

2015 Final Progress Report
Ohio Water Resources Center

Title: **Rural on-site waste treatment as a source of nutrients to a eutrophic watershed**

PI: Christopher Spiese, Ohio Northern University

Problem and Research Objectives. This project aimed to determine whether or not rural septic systems contributed to nutrient and microbial loadings in the Blanchard River. The Blanchard River is a tributary to the western Lake Erie Basin and has been heavily impacted by increased phosphorus and nitrogen concentrations. While a number of factors can potentially contribute both nutrients to the river, septic systems have been targeted as an easily regulated source. This project looked at a marker of human waste streams, caffeine, and its relationship with phosphorus and nitrogen concentrations in tile drainage effluent as well as fecal coliform and *E. coli*.

Methodologies. Standards for all chemical parameters (nitrate, ammonia, and phosphorus) were created from certified reference materials or from drug standards (caffeine). Controls, field samples, and standards were determined using at least triplicate replicates. Caffeine was quantified using isotope dilution. Additional details concerning the study site and analytical methodology are presented in the proceeding sections.

Nitrogen. Two forms of dissolved nitrogen were measured: nitrate and ammonia. Both were measured *in situ* simultaneously using an YSI ProPlus meter with ion selective electrodes (ISE) for nitrate and ammonia. This method follows Standard Method (4500-NH₃ D) for ammonia (Standard Methods, 2013) and EPA Method 9210A for nitrate (US EPA, 2007). Standardization was performed using certified standards, and each ISE was calibrated prior to use. Detection limits for nitrate were 0.1 mg N/L.

Phosphorus. Phosphorus was determined using EPA Method 365.1 (US EPA, 1993). Soluble reactive phosphorus (SRP) was determined as molybdenum blue reactive substances present after filtration through a pre-combusted 0.45µm glass fiber filter. Total phosphorus was determined using the same method on unfiltered water samples after digestion with sodium persulfate. Absorbance measurements were made on a Shimadzu UV2401-PC UV-Vis

spectrometer at 610 nm using either a 1 cm or 5 cm path length cuvette, depending on absorbance. Method detection limits were 5 µg P/L or lower for all phosphorous measurements. *Caffeine*. Caffeine was determined following the method of Buerge, *et al.* (2003). Briefly, caffeine was extracted from 1 L of water using a solid-phase extraction (SPE) cartridge filled with silica-supported C₁₈. The caffeine was then eluted from the cartridge using methanol and dichloromethane. The methanol portion was re-extracted with dichloromethane and the combined dichloromethane layers were evaporated to dryness under a gentle stream of air. The evaporated sample was then reconstituted in dichloromethane system. Quantitation was achieved using a gas chromatography-mass spectrometry (GC-MS). An internal standard (caffeine-¹⁵N₂) was employed prior to SPE in order to permit accurate quantification. Blank samples were periodically analyzed to control for any potential contamination. Spike recovery studies were carried out for method confirmation. Reported detection limits for this method were approximately 10 ng/L with a reporting limit of 100 ng/L. Previously reported caffeine concentrations observed in a neighboring western Lake Erie watershed were in excess of 100 ng/L (Wu, *et al.*, 2012).

Principal Findings and Significance. At six sites across Putnam County, Ohio, tile drainage water was sampled over the course of a year. Caffeine was found at all of the sites, at varying levels (Fig. 1). Table 1 provides the mean ± standard deviation concentrations for nutrients, suspended sediment, and caffeine observed in each site over the course of the entire research. The observed water temperature at each site ranged from 16.3 to 21.8 °C, the observed pH ranged from 7.5 to 8.0, and chloride ranged from 6.2 to 13.0 mg/L. Temperature, pH, and chloride did not demonstrate any correlation to nutrient levels or caffeine levels observed within the watershed. There were also no correlations observed between caffeine and nitrate, caffeine and ammonium, caffeine and nitrite, or caffeine and total nitrogen. However, caffeine was observed to have a strong negative correlation (Pearson's $r > -0.9$) with total phosphorous (Fig. 2).

