CONNECTICUT SEA GRANT PROJECT REPORT

Please complete this progress or final report form and return by the date indicated in the emailed progress report request from the Connecticut Sea Grant College Program. Fill in the requested information using your word processor (i.e., Microsoft Word), and e-mail the completed form to Dr. Syma Ebbin syma.ebbin@uconn.edu, Research Coordinator, Connecticut Sea Grant College Program. Do NOT mail or fax hard copies. Please try to address the specific sections below. If applicable, you can attach files of electronic publications when you return the form. If you have questions, please call Syma Ebbin at (860) 405-9278.

Please fill out all of the following that apply to your specific research or development project. Pay particular attention to goals, accomplishments, benefits, impacts and publications, where applicable.

Project #: ______________ Check one: [] Progress Report [X] Final report

Duration (dates) of entire project, including extensions: From [05/2012] to [12/2013].

a.) Project Title or Topic: Nitrogen Removal Capacity of Connecticut Estuaries: Assessing Distribution and Controls

Principal Investigator(s) and Affiliation(s):
1. Craig Tobias – University of Connecticut

2.

3.

4.

A. <u>COLLABORATORS AND PARTNERS</u>: *(List any additional organizations or partners involved in the project.)*

Bongkuen Song – Virginia Institute of Marine Science

B. PROJECT GOALS AND OBJECTIVES:

The overall goal of the project is to examine the rates and underlying factors that control dissolved inorganic nitrogen removal at representative river estuaries that discharge to Long Island Sound (LIS). We focus on both denitrification and ANAMMOX to examine potential controls on their rates resulting from changing microbial and geochemical factors. We concentrate efforts on river estuarine gradients rather than on the Sound itself for two reasons: 1) biogeochemical gradients found in the Sound (e.g. salinity, primary production, DIN concentration) are replicated in these estuaries on a compressed spatial scale thus rendering a more tractable examination of DIN removal over entire gradients; 2) working in the river estuaries targets our efforts nearest DIN sources entering the Sound via watershed discharge, where total DIN removal is most important on a system scale. Specific objectives are:

1) Quantify the N removal rates via denitrification and ANAMMOX in the Niantic river.

2) Determine how the overall N removal capacity changes throughout the estuary and map spatial and temporal N removal "hot-spots".
3) Quantify shifts in denitrification and ANAMMOX rates in response to observed changes in sediment and water chemistry along the estuarine axes.
4) Use multivariate analysis (PCA) to examine linkages among geochemical drivers, the distribution of N removal rates, and the ANAMMOX / Denitrifier microbial communities.
5) Develop and calibrate molecular based methods of estimating denitrification and ANAMMOX activity (Q-PCR and QRT-PCR) against ^{15}N tracer-based rate estimates.

C. **PROGRESS: (***Summarize progress relative to project goals and objectives. Highlight outstanding accomplishments, outreach and education efforts; describe problems encountered and explain any delays.)*

This project was funded on a pilot/scaled budget basis. The scope of work reflects this level of funding.

1) All field sampling was completed in September 2012 (Fig 1). The following rates and environmental parameters were measured: 1) Rates – Denitrification and anammox using ^{15}N tracer techniques; 2) Sediments/porewater - Dissolved inorganic nitrogen, dissolved organic carbon, extractable ammonium, ferrous iron, sulfide, sediment chlorophyll, % organic, C:N, and microbial community/ activity metrics; 3) Water column – Dissolved inorganic nitrogen/phosphorus, temperature, salinity, dissolved oxygen.
To date analysis of all these parameters with the exception of the microbial community measurements have been completed. ARCGIS software was successfully implemented to create geospatially accurate maps of ANAMMOX and denitrification, as well as maps showing the spatial distribution of sediment and water chemistry (see attached figures). We are currently using principle component analysis (PCA) to determine what chemical variables exert the greatest influence over the spatial variance of denitrification, ANAMMOX, and the ratio between these reactions.

D. **PROJECT PUBLICATIONS, PRODUCTS AND PATENTS**: *(Include published materials with complete references, as well as those which have been submitted but not yet published and those in press. Please attach electronic versions of any journal articles not previously provided.)*

The field work was only completed in Sept 2012. Sample processing and data analyses occurred in 2013.

Journal Articles: none to date / see below

Conference Papers and Presentations:
Tobias, C., Plummer, P., Cooper, C., Cady, D., Rollinson, V. Nitrogen removal capacity of Connecticut estuaries. Long Island Sound Program 2013 Meeting, Port Jefferson, NY.

Other articles, such as proceedings or book chapters: none

Web sites, Software, etc.: none

Technical Reports / Other Publications: none

Other Products (including popular articles): none

Planned Publications:
Manuscript preparation for Estuaries and Coasts or Estuarine Coastal and Shelf Science has begun and will be ready for peer review in 2014.

Patents: *(List those awarded or pending as a result of this project.)* none awarded nor pending.

