent, low-intensity fire is the dominant natural ecological force.
	East Gulf Coastal Plain Interior Upland Longleaf Pine Woodland- Open Understory Modifier	This system represents longleaf pine forests of rolling, dissected uplands of the East Gulf Coastal Plain. These stands occur inland of the coastal flatlands (sensu Peet and Allard 1993) and potentially occupy a much larger geographic area than this related system, extending landward into the Upper East Gulf Coastal Plain by about 50 miles. The characteristic species is Pinus palustris, although many stands may support only relictual individuals given a long history of exploitation and stand conversion. This system includes stands with a range of soil and moisture conditions. Mesic stands on fine-textured soils are more typical of the system, although limited xeric areas on deep sands are also present. In natural condition, fire is believed to have been frequent enough to limit development of intolerant species of hardwoods and both Pinus taeda and Pinus echinata. Although such species may be present or even common in the most mesic stands, they generally do not share dominance in the overstory unless the system has been fire-suppressed._x000D_ _x000D_ Variation: Open Undestory - Sites where fire frequency or management maintain the open aspect with herbaceous or widely scattered shrubs in the understory._x000D_ _x000D_ Other Variation(s): Loblolly, Offsite Hardwood, Scrub/Shrub.
	Atlantic Coastal Plain Fall line Sandhills Longleaf Pine Woodland- Scrub Shrub Understory Modifier	This system occurs in the Fall-Line Sandhills region of from central North Carolina extending into central Georgia. It is the predominant system in its range, covering most of the natural landscape of the region. It occurs on upland sites ranging from gently rolling, broad ridgetops to steeper side slopes, as well as locally in mesic swales and terraces. Most soils are well- to excessively drained. The vegetation is naturally dominated by longleaf pine (Pinus palustris). Most associations have an understory of scrub oaks. The herb layer is generally well-developed and dominated by grasses. Wiregrass (Aristida stricta in the north, Aristida beyrichiana in the south) dominates in most of the range, but other grasses dominate where it is absent. Forbs, including many legumes, are also abundant. Frequent, low-intensity fire is the dominant natural ecological force._x000D_ _x000D_ Variation: Scrub/Shrub - Fire supressed sites with a dense understory of scrub oaks or shrubs._x000D_ _x000D_ Other Variation(s): Loblolly, Offsite Hardwood, Open Understory.
	West Gulf Coastal Plain Upland Longleaf Pine Forest and Woodland	The common and unifying feature of this system is vegetation naturally dominated by longleaf pine. This was formerly the most extensive system within its natural range in western Louisiana and eastern Texas. In most of the region, longleaf pine is (presently) a distinctive, but rarely dominant, element of existing vegetation

		(Harcombe et al. 1993). However, this tree historically dominated the vegetation across nearly all uplands regardless of soil type or moisture (excluding wetlands), and longleaf pine forests were among the most valuable economic resources in the region at the turn of the century (Bray 1906). Typical sites included sandhills on well-drained to excessively drained soils, but also more loamy and clayey upland soils. The importance of frequent fire has been well documented for the perpetuation of this system. Unlike comparable systems east of the Mississippi River, this type lies outside the range of threeawn spp. (wiregrasses), but most stands supported open grass-dominated understories rich in species diversity.
Open Water	Open Water- Fresh	All areas of open water, generally less than 25% cover of vegetation or soil. Specifically, inland waters of streams, rivers, ponds and lakes.
Quarries, Mines, Gravel Pits and Oil Wells	Quarries, Mines, Gravel Pits, and Oil Wells	Areas of extractive mining activities with significant surface expression.
Recently Disturbed or Modified	Disturbed Successional- Grass Forb Regeneration	Areas where a relatively recent disturbance event has occurred, signs of which are still visible on the imagery (images acquired between 1999-2001) or identifiable using change detection techniques, and have regenerated to herbaceous dominated vegetation.
	Disturbed Successional- Shrub Regeneration	Areas where a relatively recent disturbance event has occurred, signs of which are still visible on the imagery (images acquired between 1999-2001) or identifiable using change detection techniques, and have regenerated to shrub or stunted tree dominated vegetation.
	Harvested Forest- Grass Forb Regeneration	Areas dominated by herbaceous ground cover following tree harvesting.
	Harvested Forest- Shrub Regeneration	Areas dominated by shrubs; less than 5 meters tall with shrub canopy typically greater than 20% of total vegetation. This class includes true shrubs, young trees in early successional stage or trees stunted from environmental conditions following a tree havesting event.
Southeastern North American Ruderal Forest & Plantation	Atlantic Coastal Plain Fall Line Sandhills Longleaf Pine Woodland- Loblolly Modifier	This system occurs in the Fall-Line Sandhills region of from central North Carolina extending into central Georgia. It is the predominant system in its range, covering most of the natural landscape of the region. It occurs on upland sites ranging from gently rolling, broad ridgetops to steeper side slopes, as well as locally in mesic swales and terraces. Most soils are well- to excessively drained. The vegetation is naturally dominated by longleaf pine (Pinus palustris). Most associations have an understory of scrub oaks. The herb layer is generally well-developed and dominated by grasses. Wiregrass (Aristida stricta in the north, Aristida beyrichiana in the south) dominates in most of the range, but other grasses dominate where it is absent. Forbs, including many legumes, are also abundant. Frequent, low-intensity fire is the dominant natural ecological force._x000D_ _x000D_ Variation: Loblolly - These stands are dominated by loblolly or slash pine as a result of past disturbance history._x000D_ _x000D_ Other Variation(s): Offsite Hardwood, Open Understory, Scrub/Shrub Understory.
	Deciduous Plantations	Even-aged, regularly spaced forest stands established by planting

		and/or seeding in the process of afforestation or reforestation where individual trees are generally > 5 meters in height. Specifically, this class refers to plantations dominated by deciduous species.
	East Gulf Coastal Plain Interior Upland Longleaf Pine Woodland- Loblolly Modifier	This system represents longleaf pine forests of rolling, dissected uplands of the East Gulf Coastal Plain. These stands occur inland of the coastal flatlands (sensu Peet and Allard 1993) and potentially occupy a much larger geographic area than this related system, extending landward into the Upper East Gulf Coastal Plain by about 50 miles. The characteristic species is Pinus palustris, although many stands may support only relictual individuals given a long history of exploitation and stand conversion. This system includes stands with a range of soil and moisture conditions. Mesic stands on fine-textured soils are more typical of the system, although limited xeric areas on deep sands are also present. In natural condition, fire is believed to have been frequent enough to limit development of intolerant species of hardwoods and both Pinus taeda and Pinus echinata. Although such species may be present or even common in the most mesic stands, they generally do not share dominance in the overstory unless the system has been fire-suppressed._x000D_ _x000D_ Variation: Loblolly - Disturbed sites where loblolly pines have invaded the site, generally higher density canopies than the native longleaf stands._x000D_ _x000D_ Other Variation(s): Offsite Hardwood, Open Understory, Scrub/Shrub.
	East Gulf Coastal Plain Interior Upland Longleaf Pine Woodland- Offsite Hardwood Modifier	This system represents longleaf pine forests of rolling, dissected uplands of the East Gulf Coastal Plain. These stands occur inland of the coastal flatlands (sensu Peet and Allard 1993) and potentially occupy a much larger geographic area than this related system, extending landward into the Upper East Gulf Coastal Plain by about 50 miles. The characteristic species is Pinus palustris, although many stands may support only relictual individuals given a long history of exploitation and stand conversion. This system includes stands with a range of soil and moisture conditions. Mesic stands on fine-textured soils are more typical of the system, although limited xeric areas on deep sands are also present. In natural condition, fire is believed to have been frequent enough to limit development of intolerant species of hardwoods and both Pinus taeda and Pinus echinata. Although such species may be present or even common in the most mesic stands, they generally do not share dominance in the overstory unless the system has been fire-suppressed._x000D_ _x000D_ Variation: Offsite Hardwood - Heavily disturbed sites where successional hardwood species dominate (sweetgum, water oak, willow oak)_x000D_ _x000D_ Other Variation(s): Loblolly, Open Understory, Scrub/Shrub.
	Evergreen Plantations or Managed Pine	Even-aged, regularly spaced forest stands established by planting and/or seeding in the process of afforestation or reforestation where individual trees are generally > 5 meters in height. Specifically, this class refers to plantations dominated by evergreen species.

Southern Coastal Plain Basin Swamp	Atlantic Coastal Plain Nonriverine Swamp and Wet Hardwood Forest- Taxodium Nyssa Modifier	This system consists of poorly drained, organic or mineral soil flats of the outer Atlantic Coastal Plain. These areas are saturated by rainfall and seasonal high water table without influence of river or tidal flooding. Fire is generally infrequent, but may be important for some associations. Vegetation consists of hardwood or mixed forests of Taxodium distichum, Nyssa spp., bottomland oaks, or other wetland trees of similar tolerance. The lower strata have affinities with pocosin or baygall systems rather than the river floodplain systems that have affinities with the canopy. The combination of canopy dominants and nonriverine, non-seepage hydrology distinguishes this system from other Coastal Plain systems._x000D_ _x000D_ Variation: Taxodium/Nyssa - Deeper water expressions of this system dominated by bald cypress and/or water tupelo and swamp blackgum._x000D_ _x000D_ Other Variation(s): Oak.
	Southern Coastal Plain Nonriverine Basin Swamp	This system occupies large, seasonally inundated basins with peaty substrates in the southern and outermost portions of the Coastal Plain of the southeastern United States. These basins are nonriverine and do not receive overbank flooding. The southern range of this system extends into central Florida especially along the Atlantic Coast in Volusia and Brevard counties (A. Johnson pers. comm.). Examples are generally forested; the vegetation is characterized by bald-cypress, swamp blackgum, evergreen "bay" shrubs and/or mixed hardwoods. Emergent slash pine may also be present. Some characteristic shrubs include black titi, titi, shining fetterbush, and blaspheme-vine.
Southern Coastal Plain Evergreen Hardwood & Conifer Swamp	Southern Coastal Plain Hydric Hammock	This system occupies flat lowlands along the southern and outermost portions of the Coastal Plain of the southeastern United States, usually over limestone substrates. Vegetation is characterized by mixed hardwood species (FNAI 1997), often with hydric oak species common (A. Johnson pers. comm.). In Florida examples of this system are often found adjacent to the floodplain of spring-fed rivers with relatively constant flows. In some areas, such as the Big Bend region, they occupy large areas of broad, shallow, mucky or seepy wetlands but generally do not receive overbank flooding (A. Johnson pers. comm.). In Alabama, this system is apparently confined to floodplains of the Mobile-Tensaw (A. Schotz pers. comm.), where examples are topographically higher than the surrounding floodplains.
	Atlantic Coastal Plain Streamhead Seepage Swamp, Pocosin, and Baygall	This system encompasses seepage-fed wetlands in dissected Coastal Plain landscapes, from southeastern Virginia to northeastern Florida. Examples are usually associated with ravines or along headwater streams. Overbank flooding is a negligible influence. Fire may be an important force in some and not in others. Vegetation consists of open to closed forest of acid-tolerant wetland hardwoods (sweetbay and swamp black gum typical) or pond pine. Generally there is a dense shrub layer consisting primarily of species shared with Atlantic Coastal Plain Peatland Pocosin and Canebrake, including inkberry, fetterbush, staggerbush, littleleaf titi, big gallberry, and honeycups.