Mean ± standard deviation caffeine concentrations across sampling sites ranged from non-detect at the control site (site #4) to 1.2±0.4 µg/L in tile drainage effluents from sites having on-site wastewater systems (site # 1-3, 5, and 6). Mean ± standard deviation concentrations observed for total nitrogen and phosphorous concentrations in all tile drains were 3.5±1.8 mg/L and 0.4±0.07 mg/L, respectively. While the overall standard deviations for all analytes were variable, the observed correlations were maintained during analysis of individual storm events. Therefore, we are confident in the observed trends indicated in the data set when taken for individual sampling events or as grouped means.

Table 1. Mean \pm standard deviation concentrations for nutrients and caffeine observed in each site over the course of the entire research project.

Site number	Ammonia (mg/L)	Nitrate (mg/L)	Total Phosphorous (mg/L)	Suspended Sediment (mg/L)	Caffeine (μ g/L)
1	0.62 \pm 1.0	3.21 \pm 3.87	0.47 \pm 0.4	21 \pm 25	0.45 \pm 0.7
2	0.26 \pm 0.2	2.47 \pm 2.19	0.45 \pm 0.4	8.2 \pm 6.0	0.43 \pm 0.3
3	0.30 \pm 0.5	0.92 \pm 0.72	0.4 \pm 0.3	16 \pm 12	0.82 \pm 1.7
5	0.14 \pm 0.1	5.7 \pm 5.2	0.31 \pm 0.3	10 \pm 14	1.2 \pm 1.4
6	0.48 \pm 0.4	2.1 \pm 1.4	0.5 \pm 0.3	15 \pm 10	0.26 \pm 0.13
control	0.17 \pm 0.04	7.3 \pm 8.6	0.38 \pm 0.3	14 \pm 8.6	BDL

The study results are interesting, because the observed caffeine-total phosphorous correlation indicates that septic effluents are not significant contributors to nutrient loadings within the rural watershed. Additionally, commonalities in nutrient fingerprints (total and speciated phosphorous and nitrogen) in groundwater and tile drainage highlight the complex relationships for nutrient and water quality management in irrigation drainage waters.

The observed trends in nutrient levels from each sampling site and the demonstrated inverse correlation with phosphorous led us to explore groundwater concentrations of the nutrients. Groundwater from a nearby well indicated a mean total phosphorous 0.39 mg P/L. A mean phosphorous tile drain concentration for the entire study was determined to be 0.4 \pm 0.07 mg P/L. Additionally, analysis of fifteen surface water samples from Riley Creek contained an average phosphorous concentration of 0.44 \pm 0.1 mg P/L. Additional groundwater samples are currently being explored to confirm a proposed pathway where property drinking water wells convey nutrient rich groundwater through the OSWT system, which then drains into the Creek via the tile. Therefore, efforts to control OSWT systems will likely not have a measurable effect on nutrient loadings unless groundwater can also be controlled or leach systems become more widely implemented. Evidence for the plausibility of the proposed nutrient pathway is also supplied by the earlier examination of the correlation between nutrients and river discharge.

The lack of a correlation between nitrate and SRP to river discharge points towards a source that is likely not coupled to rainfall. The constant use of wells throughout the region, may explain why phosphorous levels in the watershed have increased as efforts to manage nutrients in runoff have increased.