E. <u>FUNDS LEVERAGED</u>: *(If this Sea Grant funding facilitated the leveraging of additional funding for this or a related project, note the amount and source below.)*
Approximately $10Kwas leveraged from NSF-EAR-0711006.

F. <u>STUDENTS</u>: ***(****Document the number and type of students supported by this project.)*
*Note: **"Supported"** means supported by Sea Grant through financial or other means, such as Sea Grant federal, match, state and other leveraged funds. If a student volunteered time on this project, please note the number of volunteer hours below.*

Total number of **<u>new*</u>** K-12 students who worked with you: 0
Total number of **<u>new</u>** undergraduates who worked with you: 0
Total number of **<u>new</u>** Masters degree candidates who worked with you: 0
Total number of **<u>new</u>** Ph.D. candidates who worked with you: 0

Total number of **<u>continuing**</u>** K-12 students who worked with you: 0
Total number of **<u>continuing</u>** undergraduates who worked with you: 0
Total number of **<u>continuing</u>** Masters degree candidates who worked with you: 0
Total number of **<u>continuing</u>** Ph.D. candidates who worked with you: 1

Total number of volunteer hours: 0

*(Note: *<u>New</u> students are those who have <u>not</u> worked on this project previously. **<u>Continuing</u> students are those who have worked on this project previously.)*

In the case of graduate students, please list student names, degree pursued, and thesis or dissertation titles related to this project.

Student Name: Patrick Plummer
Degree Sought: PhD - Oceanography
Thesis or Dissertation Title: Nitrogen Removal in Long Island Sound Estuaries
Date of thesis <u>completion:</u> 05/05/2018
Expected date of graduation: 05/05/2018

G. PICTORIAL: Provide high resolution images/photos of personnel at work, in the field or laboratory, equipment being used, field sites, organism(s) of study. Attach images as separate files (do not embed). Include links to websites associated with the research project. Please include proper photo credits and a caption with date, location, names of people, and activity. These images are useful to document your project in future CTSG publications, websites and presentations.

FOR FINAL REPORTS ONLY, PLEASE COMPLETE THIS SECTION:

H. PROJECT OUTCOMES AND IMPACTS

RELEVANCE OF PROJECT: *(Describe briefly the issue/problem / identified need(s) that led to this work.)*

Human inputs and modifications have greatly altered the health of coastal marine environments, with estuaries often being the most effected. Estuaries serve an important role in the global ocean, removing roughly 75% of all terrestrial nitrogen prior to exchange with the coastal sea. Specific nitrogen loading into marine estuaries has increased by 2-20 fold over pre-industrial levels as a result of allochthonous sources including fertilizers, sewage treatments and fossil fuel emissions (Galloway et al., 2004). These increased nitrogen (N) levels can lead to eutrophication of estuaries, promoting overproduction of primary producers and harmful algal blooms, resulting in the creation of hypoxic zones (Burgin and Hamilton, 2007).

Long Island Sound (LIS) eutrophication is linked to nitrogen delivery from surrounding watersheds. Some of this N load is attenuated during transit from watersheds through river estuaries prior to entry into LIS. This study examines N removal via denitrification and anaerobic ammonium oxidation (ANAMMOX), as well as the competing and N recycling reaction of dissimilatory nitrite reduction to ammonium (DNRA) in one small Connecticut river estuary; the Niantic River. This river system possesses elevated nitrate concentrations seasonally, and is therefore an acceptable proxy to larger polluted systems within LIS (Klug 2006). Spatial characterization of these reactions was done in conjunction with extensive water column and sediment sampling for various geochemical analytes and microbial molecular markers, with efforts made to link rates to geochemical and/or molecular markers or possible competition with DNRA. Decoding these natural variables and linkages provides a better understanding of the ability of an estuary to naturally mitigate N pollution, and better access when an estuary may be beyond its capabilities.

RESPONSE: *(Describe briefly what key elements were undertaken to address the issue, problem or need, and who is/are the target audience(s) for the work.)*

This work integrated the ^{15}N isotope tracer based measurements of denitrification, Anammox, and DNRA rates, assessment of these bacterial communities using molecular microbial techniques, and geochemical characterization of estuarine water column and sediments. These variables were then analyzed using multivariate analysis to determining interdependency on the rates, and plotted onto geographic axes using GIS software. Information has already been presented at the Long Island Sound Conference (poster), and ultimately will be relevant for CT DEEP/NY DEP, as well as other agencies monitoring similar estuaries.

RESULTS: *(Summarize findings and significant achievements in terms of the research and any related education or outreach component; cite benefits, applications, and uses stemming from this project, including those expected in the future. Include qualitative and quantitative results.)*

The isotope tracer methods developed provide simultaneous analyses of denitrification, anammox, and DNRA on single samples. Geospatial maps were developed to understand the distribution of microbial nitrogen removal. These maps showed that denitrification and anammox are not spatially distributed in smooth gradients along the estuarine axis but rather show large variations captured by 3-4 “hotspots” (Figs 2-4).. These “hotspots”, as well as locations with minimum and median nitrogen removal were correlated to the assembled geochemical variable matrix. While certain elevated locations correspond to known areas of groundwater N input, the rates of N removal (estuary-wide) do not appear to be tightly correlated to single geochemical variables, and there appears to be differential controls on the nitrogen removal rates depending on location in the estuary.

