Nutrient enrichment effects are conditional on upstream nutrient concentrations: Implications for bioassessment in multi-use catchments

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Abstract: Human impacts on stream ecosystems are expected to intensify with population growth and climate change. Decisive information on how stream communities respond to cumulative human impacts is therefore integral for protecting streams draining multi-use catchments. To determine cumulative influences of nutrient enrichment and assess more nuanced approaches for the evaluation of human impacts, we present results from one factorial and two gradient assessment designs applied to benthic algae and macroinvertebrate data from 14 mid-order streams in southern Ontario, Canada with pre-existing human impacts (i.e., sewage effluent and agriculture). We found that among stream variability in ecological indicators measured downstream of sewage effluent outfalls confounded our generalized factorial assessment and provided inconclusive information on a known human impact. Despite our gradient assessment also not having strong statistical support, accounting for the extent of nutrient enrichment associated with differences in sewage effluent and agricultural inputs revealed that larger longitudinal changes in stream communities were associated with increased nutrient enrichment. However, re-weighting our nutrient enrichment gradient based on upstream nutrient concentrations to account for nonlinearities in the response of stream communities to nutrient enrichment produced more robust assessment results that were consistent with predicted effects of nutrients on stream ecosystems. Thus, while our factorial assessment suggests that the communities are resistant to nutrients from cumulative human impacts, our targeted gradient assessment demonstrates that the effects of nutrient enrichment are highly conditional on upstream ecosystem conditions. Future assessments may need to go beyond traditional approaches (i.e., impact presence/absence) and more explicitly consider the environmental stressors and their associated complexities related to the impact under investigation.

Keywords: diatoms; macroinvertebrates; nitrogen; phosphorus; agriculture; sewage; wastewater

1. Introduction

Streams support and regulate important ecosystem processes (e.g., nutrient cycling, water purification, and waste assimilation) and provide provisional and cultural services (e.g., food, water, and recreation) to sustain societal needs (Lowe & Likens, 2005). However, human impacts on stream ecosystems are diverse (e.g., habitat alteration and pollution) and are expected to increase with continued population growth and climate change (Søndergaard & Jeppesen, 2007). Thus, there is an ongoing need to assess the impacts of human activities to provide managers and policy makers with scientifically defensible information on how to better protect the structure and function of stream ecosystems.

One of the most effective study designs to evaluate the effects of an impact (e.g., impoundment, point source pollution, and land use change) while controlling for natural variability is the before-after control-impact (BACI) assessment (Green, 1979; Smokorowski & Randall, 2017). BACI assessments compare the temporal change in ecosystem conditions after exposure to an impact to the natural temporal variability in an unexposed stream and can readily identify the potential effect of an impact if its timing and location are known, and pre-exposure monitoring data are collected. However, researchers do not often have sufficient advance notice of when or where an impact will occur to collect pre-exposure data. Thus, suboptimal (e.g., before-after only, control-impact only) or modified (e.g., upstream-downstream control-impact) BACI assessments are often used to evaluate the effect of human impacts, despite producing less robust assessment results (Smokorowski & Randall, 2017). Moreover, BACI assessments are usually limited by the use of spatial replicates to measure ecosystem conditions within individual control-impact streams (Hurlbert, 1984), and while temporal replicates can be employed to eliminate spatial pseudo-replication (Stewart-Oaten et al., 1986), inference on the potential effect of an impact is restricted to the studied location alone (Underwood, 1991; 1994). Generalizability of cause-effect relationships from BACI assessments can be strengthened with the inclusion of multiple control-impact sites as independent replicates (*aka* multiple-BACI; Downs et al., 2002). However, in highly developed regions locating a set of suitable control-impact streams with similar environmental conditions can be near impossible and remains a common challenge for impact assessments (Stoddard et al., 2006; Herlihy et al., 2008).

Past studies have shown that although BACI assessments employ a factorial approach to provide information on how the presence of an impact influences stream ecosystems, quantitative differences in environmental stressors (e.g., chemical and sediment concentrations, and thermal pollution) associated with an impact can govern the size of the perturbation from prior ecosystem conditions (Welch et al., 1992; Chambers et al., 1997; Scrimgeour & Chambers, 2000; Bowman et al., 2005). For example, Quinn and Hickey (1993) observed that variability in the difference between benthic macroinvertebrate assemblages sampled upstream and downstream of eight sewage treatment lagoons was associated with the increase in organic matter concentration from the differential addition of effluent. Thus, the factorial evaluation of an individual impact derived from multiple study streams with differing degrees of human influence may be confounded by increased variability in the response of stream communities to specific environmental stressors. Variability in the response of ecosystem conditions to a common impact can result in decreased confidence in factorial assessment results, particularly in assessments with small sample sizes. Moreover, bioassessment in highly developed regions can be further complicated by the presence of multiple stressors from independent human impacts that may interact (i.e., additively, synergistically, or antagonistically) and accumulate along the stream continuum to contribute to a cumulative extraneous effect on ecosystem conditions (Seitz et al., 2011; Galic et al., 2018). Increased variability from cumulative and multiple stressors can therefore make it difficult to select well-matched control-impact streams that can provide decisive assessment information on the potential effects of an individual human impact.

In recognition that human impacts can have diverse effects on stream ecosystems, many studies have elected to use human activity gradients (e.g., land cover and volume of effluent discharged) to evaluate impacts at the catchment-scale or have taken a gradient approach to isolate the effects of specific stressors (e.g., nutrient and pesticide concentrations; Davies & Jackson, 2006; Yates & Bailey, 2010a; Hausmann et al., 2016). A gradient approach infers that stream ecosystems will respond to future impacts in direct accordance with identified spatial patterns in stressor-response relationships (Jarvie et al., 2013). Gradient studies have provided a more targeted understanding of how quantitative differences in a stressor affect stream communities and many have reported that stream communities respond nonlinearly to stressor gradients (Allan, 2004; Clements & Rohr, 2009; D'Amario et al., 2019). For example, stream communities often exhibit a threshold response to increased environmental stress where little to no further change in community structure is observed after a critical point has been surpassed (Groffman et al., 2006; Dodds et al., 2010; Hilderbrand & Utz, 2015). However, because human impacts in highly developed regions often occur on streams exposed to existing environmental stressors, these nonlinearities in stressor-response relationships have the potential to mask the cumulative effects of additional human impacts on stream conditions in factorial before-after comparisons (Clements et al., 2016). Indeed, although local stream communities can be resistant to additional degradation, cumulative human impacts may decrease the resilience of stream communities and have farther-reaching consequences throughout the stream network. Moreover, ill-informed management decisions from suboptimal impact assessments may promote environmental degradation across larger spatial scales. Thus, in order to improve and further protect imperilled stream ecosystems, there is a need for a more robust understanding of how cumulative impacts from human activities influence streams draining highly developed regions.

Nutrient (i.e., nitrogen and phosphorus) enrichment is a pervasive chemical stressor that affects many streams draining developed regions (Dodds & Smith, 2016; Wurtsbaugh et al., 2019). Human impacts from point (e.g., sewage effluent) and nonpoint (e.g., agriculture) sources of pollution can cumulatively increase instream nitrogen and phosphorus concentrations and degrade the structure and function of stream ecosystems through accelerated eutrophication (Mainstone & Parr, 2002; Withers & Jarvie, 2008). Eutrophication is characterized by excessive primary production (i.e., sestonic and benthic algae, and macrophytes) and while enriched nutrient concentrations can increase the biomass of primary producers, past studies have often reported threshold or breakpoint type relationships between stream degradation by eutrophication and nutrient concentrations (Evans-White et al., 2013; Jarvie et al., 2013; Heiskary & Bouchard, 2015). The ecological implications of stream nutrient enrichment have been well established (e.g., Biggs, 2000; Friberg et al., 2010; Woodward et at., 2012) and because point sources can be readily identified, there has been a substantive amount of research on the nutrient impacts of treated sewage effluents (see Hamdhani et al., 2020). For example, many studies have reported an increase in tolerant and decrease in sensitive benthic macroinvertebrate taxa downstream of sewage outfalls compared to upstream reference reaches (e.g., Quinn & Hickey, 1993; Ortiz et al., 2005; Englert et al., 2013). Assemblage-level differences have also been observed in benthic algal communities (e.g., Chambers et al., 1997; Scrimgeour & Chambers, 2000; Bowman et al., 2005). Most of these past studies occurred in streams with either low upstream nutrient concentrations or a pronounced spike in concentration due to input of sewage with little or no treatment. However, in developed regions sewage effluent is often discharged into streams already enriched with nutrients from upstream nonpoint sources, such as agricultural lands, resulting in more pronounced cumulative effects and nonlinear responses. The point source discharge of effluent into agricultural streams thus provides an opportunity to determine if the cumulative effects of nutrient enrichment can be detected with general and more nuanced assessment approaches that account for nonlinearities associated with environmental stressors.

Our goal was to demonstrate the challenges of impact assessments in highly developed regions by evaluating the cumulative effects of nutrient enrichment on instream ecological communities using both a general and more targeted study design. First, a multiple before-after (upstream-downstream) control-impact assessment was used to conduct a factorial evaluation of the generalized impact that treated sewage effluent has on ecological communities (i.e., benthic diatoms and macroinvertebrates) of agricultural streams. Second, a targeted nutrient enrichment gradient assessment was used to evaluate the association between upstream-downstream differences in stream communities and the extent of nitrogen and phosphorus enrichment from human nutrient sources in stream catchments. Third, we tested if accounting for nonlinear stressor-response relationships when evaluating cumulative effects would provide a more robust understanding of how increased nutrient enrichment influences ecological communities in streams draining highly developed regions.

2. Methods

2.1 Study Area and Design

We assessed the cumulative impact of nutrients from agriculture and sewage effluent on 14 streams in the mixed-wood plains ecozone (Crins et al., 2009) of southern Ontario, Canada (Figure 1). Southern Ontario is characterized by a humid, temperate climate with an average annual temperature between 4.9 and 7.8 ^{o}C and total precipitation from 759 to 1087 mm (Crins et al., 2009). The underlying physiography consists of post-glacial surface geologies dominated by mixed agricultural land covers (i.e., crop and livestock operations) with patches of natural (i.e., forest and wetland) and urban areas throughout the landscape (Yates & Bailey, 2010b).