Southern Floodplain Hardwood Forest	Atlantic Coastal Plain Blackwater Stream Floodplain Forest- Forest Modifier	This Atlantic Coastal Plain system, which is apparently most abundant in the Carolinas, occurs in floodplains of small streams that carry little mineral sediment (blackwater streams). These streams have their headwaters in sandy portions of the Coastal Plain. The water is usually strongly stained by tannins but has little suspended clay and is not turbid. Depositional landforms may be absent or present only in limited variety and of small size. Soils are usually strongly acidic. Flooding ranges from semipermanent in the wettest floodplains to intermittent and short in higher gradient streams. Some small blackwater streams have most of their flow from sandhill seepage and have limited fluctuation in water levels. Vegetation consists almost entirely of forests of wetland trees. Wetter examples are strongly dominated by Taxodium distichum and Nyssa biflora. Other examples have mixtures of these species with Quercus spp. and other bottomland hardwoods tolerant of blackwater conditions. Species richness ranges from low to moderate, but is lower than in comparable brownwater systems. Flooding is an important ecological factor in this system and may be the most important factor separating it from adjacent systems. Flooding brings nutrients and excludes non-flood-tolerant species. Unlike river systems, flooding tends to be variable and of shorter duration._x000D_ _x000D_ Variation: Forest - Canopy forest present._x000D_ _x000D_ Other Variation(s): Herbaceous.
	Atlantic Coastal Plain Small Blackwater River Floodplain Forest	This system encompasses the floodplains of small to medium blackwater rivers, intermediate between the smaller streams and the largest rivers. Blackwater rivers originate in the sandy areas of the Coastal Plain and have less well-developed depositional alluvial landforms. Soils are sandy or mucky, acidic, and infertile. Vegetation is a mosaic of cypress and gum swamps and bottomland hardwoods of a limited set of oaks and other species. In general vegetation is low in species richness.
	East Gulf Coastal Plain Small Stream and River Floodplain Forest	This is a predominantly forested system of the East Gulf Coastal Plain associated with small brownwater rivers and creeks. In contrast to ~East Gulf Coastal Plain Large River Floodplain Forest (CES203.489)$$, it has fewer major geomorphic floodplain features typically associated with large river floodplains. Those features that are present tend to be smaller and more closely intermixed with one another, resulting in less obvious vegetational zonation. Bottomland hardwood tree species are typically important and diagnostic, although mesic hardwood species are also present in areas with less inundation, such as upper terraces and possibly second bottoms. As a whole, flooding occurs annually, but the water table usually is well below the soil surface throughout most of the growing season. Areas impacted by beaver impoundments are also included in this system.
Southern Mixed Deciduous-Evergreen Broadleaf Forest	Southern Coastal Plain Dry Upland Hardwood Forest	This is one of three hardwood-dominated systems found in the East Gulf Coastal Plain and adjacent areas of central Florida. This type is found in the Southern Coastal Plain and Southeastern Plains (EPA Level III Ecoregion 75 and parts of 65). Examples attributable to this type are typically deciduous or mixed evergreen oak-dominated forests, often with a pine component present. Although the southern portion of the range of this system overlaps

		~Southern Coastal Plain Oak Dome and Hammock (CES203.494)$$, the latter is dominated by evergreen oak species, and the two should not be confused. The core range of this type extends northward to the approximate historical range of longleaf pine; although most deciduous species do not mimic this range, this boundary does appear to be a reasonable demarcation boundary north of which white oak becomes more abundant and south of which sand laurel oak is more diagnostic. Like all hardwood systems of this region, examples occur within a landscape matrix historically occupied by pine-dominated uplands and consequently only occurred in fire-sheltered locations in naturally small to large patches. Examples of this system tend to occur on sites intermediate in moisture tendency (mostly dry to dry-mesic), although occasional xeric stands may also be included. Toward the northern range limits of this system, it may have been less restricted to small patches in fire-protected locations, and may have been formerly more prevalent on the landscape even in areas heavily influenced by fire. Important tree species vary geographically and according to previous disturbance. Sand laurel oak is a typical species in many examples, with post oak, southern red oak, and white oak less frequently encountered, but dominant in some stands. The overstory of some examples may be quite diverse, with hickories and other hardwood species often present. Typically mesic sites, as indicated by species indicative of these conditions, are covered under other systems. Loblolly pine is sometimes present, but it is unclear if it is a natural component or has entered only as a result of past cutting. Spruce pine or shortleaf pine may also be present in some examples. Stands may be found on slopes above rivers and adjacent to sinkholes, as well as other fire-infrequent habitats.
Southern-Central Oak-Hardwood & Pine Forest	Atlantic Coastal Plain Dry and Dry Mesic Oak Forest	This system encompasses oak-dominated forests of somewhat fire-sheltered dry to dry-mesic sites in the coastal plain from southeastern Virginia to Georgia. It occurs in areas somewhat protected from most natural fires by some combination of steeper topography, isolation from the spread of fire, and limited flammability of the vegetation. If fires were more frequent, the vegetation would likely be replaced by more fire-tolerant southern pines, especially longleaf pine.
	Atlantic Coastal Plain Fall line Sandhills Longleaf Pine Woodland-Offsite.Hardwood Modifier	This system occurs in the Fall-Line Sandhills region of from central North Carolina extending into central Georgia. It is the predominant system in its range, covering most of the natural landscape of the region. It occurs on upland sites ranging from gently rolling, broad ridgetops to steeper side slopes, as well as locally in mesic swales and terraces. Most soils are well- to excessively drained. The vegetation is naturally dominated by longleaf pine (Pinus palustris). Most associations have an understory of scrub oaks. The herb layer is generally well-developed and dominated by grasses. Wiregrass (Aristida stricta in the north, Aristida beyrichiana in the south) dominates in most of the range, but other grasses dominate where it is absent. Forbs, including many legumes, are also abundant. Frequent, low-intensity fire is the dominant natural ecological force._x000D_ _x000D_ Variation: Offsite Hardwoods - Sites that have been heavily disturbed and dominated by offsite hardwoods (water oak,

		sweetgum, red maple, tulip poplar)._x000D_ _x000D_ Other Variation(s): Loblolly, Open Understory, Scrub/Shrub Understory.
Wet Longleaf Pine & Southern Flatwoods	Southern Coastal Plain Nonriverine Cypress Dome	This system consists of small forested wetlands, typically dominated by pond-cypress, with a characteristic and unique dome-shaped appearance in which trees in the center are higher than those around the sides (Monk and Brown 1965). Examples are known from the Southern Coastal Plain (Omernik Ecoregion 75 and adjacent 65) (EPA 2004) of Florida and Georgia, extending into Alabama, Mississippi and Louisiana. Examples occupy poorly drained depressions which are most often embedded in a matrix of pine flatwoods. The oldest and largest individual trees typically occupy the center of these domed wetlands, with smaller and younger individuals around the margins. Pools of stagnant, highly acidic water may stand in the center of these depressions ranging from 1-4 feet in depth, but becoming increasingly shallow along the margins. These sites are underlain by an impervious clay pan which impedes drainage and traps precipitation. Some examples may have thick (50-100 cm) organic layers. In addition to pond-cypress, other woody species may include swamp blackgum, Chapman's St. John's-wort, myrtleleaf St. John's-wort, myrtle dahoon, swamp doghobble, wax-myrtle, common buttonbush, sweetgum, coastal sweet-pepperbush, shining fetterbush, and downy snowbell.
	Central Atlantic Coastal Plain Wet Longleaf Pine Savanna and Flatwoods	This system of wet pond pine -dominated savannas and flatwoods ranges from southern Virginia through South Carolina. It was once one of the most extensive systems in the coastward part of its range. It is characterized by wet, seasonally saturated, mineral soils and exposure to frequent fire. It occurs on a wide range of soil textures, which is an important factor in variation in cover and species. The vegetation is naturally dominated by pond pine. There is a dense ground cover of herbs and low shrubs; grasses dominate but there is often a large diversity of other herbs. Frequent, low-intensity fire is the dominant natural ecological force.

Chapter 5: Disturbance deterministically assembles macroinvertebrate stream communities: an experimental manipulation (Formatted for submission to Ecology)

Running Head: Deterministic stream communities

Disturbance deterministically assembles macroinvertebrate stream communities: an experimental manipulation

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ABSTRACT

Expanding global biodiversity declines require a heightened understanding of community assembly. Neutral theory and species sorting represent ends of a stochastic to deterministic gradient of community assembly in a metacommunity, with empirical work showing contrasting patterns of assembly in terrestrial and aquatic ecosystems in the face of disturbance. We conducted a field experiment in a sandy-bottom stream in the southeastern US to assess how communities are assembled under differing disturbance intensity. Benthic macroinvertebrate assemblage composition (taxa and trait) was subjected to 5 disturbance regimes simulating bed disturbance intensity. We found that the streambed and a moderate-high level of disturbance satisfied 3 conditions necessary to show deterministic assembly: 1) lower β–diversity, 2) higher nestedness, and 3) similar mean trait values when compared to low/high disturbance treatments. Interestingly, predators were more common in the low/high disturbance treatments, and could be causing stochastic assembly under low disturbances, and occur in high disturbances through priority effects. Our results suggested stream beds were deterministically assembled at moderate-high levels of disturbance, and stochastic processes dominated at low/high levels of disturbance. This suggests that niche assembly on functional traits through the mechanism of species sorting was acting to assemble macroinvertebrate communities in the study stream.

Keywords: community assembly; experiment; niche assembly; deterministic assembly; stochastic assembly.

INTRODUCTION

Predicted declines in global biodiversity over the next 100 years make it essential to understand the mechanisms regulating biotic distributions (Sala et al. 2000). Community assembly "deletion rules" have been posited as a way to predict community patterns by conceptualizing the community assembly process as a filter that organisms from the regional species pool have to "pass" based on their traits to be a member of the local community (sensu Keddy [1992]). Both biotic and abiotic constraints (e.g., predation or disturbance) can filter organisms from the regional species pool (Belyea and Lancaster 1999). Two ways to quantify the response to the filtering processes are: 1) species identities, and 2) species traits. With regards to species identity, Whittaker (1972) partitioned diversity into local (α; α-div), between site (β; β-div), and regional diversity (γ; γ-div), and suggested they are linked multiplicatively (i.e., γ-div/α-div=β-div). Many studies have used β-div to investigate community assembly's relationship with disturbance in both aquatic (Chase 2007, Jiang and Patel 2008, Lepori and Malmqvist 2009, Tonkin and Death 2013, Vanschoenwinkel et al. 2013) as well as terrestrial systems (Vellend et al. 2007, Myers and Harms 2011, Guo et al. 2014, Myers et al. 2015). With regards to trait responses, empirical and theoretical research has focused on how traits are filtered by habitat constraints in terrestrial and aquatic systems (Poff 1997, Garnier et al. 2004, Shipley et al. 2006, de Bello et al. 2012). Although there have been many studies investigating the relationship of disturbance with community assembly, there are no generalizable assembly rules for disturbed communities across ecosystems. Thus, knowing if there are predictable filters for organismal distribution can add useful insights into the mechanisms controlling community assembly.

The metacommunity concept explains community assembly as sets of interacting species assemblages linked through dispersal, resulting in local communities that are subsets of the regional species pool (reviewed in Leibold et al. 2004). These subset communities are often seen as resulting from the interplay of deterministic (non-random) and stochastic(random)processes (Chase and Myers 2011). Two potential mechanisms of metacommunity assembly are deterministic species sorting and stochastic neutral theory. First, similar to the classic niche concept (Hutchinson 1957), deterministic species sorting posits that local abiotic or biotic factors act on species traits to filter organisms from the regional species pool (Keddy 1992, Poff 1997). In contrast, neutral theory assumes organisms are functionally equivalent, and that local stochastic demographic processes (i.e., ecological drift; sensu Hubbell [2005]) produce observed assemblages (Hubbell 2005). Chase (2007) proposed a synthetic view that both deterministic and stochastic processes influence assemblage composition with deterministic processes becoming more important than neutral processes in harsher environments because niche assembly favors organisms with traits that are suited to the environment (see also Jiang and Patel [2008] and Menge and Sutherland [1987]). Chase's (2007) synthetic view of community assembly provides testable predictions to investigate community assembly in freshwater ecosystems. Streams offer an appropriate model system to test assembly processes because of the primacy of hydrologic disturbance as a primary organizing force (Resh et al. 1988).