The nitrogen removal processes of denitrification and anammox were shown to be strongly correlated throughout the river. Denitrification dominated anammox but multiple variables exhibited strong controls of both microbial communities (Fig 5). The magnitude of these controls were not constant for each community, with more controls being identified for anammox compared to denitrification. There does appear to be competition between DNRA and nitrogen removal; the highest areas of denitrification and Anammox were inversely correlated the DNRA rates. Conversely, the areas of highest DNRA do not demonstrate any correlation to denitrification or anammox. Thus it seems apparent that DNRA and nitrogen removal are controlled by a different set of variables, and that DNRA will outcompete in areas where the variables conflict. One of the environmental benefits is that the rates coupled with the spatial distribution permit calculation of the overall estuary scale NOx removal (denitrification + anammox) vs. NOx retention (DNRA).

The areas of highest denitrification (the largest removal reaction) were corresponded with the locations with the highest water column carbon and nitrogen concentrations, with a strong inverse correlation to the $\delta^{13}C$ and $\delta^{15}N$ values; this suggests that likely electron donor for denitrification within the river is C3 upland plants from terrestrial sources (Figs 6-7). The highest denitrification values also correlated to areas of lower salinity, which further suggests the terrestrial signature of the substrate. There is a lesser, but still significant positive control of denitrification exerted by both the chlorophyll a and phaeophytin concentrations below 1 cm of depth. This may suggest the use of benthic algae as an electron donor, but does not correlate with other sediment variables tested. The lack of response to chlorophyll a and phaeophytin in the 1st cm of sediment, coupled with the lack of a correlation to carbon or nitrogen percentage in sediments, only repetition of inverse relationship to the $\delta^{13}C$ value, further suggests terrestrial organic matter buried in the anoxic sediments. The perceived organic carbon type controls on denitrification suggest links between watershed carbon management and downstream nitrogen removal efficiency.