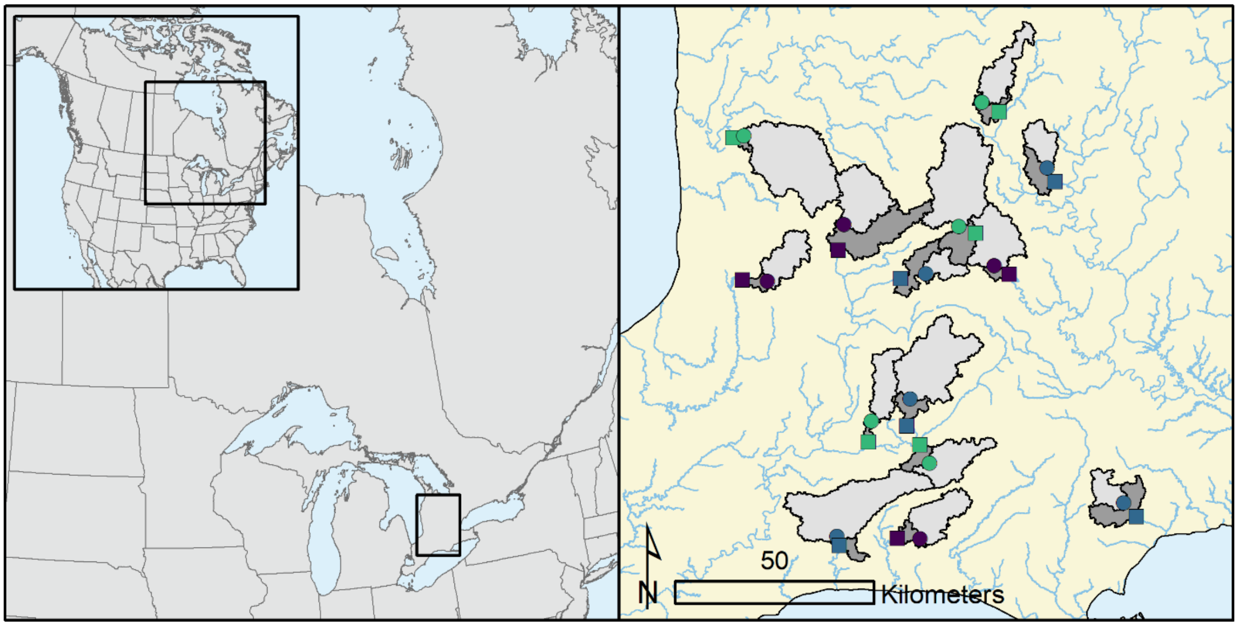

Figure 1: Location of the 14 study streams in southern Ontario, Canada. Upstream sampling locations are symbolized by circles and light grey catchments. Downstream sampling locations are symbolized by squares and dark grey catchments correspond to the additional drainage area captured by the nested stream reach. Green, purple, and blue symbols correspond to watersheds exposed to agriculture, agriculture + passively treated lagoon sewage effluent, and agriculture + actively treated mechanical sewage effluent, respectively.

Study streams were selected to have comparable catchment sizes, land cover composition, and surface geology (glacial till), but differ in their exposure to anthropogenic sources of nutrients (Table 1). All 14 streams were exposed to nutrients from agricultural activities upstream in study catchments with 9 of those streams also being exposed to nutrients from point sources of municipal sewage effluent. Of the 9 effluent receiving streams, 5 streams were exposed to actively treated sewage effluent from mechanical sewage treatment plants and 4 streams were exposed to passively treated sewage effluent from sewage lagoons (a.k.a. wastewater stabilization ponds). Operational differences between active and passive sewage treatment plants are described in Pearce et al. (2020).

To evaluate the potential impact of anthropogenic nutrient enrichment, two paired sampling sites were established on each study stream. Sites were located upstream and downstream of identified point sources or a comparable longitudinal distance apart along agricultural streams (without point sources). Catchment size was on average (± standard deviation) 170 ± 107 km^2 for upstream sites and 198 ± 123 km^2 for downstream sites. Average (± standard deviation) land cover composition was largely similar between upstream and downstream study catchments (agriculture upstream = 85 ± 6 % versus downstream = 83 ± 8 %; urban upstream = 2 ± 2 % versus downstream = 4 ± 4 %; natural upstream = 13 ± 6 % versus downstream = 13 ± 5 %).

Table 1: Properties of upstream and downstream sampling locations on the 14 study streams in southern Ontario, Canada. Distance (Dist.) between sampling locations reflects longitudinal distance along the stream network and downstream distance of sampling locations from identified point sources is provided in brackets. Agriculture (Agri.), urban (Urb.), and natural (Nat.) land cover was summarized from the Agriculture and Agri-Food Canada Annual Crop Inventory (2013) dataset.

		Upstream				Downstream			
Stream Name	Dist.	Area	Agri.	Urb.	Nat.	Area	Agri.	Urb.	Nat.
Agriculture	(km)	(km^2)	(%)	(%)	(%)	(km^2)	(%)	(%)	(%)
Mallet River	6.7	111.6	84.2	1.2	14.5	144.1	84.4	1.2	14.4
Reynolds Creek	5.9	123.4	85.9	0.7	12.9	153.0	86.0	0.8	12.8
S. Maitland River	4.4	359.4	87.8	1.1	10.1	371.5	87.6	1.1	10.3
Upper Nith River	6.4	311.8	89.5	1.3	9.3	377.2	88.0	1.3	10.6
Waubuno Creek	7.3	98.6	87.1	0.5	12.1	106.4	86.4	1.0	12.3
Mean	*6.11*	*201.0*	*86.9*	*0.9*	*11.8*	*230.4*	*86.5*	*1.1*	*12.1*
Standard Deviation	*1.1*	*124.4*	*2.0*	*0.3*	*2.1*	*132.5*	*1.5*	*0.2*	*1.7*
Lagoon									
Ausable River	7.1 (1.2)	100.5	88.5	0.7	10.7	115.8	86.4	3.0	10.3
Catfish Creek	8.7 (2.3)	119.1	86.9	2.2	10.8	141.8	84.3	5.1	10.2
Lower Nith River	12.2 (1.1)	217.4	79.4	2.4	18.0	546.4	84.4	2.7	12.9
North Thames River	7.1 (2.6)	166.6	93.7	0.8	5.5	323.6	91.4	2.1	6.4
Mean	*8.8 (1.8)*	*150.9*	*87.1*	*1.5*	*11.2*	*281.9*	*86.6*	*3.2*	*10.0*
Standard Deviation	*2.4 (0.8)*	*52.3*	*5.9*	*0.9*	*5.1*	*199.1*	*3.3*	*1.3*	*2.7*
Mechanical									
Avon River	6.7 (1.1)	53.6	83.6	2.7	13.3	115.6	71.2	14.8	13.5
Canagagigue Creek	5.7 (1.6)	65.4	85.3	0.6	13.1	115.9	81.8	6.3	11.2
Kettle Creek	4.0 (1.4)	329.3	80.9	6.5	12.4	354.5	78.3	9.1	12.3
Lynn River	7.2 (1.6)	59.1	67.2	1.8	30.6	138.0	61.7	9.0	29.1
Middle Thames River	9.1 (2.4)	269.7	84.4	0.6	14.9	304.9	84.0	1.5	14.5
Mean	*6.5 (1.6)*	*155.4*	*80.3*	*2.4*	*16.9*	*205.8*	*75.4*	*8.1*	*16.1*
Standard Deviation	*1.9 (0.5)*	*133.3*	*7.5*	*2.5*	*7.7*	*114.8*	*9.1*	*4.8*	*7.4*
Mean	7.0 (1.7)	170.4	145.4	3.3	21.1	236.3	198.2	8.5	29.0
Standard Deviation	2.0 (0.6)	106.9	92.4	5.5	13.0	140.1	123.0	8.4	17.3

2.2 Sample Collection and Processing

Grab water samples were collected at each sampling site every three weeks from May to September of 2013 (n = 8). Samples were collected in the thalweg of each stream at 60% depth and stored at 4 ^{o}C prior to overnight transport to the National Laboratory for Environmental Testing (Environment and Climate Change Canada) for colorimetric determination of total phosphorus (TP) and total nitrogen concentrations (TN). Detection limits were 0.0005 mg P L^{-1} for TP and 0.015 mg N L^{-1} for TN.

Benthic diatom and macroinvertebrate assemblages were sampled in September 2013. Diatoms were sampled by collecting biofilm from 10 randomly selected cobbles throughout the defined sampling reach. Composite biofilm samples were preserved in dark bottles with Lugols iodine (~ 1% v/v) and subsampled for taxonomic identification. Biofilm subsamples were digested in 800 µL of 100% (v/v) nitric acid for 48 hours and 200 µL of hydrogen peroxide 30% (v/v) for an additional 48 hours. Digested samples were rinsed to remove nitric acid and were mounted with Naphrax® on prepared microscope slides (refractive index: 1.74; Brunel microscopes Ltd.,Wiltshire, UK). Diatoms valves were enumerated (minimum 400) with use of a Reichert-Jung Polyvar microscope equipped with differential interference contrast (magnification 1250x). Diatom frustules were identified to lowest possible taxonomic, usually species, following Lavoie et al. (2008b).

Benthic macroinvertebrates were sampled following the Canadian Aquatic Biomonitoring Network (CABIN) protocols (Reynoldson et al., 2012), which consisted of a 3-minute traveling kick sample (400 micron D-frame net) over a defined sampling reach (six times the bankfull width). Macroinvertebrates were fixed with 10% buffered formalin and preserved in 75% ethanol. Collected macroinvertebrate samples were subsampled at random with the use of a

Marchant box until a minimum of 300 individuals were enumerated. Subsampled macroinvertebrates were identified to genus or family.

2.3 Data Analysis

Consistency in taxonomic resolution was achieved for benthic macroinvertebrate samples where individuals were enumerated at the family and genus levels following Velk et al. (2004). In brief, if greater than 20% of individuals in a taxon were identified to the family level, then all individuals from the lower genus levels would be included in the family level count. However, if less than 20% of individuals were identified to the family level, and all samples had at least one individual enumerated at the genus level for that taxon, then the family level data was removed from analysis. In cases where less than 20% of individuals were identified to the family level, but not all samples had individuals at the genus level, all individuals were adjusted to the family level. Following taxonomic adjustments, any taxon that was present in less than 5% of the samples was declared as rare and was removed for dissimilarity analyses, unless the number of individuals for the taxon within an individual sample was greater than 5% of total individuals counted. Removal of rare taxa was completed for both benthic diatom and macroinvertebrate assemblages. Rare taxa were not removed in the calculation of community composition indices.