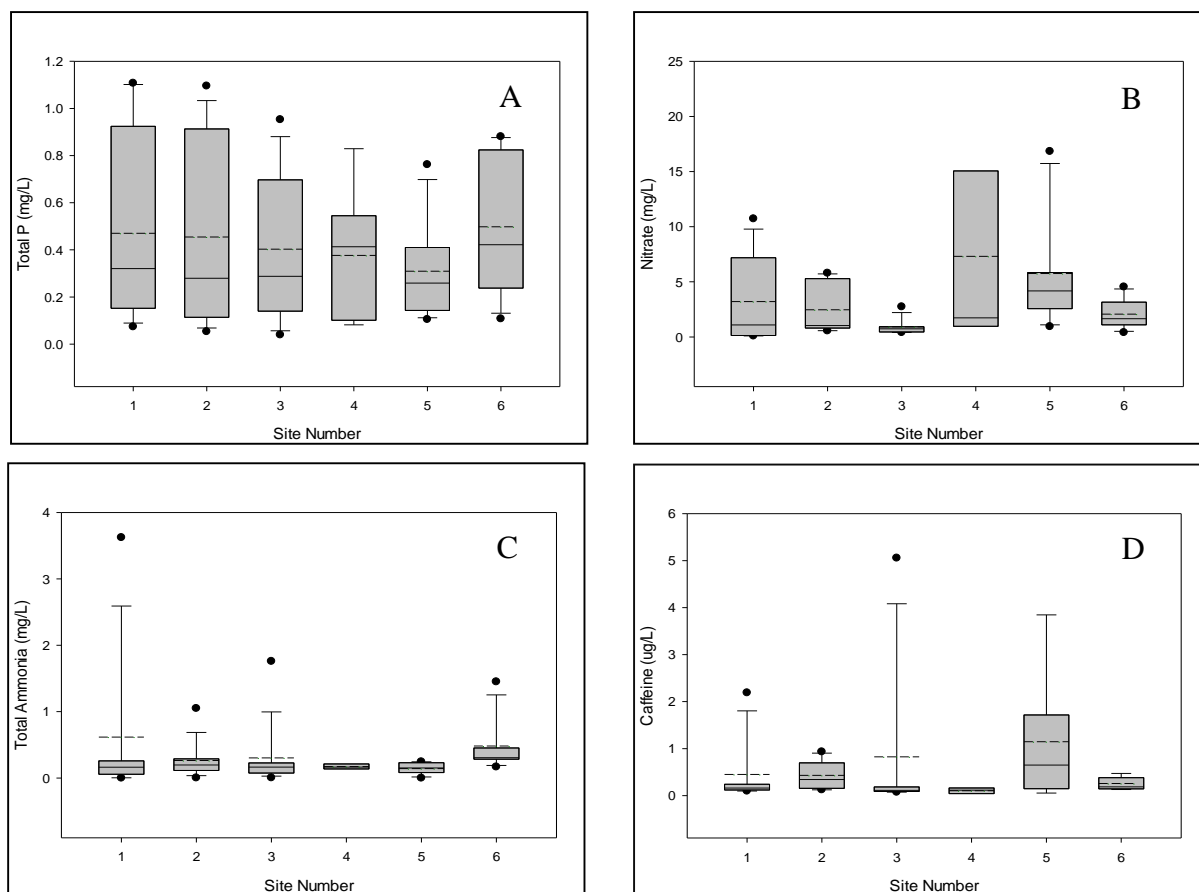
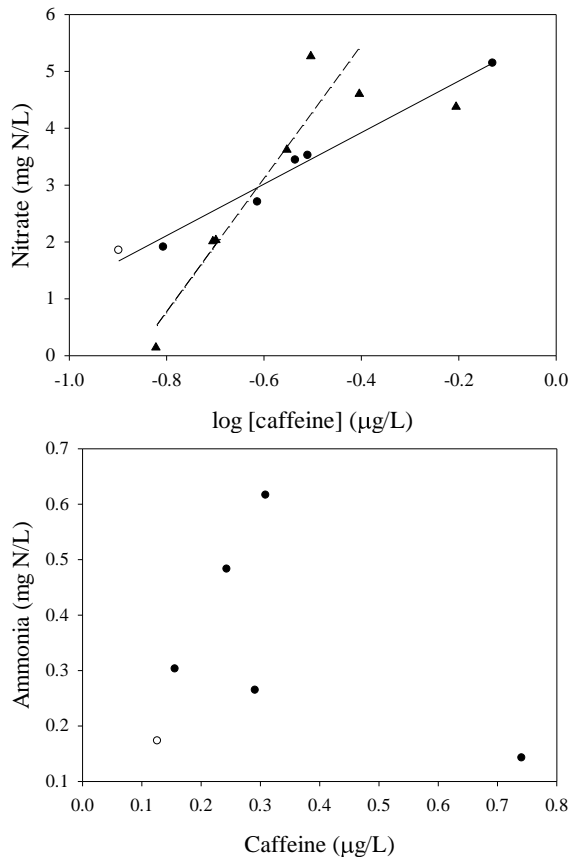