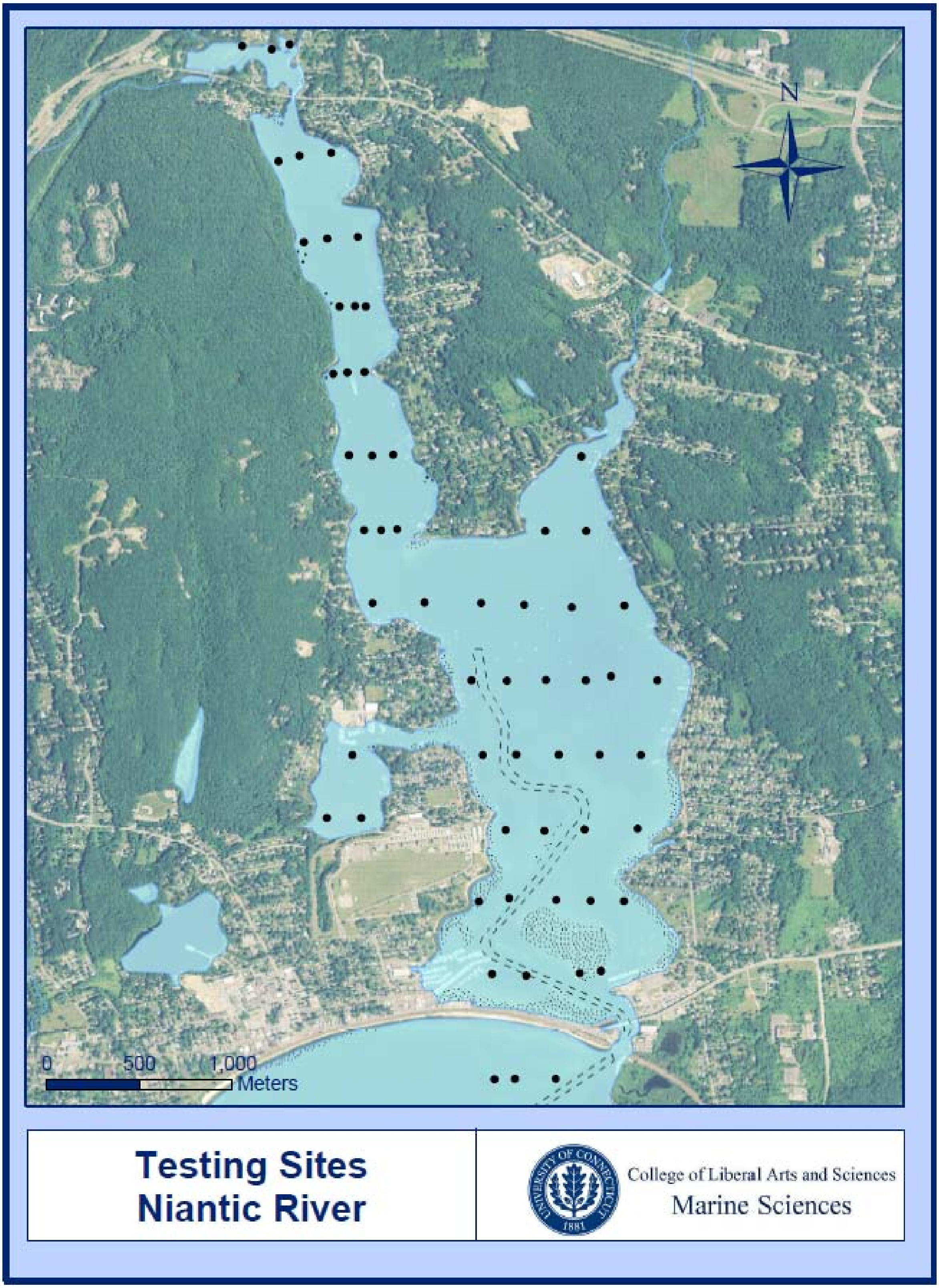

Figure 1. Niantic River sampling locations.

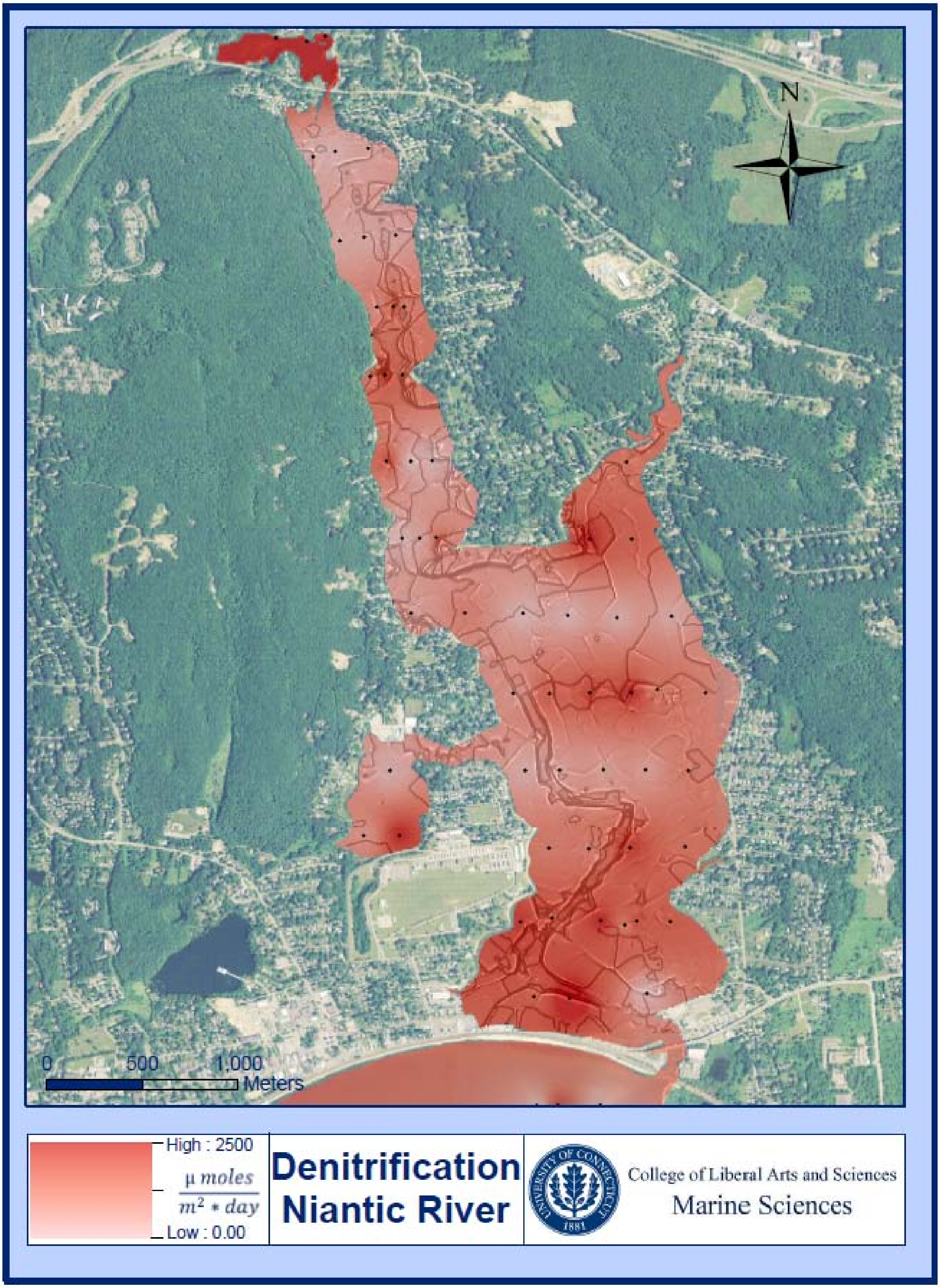

Figure 2. Niantic River denitrification distribution.