2.4 Diatom and Macroinvertebrate Indices

The composition of benthic diatom and macroinvertebrate assemblages were described using common bioassessment indices (see Barbour et al., 1999; Table 2). Diatom assemblages were described by the Eastern Canadian Diatom Index (IDEC; Lavoie et al., 2014), pollution tolerance index (PTI_D; Barbour et al., 1999; Muscio, 2002), percent abundance of high nutrient taxon (%high-NP; Potapova & Charles, 2007), percent abundance of low nutrient taxon (%low-

NP; Potapova & Charles, 2007), taxon richness (s_D), and Bray-Curtis dissimilarity ($dissimilarity_D$). Benthic macroinvertebrate assemblages were described using the Hilsenhoff Family Biotic Index (FBI; Hilsenhoff, 1998), pollution tolerance index (PTI_{BMI}; *aka* enrichment tolerance in Krynak & Yates, 2018), percent Ephemeroptera, Plecoptera, and Tricoptera abundance (%EPT), percent Diptera abundance (%Dipt.), taxon richness (s_{BMI}), and Bray-Curtis dissimilarity ($dissimilarity_{BMI}$). Bray-Curtis dissimilarity matrices were calculated for benthic diatom and macroinvertebrate assemblages from Hellinger transformed taxon abundance data with the *vegan* package in R (Oksanen et al., 2019). Pairwise dissimilarities between sampling locations on each stream were used to evaluate the effect of different human impact categories.

Table 2: Description of benthic diatom and macroinvertebrate indices used to evaluate nutrient enrichment. Predicted direction of association between nutrient enrichment and each ecological indicator is denoted by positive (+) and negative (–) symbols.

Indicator	Description	Nutrient Enrichment Effect
Diatoms		
IDEC	Eastern Canadian Diatom Index used to assess biological integrity associated with stream eutrophication	–
PTI_D	Metric summarizing the pollution sensitivity of taxa present in an assemblage	–
%high-NP	Proportional abundance of taxon associated with nutrient enriched environments	+
%low-NP	Proportional abundance of taxon associated with nutrient poor environments	–
s_D	Taxon richness or number of unique taxa at lowest possible taxonomic resolution	–
$Dissimilarity_D$	Pairwise Bray-Curtis dissimilarity between assemblages	+
Macroinvertebrates		
FBI	Hilsenhoff Family Biotic Index used to assess biological integrity associated with organic pollution	+
PTI_{BMI}	Metric summarizing the pollution sensitivity of taxa present in an assemblage	+
%EPT	Proportional abundance of Ephemeroptera, Plecoptera, and Tricoptera taxa	–
%Dipt.	Proportional abundance of Diptera taxa	+
s_{BMI}	Taxon richness or number of unique taxa at lowest possible taxonomic resolution	–
$Dissimilarity_{BMI}$	Pairwise Bray-Curtis dissimilarity between assemblages	+

2.5 Statistical Analyses

Two statistical approaches were used to evaluate the cumulative impact of nutrients from agriculture and sewage effluent on instream ecological communities. First, a multiple BACI assessment model was used to evaluate differences ($\alpha = 0.05$) in the mean of each dependent variable (TN, TP, IDEC, PTI_D, %high-NP, %low-NP, and s_D, FBI, PTI_{BMI}, %EPT, %Dipt., s_{BMI},) associated with sampling location (i.e., upstream or before, and downstream or after) and human impact (i.e., agriculture or control, and passive/active effluent or impact). Human impact was evaluated in separate analyses (1) as a three-level factor accounting for the operational differences of passive and active sewage treatment facilities and (2) as a two-level factor where effluent treatment was amalgamated to increase statistical power. An interaction term (sampling location: human impact) was included in the model to determine if differences in measured variables between sampling locations depended on the presence/type of human impact. Stream ID was included as a random effect term to account for site nestedness. Mixed model analyses of variance for BACI assessments were performed with the *nlme* package in R version 3.6.1 (Pinherio et al., 2020). The sampling location : human impact interaction term represents the BACI contrast and is equal to an analysis of variance between the stream specific differences (downstream – upstream) in each dependent variable and human impact (i.e., agriculture, passive effluent, and active effluent). Because measures of Bray-Curtis dissimilarity correspond to stream specific differences in assemblage composition, a one factor analysis of variance was performed between the pairwise dissimilarity ($\text{dissimilarity}_{\text{D}}$ and $\text{dissimilarity}_{\text{BMI}}$) measures for each study stream and human impact. For all models, Cohen's d effect size and 90% confidence intervals were calculated from the stream specific differences (downstream – upstream) in each dependent variable to discern differences in the cumulative effects of sewage effluent (i.e., all sewage effluent, passive sewage effluent, active sewage effluent) on agricultural rivers. Analyses were performed with the *stats* and *effsize* packages in R (R Core Team, 2020; Torchiano, 2020).

Second, the potential cumulative effects of increased nitrogen and phosphorus concentrations on the upstream-downstream differences in stream communities were evaluated through a gradient assessment approach with data from streams exposed to agriculture only and sewage effluent. Multiple regression analyses were used to determine the association ($\alpha = 0.05$) between longitudinal differences (downstream – upstream) in TP and TN (independent variables) and longitudinal differences in individual ecological indicators (dependent variables). The downstream cumulative effects of TP and TN were assessed directly based on the absolute difference in concentration. However, stream communities, particularly benthic algae, have been commonly found to exhibit nonlinear (threshold) response to low-level nutrient enrichment (e.g., Biggs, 2000; Stevenson et al., 2008; Black et al., 2011; Taylor et al., 2014) and thus the effect of increased nutrient enrichment may be conditional on upstream ecosystem conditions (Lacoursière et al., 2011; Clements et al., 2016). Thus, in addition to absolute differences in concentration, a separate assessment was conducted with TP and TN concentrations that were inverse (reciprocal) transformed prior to differencing (1/downstream – 1/upstream) to increased the numerical weight of nutrient inputs into streams that had lower upstream nutrient concentrations (Figure 2). For example, the absolute difference in concentration would be the same for a stream that increased from 0.01 mg L^{-1} TP to 0.03 mg L^{-1} TP as a stream that increased from 0.05 mg L^{-1} TP to 0.07 mg L^{-1} TP, but the inverse transformed differences would be about 11-fold greater for the stream with the lower upstream TP concentration. Independent variables were normalized prior to analysis such that greater values correspond to increased cumulative effects of nutrients. Statistical analyses were performed in R with the *stats* package (R Core Team, 2020).

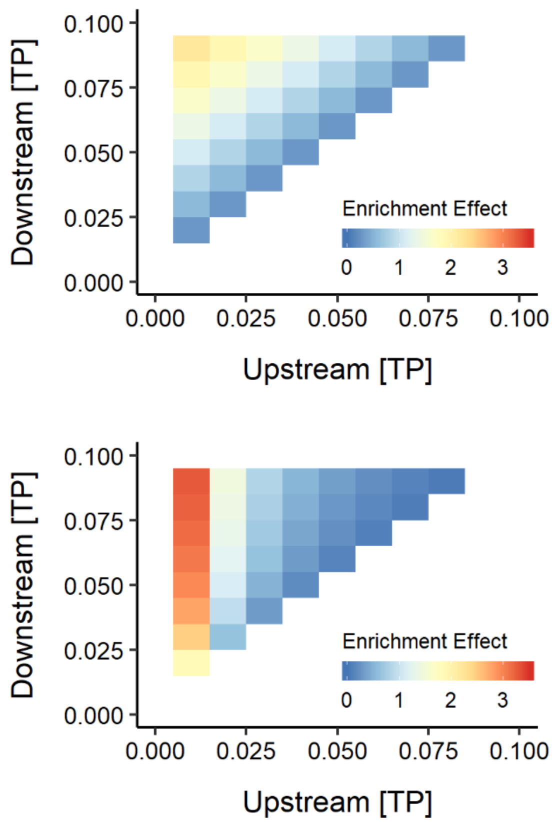

Figure 2: Illustration of the mean normalized enrichment effect of nutrients (colour gradient) based on (a) absolute concentration differencing and (b) inverse concentration differencing of hypothetical total phosphorus (TP) concentrations (mg L^{-1}) between upstream and downstream study sties.

3. Results and Discussion

3.1 Multiple Before-After Control-Impact Assessment

Nutrient concentrations in agricultural streams and at upstream sampling locations in effluent impacted streams were on average (± standard deviation) 0.055 ± 0.034 mg P L^{-1} for TP and 5.25 ± 1.27 mg N L^{-1} for TN (Figure 3). Past studies in southern Ontario have reported similar total nutrient concentrations in streams that drain agricultural catchments (Raney & Eimers, 2014; Thomas et al., 2018; DeBues et al., 2019; Pearce et al., 2020) and compared to regional nutrient criteria (0.024 mg P L^{-1} for TP; 1.07 mg N L^{-1} for TN) most control streams in our study had enriched nutrient concentrations (Chambers et al., 2012). Sewage effluent was observed to cumulatively increase stream nutrient concentrations at downstream sampling locations. Mean nutrient concentrations were 0.144 ± 0.136 mg P L^{-1} for TP and 6.40 ± 1.68 mg N L^{-1} for TN downstream of lagoon sewage effluent, and 0.104 ± 0.085 mg P L^{-1} for TP and 6.81 ± 2.64 mg N L^{-1} for TN downstream of mechanically treated sewage effluent (Figure 3). Many studies have shown increased nutrient concentrations in stream reaches that receive effluent compared to upstream reaches (see Carey & Migliaccio, 2009; Hamdhani et al., 2020). However, while we observed an increase in mean nutrient concentration downstream of sewage outfalls, a significant ($p < 0.05$) sampling location : human impact interaction term was not detected in multiple BACI assessment models of TN and TP (Table S1) suggesting that sewage effluent was not impacting nutrient concentrations of agricultural streams.