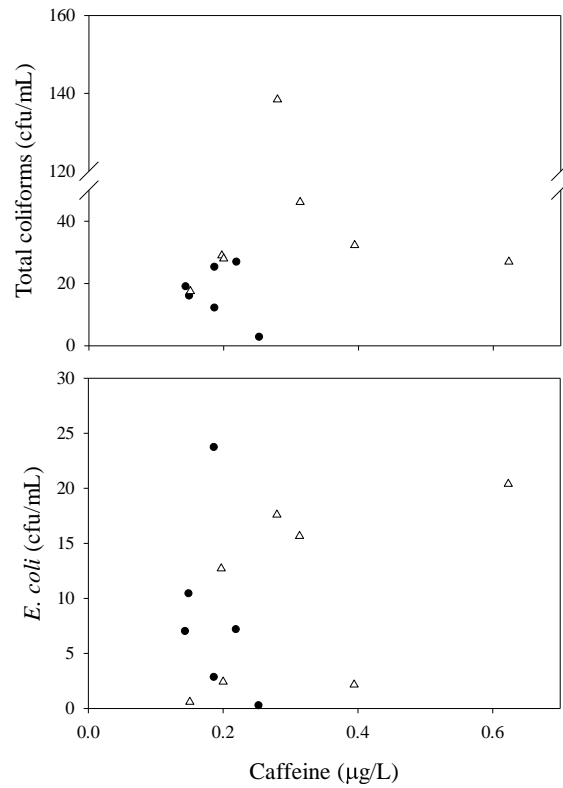
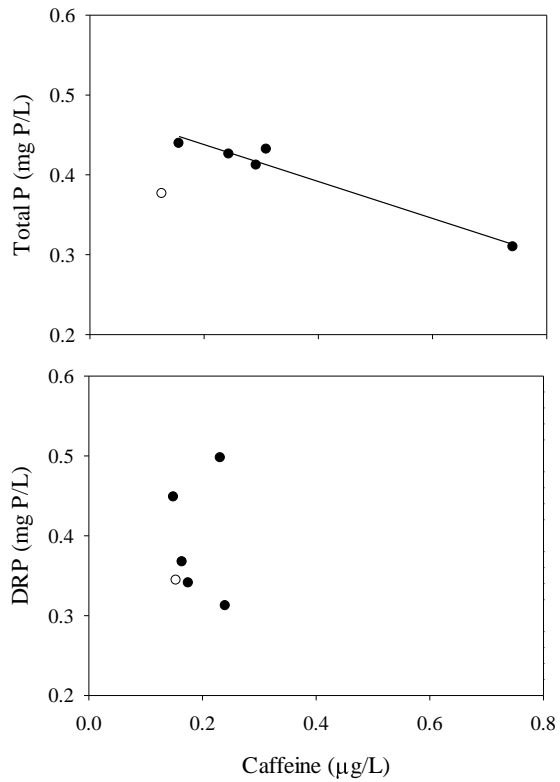