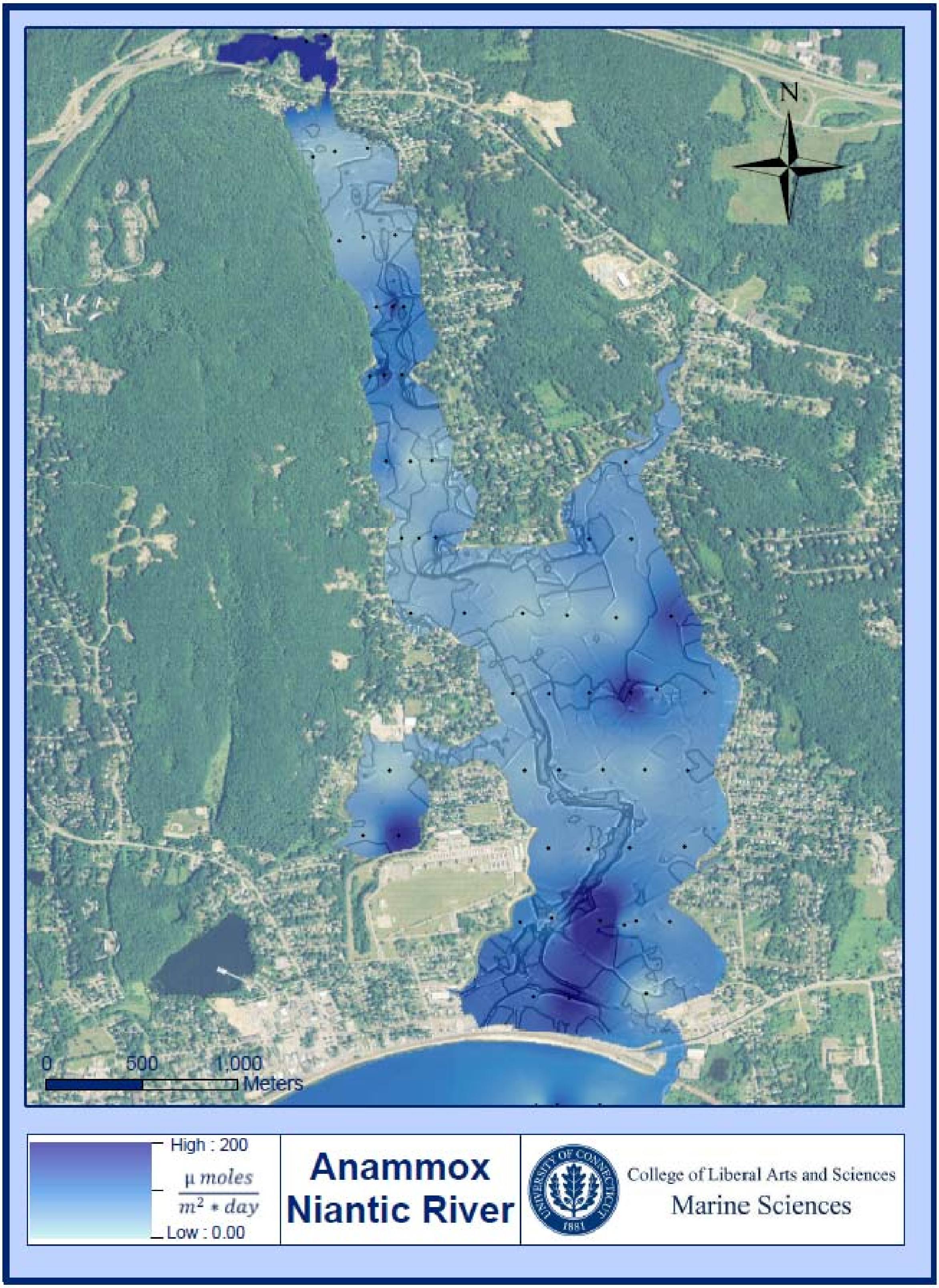

Figure 3. Niantic River anammox distribution.