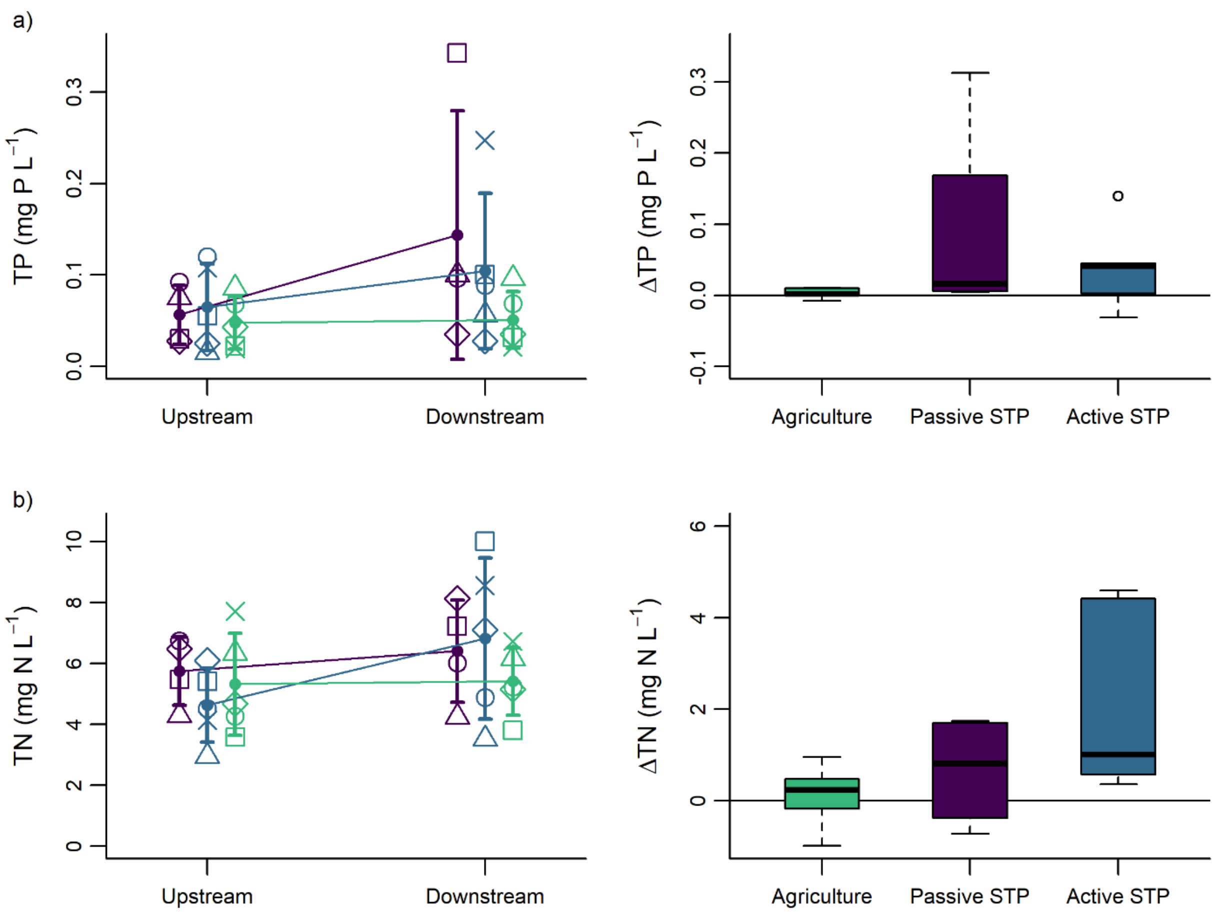

Figure 3: Mixed model analysis of variance for multiple before-after (upstream-downstream) control-impact (agriculture-sewage effluent) assessment of (a) total phosphorus (TP) and (b) total nitrogen (TN) concentrations. Green, purple, and blue colours correspond to streams exposed to agriculture, agriculture + passively treated lagoon sewage effluent, and agriculture + actively treated mechanical sewage effluent, respectively. Open symbols correspond to nutrient concentrations for individual study streams and filled points represent the mean ± one standard deviation within each human impact category. Longitudinal differences (downstream – upstream) in nutrient concentrations are summarized by boxplots (min, 25th percentile, 50th percentile, 75th percentile, max) for each human impact category.

Longitudinal differences (downstream – upstream) in nutrient concentrations were highly variable among streams that received sewage effluent (-0.03 to 0.31 mg P L^{-1} for TP; -0.73 to 4.60 mg N L^{-1} for TN) compared to agricultural streams (-0.008 to 0.01 mg P L^{-1} for TP; -0.99 to 0.95 mg N L^{-1} for TN) that received no point source inputs of effluent (Figure 3). Variable water quality impacts of sewage effluent have also been reported in past studies (Quinn & Hickey, 1993; Chambers et al., 1997; Scrimgeour & Chambers, 2000; Bowman et al., 2005) and are often associated with operational differences (e.g., effluent quality, compliance limits, discharge volume, and dilution factor) in sewage treatment facilities (Carey & Migliaccio, 2009). For example, Welch et al., (1992) observed a 1 to 103-fold increase in dissolved reactive phosphorus and a 1 to 176-fold increase in dissolved inorganic nitrogen among seven streams in New Zealand that received effluent from point sources. Although primary operational differences in sewage treatment facilities were accounted for in our three-level study design (i.e., passive versus active treatment), high variability in the sewage nutrient load and its dilution appears to have confounded our ability to detect a generalizable cumulative effect of sewage effluent on the nutrient concentrations of agricultural streams when summarised categorically.

Among the 12 ecological indices evaluated in our study (IDEC, PTI_D, %high-NP, %low-NP, s_D, $dissimilarity_D$, FBI, PTI_{BMI}, %EPT, %Dipt., s_{BMI}, and $dissimilarity_{BMI}$), no significant BACI interaction effects were observed in three-level human impact models (Table S1). Our finding of no difference in any of the ecological indices suggests that the discharge of sewage effluent had no influence on longitudinal patterns in benthic diatom and macroinvertebrate assemblages of agricultural streams. All upstream reaches in our study, as well as downstream reaches in streams without point sources, were affected by agricultural nutrient enrichment and thus may have been comprised of tolerant communities resistant to further changes from the addition of sewage effluent (Lavoie et al., 2008a). Although it is not uncommon to observe community tolerance to cumulative stress (Gücker et al., 2006; Burdon et al., 2016; 2019), like TP and TN concentrations, we observed high variability in the differences of measured ecological indicators between sampling locations within all three human impact categories (Figure 4; Figure S-1). Given the known effects of sewage effluent on instream biological communities (e.g., Quinn & Hickey, 1993; Scrimgeour & Chambers, 2000; Bowman et al., 2005;

Englert et al., 2013), it appears more likely that high variability in measured ecological indicators at the categorical level masked any generalizable impact of exposure to sewage effluent.

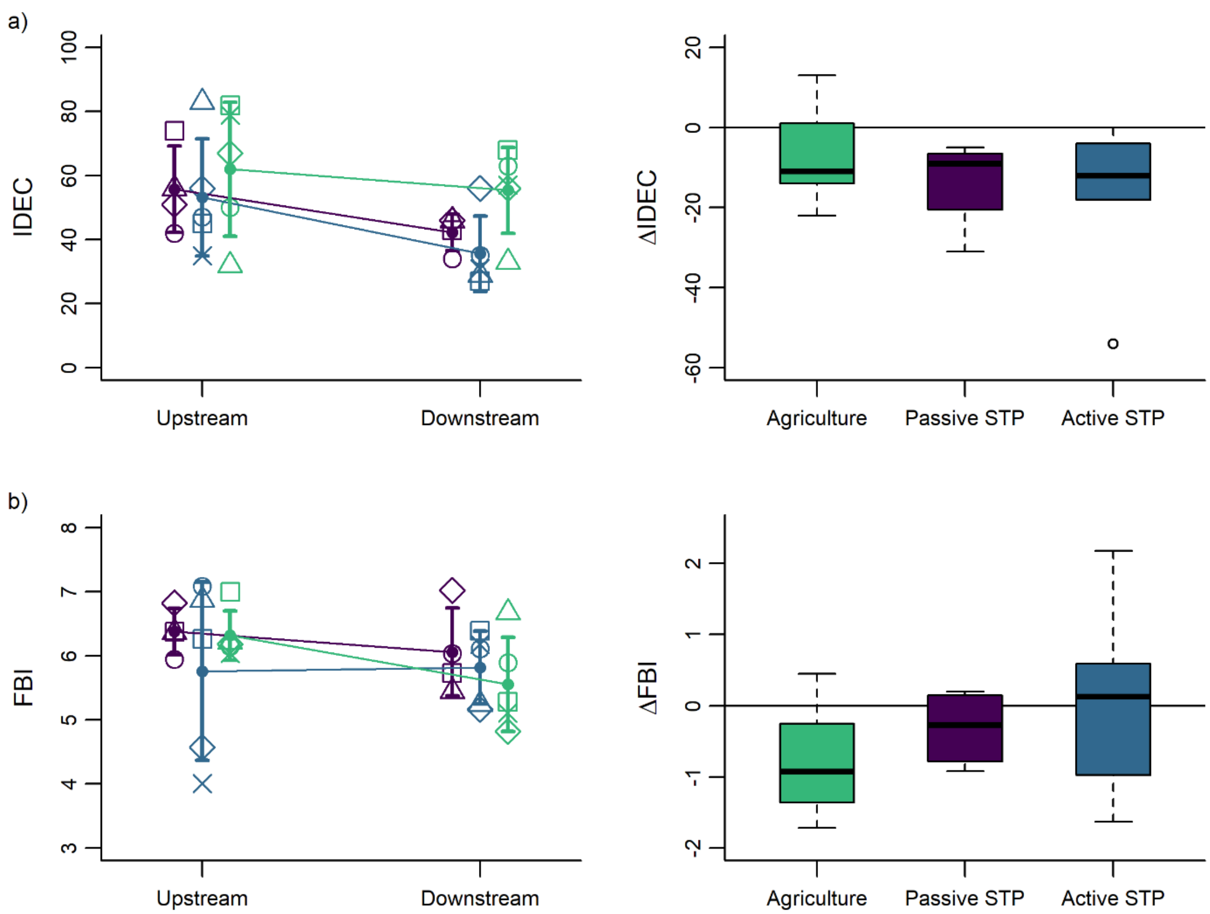

Figure 4: Mixed model analysis of variance for modified before-after (upstream-downstream) control-impact (agriculture-sewage effluent) assessment of (a) the eastern Canadian diatom index (IDEC) and (b) the Hilsenhoff family biotic index (FBI). Green, purple, and blue colours correspond to streams exposed to agriculture, agriculture + passively treated lagoon sewage effluent, and agriculture + actively treated mechanical sewage effluent, respectively. Open symbols correspond to indicator values for individual study streams and filled points represent the mean ± one standard deviation within each human impact category. Longitudinal differences (downstream – upstream) in ecological indicators are summarized by boxplots (min, 25th percentile, 50th percentile, 75th percentile, max) for each human impact category. Results for other ecological indicators are depicted in the supplementary material (Figure S1).