Empirical evidence from freshwater ecosystems suggests that deterministic (Lamouroux et al. 2002, Urban 2004, Brown and Swan 2010) as well as stochastic mechanisms (Thompson and Townsend 2006) influence community assembly in streams. Hydrologic disturbance could have an effect on stream organisms by either direct effects of organism removal (McCabe and Gotelli 2000), indirect effects such as habitat modification (Burcher et al. 2007), or modifying competitive interactions (Feminella and Resh 1990). Disturbance results in deterministic community assembly if disturbance filters a predictable subset of the macroinvertebrate community based on their traits. In contrast, disturbance could also result in stochastic community assembly by randomly removing organisms or modifying habitat that results in a non-predictable subset of the community. In a field study in Scandinavian streams, Lepori and Malmqvist (2009) found determinism to be highest at intermediate levels of hydrologic disturbance, suggesting that Chase's (2007) hypothesis be amended to include stochastic effects at low levels in addition to high levels of disturbance. In contrast, Milner et al. (2011) found deterministic assembly to increase with environmental harshness, similar to Chase (2007), in a field study in an Alaskan stream.

In streams, hydrologic disturbance from storm events is considered a primary organizing force for benthic communities (Power et al. 1995, Hart and Finelli 1999, Lake 2000, Bunn and Arthington 2002). One way hydrologic disturbance influences benthic biota is through increases in near-bed shear stress and resulting bed movement with increasing storm flows. Empirical evidence suggests that intensity of bed disturbance is a strong determinant of benthic organismal distribution (Townsend et al. 1997), and organisms show hydraulic preferences related to shear stress (Doledec et al. 2006). Bed movement may be even more important in low-gradient sandbed streams because the bed is mobile at discharges as low as the annual mean (Soar and Thorne 2001) and benthic habitats are disrupted. Thus, highly variable sandbed streams should represent a unique opportunity to investigate the role of disturbance on assembling communities.

Because observed pattern does not suggest generalizable mechanisms of assembly in the face of disturbance among ecosystems, or when restricted to aquatic systems, we conducted an experiment to investigate how stream communities are assembled in the face of disturbance. In order to demonstrate that communities are deterministically-assembled the following 3 conditions must be met: 1) β-div will be lowest when communities are deterministically assembled under similar disturbance regimes, 2) assemblages that are deterministically assembled will be a nested subset of the regional species pool, and 3) communities that are deterministically assembled will have organisms that are filtered from the regional species pool based on the traits they possess. The first and second conditions are necessary to show that communities are deterministically assembled (Chase 2007). The third condition, implicit to deterministic assembly, is derived from the species sorting paradigm of metacommunity theory (Leibold et al. 2004), and has not been explicitly investigated in parallel with deterministic assembly until this study (e.g., Chase 2007, Jiang and Patel 2008, Lepori and Malmqvist 2009). To investigate deterministic assembly in stream communities in the face of disturbance, we applied a disturbance intensity gradient to benthic macroinvertebrate assemblages.

METHODS

Study area and experimental design

We conducted the experiment in Sandy Creek (32.76N, -85.59W; Fig. 1), a 3rd-order, low-gradient sandbed stream draining a small -forested watershed (watershed area=56.69 km^2) in the Tallapoosa River drainage in the outer Piedmont of east Alabama. While Sandy Creek has had historic impacts such as row cropping, the stream is not incised (an indicator of no hydrologic alteration), has access to well forested, broad floodplains (Singer and Gangloff 2011), and contemporary impacts are minimal making this stream suitable for this study.

We deployed the experiment on 9 November 2013, allowed it to equilibrate for 4 d, and ran it for 1 mo (13 Nov-12 Dec 2013). We used instream experimental enclosures (enclosures) as experimental units to quantify macroinvertebrate colonization at different levels of disturbance intensity. Enclosures (17.5 cm L x 17.5 cm W x 9.5 cm H, total volume=2909.4 cm^3) were constructed from ~1.5 cm^2 square black plastic-mesh, and filled with ~3.9 kg acid-washed play sand (particle size ≤ 2 mm, immersed in 1% acetic acid for ~ 24 h) to remove nutrients and microorganisms. Five treatments (i.e., levels of disturbance intensity, see below) and 1 streambed control (**S**, unmanipulated area of stream bed of similar size) were randomly assigned within each of 6 blocks deployed in a single large stream run (20 m L x 10 m W, Fig. 1A) using a randomized complete block design to control for microhabitat variation (Longnecker and Ott 2001). Enclosures were deployed 15 cm apart within blocks, with adjacent blocks separated by at least 1.57 m perpendicular to the flow, and 4 m in the downstream direction (Fig. 1B; mean deployment±1SD; velocity=0.10±0.06 m/s; depth=0.19±0.04 m).

We simulated disturbance intensity by applying 5 levels of bed disturbance using a hand-cranked mixing impeller approximately weekly over a 4-wk period (13 Nov – 5 Dec 2013). We used 5 levels of disturbance intensity: no disturbance (0 turns; **0**), low intensity (1 turn; **1**), moderate-low intensity (2 turns; **2**), moderate-high intensity (3 turns; **3**), and high intensity (4 turns; **4**). Disturbance was applied by embedding the mixer (mixing impeller 10 cm diam x 9.5 cm H; estimated manipulated area ~746.1 cm^3; ~26%) into the center of the sand substrate of each the enclosures to a depth of 9.5 cm, and then rotating the impeller clockwise one or more complete revolutions (below) in 1 second. For each disturbance event we placed a 250 μm D-net downstream of the enclosure, removed all macroscopic organic matter accumulated above the enclosure, and then disturbed the substrate with the mixing device. The OM removed from enclosures was removed from the stream; in this way, undesired treatment effects on downstream enclosures were minimized. Water velocity and depth were measured during each disturbance event (downstream edge of enclosures and **S**). At the end of the experiment the entire enclosure was retrieved by placing into a 250 µm D-net to minimize loss of material. Streambed controls (**S**) were sampled with a modified Surber sampler (250 µm) and hand raked to approximate enclosure volume. Samples were collected and preserved in 95% ethanol in the field and stored in the laboratory at 0 ºC until processed.

In the laboratory, we elutriated each benthic sample to separate and remove all macroinvertebrates and organic matter from heavier inorganic matter (Feminella 1996). Macroinvertebrates were sorted , identified and enumerated to the lowest practical taxonomic level (typically genus or species, including Chironomidae) (Brigham et al. 1982, Kowalyk 1985, Epler 2001, Merritt et al. 2008, Thorp and Covich 2009, Epler 2010). We resolved ambiguous taxa by adding abundances of ambiguous parent taxa (e.g., tribe Chironomini) to children abundances (e.g., all taxa in a sample in the tribe Chironomini) within a sample proportional to the relative abundances of children (Cuffney et al. 2007). In other words, we multiplied ambiguous parent numbers by proportional abundance of children, and added the number to the child's abundance. To quantify macroinvertebrate food resources, OM collected along with enclosures was divided into 2 size fractions (≥2 mm, <2 to 250 µm), dried to a constant mass at 65 ºC, and then combusted for 6 h at 550 ºC to determine ash free dry mass (AFDM, Wallace and Grubaugh 1996).

Stream hydrology

We quantified stream hydrology (water level or stage) to describe natural variability in stream flow during the experiment. We estimated stage every 15 min using Solinst Levelogger Junior pressure transducers (Model 3001, Solinst Canada Ltd., Canada) adjusted for ambient atmospheric pressure using barometric pressure from Auburn-Opelika airport (USAF 722284) weather station. The pressure transducer was deployed at the end of the run in a temporary stilling well constructed of schedule-40 PVC (3.81 cm ID), which was perforated on the downstream side to allow stage equilibration.

Statistical Analysis

Our general modeling approach was to investigate differences among treatments for β-div, Nestedness (Nest), and OM was to use a linear mixed effects model framework. We included OM as a factor because of its importance as a food source to benthic invertebrate colonization in sandbed streams (Yamamuro and Lamberti 2007), and to investigate whether food resources and associated macroinvertebrate distributions were affected by disturbance intensity. All 3 of these models included treatment and the median velocity at each experimental enclosure as fixed effects. In addition, we included a random intercept, and a random slope of median velocity nested within block as random effects to account for changes to treatment means related to median velocity and block. We fit these models with the lmer function in the lme4 R package (Bates et al. 2015). We inspected models for adherence to regression assumptions with residual plots, and, as a result, only ln-transformed OM data. We used a likelihood ratio test to assess the significance of all models, and whether a model including disturbance treatment explained more variation in the response than a null model excluding treatment. If the likelihood ratio test was significant, then we used planned pairwise comparisons (glht; package multcomp [Hothorn et al. 2008]) to investigate differences between **S** (reference level) and all other treatments. *P*-values were corrected for multiple testing with Holm's step-down method (Holm 1979).

To test condition 1 (β-div will be lowest in deterministically assembled communities related to disturbance intensity), we used a modified Raup-Crick dissimilarity measure of a taxa presence/absence matrix and 9999 permutations with R code provided by Chase et al. (2011).

We chose this measure because it uses a null model to decouple changes in α-div, which was variable in our experiment, with β-div (Chase et al. 2011). We used the unscaled version (i.e., 0 to 1 with all other arguments of the software remaining the default [Chase et al. 2011]) of the dissimilarity index to test for differences in β-div (i.e., within treatment distance to the multivariate median) between treatments and **S** (Anderson et al. 2006, Anderson et al. 2011). We calculated β-div with the betadisper function in the R package vegan (Oksanen et al. 2007).

To test condition 2 (presence of nested subsets of the macroinvertebrate assemblage related to disturbance intensity), we used BINMATNEST to calculate the maximally nested matrix, matrix temperature (a metric of unexpected occurrences with regards to the maximally nested matrix; [Atmar and Patterson 1993]) and its significance, and site rank order (ranked by species richness) (Rodríguez-Gironés and Santamaría 2006). First, we reordered the taxa presence/absence matrix to be maximally nested by ordering taxa from most to least frequent occurrence (as columns), and disturbance treatments from most to least species rich (as rows). Next, we assessed if treatments were nested by calculating matrix temperature of the taxa presence/absence matrix and assessed the probability that the observed matrix temperature would occur by random chance with NULL model 3 (this null model follows the recommendations of Bascompte et al. [2003] reported in Rodrigues-Girones and Santamaria [2006]) and 9999 permutations (Rodríguez-Gironés and Santamaría 2006). Last, we used the nested rank order of sites in the maximally nested matrix to investigate if Nest was related to disturbance intensity (similar to Lepori and Malmqvist 2009).

To test condition 3 (organisms will be filtered from the regional species pool based on the traits they possess in relation to disturbance intensity), we acquired macroinvertebrate trait information for the continental US (Vieira et al. 2006), summed trait values for all taxa in a genus, and then divided by the total for a given trait (Chevenet et al. 1994). For example, occurrence in the drift has 3 modalities (rare, common, and abundant), so we summed all values for the taxon in a genus for each modality and then divided these values by the total of all modalities. We summarized trait and taxon relative abundances by calculating mean trait values (mT; [Garnier et al. 2004]). Our experimental manipulation was designed to mimic hydrologic disturbance, so we chose feeding, locomotion, morphology, and dispersal traits, each of which is putatively related to streambed disturbance of benthic habitats. Specifically, we chose functional feeding group (Sefick et al. unpublished data and Burcher et al. [2007]), occurrence in the drift, larval dispersal, body armoring, shape, size, and habit (Stazner and Beche [2010]; Table 1). To investigate the relationship between treatment and mTs, we used a partial redundancy analysis (pRDA; similar to Kleyer et al. [2012]), which controlled for the effect of block and median velocity (i.e., partial out the effect) on mT and thus maintained consistency among analyses (rda function; R package vegan [Oksanen et al. 2007]). We tested the significance of the pRDA, and then sequentially tested the axes for significance with a permutation procedure (9999 permutations) constrained by block to incorporate the experimental design into the randomization (see Spackova et al. [1998] and Legendre et al. [2011]). Last, we tested differences in mean rank along pRDA 1 among treatments with an ANOVA because standard transformations (e.g., square root) were unable to make the unranked data’s residual errors satisfy test assumptions (assessed with residual plots), and followed by a planned mean comparison test, similar to β-div and Nest. For all analyses, alpha was set at 0.05, and unless otherwise indicated were coded in the R language for statistical computing (Ihaka and Gentleman 1996, R Core Team 2015).