Figure 1. Compiled data for 2013 for A) total phosphorus, B) Nitrate, C) total ammonia, and D) caffeine. The boxes denote 25th through 75th quartiles, with the solid line at the median value and the dashed line at the mean. Error bars denote 95% confidence intervals and points denote outliers.

Initial results are available regarding microbial loadings tied to caffeine concentrations (Fig. 3). At nearly all sites tested, both fecal coliform bacteria and *E. coli* were detected. Both microbe types were correlated with caffeine concentration, indicating a common source. This is particularly true for *E. coli*, where the correlation is statistically significant ($p = 0.015$), and there is precedent for *E. coli* being linked directly to human waste streams (Frenzel and Couvillion 2002).

Taken together, the role of OSWT in loadings of nutrients and bacteria is complex. In this agricultural watershed, OSWT does not contribute significantly to nutrients, but do appear to be a source of bacteria. These results urge caution when making policy decisions related to nutrient reduction by targeting residential OSWT systems.



(left) Figure 2. Relationship between caffeine and total phosphorus, dissolved reactive phosphorus, nitrate, and ammonia. Points denote the mean for each sampling site ($n = 4 - 15$ for caffeine and TP, 2 - 10 for DRP), and the open circle indicates the control site (no OSWT). For TP, the line represents the linear regression for all sites with OSWT present within the drainage area ($[TP] = (-0.2303 \text{ mg P/mg caffeine}) \times [\text{caffeine}] + (0.4839 \text{ mg P/L}), r^2 = 0.957$). Lines denote the best fit for each data set. Putnam County (solid line): $[NO_3^-] = (4.5308 \text{ mg N/L}) \times \log [\text{caffeine}] + 5.7358 \text{ mg N/L}, r^2 = 0.9796$. Hardin County: $[NO_3^-] = (11.714 \text{ mg N/L}) \times \log [\text{caffeine}] + 10.146 \text{ mg N/L}, r^2 = 0.8975$.

(above) Figure 3. Relationship between caffeine and (top) total coliform loading or (bottom) *E. coli* loading at Putnam County sites (circles) and Hardin County sites (triangles). Points denote the mean for each sampling site ($n = 1 - 9$).

References

Buerge IJ, Poiger T, Müller MD & Buser H-R (2003) Caffeine, an anthropogenic marker from wastewater contamination of surface waters. *Env. Sci. Tech.* **37**: 691-700.

Dolan DM & Chapra SC (2012) Great Lakes total phosphorus revisited: 1. Loading analysis and update (1994-2008). *J. Great Lakes Res.* **38**: 730-740.

Frenzel SA & Couvillion CS (2002) Fecal-indicator bacteria in streams along a gradient of residential development. *J. Am. Water Resour. Assn.* **30**: 265-273.

North RL, Guildford SJ, Smith REH, Havens SM & Twiss MR (2007) Evidence for phosphorus, nitrogen, and iron colimitation of phytoplankton communities in Lake Erie. *Limnol. Oceanogr.* **52**: 315-328.

Peeler KA, Opsahl SP & Chanton JP (2006) Tracking anthropogenic inputs using caffeine, indicator bacteria and nutrients in rural freshwater and urban marine systems. *Env. Sci. Tech.* **40**: 7616-7622.

Seiler R, Zaugg SD, Thomas JM & Howcroft DL (1999) Caffeine and pharmaceuticals as indicators of water contamination in wells. *Ground Water* **37**: 405-410.

Standard Methods (2013) Standard Method 4500-NH₃ D. Ammonia-Selective Electrode Method. Standard Methods Online -- Standard Methods for the Examination of Water and Wastewater. <http://standarmethods.org>, last accessed Jan 16, 2014

Starr JL & Sawhney BL (1980) Movement of nitrogen and carbon from a septic system drainfield. *Water Air Soil Pollut.* **13**: 113-123.

US EPA (2013) <http://water.epa.gov/infrastructure/septic/>, last accessed Jan 16, 2014

US EPA (2007) EPA Method 9210A Potentiometric Determination of Nitrate in Aqueous Samples with an Ion-Selective Electrode

US EPA (1993) EPA Method 365.1 Determination of Phosphorous By Semi-Automated Colorimetry

Wu C, Witter JD, Spongberg AL & Czajkowski KP (2012) Occurrence of selected pharmaceuticals in an agricultural landscape, western Lake Erie basin. *Wat. Res.* **43**: 3407-3416.