Our study was limited to a small number of potential sampling locations within each human impact category in order to minimize variation in catchment physiography, land cover composition, and reach characteristics, in addition to other logistical constraints (e.g., site access). Small sample sizes likely accentuated the variability observed in nutrient concentrations downstream of sewage treatment facilities leading to large variation in ecological indices among sampling locations. Increased variability in stream conditions within human impact categories likely resulted in the lack of significant BACI interaction effects among streams. Although the multiple BACI experimental design is an effective method to establish generalizable conclusions on the effects of an identifiable impact on stream ecosystem conditions (e.g., Keough & Quinn, 2000; Ried & Quinn, 2004; Roberts et al., 2007; Moreno et al., 2007), suboptimal study designs or assessments with high variability have the potential to result in the false conclusion of an effect (i.e., false positive/type I error) or the false conclusion that there is no effect (i.e., false negative/type II error) (Smokorowski & Randall, 2016). Because of the importance of impact assessments in guiding management interventions, both types of error can have severe implications for environmental management (Keough & Mapstone, 1997). For example, falsely concluding that there is no effect of an impact can result in the further degradation of ecological conditions. Post hoc improvements to study designs (i.e., amalgamation of related human impact levels) and increasing the alpha value (i.e., probability of type I error) in hypothesis tests can lower the probability of type II error and increase the statistical power to detect differences in ecological variables. Where study design is limited by sample size, post hoc leniency is common when extra caution regarding an environmental impact is warranted (Hanson, 2011; Murtaugh, 2014). Thus, it is important to critically interpret the results from BACI experiments, especially experiments with suboptimal study designs or high variability (Smokorowski & Randall, 2016).

Evaluating our three-level BACI assessment results at an alpha value of 0.1, we found that two ecological indices had significant BACI interaction terms: s_{BMI} (Sampling Location : Human Impact, $F_{(2,11)} = 3.27$, $p = 0.077$) and dissimilarity$_D$ (Sampling Location : Human Impact,

$F_{(2,11)} = 3.34$, $p = 0.073$). No additional significant BACI interaction terms were identified in two-level models that combined passive and active sewage effluent impact categories (Table S1), but the probability of type I error decreased for both s_{BMI} (Sampling Location : Human Impact, $F_{(1,12)} = 3.90$, $p = 0.072$) and dissimilarity$_D$ (Sampling Location : Human Impact, $F_{(1,12)} = 6.60$, $p = 0.025$). Richness of benthic macroinvertebrate assemblages (s_{BMI}) decreased downstream of point sources with streams that received actively treated sewage effluent having a significantly larger reduction in richness compared to streams exposed to agricultural activities alone (Cohen's d, 1.451 [0.129, 2.773]; Table S2). However, longitudinal differences in benthic macroinvertebrate richness in agricultural streams did not differ in comparison to streams that received passively treated sewage effluent. Likewise, Bray Curtis dissimilarity of diatom assemblages (dissimilarity$_D$) increased downstream of point sources with streams that received actively treated sewage effluent having assemblages that were significantly more dissimilar than streams exposed to agricultural activities alone (Cohen's d, 1.833 [0.432, 3.235]), but longitudinal differences in agricultural streams again did not differ from streams that received passively treated effluent. Although the response observed in these two ecological indicators corresponds to the predicted effect of increased nutrient enrichment, our findings do not provide strong support for a generalizable conclusion on the cumulative impact of sewage effluent on ecological communities of agricultural streams.

Past studies on the impact of sewage treatment facilities have commonly attributed variation in the response of stream communities to the concentration and bioavailability of effluent downstream of point sources (Welch et al., 1992; Chambers et al., 1997; Scrimgeour & Chambers, 2000; Bowman et al., 2005). For example, Quinn and Hickey (1993) found that the density of common benthic macroinvertebrate taxa sampled upstream and downstream of eight sewage treatment lagoons differed from about 75 to 15% in association with a 6 to 484-fold difference in effluent dilution factor of downstream reaches. Although upstream sampling locations in these past studies often had lower nutrient concentrations than were measured in our study, the among stream variation that we observed in the longitudinal patterns of benthic diatom and macroinvertebrate assemblages may also be explained by the extent that downstream changes in agricultural activities and the discharge of sewage effluent increase stream nutrient concentrations. Therefore, a gradient study design targeting quantitative changes in stream nutrient enrichment may be better suited to evaluate the cumulative effects of sewage effluent on ecological communities of agricultural streams than a more generalized factorial approach.

3.2 Nutrient Enrichment Gradient

Multiple regression analyses between longitudinal differences (downstream – upstream) in absolute nutrient concentrations (normalized) and measured ecological indicators revealed two significant ($\alpha = 0.05$) models (Table S3; Figure 5; Figure S2). Increased inputs of TP were negatively associated with the diatom pollution tolerance index (PTI_D), indicative of a greater increase in abundance of pollution tolerant taxa ($p = 0.008$, adjusted $R^2 = 0.40$). Likewise, richness of benthic macroinvertebrate assemblages (s_{BMI}) was observed to decrease downstream of cumulative TN inputs ($p = 0.027$, adjusted $R^2 = 0.35$). Increasing the leniency in model evaluation ($\alpha = 0.1$), two other ecological indicators were found to be associated with increased nutrient enrichment. In contrast to benthic macroinvertebrate assemblages, diatom assemblage richness (s_D) increased in association with TP enrichment ($p = 0.065$, adjusted $R^2 = 0.15$), but assemblages were observed to be more dissimilar ($Dissimilarity_D$) between sampling locations with greater differences in TP concentration ($p = 0.071$, adjusted $R^2 = 0.35$). Longitudinal differences in the Hilsenhoff family biotic index (FBI) also increased (indicative of increased organic pollution) with TN enrichment ($p = 0.052$, adjusted $R^2 = 0.21$). Indeed, our finding of multiple significant associations between stream nutrient enrichment and longitudinal differences in ecological indicators provides greater support for the cumulative effects of nutrients on instream ecological communities compared to the factorial evaluation of human impact categories. However, the statistical support for multiple regression models between absolute nutrient concentrations and measured ecological indicators is likely not strong enough to provide defensible conclusions on the effects of nutrient enrichment due to the relaxed error assumptions in the majority of models that were considered significant.

A potential explanation for weak statistical models between stream specific differences in nutrient concentration and ecological indicators is the nonlinear response of instream ecological communities to environmental stressors. Many past studies have reported threshold or breakpoint type relationships between ecological communities and stream nutrient concentrations or associated land use gradients (e.g., Stevenson et al., 2008; Black et al., 2011; Evans-White et al., 2013; Heiskary & Bouchard, 2015; Grimstead et al., 2019). For example, Taylor et al., (2014) observed increased dissimilarity in diatom assemblage composition associated with increasing TP concentrations among oligotrophic to mesotrophic streams, but no difference in dissimilarity among mesotrophic to eutrophic streams. Longitudinal differences in absolute nutrient concentrations do not account for the background or ambient nutrient status of streams that receive nutrient enrichments. Thus, the expectation that larger nutrient inputs should be associated with greater differences in ecological conditions may be confounded by the pre-existing effect of upstream nutrient concentrations on stream communities (Lacoursière et al., 2011; Taylor et al., 2018). For example, Burdon et al. (2016) found that benthic macroinvertebrate communities in streams that drained catchments with intensive agricultural land uses were more resistant to eutrophication-mediated disturbance from sewage effluent. Therefore, the environmental context of upstream, pre-exposed reaches may need to be quantitatively accounted for to fully understand the anthropogenic pressures associated with nutrient enrichment on stream ecosystems.

Inverse (reciprocal) transformation of stream nutrient concentrations prior to upstream-downstream differencing was used to increase the numerical weight of cumulative nutrient inputs to streams with lower upstream concentrations compared to the same cumulative nutrient input in streams with enriched upstream nutrient concentrations. Multiple regression analyses between longitudinal differences in inverse transformed nutrient concentrations and measured ecological indicators revealed seven significant ($\alpha = 0.05$) models with one addition ecological indicator having a significant model at an alpha value of 0.1 (Table S3; Figure 5; Figure S2).