RESULTS

Experimental conditions

Stream velocity and depth variation among enclosures over the experiment were high (velocity range 0 to 0.9 m/s; median 0.15 m/s and maximum increase in depth of 0.32 m), mostly from one storm event that showed a marked increase above median stage for ~5 d (~11/26-11/30; Fig. A2). This event did result in moderate substrate turnover within enclosures, but appeared similar among blocks (Sefick, personal observations). There were no differences in combined coarse and fine size fractions of OM means among treatments (*P*=0.25; results for separate size fractions showed no trends, and omitted for brevity).

Taxonomic Characterization and Disturbance effects on nestedness and β-diversity

We identified 6069 individuals representing 85 taxa and 39 families of macroinvertebrates. Of the macroinvertebrates identified, 66% of specimens were in the order Diptera, with most of those in the family Chironomidae (55% of total macroinvertebrates). Mollusks (bivalves and snails) composed 10% of the total whereas Ephemeroptera, Plecoptera, and Trichoptera taxa composed 9, 6, and 6% of the total, respectively.

Macroinvertebrate assemblages were significantly nested (matrix temperature=25.13; *P*<0.0001; FIG. A3). Disturbance intensity had a significant effect on Nest (*P*≤0.0244), with mean rank being significantly different (*P*<0.0305) and lower (less nested) than **S** for all treatments except **3,** which did not differ from **S** (TABLE 2; FIG. 2A). β-div significantly differed with disturbance intensity (*P*≤0.0029), which was higher than **S** (*P*≤0.0035) for all treatments except **3** (TABLE 2; FIG. 2B).

Disturbance effects on macroinvertebrate traits

Because there was a multivariate outlier, we square-root transformed mT data to down-weight extreme values before the final pRDA was done (Appendix A: Fig. A1). We chose to down-weight and not remove this observation because it was only an outlier in the mT analysis. The overall pRDA ordination configuration was significant (*P*=0.0246), and only pRDA axis 1 was significant (*P*=0.0001). As a result we only used this axis in subsequent analyses. There was a significant difference in mean rank along pRDA 1 among disturbance treatments (62% of the constrained variance, *P*=0.000016), with mean rank being higher than **S** for all treatments except **3** (TABLE 2; FIG. 3A). Mean macroinvertebrate trait loadings on pRDA 1 showed contrasting suites of traits characterizing **S** and **3** when compared to **0**, **1**, **2**, and **4** (FIG. 3B). Specifically, taxa that were shredders, burrowers, and climbers and were dorsoventrally flattened, characterized **S** and treatment **3**, whereas, taxa that were predators, sprawlers, filterers, and clingers, and often occurred in the drift, characterized treatments **0**, **1**, **2**, and **4**. Interestingly, treatment **0** was intermediate in mT space between **S** and **3** and **1**, **2**, and **4**.

DISCUSSION

Our study has shown that physical disturbance, as mimicked in our field experiment, strongly influenced community assembly by acting as a filter on which macroinvertebrate taxa and traits varied. The 2 conditions required to show that communities were deterministically and not neutrally assembled, conditions 1 and 2, based on niche assembly (Chase 2007), were observed in this experiment: 1) β-div was lower in **S** and in the moderate-high disturbance treatment (**3**), and 2) macroinvertebrate assemblages in **S** and **3** were nested subsets of the species pool. In addition to fulfilling the conditions for deterministic assembly, we found that the traits in **S** and **3** assemblages were similar and strongly divergent from all other treatments, supporting condition 3. This study represents the first experimental demonstration of deterministic sorting of macroinvertebrate traits within a stream metacommunity.

As this was a field experiment, there are necessary tradeoffs between realism and experimental control. First, one such uncontrolled variable was the storm event that occurred during the experimental period. This event likely did not have differential effects on enclosures because observations in the field did not suggest that there were any appreciable differences in organic matter accumulation or sediment composition among enclosures. In addition, our results for organic matter suggest no differences in accumulated organic matter. Second, we accounted for block heterogeneity by including both block and velocity as random variables in our models effectively adjusting mean effect by environmental heterogeneity at the block scale. In addition, we statistically controlled for the effect of these variables in the pRDA analysis. Last, our disturbance treatments successfully mimicked a hydrologic disturbance intensity gradient. We conclude this because there were large differences in β-div, Nest, and mT among **S** and **3** and **2** and **4** with only small decrease/increases in disturbance intensity (see FIG. 2 and 3). We believe that these potential confounding effects do not interfere with interpretation of our experiment..

Our results suggest that disturbance acts as a strong filter on traits, and that species sorting was acting to assemble communities at moderate-high disturbances similar to Lepori and Malmqvist (2009), and in contrast to other studies (e.g., Chase 2007, Jiang and Patel 2008, Milner et al. 2011). In contrast to Lepori and Malmqvist (2009) which did not control for γ-div due to logistic constraints, this study controlled for γ-div, i.e., utilizing a single stream, differences which have been shown to affect β-div, and need to be accounted for in studies of community assembly (Kraft et al. 2011). Thus, the inferences drawn from this study are strong with regards to community assembly mechanisms. These results were concordant with other studies finding that sorting is the most often occurring community assembly mechanism in streams (Campbell et al. 2015, Heino et al. 2015). In a meta-analysis of terrestrial and aquatic ecosystems, Cottenie (2005) reported that species sorting was the dominant mechanism assembling communities in aquatic and terrestrial ecosystems. The pattern observed in our study was not likely due to mass effects, a related mechanism where dispersal overcomes local species sorting (reviewed in Logue et al. 2011), because our experimental design limited spatial differences between enclosures, and this mechanism would have resulted in communities having higher β-div in **S** and **3** because dispersal would have overcome the effects of species sorting (Souffreau et al. 2014). As **S** and **3** showed similar β-div and putatively were assembled in the same manner, we infer that the stream bed naturally experienced disturbance at a level similar to our moderate-high disturbance treatment (**3**).

In contrast to moderate-high disturbance treatments (i.e., **3**), both low and high levels of disturbance showed: 1) higher β-div, 2) lower nestedness, and 3) a higher number of mT values, suggesting that these treatments were assembled stochastically. The most parsimonious explanation for this pattern was that low/high disturbances were being assembled in a neutral manner with only ecological drift resulting in observed patterns (sensu Hubell [2005]).

Although our study was not designed to investigate biotic interactions,the higher prevalence of predators, as evidenced by a high loading on pRDA axis 1 in the low/high disturbance direction, could be related to developing biotic interactions, but we cannot separate this potential mechanism from neutral assembly. Other studies directly investigating predator relationships with community assembly have found that β-div increases with increased predation (Chase et al. 2009, Stier et al. 2013, Stier et al. 2014). In our study, a possible mechanism could be the indiscriminant predation by benthic macroinvertebrate predators. However, the Harsh-

Benign hypothesis for stream ecosystems (Peckarsky et al. 1990) would suggest that under low disturbance (**0**, **1**, and **2**) biotic interactions are more important and predation is driving the stochastic assembly, and at higher levels of disturbance (**4**) disturbance is driving stochastic assembly with predator occurrence being driven by priority effects. More research would have to be undertaken to investigate the interaction of predators and disturbance on community assembly in stream ecosystems.

It is of note that treatment **0**, albeit statistically different from **S**, was intermediate in mT space between the low and high disturbances and **S** and **3**. This pattern would suggest that **0** had traits representing a mix of both deterministically assembled (**S** and **3**) and stochastically assembled (**1**, **2**, and **4**) treatments, which may suggest that some taxa are being sorted based on their traits while others are colonizing this low level of disturbance at random. Treatment **0,** likely represented a high-flow refugium for macroinvertebrate colonizers because of lower velocity and low manipulated disturbance at the enclosure scale, which, if true, produced a lower level of disturbance than **S** and **3**. Trait data for treatment **0** was intermediate to all other treatments on pRDA 1, suggesting that the assemblage had traits that were a subset of all other treatments.

The large differences in response between treatments **3** and **2** and **3** and **4** were surprising as disturbance intensity in **2** and **4** was only slightly lower and higher, respectively, than **3**, suggesting a non-linear or stepped response to disturbance. A recent study showed that, compared to streams with minimal anthropogenic disturbance, disturbed streams showed higher β-div (Hawkins et al. 2015). Our investigation suggests streams with minimal anthropogenic disturbance were similar to our **S** and **3,** and stochastic assembly was responsible for the increase in β-div with increasing disturbance observed in Hawkins et al. (2015). Other studies in ponds have, also, shown an increase in β-div with regards to increasing disturbance with one potential explanation being stochastic colonization and priority effects (Vanschoenwinkel et al. 2013). This explanation may apply to our study in the sense that predators arrived first, and are a result the stochastic assembly process in **4**.

Although treatments **S** and **3** were statistically indistinguishable in terms of Nest, β-div, and mT, there were important biotic differences. With regards to mT values, shredders were highly loaded in the **S** and **3** direction (i.e., strong positive loadings on Fig. 3B), but **S** scores were more highly separated from all of the other treatments than the scores for **3** (Fig. 3B). The conditions of the stream bed and the enclosures were not exactly the same and so did not filter the exact same assemblages from the regional species pool. However, despite these differences, **S** and **3** were more similar to each other than all of the other treatments with regards to β-div, Nest, and mT composition. This was likely a result of both **S** and **3** experiencing a similar intensity of disturbance (moderate-high). We infer from this that a moderate-high disturbance showed more deterministic sorting than either high or low disturbances. For stream specific studies, this result is in contrast to Milner et al. (2011), and concordant with Lepori and Malmqvist's (2009) investigation, but with stronger experimental control over possible confounding variables (i.e., γ-div, spatial structure, etc.). In addition, our results suggest that specific traits are responsible for deterministic assembly under moderate-high disturbance in sand-bed streams.

Conclusion

Our study suggests that stream assemblages were deterministically assembled at moderate-high disturbance from the regional species pool based on the traits that they possess, and that Chase's (2007) hypothesis should be amended to included low as well as high levels of disturbance resulting in stochastic assembly in streams. Our study suggests that stochastic assembly at low levels of disturbance may be related to biotic agents of stochastic assembly while higher levels of stochastic assembly are dominated by abiotic processes. We suggest that future studies investigate a broader range of disturbances to determine community assembly responses at higher levels of disturbance, and specifically investigate the interaction of biotic interactions with physical disturbance as they relate to community assembly. In addition, other system types, including upland cobble bottom streams (as well as non-stream ecosystems) should be investigated in order to ascertain whether deterministic assembly at moderate-high levels of disturbance is a general pattern for system, disturbance, and community type.

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LITERATURE CITED

Anderson, M. J., T. O. Crist, J. M. Chase, M. Vellend, B. D. Inouye, A. L. Freestone, N. J. Sanders, H. V. Cornell, L. S. Comita, K. F. Davies, S. P. Harrison, N. J. B. Kraft, J. C. Stegen, and N. G. Swenson. 2011. Navigating the multiple meanings of β diversity: a roadmap for the practicing ecologist. Ecology letters **14**:19-28.

Anderson, M. J., K. E. Ellingsen, and B. H. McArdle. 2006. Multivariate dispersion as a measure of beta diversity. Ecology letters **9**:683-693.

Atmar, W. and B. D. Patterson. 1993. The measure of order and disorder in the distribution of species in fragmented habitat. Oecologia **96**:373-382.

Bascompte, J., P. Jordano, C. J. Melian, and J. M. Olesen. 2003. The nested assembly of plant-animal mutualistic networks. Proceedings of the National Academy of Sciences **100**:9383-9387.

Bates, D., M. Machler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software **67**:1-48.

Belyea, L. R. and J. Lancaster. 1999. Assembly rules within a contingent ecology. Oikos:402-416.

Brigham, A. R., W. U. Brigham, and A. Gnilka. 1982. Aquatic insects and oligochaetes of North and South Carolina. Midwest Aquatic Enterprises, Illinois.

Brown, B. L. and C. M. Swan. 2010. Dendritic network structure constrains metacommunity properties in riverine ecosystems. Journal of Animal Ecology **79**:571-580.