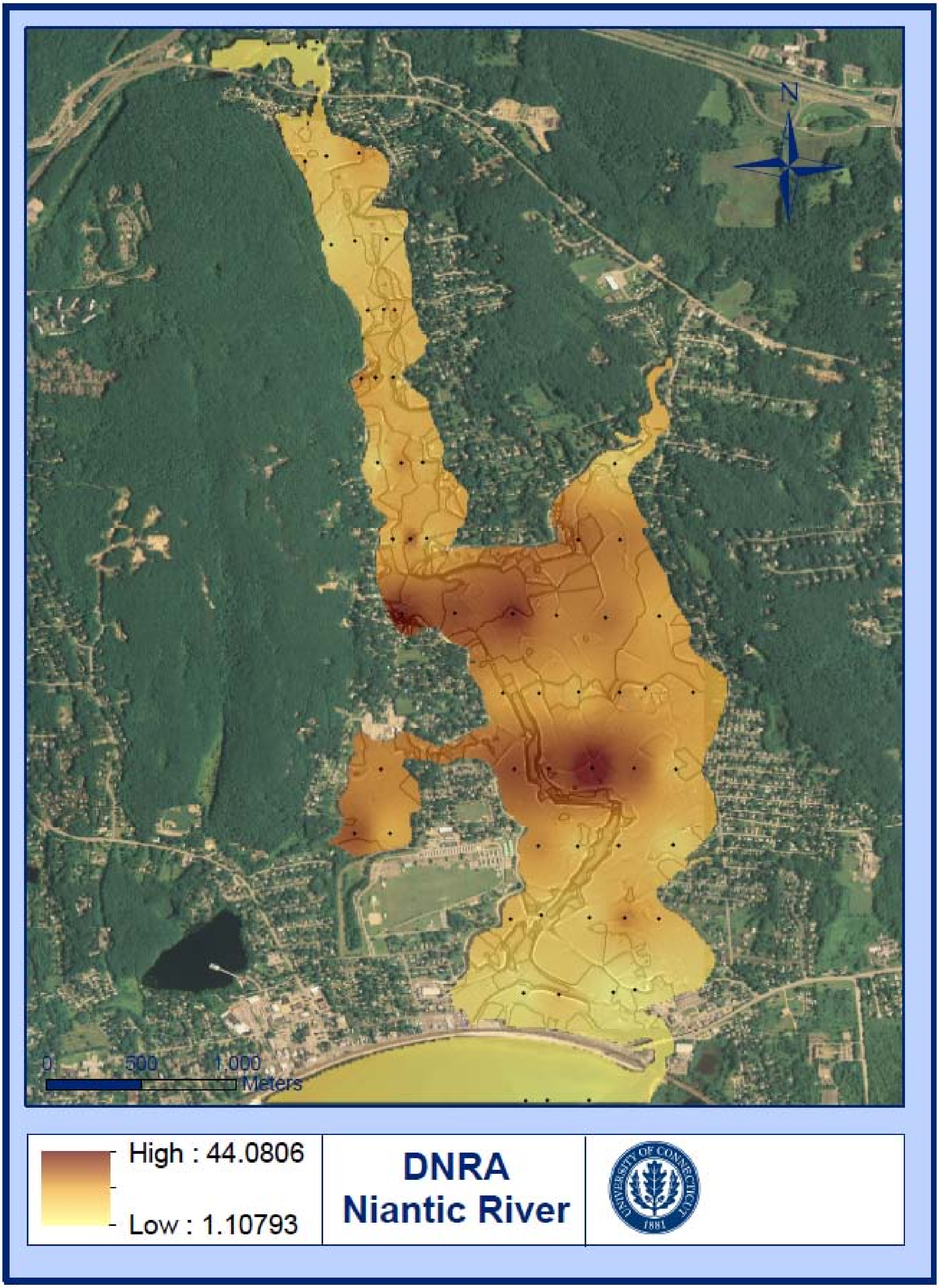

Figure 4. Niantic River DNRA distribution.