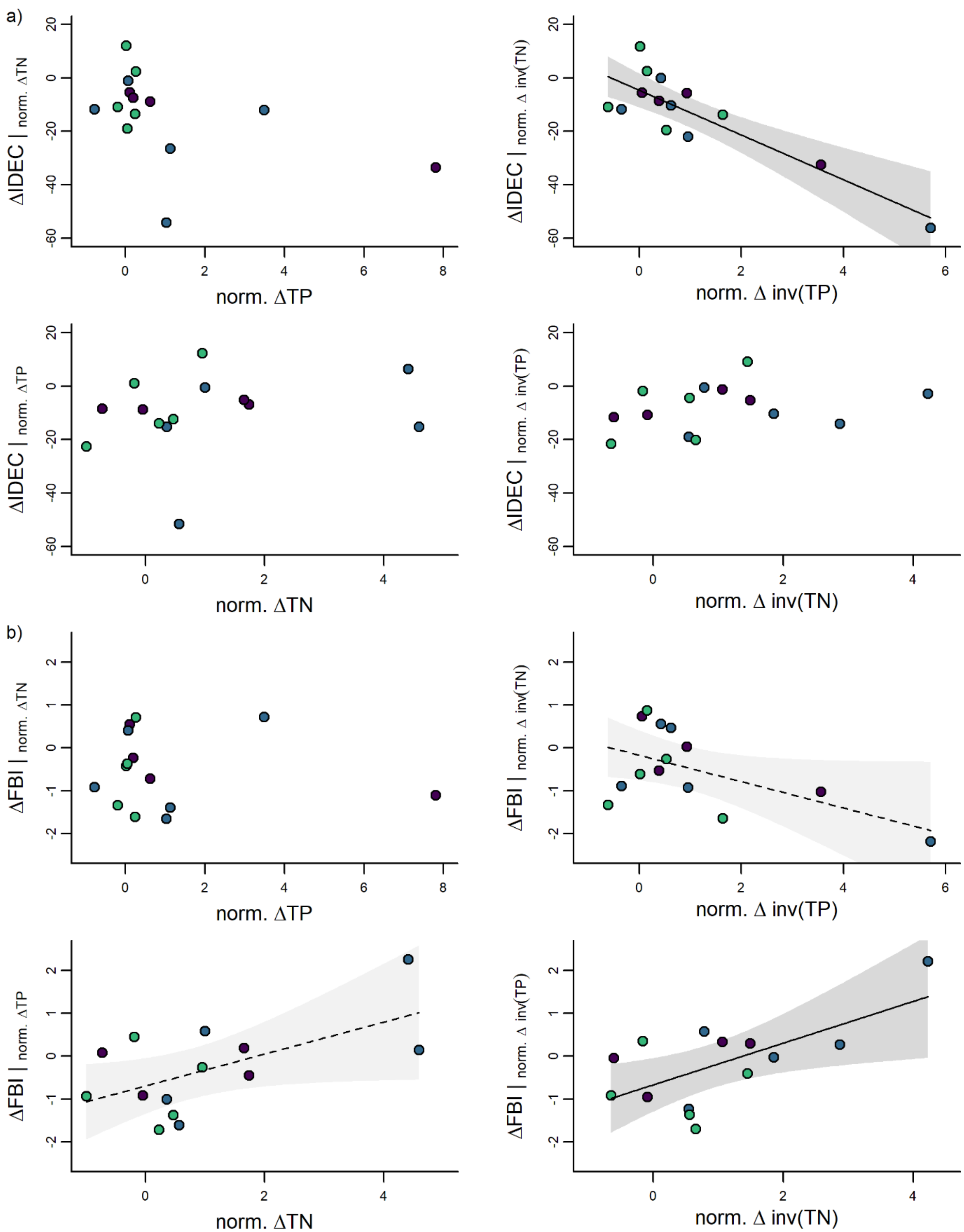

Figure 5: Multiple regression models of the association between normalized (norm.) longitudinal differences (downstream – upstream) in untransformed and inverse transformed (inv.) total phosphorus (TP) and total nitrogen (TN) concentrations and measures of (a) the eastern Canadian diatom index (IDEC) and (b) the Hilsenhoff family biotic index (FBI). Green, purple, and blue points correspond to streams exposed to agriculture, agriculture +

passively treated lagoon sewage effluent, and agriculture + actively treated mechanical sewage effluent, respectively. Solid trend lines correspond to significant associations at $\alpha = 0.05$ and dashed trend lines correspond to significant associations at $\alpha = 0.10$. Figures for other ecological indices are located in the supplementary material (Figure S2).

Three indicators of diatom assemblage composition had significant models: IDEC, %high-NP, and Dissimilarity_D. Longitudinal differences in the IDEC were negatively associated with greater enrichment of TP and indicative of increased pollution ($p < 0.001$, adjusted $R^2 = 0.68$). In contrast, longitudinal differences in the relative abundance of high nutrient taxa (%high-NP; $p = 0.052$, adjusted $R^2 = 0.32$) and assemblage dissimilarity (Dissimilarity_D; $p = 0.024$, adjusted $R^2 = 0.44$) were positively associated with cumulative inputs of TP.

Five indicators of benthic macroinvertebrate assemblage composition had significant models: FBI, PTI_{BMI}, %EPT, %Dipt., and s_{BMI}. Longitudinal differences in the Hilsenhoff family biotic index (FBI; $p = 0.021$, adjusted $R^2 = 0.36$), pollution tolerance index (PTI_{BMI}; $p = 0.037$, adjusted $R^2 = 0.24$), and Diptera relative abundance (%Dipt.; $p = 0.038$, adjusted $R^2 = 0.24$) were positively associated with TN enrichment and indicative of poorer ecological conditions. Similarly, TN enrichment was associated with poorer ecological conditions through negative associations with longitudinal differences in Ephemeroptera, Plecoptera, and Trichoptera relative abundance (%EPT; $p = 0.038$, adjusted $R^2 = 0.32$) and richness (s_{BMI}; $p = 0.014$, adjusted $R^2 = 0.34$). However, in contrast to the associations observed with TN, TP enrichment was negatively associated with longitudinal differences in the Hilsenhoff family biotic index (FBI; $p = 0.056$, adjusted $R^2 = 0.36$) and positively associated with differences in the relative abundances of Ephemeroptera, Plecoptera, and Trichoptera taxa ($p = 0.052$, adjusted $R^2 = 0.32$). However, contrasting directions of association within these two models may be a result of leniency in error assumptions as both associations with TP had increased probabilities of concluding a false positive (type I error).

Accounting for nonlinearities in associations with cumulative effects of nutrients resulted in an improvement in the number and strength of significant models between longitudinal differences in nutrient enrichment and ecological indicators (Table 3). In contrast to more general approaches used to evaluate our dataset, the inverse transformed nutrient gradient provided stronger evidence to support the negative effects of cumulative nutrient enrichment in streams. Our finding that ecological indicators measured in streams of lower nutrient status changed more substantially downstream of nutrient inputs compared to streams of higher nutrient status is consistent with the nonlinear response of stream communities to increased stress (Burdon et al., 2016; 2019). Therefore, the cumulative effects of sewage effluent and/or increased nutrient loading from agricultural activities appear to be context dependent based on ambient (upstream) stream conditions.

Table 3: Summary of significant models for the three statistical approached used to evaluate the effect of nutrient enrichment and on indices of benthic diatom and macroinvertebrate assemblages. Positive (+) and negative (–) symbols indicate the direction of association for significant models with one and two symbols indicating significance at $\alpha = 0.10$ and $\alpha = 0.05$, respectively.

Indicator	Multiple-BACI	Untransformed Enrichment Gradient		Inv. Transformed Enrichment Gradient	
		TP	TN	TP	TN
Diatoms					
IDEC				– –	
PTI_D		– –			
%high-NP				+	
%low-NP					
S_D		+			
$Dissimilarity_D$	+	+		+ +	
Macroinvertebrates					
FBI			+	–	+ +
PTI_{BMI}					+ +
%EPT				+	– –
%Dipt.					+ +
S_{BMI}	–		– –		– –
$Dissimilarity_{BMI}$					

3.3 Summary and Conclusions

We demonstrated that cumulative nutrient inputs to streams can negatively influence biological communities, but that this conclusion would not have been realized with more general assessment approaches. Results form our multiple BACI assessment suggested that sewage effluent had no effect on measured ecological indicators and that upstream agricultural activities likely resulted in degradation to stream communities (e.g., loss of sensitive taxa). However, we expect that quantitative differences in environmental stressors associated with the operation of sewage treatment facilities resulted in our inability to qualitatively detect a generalizable, system-wide impact of sewage effluent. Moreover, although results from our gradient assessment that quantitatively evaluated the influence of longitudinal differences in nitrogen and phosphorus concentrations on stream communities adhered to the anticipated effects of nutrient enrichment, these findings also did not provide strong statistical support to make defensible conclusions regarding ecological impacts of nutrient enrichment. Given the well-established nonlinear effect of nutrient enrichment on stream communities (e.g., Evans-White et al., 2013; Heiskary & Bouchard, 2015), we further refined our gradient assessment and transformed our nutrient enrichment gradient to increase the numerical weight of nutrient inputs that occurred in streams with low ambient nutrient concentrations. Accounting for the nonlinearity of nutrient enrichment effects reduced the unexplained variability observed in longitudinal differences in ecological indicators among streams and provided a more robust understanding of how stream communities respond to cumulative increases in nutrient concentrations. However, our inverse transformed nutrient enrichment gradient best represents ecological indicators that have a logarithmic-like stressor-response relationship. Different stressors or ecological indicators that have alternative nonlinear associations with increased nutrient concentrations (e.g., state shift) may therefore require independently refined statistical consideration in cumulative effects assessments.

Ecological monitoring and assessment studies are needed to evaluate human impacts on stream ecosystems and provide defensible information to guide mitigation actions. Even though considerable effort was taken during site selection to isolate the impact of sewage effluent, variability associated with complex, multi-use catchments in highly developed regions appears to have limited the ability of factorial assessment approaches to detect differences in diatom and benthic macroinvertebrate indices. Potential differences in other sewage effluent contaminants (e.g., micropollutants, synthetic chemicals, metals, and organic matter) and study design constraints (e.g., sample size and distance between sampling locations) may have further contributed to the residual variance in assessment models and resulted in disconcerted conclusions. However, despite potential covariates, we did observe that nutrient enrichment from sewage effluent was associated with the downstream degradation of biological community structure independent of human activity categories in our gradient assessment. Simple BACI assessments conducted on ecological indicators replicated spatially or temporally within individual impact streams would therefore have likely yielded significant BACI interaction effects for some of the effluent receiving streams in our study. However, these simple assessments would be subject to limitations associated with pseudo-replication, restricted generalizability, and diagnostic interpretation. To provide basin-wide management recommendations on the effects of human impacts in highly developed regions, researchers may therefore need to go beyond standard assessment approaches and more explicitly consider the environmental stressors and their associated complexities related to the impact under investigation (e.g., Burdon et al., 2016).

From a management perspective, our finding that nutrient enrichment effects were only evident in our transformed nutrient enrichment gradient assessment indicates that the cumulative effects of nutrient enrichment are highly conditional on upstream ecosystem conditions. Thus, while communities of more degraded streams may be comprised of tolerant taxa and unaffected by further nutrient enrichment, communities of less degraded streams may change dramatically from increased nutrient inputs. Managers should therefore consider the trophic status of streams on a case-by-case basis to ensure that the most effective strategies are employed to conserve or improve stream ecosystem conditions. For example, nutrient load reductions from point sources (e.g., sewage treatment plants) would provide the most benefit to communities of streams with lower nutrient concentrations (oligotrophic/mesotrophic), but in streams with high nutrient concentrations (eutrophic) prioritizing the reduction of nutrient loading from nonpoint sources (e.g., agriculture) may be required before the benefits of cumulative load reductions can be observed in local stream communities.

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References

Allan, J.D., 2004. Landscapes and riverscapes: The influence of land use on stream ecosystems. Annu. Rev. Ecol. Evol. Syst. 35, 257–284. https://doi.org/10.1146/annurev.ecolsys.35.120202.110122

Álvarez-Cabria, M., Barquín, J., Antonio Juanes, J., 2010. Spatial and seasonal variability of macroinvertebrate metrics: Do macroinvertebrate communities track river health? Ecol. Indic. 10, 370–379. https://doi.org/10.1016/j.ecolind.2009.06.018

Angeler, D.G., Moreno, J.M., 2007. Zooplankton community resilience after press-type anthropogenic stress in temporary ponds. Ecol. Appl. 17, 1105–1115. https://doi.org/10.1890/06-1040

Barbour, M.T., Gerritsen, J., Snyder, B.D., Stribling, J.B., 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.