Bunn, S. E. and A. H. Arthington. 2002. Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. Environmental Management **30**:492-507.

Burcher, C. L., H. M. Valett, and E. F. Benfield. 2007. The land-cover cascade: relationships coupling land and water. Ecology **88**:228-242.

Campbell, R. E., M. J. Winterbourn, T. A. Cochrane, and A. R. McIntosh. 2015. Flow-related disturbance creates a gradient of metacommunity types within stream networks. Landscape Ecology **30**:667-680.

Chase, J. M. 2007. Drought mediates the importance of stochastic community assembly. Proceedings of the National Academy of Sciences **104**:17430-17434.

Chase, J. M., E. G. Biro, W. A. Ryberg, and K. G. Smith. 2009. Predators temper the relative importance of stochastic processes in the assembly of prey metacommunities. Ecology letters **12**:1210-1218.

Chase, J. M., N. J. B. Kraft, K. G. Smith, M. Vellend, and B. D. Inouye. 2011. Using null models to disentangle variation in community dissimilarity from variation in β-diversity. Ecosphere **2**:art24.

Chase, J. M. and J. A. Myers. 2011. Disentangling the importance of ecological niches from stochastic processes across scales. Philosophical transactions of the Royal Society B: Biological sciences **366**:2351-2363.

Chevenet, F., S. Doledec, and D. Chessel. 1994. A fuzzy coding approach for the analysis of long-term ecological data. Freshwater Biology **31**:295-309.

Cottenie, K. 2005. Integrating environmental and spatial processes in ecological community dynamics. Ecology letters **8**:1175-1182.

Cuffney, T. F., M. D. Bilger, and A. M. Haigler. 2007. Ambiguous taxa: effects on the characterization and interpretation of invertebrate assemblages. Journal of the North American Benthological Society **26**:286-307.

de Bello, F., J. N. Price, T. Munkemuller, J. Liira, M. Zobel, W. Thuiller, P. Gerhold, L. Gotzenberger, S. Lavergne, and J. Leps. 2012. Functional species pool framework to test for biotic effects on community assembly. Ecology **93**:2263-2273.

Doledec, S., N. Lamouroux, U. Fuchs, and S. Merigoux. 2006. Modelling the hydraulic preferences of benthic macroinvertebrates in small European streams. Freshwater Biology **52**:145-164.

Epler, J. 2010. The water beetles of Florida, an identification manual for the families Chrysomelidae. Curculionidae, Dryopidae, Elmidae, Gyrinidae, Haliplidae, Helophoridae, Hydraenidae, Hydrochidae, Hydrophilidae, Noteridae, Psephenidae, Ptilodactylidae and Scirtidae. Florida Department of Environmental Protection, Tallahassee, Florida. (Avaliable from: http://www.floridadep.org/labs/cgi-bin/sbio/keys.asp).

Epler, J. H. 2001. Identification Manual for the larval Chironomidae (Diptera) of North and South Carolina. A guide to the taxonomy of the midges of the southeastern United States including Florida. Special Publication SJ2001-SP13, North Carolina Department of Environmental and Natural Resources, Raleigh, NC, and St. Johns River Water Managment district, Palatka, FL. (Avaliable from: http://www.floridadep.org/labs/cgi-bin/sbio/keys.asp).

Feminella, J. W. 1996. Comparison of benthic macroinvertebrate assemblages in small streams along a gradient of flow permanence. Journal of the North American Benthological Society **15**:651-669.

Feminella, J. W. and V. H. Resh. 1990. Hydrologic influences, disturbance, and intraspecific competition in a stream caddisfly population. Ecology:2083-2094.

Garnier, E., J. Cortez, G. Billes, M.-L. Navas, C. Roumet, M. Debussche, G. Laurent, A. Blanchard, D. Aubry, A. Bellmann, C. Neill, and J.-P. Toussaint. 2004. Plant functional markers capture ecosystem properties during secondary succession. Ecology **85**:2630-2637.

Guo, H., K. Wieski, Z. Lan, and S. C. Pennings. 2014. Relative influence of deterministic processes on structuring marsh plant communities varies across an abiotic gradient. Oikos **123**:173-178.

Hart, D. D. and C. M. Finelli. 1999. Physical-biological coupling in streams: the pervasive effects of flow on benthic organisms. Annual Review of Ecology and Systematics **30**:363-395.

Hawkins, C. P., H. Mykra, J. Oksanen, and J. J. Vander Laan. 2015. Environmental disturbance can increase beta diversity of stream macroinvertebrate assemblages. Global Ecology and Biogeography **24**:483-494.

Heino, J., A. S. Melo, T. Siqueira, J. Soininen, S. Valanko, and L. M. Bini. 2015. Metacommunity organisation, spatial extent and dispersal in aquatic systems: patterns, processes and prospects. Freshwater Biology **60**:845-869.

Holm, S. 1979. A simple sequentially rejective multiple test procedure. Scandinavian journal of statistics:65-70.

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models.

Hubbell, S. P. 2005. Neutral theory in community ecology and the hypothesis of functional equivalence. Functional ecology **19**:166-172.

Hutchinson, G. E. 1957. Concluding remarks. Cold Spring Harbor Symposium on Quantitative Biology **22**:415-427.

Ihaka, R. and R. Gentleman. 1996. R: A language for data analysis and graphics. Journal of Computational and Graphical Statistics **5**:299-314.

Jiang, L. and S. N. Patel. 2008. Community assembly in the presence of disturbance: a microcosm experiment. Ecology **89**:1931-1940.

Keddy, P. A. 1992. Assembly and response rules: two goals for predictive community ecology. Journal of Vegetation Science **3**:157-164.

Kleyer, M., S. p. Dray, F. Bello, J. LepÅ¡, R. J. Pakeman, B. Strauss, W. Thuiller, and S. Lavorel. 2012. Assessing species and community functional responses to environmental gradients: which multivariate methods? Journal of Vegetation Science **23**:805-821.

Kowalyk, H. E. 1985. The larval cephalic setae in the Tanypodinae (Diptera: Chironomidae) and their importance in generic determinations. The Canadian Entomologist **117**:67-106.

Kraft, N. J., L. S. Comita, J. M. Chase, N. J. Sanders, N. G. Swenson, T. O. Crist, J. C. Stegen, M. Vellend, B. Boyle, and M. J. Anderson. 2011. Disentangling the drivers of β-diversity along latitudinal and elevational gradients. Science **333**:1755-1758.

Lake, P. S. 2000. Disturbance, patchiness, and diversity in streams. Journal of the North American Benthological Society **19**:573-592.

Lamouroux, N., N. L. Poff, and P. L. Angermeier. 2002. Intercontinental convergence of stream fish community traits along geomorphic and hydraulic gradients. Ecology **83**:1792-1807.

Legendre, P., J. Oksanen, and C. J. ter Braak. 2011. Testing the significance of canonical axes in redundancy analysis. Methods in Ecology and Evolution **2**:269-277.

Leibold, M. A., M. Holyoak, N. Mouquet, P. Amarasekare, J. M. Chase, M. F. Hoopes, R. D. Holt, J. B. Shurin, R. Law, D. Tilman, M. Loreau, and A. Gonzalez. 2004. The metacommunity concept: a framework for multi-scale community ecology. Ecology letters **7**:601-613.

Lepori, F. and B. Malmqvist. 2009. Deterministic control on community assembly peaks at intermediate levels of disturbance. Oikos **118**:471-479.

Logue, J. r. B., N. Mouquet, H. Peter, H. Hillebrand, and M. W. Group. 2011. Empirical approaches to metacommunities: a review and comparison with theory. Trends in Ecology & Evolution **26**:482-491.

Longnecker, M. and R. Ott. 2001. An introduction to statistical methods and data analysis. ISBN-13 **854576151**.

McCabe, D. J. and N. J. Gotelli. 2000. Effects of disturbance frequency, intensity, and area on assemblages of stream macroinvertebrates. Oecologia **124**:270-279.

Menge, B. A. and J. P. Sutherland. 1987. Community regulation: variation in disturbance, competition, and predation in relation to environmental stress and recruitment. American Naturalist:730-757.

Merritt, R. W., K. W. Cummins, and M. B. Berg. 2008. An Introduction to the Aquatic Insects of North America. 4th edition. Kendall Hunt Publishing, Dubuque, Iowa.

Milner, A. M., A. L. Robertson, L. E. Brown, S. H. SÃ¸nderland, M. McDermott, and A. J. Veal. 2011. Evolution of a stream ecosystem in recently deglaciated terrain. Ecology **92**:1924-1935.

Myers, J. A., J. M. Chase, R. M. Crandall, and I. n. JimÃ©nez. 2015. Disturbance alters beta-diversity but not the relative importance of community assembly mechanisms. Journal of Ecology **103**:1291-1299.

Myers, J. A. and K. E. Harms. 2011. Seed arrival and ecological filters interact to assemble high-diversity plant communities. Ecology **92**:676-686.

Oksanen, J., R. Kindt, P. Legendre, B. O'Hara, M. H. H. Stevens, M. J. Oksanen, and M. Suggests. 2007. The vegan package. community ecology package version 1.17-10. (http://cran.r-project.org).

Peckarsky, B. L., S. C. Horn, and B. Statzner. 1990. Stonefly predation along a hydraulic gradient: a field test of the harsh-benign hypothesis. Freshwater Biology **24**:181-191.

Poff, N. L. 1997. Landscape filters and species traits: Towards mechanistic understanding and prediction in stream ecology. Journal of the North American Benthological Society **16**:391-409.

Poff, N. L., J. D. Olden, N. K. M. Vieira, D. S. Finn, M. P. Simmons, and B. C. Kondratieff. 2006. Functional trait niches of North American lotic insects: traits-based ecological applications in light of phylogenetic relationships. Journal of the North American Benthological Society **25**:730-755.

Power, M. E., A. Sun, G. Parker, W. E. Dietrich, and J. T. Wootton. 1995. Hydraulic food-chain models. BioScience **45**:159-167.

R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Resh, V. H., A. V. Brown, A. P. Covich, M. E. Gurtz, H. W. Li, G. W. Minshall, S. R. Reice, A. L. Sheldon, J. B. Wallace, and R. C. Wissmar. 1988. The role of disturbance in stream ecology. Journal of the North American Benthological Society **7**:433-455.

Rodríguez-Gironés, M. A. and L. Santamaría. 2006. A new algorithm to calculate the nestedness temperature of presence–absence matrices. Journal of Biogeography **33**:924-935.

Sala, O. E., F. S. Chapin III, J. J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L. F. Huenneke, R. B. Jackson, A. Kinzig, R. Leemans, D. M. Lodge, H. A. Mooney, M. Oesterheld, N. L. Poff, M. T. Sykes, B. H. Walker, M. Walker, and D. H. Wall. 2000. Biodiversity - global biodiversity scenarios for the year 2100. Science **287**:1770-1774.

Shipley, B., D. Vile, and E. Garnier. 2006. From plant traits to plant communities: a statistical mechanistic approach to biodiversity. Science **314**:812-814.

Singer, E. E. and M. M. Gangloff. 2011. Effects of a small dam on freshwater mussel growth in an Alabama (USA) stream. Freshwater Biology **56**:1904-1915.

Soar, P. J. and C. R. Thorne. 2001. Channel restoration design for meandering rivers. DTIC Document.

Souffreau, C., B. Pecceu, C. Denis, K. Rummens, and L. De Meester. 2014. An experimental analysis of species sorting and mass effects in freshwater bacterioplankton. Freshwater Biology **59**:2081-2095.

Spackova, I., I. Kotorova, and J. Leps. 1998. Sensitivity of seedling recruitment to moss, litter and dominant removal in an oligotrophic wet meadow. Folia Geobotanica **33**:17-30.

Statzner, B. and L. A. Beche. 2010. Can biological invertebrate traits resolve effects of multiple stressors on running water ecosystems? Freshwater Biology **55**:80-119.