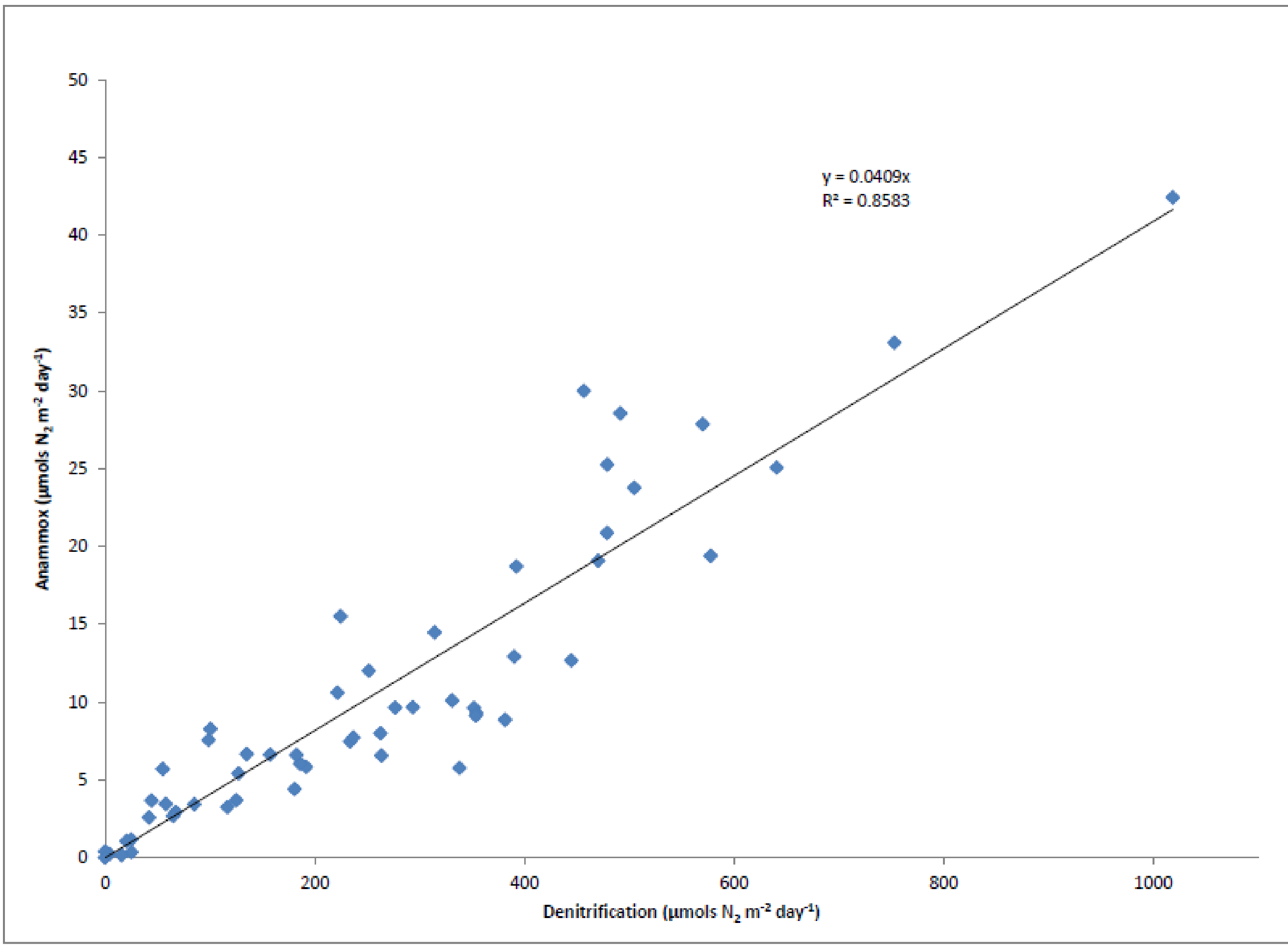

Figure 5. Anammox and denitrification regression. All data.