Biggs, B.J.F., 2000. Eutrophication of streams and rivers: dissolved nutrient-chlorophyll relationships for benthic algae. J. North Am. Benthol. Soc. 19, 17–31. https://doi.org/10.2307/1468279

Black, R.W., Moran, P.W., Frankforter, J.D., 2011. Response of algal metrics to nutrients and physical factors and identification of nutrient thresholds in agricultural streams. Environ. Monit. Assess. 175, 397–417. https://doi.org/10.1007/s10661-010-1539-8

Bowman, M.F., Chambers, P.A., Schindler, D.W., 2005. Epilithic algal abundance in relation to anthropogenic changes in phosphorus bioavailability and limitation in mountain rivers. Can. J. Fish. Aquat. Sci. 62, 174–184. https://doi.org/10.1139/f04-244

Burdon, F.J., Munz, N.A., Reyes, M., Focks, A., Joss, A., Räsänen, K., Altermatt, F., Eggen, R.I.L., Stamm, C., 2019. Agriculture versus wastewater pollution as drivers of macroinvertebrate community structure in streams. Sci. Total Environ. 659, 1256–1265. https://doi.org/10.1016/j.scitotenv.2018.12.372

Burdon, F.J., Reyes, M., Alder, A.C., Joss, A., Ort, C., Räsänen, K., Jokela, J., Eggen, R.I.L., Stamm, C., 2016. Environmental context and magnitude of disturbance influence trait-mediated community responses to wastewater in streams. Ecol. Evol. 6, 3923–3939. https://doi.org/10.1002/ece3.2165

Carey, R.O., Migliaccio, K.W., 2009. Contribution of wastewater treatment plant effluents to nutrient dynamics in aquatic systems: A review. Environ. Manage. 44, 205–217. https://doi.org/10.1007/s00267-009-9309-5

Chambers, P.A., Allard, M., Walker, S.L., Marsalek, J., Lawrence, J., Servos, M., Busnarda, J., Munger, K.S., Adare, K., Jefferson, C., Kent, R.A., Wong, M.P., 1997. Impacts of municipal wastewater effluents on canadian waters: A review. Water Qual. Res. J. 32, 659–714. https://doi.org/10.2166/wqrj.1997.038

Clements, W.H., Kashian, D.R., Kiffney, P.M., Zuellig, R.E., 2016. Perspectives on the context-dependency of stream community responses to contaminants. Freshw. Biol. 61, 2162–2170. https://doi.org/10.1111/fwb.12599

Clements, W.H., Rohr, J.R., 2009. Community responses to contaminants: using basic ecological principles to predict ecotoxicological effects. Environ. Toxicol. Chem. 28, 1789–800. https://doi.org/10.1897/09-140.1

Crins, W.J., Gray, P.A., Uhlig, P.W., Wester, M.C., 2009. The Ecosystems of Ontario, Part 1: Ecozones and Ecoregions. Minist. Nat. Resour. Sci. Inf. Branch, Invent. Monit. Assess. Sect. Tech. Rep. SIB TER IMA TR-01 1–76.

D'Amario, S.C., Rearick, D.C., Fasching, C., Kembel, S.W., Porter-Goff, E., Spooner, D.E., Williams, C.J., Wilson, H.F., Xenopoulos, M.A., 2019. The prevalence of nonlinearity and detection of ecological breakpoints across a land use gradient in streams. Sci. Rep. 9, 3878. https://doi.org/10.1038/s41598-019-40349-4

Davies, S.P., Jackson, S.K., 2006. The biological condition gradient: A descriptive model for interpreting change in aquatic ecosystems. Ecol. Appl. 16, 1251–1266. https://doi.org/10.1890/1051-0761(2006)016[1251:TBCGAD]2.0.CO;2

DeBues, M.J., Eimers, M.C., Watmough, S.A., Mohamed, M.N., Mueller, J., 2019. Stream nutrient and agricultural land-use trends from 1971 to 2010 in Lake Ontario tributaries. J. Great Lakes Res. 45, 752–761. https://doi.org/10.1016/j.jglr.2019.05.002

Dodds, W.K., Clements, W.H., Gido, K., Hilderbrand, R.H., King, R.S., 2010. Thresholds, breakpoints, and nonlinearity in freshwaters as related to management. J. North Am. Benthol. Soc. 29, 988–997. https://doi.org/10.1899/09-148.1

Dodds, W., Smith, V., 2016. Nitrogen, phosphorus, and eutrophication in streams. Inl. Waters 6, 155–164. https://doi.org/10.5268/IW-6.2.909

Downes, B.J., Barmuta, L.A., Fairweather, P.G., Faith, D.P., Keough, M.J., Lake, P.S., Mapstone, B.D., Quinn, G.P., 2002. Monitoring Ecological Impacts: Concepts and Practice in Flowing Waters. Cambridge University Press, Cambridge.

Englert, D., Zubrod, J.P., Schulz, R., Bundschuh, M., 2013. Effects of municipal wastewater on aquatic ecosystem structure and function in the receiving stream. Sci. Total Environ. 454–455, 401–410. https://doi.org/10.1016/j.scitotenv.2013.03.025

Evans-White, M.A., Haggard, B.E., Scott, J.T., 2013. A review of stream nutrient criteria development in the united states. J. Environ. Qual. 42, 1002–1014. https://doi.org/10.2134/jeq2012.0491

Friberg, N., Skriver, J., Larsen, S.E., Pedersen, M.L., Buffagni, A., 2009. Stream macroinvertebrate occurrence along gradients in organic pollution and eutrophication. Freshw. Biol. 55, 1405–1419. https://doi.org/10.1111/j.1365-2427.2008.02164.x

Galic, N., Sullivan, L.L., Grimm, V., Forbes, V.E., 2018. When things don’t add up: quantifying impacts of multiple stressors from individual metabolism to ecosystem processing. Ecol. Lett. 21, 568–577. https://doi.org/10.1111/ele.12923

Green, R.H., 1979. Sampling design and statistical methods for environmental biologist. Wiley Interscience, Chichester, UK.

Grimstead, J.P., Krynak, E.M., Yates, A.G., 2018. Scale-specific land cover thresholds for conservation of stream invertebrate communities in agricultural landscapes. Landsc. Ecol. 33, 2239–2252. https://doi.org/10.1007/s10980-018-0738-5

Groffman, P.M., Baron, J.S., Blett, T., Gold, A.J., Goodman, I., Gunderson, L.H., Levinson, B.M., Palmer, M.A., Paerl, H.W., Peterson, G.D., Poff, N.L., Rejeski, D.W., Reynolds, J.F., Turner, M.G., Weathers, K.C., Wiens, J., 2006. Ecological thresholds: The key to successful environmental management or an important concept with no practical application? Ecosystems 9, 1–13. https://doi.org/10.1007/s10021-003-0142-z

Gücker, B., Brauns, M., Pusch, M.T., 2006. Effects of wastewater treatment plant discharge on ecosystem structure and function of lowland streams. J. North Am. Benthol. Soc. 25, 313–329. https://doi.org/10.1899/0887-3593(2006)25[313:EOWTPD]2.0.CO;2

Hamdhani, H., Eppehimer, D.E., Bogan, M.T., 2020. Release of treated effluent into streams: A global review of ecological impacts with a consideration of its potential use for environmental flows. Freshw. Biol. 65(9), 1657–1670. https://doi.org/10.1111/fwb.13519

Hanson, N., 2011. Using biological data from field studies with multiple reference sites as a basis for environmental management: The risks for false positives and false negatives. J. Environ. Manage. 92, 610–619. https://doi.org/10.1016/j.jenvman.2010.09.026

Hausmann, S., Charles, D.F., Gerritsen, J., Belton, T.J., 2016. A diatom-based biological condition gradient (BCG) approach for assessing impairment and developing nutrient criteria for streams. Sci. Tot. Environ. 562, 914–927. https://doi.org/10.1016/j.scitotenv.2016.03.173

Heiskary, S.A., Bouchard, R.W., 2015. Development of eutrophication criteria for Minnesota streams and rivers using multiple lines of evidence. Freshw. Sci. 34, 574–592. https://doi.org/10.1086/680662

Herlihy, A.T., Paulsen, S.G., Sickle, J. Van, Stoddard, J.L., Hawkins, C.P., Yuan, L.L., 2008. Striving for consistency in a national assessment: the challenges of applying a reference-condition approach at a continental scale. J. North Am. Benthol. Soc. 27, 860–877. https://doi.org/10.1899/08-081.1

Hillebrand, R.H., Utz, R.M., 2015. Ecological thresholds and resilience in streams. In: Rowinski, P., Radecki-Pawlik, A. (Eds.), Rivers – Physical, fluvial and environmental processes (pp. 461–478). Springer, New York, NY.

Hilsenhoff, W.L., 1988. Rapid field assessment of organic pollution with a family-level biotic index. J. North Am. Benthol. Soc. 7, 65–68. https://doi.org/10.2307/1467832

Hurlbert, S.H., 1984. Pseudoreplication and the design of ecological field experiments stuart. Ecol. Monogr. 54, 187–211.

Ibáñez, C., Caiola, N., Sharpe, P., Trobajo, R., 2005. Ecological Indicators to Assess the Health of River Ecosystems. In: Jørgensen, S., Xu, L., Constanza, R. (eds.) Handbook of Ecological Indicators for Assessment of Ecosystem Health. CRC Press Taylor and Francis Group, Boca Raton, FL.

Jarvie, H.P., Sharpley, A.N., Withers, P.J.A., Scott, J.T., Haggard, B.E., Neal, C., 2013. Phosphorus mitigation to control river eutrophication: Murky waters, inconvenient truths, and “postnormal” science. J. Environ. Qual. 42, 295–304. https://doi.org/10.2134/jeq2012.0085

Keough, M.J., Mapstone, B.D., 1997. Designing environmental monitoring for pulp mills in Australia. Water Sci. Technol.