Stier, A. C., S. W. Geange, K. M. Hanson, and B. M. Bolker. 2013. Predator density and timing of arrival affect reef fish community assembly. Ecology **94**:1057-1068.

Stier, A. C., K. M. Hanson, S. J. Holbrook, R. J. Schmitt, and A. J. Brooks. 2014. Predation and landscape characteristics independently affect reef fish community organization. Ecology **95**:1294-1307.

Thompson, R. and C. Townsend. 2006. A truce with neutral theory: local deterministic factors, species traits and dispersal limitation together determine patterns of diversity in stream invertebrates. Journal of Animal Ecology **75**:476-484.

Thorp, J. H. and A. P. Covich. 2009. Ecology and classification of North American freshwater invertebrates. 3rd edition. Academic Press, Burlington, MA.

Tonkin, J. D. and R. G. Death. 2013. Scale dependent effects of productivity and disturbance on diversity in streams. Fundamental and Applied Limnology/Archiv fur Hydrobiologie **182**:283-295.

Townsend, C. R., S. Doledec, and M. R. Scarsbrook. 1997. Species traits in relation to temporal and spatial heterogeneity in streams: a test of habitat templet theory. Freshwater Biology **37**:367-387.

Urban, M. C. 2004. Disturbance heterogeneity determines freshwater metacommunity structure. Ecology **85**:2971-2978.

Vanschoenwinkel, B., F. Buschke, and L. Brendonck. 2013. Disturbance regime alters the impact of dispersal on alpha and beta diversity in a natural metacommunity. Ecology **94**:2547-2557.

Vellend, M., K. Verheyen, K. M. Flinn, H. Jacquemyn, A. Kolb, H. Van Calster, G. Peterken, B. J. Graae, J. Bellemare, and O. Honnay. 2007. Homogenization of forest plant communities and weakening of species-environment relationships via agricultural land use. Journal of Ecology **95**:565-573.

Vieira, N. M. K., N. L. M. Poff, D. M. Carlisle, S. R. Moulton II, M. L. Koski, and B. C. Kondratieff. 2006. A database of lotic invertebrate traits for North America. U.S. Geological Survey.

Whittaker, R. H. 1972. Evolution and measurement of species diversity. Taxon:213-251.

Yamamuro, A. M. and G. A. Lamberti. 2007. Influence of organic matter on invertebrate colonization of sand substrata in a northern Michigan stream. Journal of the North American Benthological Society **26**:244-252.

TABLE 1. Benthic macroinvertebrate traits, trait modalities, and abbreviations used in the analysis (see Poff et al. [2006]).

Trait	Trait modality	Abbreviation
Trophic		
Feeding Habit	Collector-Filterer	ffg1
	Collector-Gatherer	ffg2
	Predator	ffg4
	Scraper/Grazer	ffg5
	Shredder	ffg6
Locomotion		
Habit	Burrower	hab1
	Climber	hab2
	Clinger	hab3
	Sprawler	hab4
	Swimmer	hab5
Morphology		
Size	< 9mm	siz1
	9-16 mm	siz2
	> 16 mm	siz3
Shape	Bluff/Blocky	shp1
	Round/Humped	shp2
	Tubular	shp3
	Streamlined/Fusiform	shp4
	Dorsoventrally Flattened	shp5
Armoring	Soft	arm1
	Partly Sclerotized	arm2
	Hard Shelled	arm3
	All Sclerotized	arm4
Dispersal		
Occurrence in drift	Rare	drf1
	Common	drf2
	Abundant	drf3
Larval dispersal	< 1 m	lds1
	1-10 m	lds2
	11-100 m	lds3

TABLE 2. Mixed-effects, ANOVA, and posthoc test results for β-div, Nest, and mT.

		Comparison	**Estimate**	**SE**	***z***	***P***
	Likelihood Ratio Test (*P*)					
β	0.002924	S-0	-0.2548	0.0686	-3.7140	**0.00102**
		S-1	-0.2235	0.0669	-3.3400	**0.00335**
		S-2	-0.2159	0.0675	-3.1980	**0.00354**
		S-3	-0.1048	0.0673	-1.5580	0.11919
		S-4	-0.2201	0.0678	-3.2440	**0.00354**
Nest	0.024380	S-0	15.1500	5.5670	2.7210	**0.02580**
		S-1	16.6800	5.5150	3.0250	**0.01240**
		S-2	15.0940	5.5410	2.7240	**0.02580**
		S-3	9.0720	5.5270	1.6410	0.10070
		S-4	13.4650	5.5480	2.4270	**0.03050**
	ANOVA (*P*)					
Trait	0.000016	S-0	12.3330	4.0790	3.0240	**0.01015**
		S-1	22.6670	4.0790	5.5570	**0.00002**
		S-2	19.1670	4.0790	4.6990	**0.00016**
		S-3	8.0000	4.0790	1.9610	0.05918
		S-4	21.8330	4.0790	5.3530	**0.00003**

Notes:SE is the standard error of the Estimate, z is the z-statistic for multiple comparisons.

Bolded P-values represent significance at α=0.05. See text for other abbreviations.

FIG. 1. A) Schematic showing design of artificial disturbance experiment in Sandy Creek, near Waverly, Alabama. Numbers above grouping of 5 treatments and streambed control represent treatment blocks, black squares represent individual experimental enclosures, and numbers inside squares are the disturbance treatments, and refer to the number of revolutions applied to the substrate by the paint mixer (stream-bed control (0 turns; **S**), (0 turns; **0**), low intensity (1 turn; **1**), moderate-low intensity (2 turns; **2**), moderate-high intensity (3 turns; **3**), and high intensity (4 turns; **4**)). **B)** The study stream location (Sandy Creek) near Waverly, Alabama (inset). **C)** An empty EE with disturbance device inside.

FIG. 2. Nested rank order (**A**) and β-div (**B**) compared among treatments. * represents least squares means that are significantly different ($P \leq 0.031$) from **S**. Means (●) plotted with 95% confidence intervals (bar).

FIG. 3. Partial pRDA scores (A) and mT loadings (B). A) are unranked scores in order to allow comparison with mT loadings (B), ordered in terms of decreasing absolute loading score. * represents ranked treatment means that are significantly different from **S**. Trait abbreviations defined in TABLE 1. A and B are on the same scale and are scores from significant pRDA axis 1 (see text for detail).

FIGURE 1

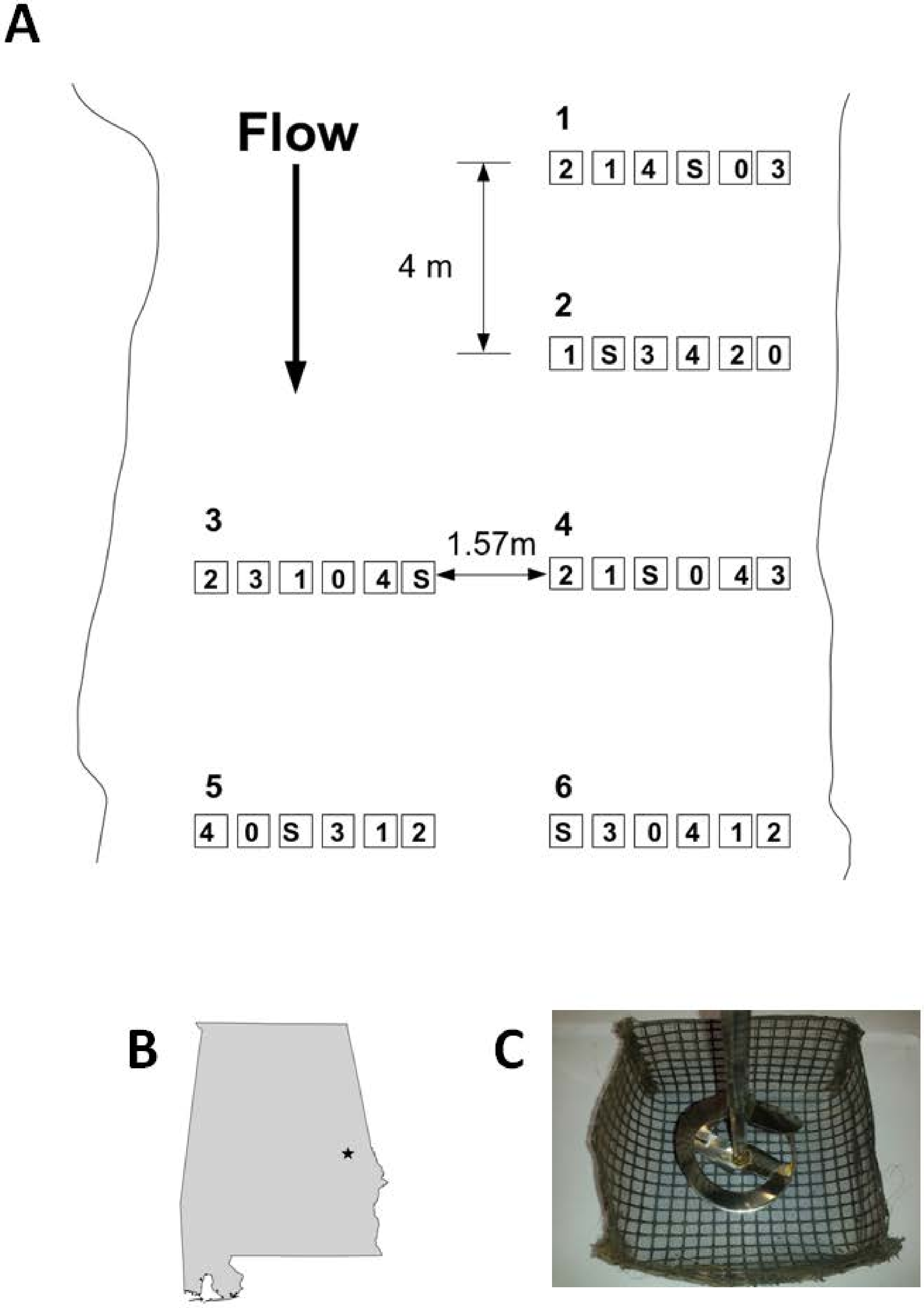

A
Flow
1
2 1 4 S 0 3
4 m
2
1 S 3 4 2 0
3
2 3 1 0 4 S
1.57m
4
2 1 S 0 4 3
5
4 0 S 3 1 2
6
S 3 0 4 1 2
B
C