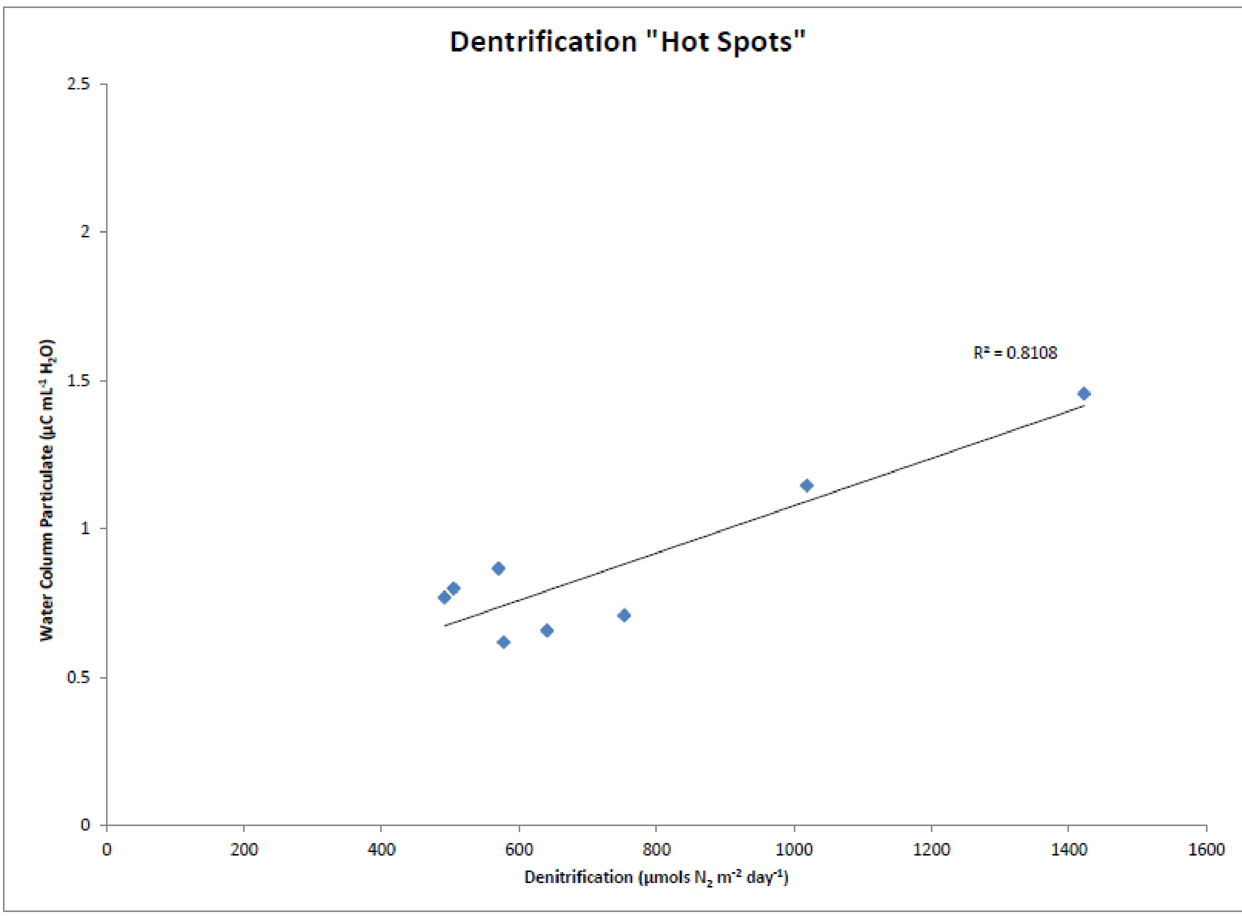

Figure 6. Relationship between elevated denitrification rates and water column particulate carbon. Hot-spots defined as rates > 2 standard deviations above the mean.

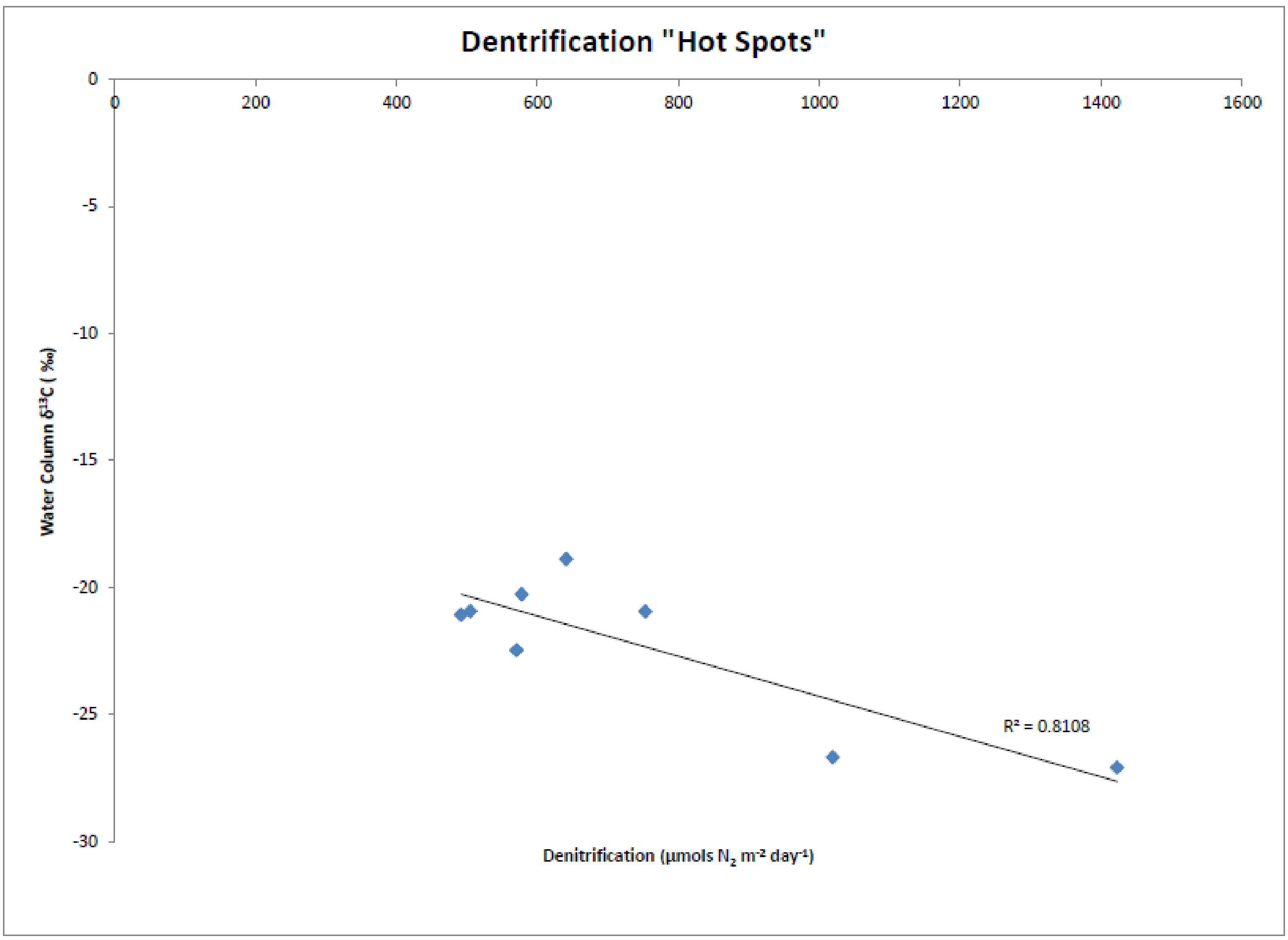

Figure 7. Increasing denitrification rates related to the presence of isotopically light carbon. The watershed likely delivers carbon with $\delta^{13}C$ < 25 per mil.