FIGURE 2

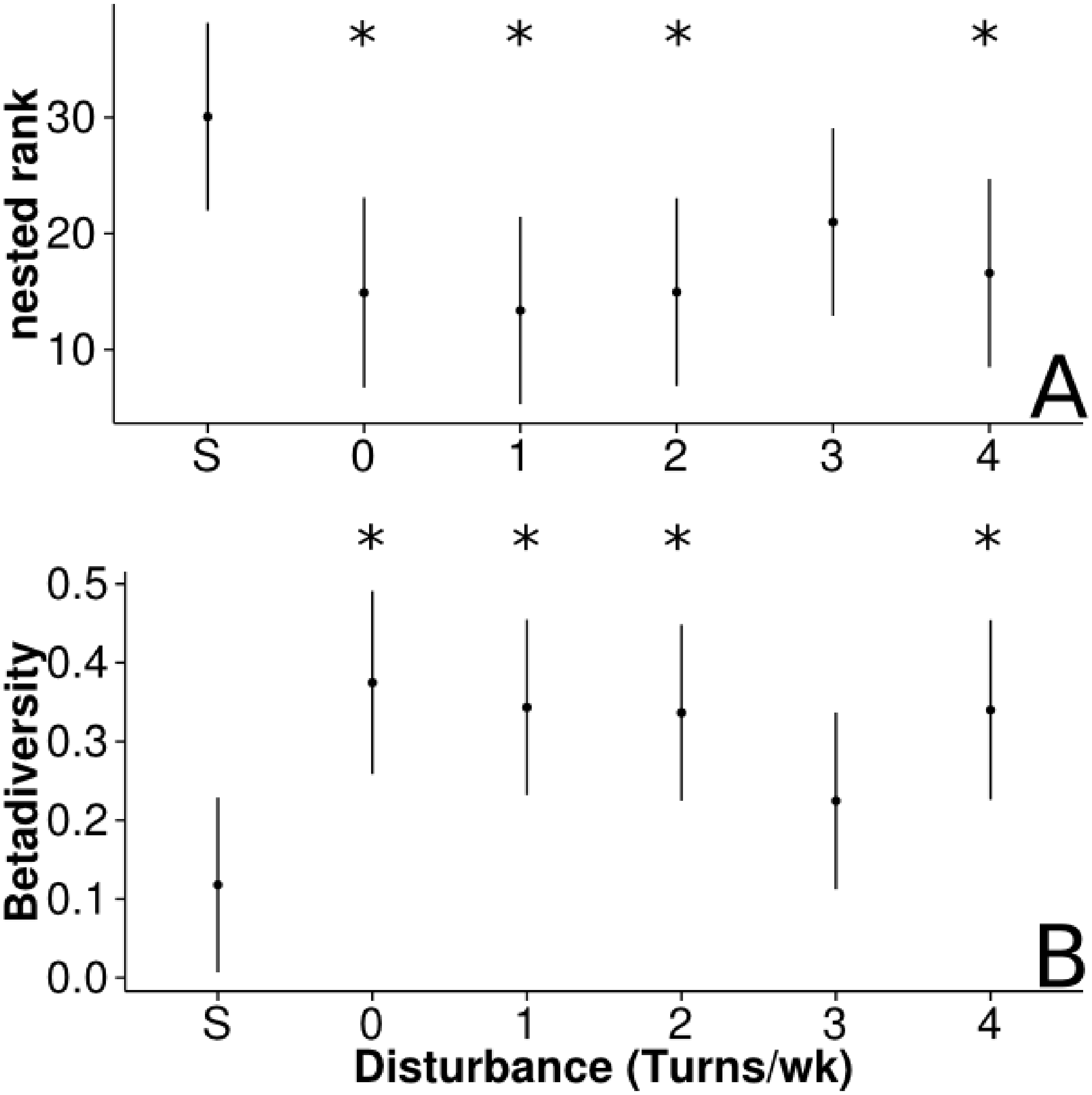
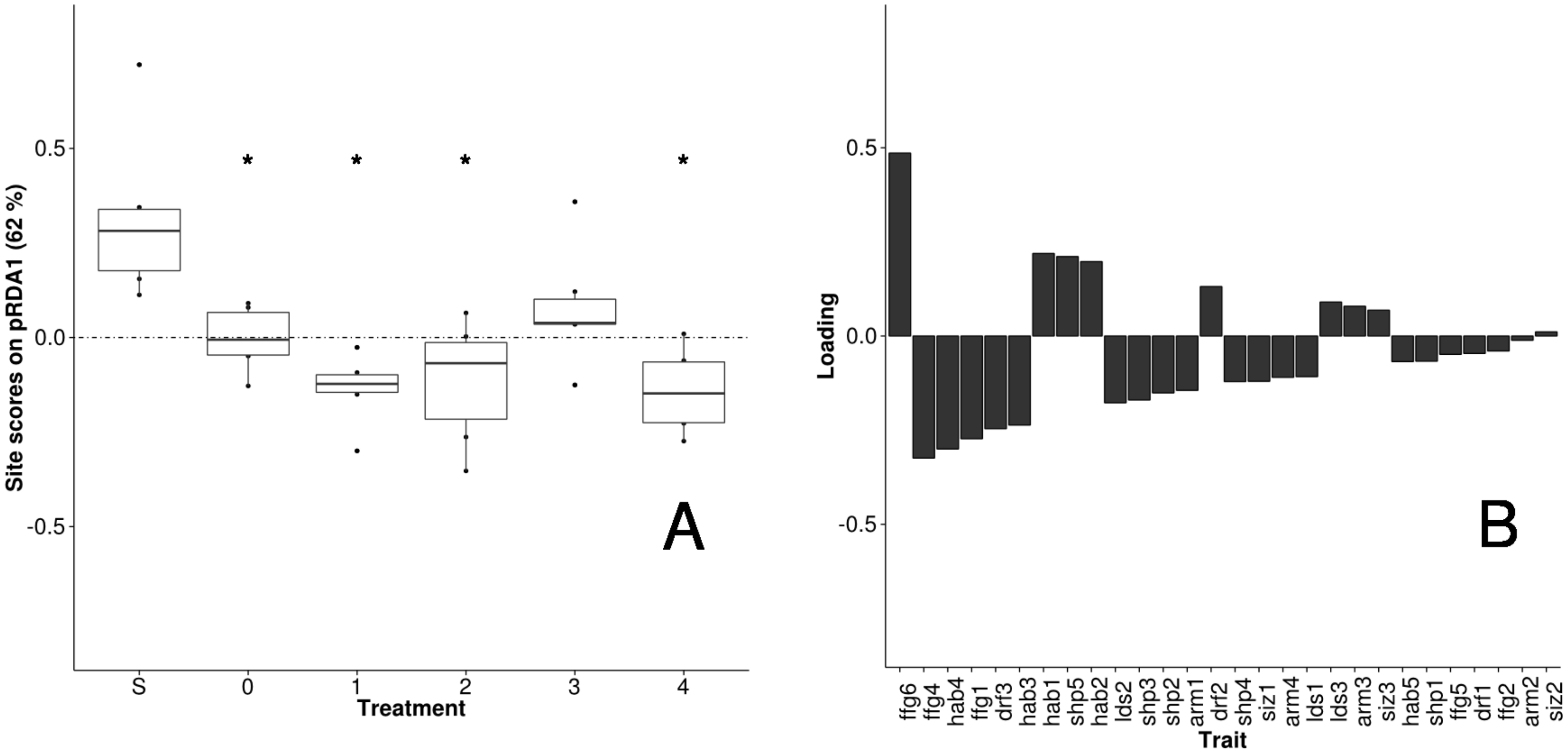

nested rank
30
20
10
A
S
0
1
2
3
4
*
*
*
*
Betadiversity
0.5
0.4
0.3
0.2
0.1
0.0
B
Disturbance (Turns/wk)

FIGURE 3



Site scores on pRDA1 (62 %)
0.5
0.0
-0.5
*
*
*
*
S
0
1
2
3
4
Treatment
A
Loading
0.5
0.0
-0.5
ffg6
ffg4
hab4
ffg1
drf3
hab3
hab1
shp5
hab2
lds2
shp3
shp2
arm1
drf2
shp4
siz1
arm4
lds1
lds3
arm3
siz3
hab5
shp1
ffg5
drf1
ffg2
arm2
siz2
Trait
B

APPENDIX A

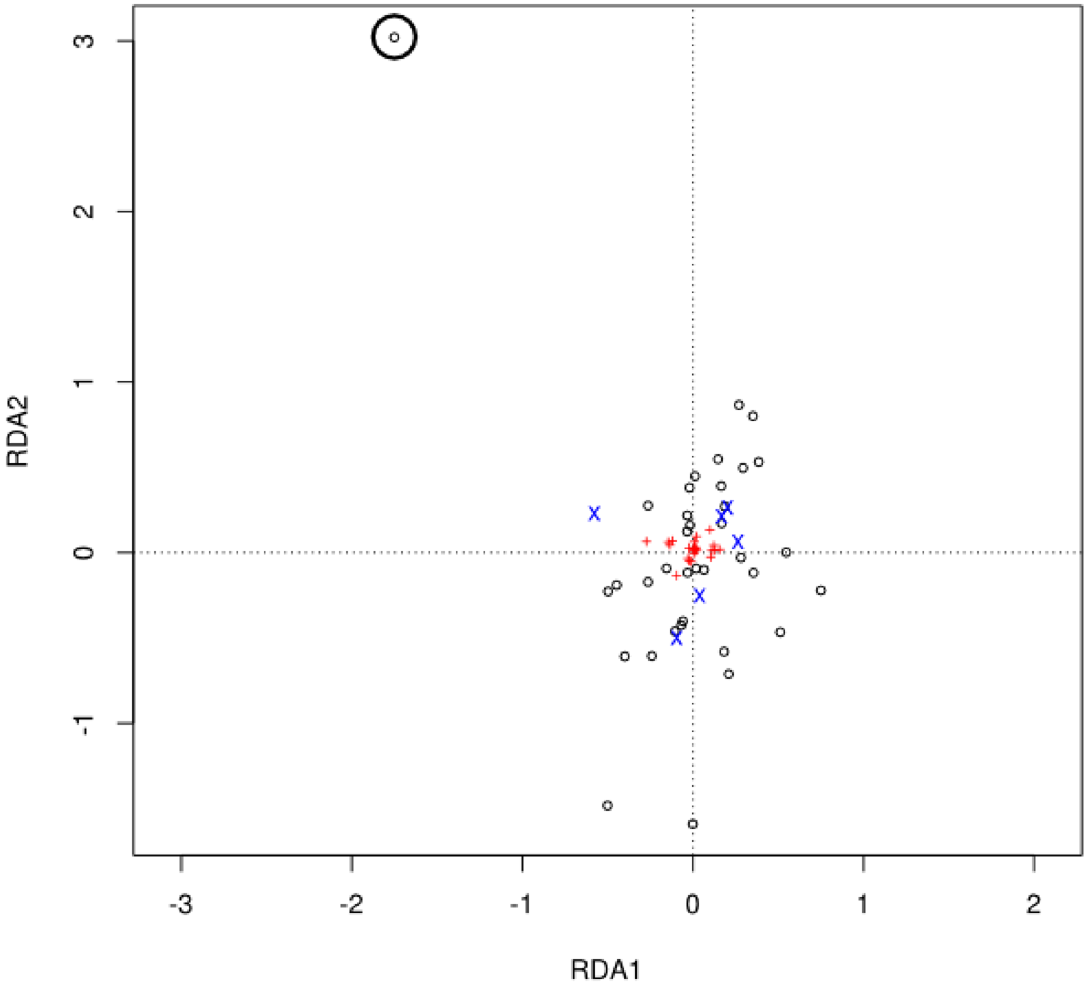


FIG. A1. Redundancy analysis on original data (not square-root transformed). X's are treatment centroids, o's are individual sites, and +s are mT loadings. The multivariate outlier in mT space is circled.

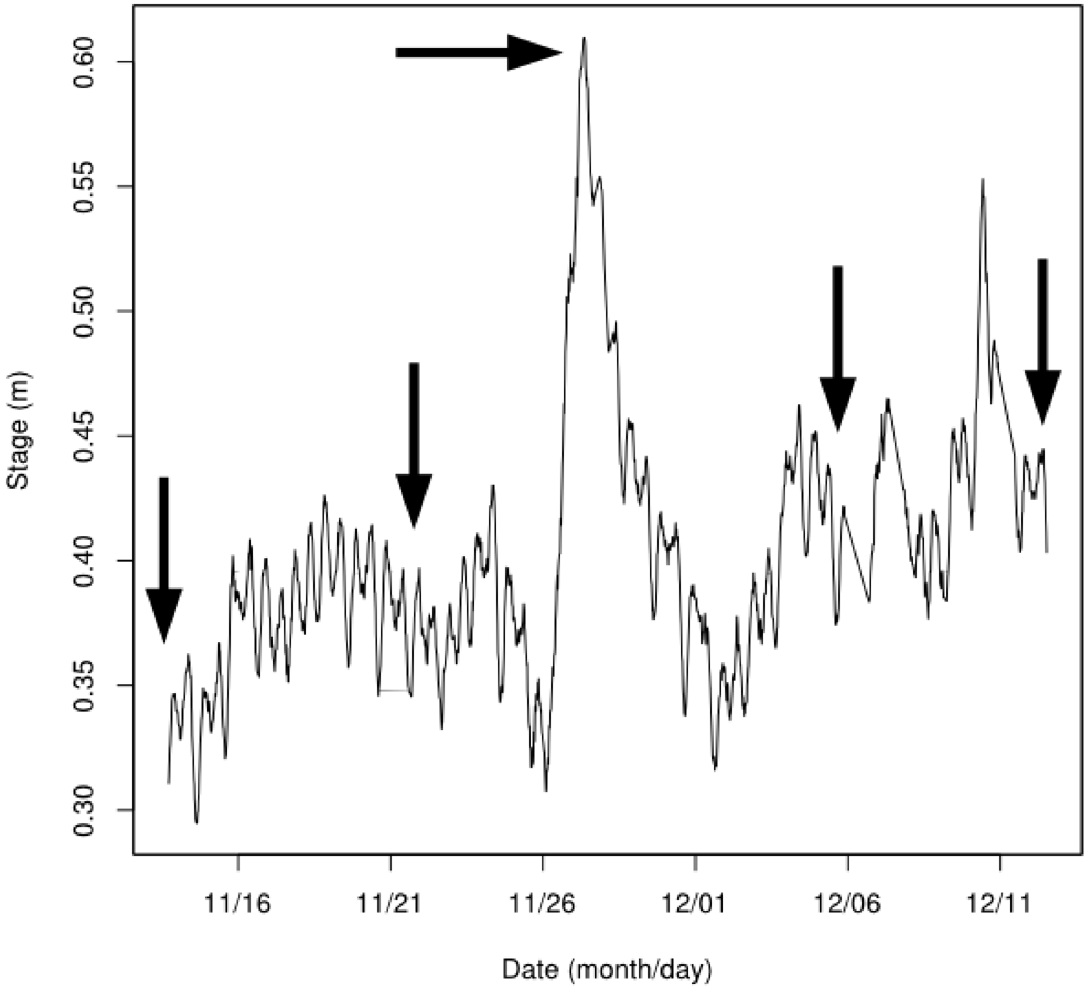


FIG. A2. Stream stage (water level) over the experiment (Nov. 13-Dec. 12, 2013; grey line). The arrows represent the days artificial disturbances were administered (first 4 arrows) and when the experiment was retrieved (5th arrow). The storm event that showed a marked increase above median stage for approximately 5 days (~11/26-11/30) is the 3rd black arrow.

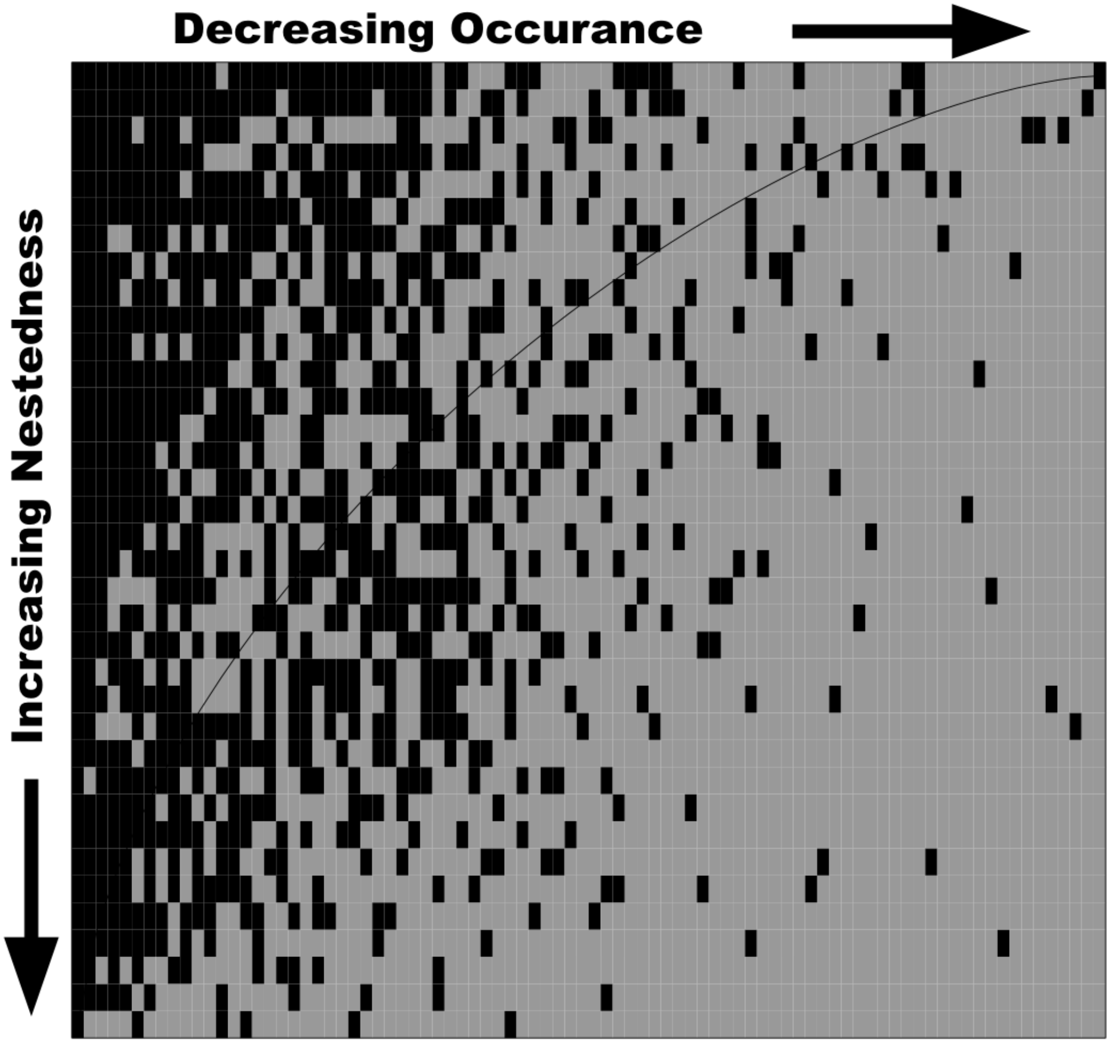


FIG. A3. Maximally packed presence/absence matrix. Black represents presence and grey represents absence. The curved line is the maximally packed isocline. Complete nestedness would be represented by all occurrences above the isocline line.

Chapter 6: Conclusions and Future Directions (Formatted for Ecological Applications)

Conclusions

My dissertation is an important contribution to understanding many facets of disturbance and hydraulic features of stream ecosystems. Particularly, this dissertation uses sand-bed streams as the stream system of study. Sand-bed streams are, as highlighted specifically in Chapter 2-5, different than gravel/cobble streams in hydrologic, geomorphic, habitat, and biological variables. This dissertation is thus valuable in understanding sand-bed streams, and the effect of hydrologic disturbance on sand-bed stream assemblages. Below I reiterate conclusions that are argued, with greater detail, in each of the individual chapters. After providing broad conclusions, I explore possible future research directions (**6.2**).

With regards to chapter 2, there is a different relationship of discharge and channel morphology in sand-bed streams than in gravel/cobble stream beds. In particular, the model that I developed and validated should be used to predict instream discharge in sand-bed streams of the Southeastern Plains as it shows better prediction ability than all other similar, published models validated with a large number of USGS gauging stations. This represents a new tool for resource managers, practitioners, and ecologists when gauging Southeastern Plains sand-bed steams, and may represent a generally applicable equation for discharge prediction in sand-bed streams. However, this would need to be investigated in other sand-bed streams before this could be suggested.

With regards to chapter 3, there is a biologically and physico-chemically relevant reference condition based on the deviation in channel morphology from that expected given watershed area that has not, before this work, been identified and validated in Sand Hills streams. The reference condition itself and associated physicochemical and macroinvertebrate trait relationships have broad implications for management in Southeastern Plains streams. Further, this type of reference condition has not be identified and validated for streams more generally. Thus, the methodology could have broader impacts than just for management in Southeastern Plains streams, with possible application to other sand-bed streams, and possibly to streams that are in different geomorphic settings.

With regards to chapter 4, the Hydrology as a master variable model represents a different translation of the landcover to benthic biota in sand-bed streams of the Southeastern Plains. Of particular interest is the primacy of hydrology as the major variable with highest direct effects on macroinvertebrate functional composition, and, in addition, the highest indirect effects of landuse on functional composition through hydrology. This is in contrast to the original LCC where geomorphology was the major driver of functional compositional change (Burcher et al. 2007). This likely has implications for other systems because, in contrast to the original LCC, I quantified higher resolution hydrology data that resulted in better quantifying hydrologic disturbance by better characterizing hydrologic disturbance, which was a stated deficiency in the original paper. In the Southeastern Plains, the results of this study suggest that restoration of long leaf pine ecosystems do not only provide benefit for reasons not explored in this dissertation, but have important relationships with hydrologic disturbance and important consequences for instream functional composition. Of broader implications, the results of this study suggest that simple cascade models, likely, do not capture the more complex relationships of landcover with instream functional composition in sand-bed streams, and, likely, streams in general. As a result, stream ecologist should reinvestigate which variables are most likely driving instream functional change. This has broad impacts to stream ecosystems in general.

With regards to Chapter 5, my results suggest that stream ecosystems are deterministically assembled at intermediate levels of disturbance. Further, the results of this study suggest that Chases's (2007) hypothesis should be amended to intermediate levels of disturbance resulting in deterministic assembly in stream ecosystems. In addition, my study represents the first time that the conditions for deterministically assembly have been satisfied, and that functional traits have been shown to be filtered from the regional species pool. This condition, while not being previously shown, is implicit in deterministic assembly of communities. Thus, this study has shown that macroinvertebrates are assembled from the regional species pool based on the traits they possess in concordance with the way that Keddy (1992) envisions this process.

Future Directions

I believe a lot of what I will do in the future will depend on the colleagues I find myself near (both proximity and intellectually), the funding sources that are available, and a large dollop of serendipity. First, I would like to look at fish traits in relation to the HGM reference condition from the SERDP project that forms the bulk of my dissertation. Second, I would like to submit both the working MSs from my master's work and the MS that would serve as the citable documentation for the R package that I wrote during my tenure at Southeastern Natural Sciences Academy that have taken a back seat while I have been working on my dissertation.

Broadly, I would like to continue working at the intersection of the physical environment and the distributions of aquatic macroinvertebrates compositional and functional distributions. Hydrologic disturbance is an important organizing force in stream ecosystems, and I would like to continue working in this area of inquiry. These have both basic and applied avenues that could be explored in future research.

I believe that, along with traditional macroinvertebrate compositional metrics, functional aspects of macroinvertebrate assemblages can offer important insights into potential mechanistic explanations for specific stressors (Keddy 1992, Poff 1997). Along these lines, I could see a potential avenue of basic research quantifying regional specific functional trait values for aquatic macroinvertebrates. For instance, measurement of velocity thresholds or temperature tolerances for various macroinvertebrate taxa, and use these to make predictions for assemblage structure at field sites. These specific traits would have applications to hydrologic alteration and climate change, respectively.

As an extension of chapter 2, I would like to explore the hydrogeomorphic reference approach (HGM) in other stream types. The most directly applicable would be other sand-bed streams in the lower coastal plains and the plains of the mid-west, and, more generally, in other sand-bed streams. Is there a predictable, physico-chemical and biological HGM reference condition in all sand bed streams based on deviation of channel morphology variables given watershed size? Another extension of Chapter 2 could be to look at whether this condition is predictable based on land use. In this way, reference stream sites might be able to be screened in Sand Hills streams from a GIS context allowing for setting broad conservation prioritization across the ecoregion.

Literature cited

Burcher, C. L., H. M. Valett, and E. F. Benfield. 2007. THE LAND-COVER CASCADE: RELATIONSHIPS COUPLING LAND AND WATER. Ecology 88:228-242.

Chase, J. M. 2007. Drought mediates the importance of stochastic community assembly. Proceedings of the National Academy of Sciences 104:17430-17434.

Keddy, P. A. 1992. Assembly and response rules: two goals for predictive community ecology. Journal of Vegetation Science 3:157-164.

Lepori, F., and B. Malmqvist. 2009. Deterministic control on community assembly peaks at intermediate levels of disturbance. Oikos 118:471-479.

Poff, N. L. 1997. Landscape filters and species traits: towards mechanistic understanding and prediction in stream ecology. Journal of the North American Benthological Society 16:391-409.