Keough, M.J., Quinn, G.P., 2000. Legislative vs. Practical protection of an intertidal shoreline in southeastern Australia. Ecol. Appl. 10, 871–881. https://doi.org/10.1890/1051-0761(2000)010[0871:LVPPOA]2.0.CO;2

Krynak, E.M., Yates, A.G., 2018. Benthic invertebrate taxonomic and trait associations with land use in an intensively managed watershed: Implications for indicator identification. Ecol. Indic. 93, 1050–1059. https://doi.org/10.1016/j.ecolind.2018.06.002

Lacoursière, S., Lavoie, I., Rodríguez, M.A., Campeau, S., 2011. Modeling the reponse time of diatom assemblages to simulated water quality imporvement and degradation in running waters. Can. J. Fish. Aquat. Sci. 68(3), 487–497. https://doi.org/10.1139/F10-162

Lavoie, I., Campeau, S., Darchambeau, F., Cabana, G., Dillon, P.J., 2008a. Are diatoms good integrators of temporal variability in stream water quality? Freshw. Biol. 53, 827–841. https://doi.org/10.1111/j.1365-2427.2007.01935.x

Lavoie, I., Campeau, S., Zugic-Drakulic, N., Winter, J.G., Fortin, C., 2014. Using diatoms to monitor stream biological integrity in Eastern Canada: An overview of 10 years of index development and ongoing challenges. Sci. Tot. Environ. 475, 187–200. https://doi.org/10.1016/j.scitotenv.2013.04.092

Lavoie I., Hamilton, P.B., Campeau, S., Grenier, M., Dillon, P.J., 2008b. Guide d'identification des diatomées des riviéres de l'est du Canada, ed. Presses de l'Université du Québec (PUQ).

Lowe, R.L., Likens, G.E., 2005. Moving headwater streams to the head of the class. Bioscience 55, 196–197. https://doi.org/https://doi.org/10.1641/0006-3568(2005)055[0196:MHSTTH]2.0.CO;2

Mainstone, C.P., Parr, W., 2002. Phosphorus in rivers — ecology and management. Sci. Total Environ. 282–283, 25–47. https://doi.org/10.1016/S0048-9697(01)00937-8

Murtaugh, P.A., 2014. In defense of P values. Ecology 95, 611–617. https://doi.org/10.1093/jncics/pkaa012

Muscio, C., 2002. The diatom pollution tolerance index: Assigning tolerance values. City of Austin-Watershed Protection Development Review Department. SR-02-02.

Niemi, G.J., McDonald, M.E., 2004. Application of ecological indicators. Annu. Rev. Ecol. Evol. Syst. 35, 89–111. https://doi.org/10.1146/annurev.ecolsys.35.112202.130132

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2019. vegan: community ecology package. Version 2.5-6.

Ortiz, J.D., Martí, E., Puig, M.À., 2005. Recovery of the macroinvertebrate community below a wastewater treatment plant input in a Mediterranean stream. Hydrobiologia 545, 289–302. https://doi.org/10.1007/s10750-005-3646-z

Pearce, N.J.T., Yates, A.G., 2020. Spatial and temporal patterns in macronutrient concentrations and stoichiometry of tributaries draining the lower Great Lakes-St. Lawrence basin. J. Great Lakes Res. https://doi.org/10.1016/j.jglr.2020.05.002

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., 2020. nlme: Linear and nonlinear mixed effects models. Version 3.1-148.

Potapova, M., Charles, D.F., 2007. Diatom metrics for monitoring eutrophication in rivers of the United States. Ecol. Indic. 7, 48–70. https://doi.org/10.1016/j.ecolind.2005.10.001

Quinn, J.M., Hickey, C.W., 1993. Effects of sewage waste stabilization lagoon effluent on stream invertebrates. J. Aquat. Ecosyst. Heal. 2, 205–219. https://doi.org/10.1007/BF00047770

R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Raney, S.M., Eimers, M.C., 2014. Unexpected declines in stream phosphorus concentrations across southern Ontario. Can. J. Fish. Aquat. Sci. 71, 337–342. https://doi.org/10.1139/cjfas-2013-0300

Reid, M.A., Quinn, G.P., 2004. Hydrologic regime and macrophyte assemblages in temporary floodplain wetlands: Implications for detecting responses to environmental water allocations. Wetlands 24, 586–599. https://doi.org/10.1672/0277-5212(2004)024[0586:HRAMAI]2.0.CO;2

Reynoldson, T.B., Logan, C., Pascoe, T., Thompson, S.P., Strachan, S., Mackinlay, C., McDermott, H., Paull, T., 2012. Canadian Aquatic Biomonitoring Network field manual wadeable streams. Environment Canada, Dartmouth, Nova Scotia.

Roberts, D.A., Poore, A.G.B., Johnston, E.L., 2007. MBACI sampling of an episodic disturbance: Stormwater effects on algal epifauna. Mar. Environ. Res. 64, 514–523. https://doi.org/10.1016/j.marenvres.2007.04.005

Scrimgeour, G.J., Chambers, P.A., 2000. Cumulative effects of pulp mill and municipal effluents on epilithic biomass and nutrient limitation in a large northern river ecosystem. Can. J. Fish. Aquat. Sci. 57, 1342–1354. https://doi.org/10.1139/f00-057

Seitz, N.E., Westbrook, C.J., Noble, B.F., 2011. Bringing science into river systems cumulative effects assessment practice. Environ. Impact Assess. Rev. 31, 172–179. https://doi.org/10.1016/j.eiar.2010.08.001

Smokorowski, K.E., Randall, R.G., 2017. Cautions on using the Before-After-Control-Impact design in environmental effects monitoring programs. FACETS 2, 212–232. https://doi.org/10.1139/facets-2016-0058

Søndergaard, M., Jeppesen, E., 2007. Anthropogenic impacts on lake and stream ecosystems, and approaches to restoration. J. Appl. Ecol. 44, 1089–1094. https://doi.org/10.1111/j.1365-2664.2007.01426.x

Stevenson, R.J., Hill, B.H., Herlihy, A.T., Yuan, L.L., Norton, S.B., 2008. Algae–P relationships, thresholds, and frequency distributions guide nutrient criterion development. J. North Am. Benthol. Soc. 27, 783–799. https://doi.org/10.1899/07-077.1

Stewart-Oaten, A., Murdoch, W.W., Parker, K.R., 1986. Environmental impact assessment: “pseudoreplication” in time? Ecology 67, 929–940. https://doi.org/10.2307/1939815

Stoddard, J.L., Larsen, D.P., Hawkins, C.P., Johnson, R.K., Norris, R.H., 2006. Setting expectations for the ecological condition of streams: The concept of reference condition. Ecol. Appl. 16, 1267–1276. https://doi.org/10.1890/1051-0761(2006)016[1267:SEFTEC]2.0.CO;2

Taylor, J.M., Black, J.A., Brooks, B.W., King, R.S., 2018. Spatial, temporal and experimental: three study design cornerstones for establishing defensible numeric criteria in freshwater ecosystems. J. Appl. Ecol. 55(5), 2114–2123. https://doi.org/10.1111/1365-2664.13150

Taylor, J.M., King, R.S., Pease, A.A., Winemiller, K.O., 2014. Nonlinear response of stream ecosystem structure to low-level phosphorus enrichment. Freshw. Biol. 59, 969–984. https://doi.org/10.1111/fwb.12320

Thomas, K.E., Lazor, R., Chambers, P.A., Yates, A.G., 2018. Land-use practices influence nutrient concentrations of southwestern Ontario streams. Can. Water Resour. J. 43, 2–17. https://doi.org/10.1080/07011784.2017.1411211

Torchiano, M., 2020. effsize: Efficient effect size computation. Version 0.8.1.

Underwood, A.J., 1991. Beyond BACI: Experimental designs for detecting human environmental impacts on temporal variations in natural populations. Mar. Freshwater Res. 42, 569–587. https://doi.org/10.1071/MF9910569

Underwood, A.J., 1994. On beyond baci: Sampling designs that might reliably detect environmental disturbances. Ecol. Appl. 4, 3–15. https://doi.org/10.2307/1942110

Vlek, H.E., Verdonschot, P.F.M., Nijboer, R.C., 2004. Towards a multimetric index for the assessment of Dutch streams using benthic macroinvertebrates. Hydrobiologia 516, 173–189. https://doi.org/10.1023/B:HYDR.0000025265.36836.e1

Welch, E.B., Quinn, J.M., Hickey, C.W., 1992. Periphyton biomass related to point-source nutrient enrichment in seven New Zealand streams. Water Res. 26, 669–675. https://doi.org/10.1016/0043-1354(92)90243-W

Withers, P.J.A., Jarvie, H.P., 2008. Delivery and cycling of phosphorus in rivers: A review. Sci. Total Environ. 400, 379–395. https://doi.org/10.1016/j.scitotenv.2008.08.002

Woodward, G., Gessner, M.O., Giller, P.S., Gulis, V., Hladyz, S., Lecerf, A., Malmqvist, B., McKie, B.G., Tiegs, S.D., Cariss, H., Dobson, M., Elosegi, A., Ferreira, V., Graca, M.A.S., Fleituch, T., Lacoursiere, J.O., Nistorescu, M., Pozo, J., Risnoveanu, G., Schindler, M., Vadineanu, A., Vought, L.B.-M., Chauvet, E., 2012. Continental-scale effects of nutrient pollution on stream ecosystem functioning. Science (80-.). 336, 1438–1440. https://doi.org/10.1126/science.1219534

Wurtsbaugh, W.A., Paerl, H.W., Dodds, W.K., 2019. Nutrients, eutrophication and harmful algal blooms along the freshwater to marine continuum. Wiley Interdiscip. Rev. Water 6, 1–27. https://doi.org/10.1002/wat2.1373

Yates, A.G., Bailey, R.C., 2010a. Covarying patterns of macroinvertebrate and fish assemblages along natural and human activity gradients: Implications for bioassessment. Hydrobiol. 637, 87–100. https://doi.org/10.1007/s10750-009-9987-2

Yates, A.G., Bailey, R.C., 2010b. Improving the description of human activities potentially affecting rural stream ecosystems. Landsc. Ecol. 25, 371–382. https://doi.org/10.1007/s10980-009-9413-1

Supplementary Material

Table S1: Mixed model analysis of variance for modified before-after (Location) control-impact (Human Impact) assessment of nutrient concentrations, diatom assemblage indicators, and macroinvertebrate assemblage indicators. Analysis of variance results are reported for models with three (i.e., agriculture, passive sewage effluent, and active sewage effluent) and two (i.e., agriculture and sewage effluent) human impact factor levels. F and p values in bold represent significant models at α = 0.05 (*) and α = 0.10 (italic).

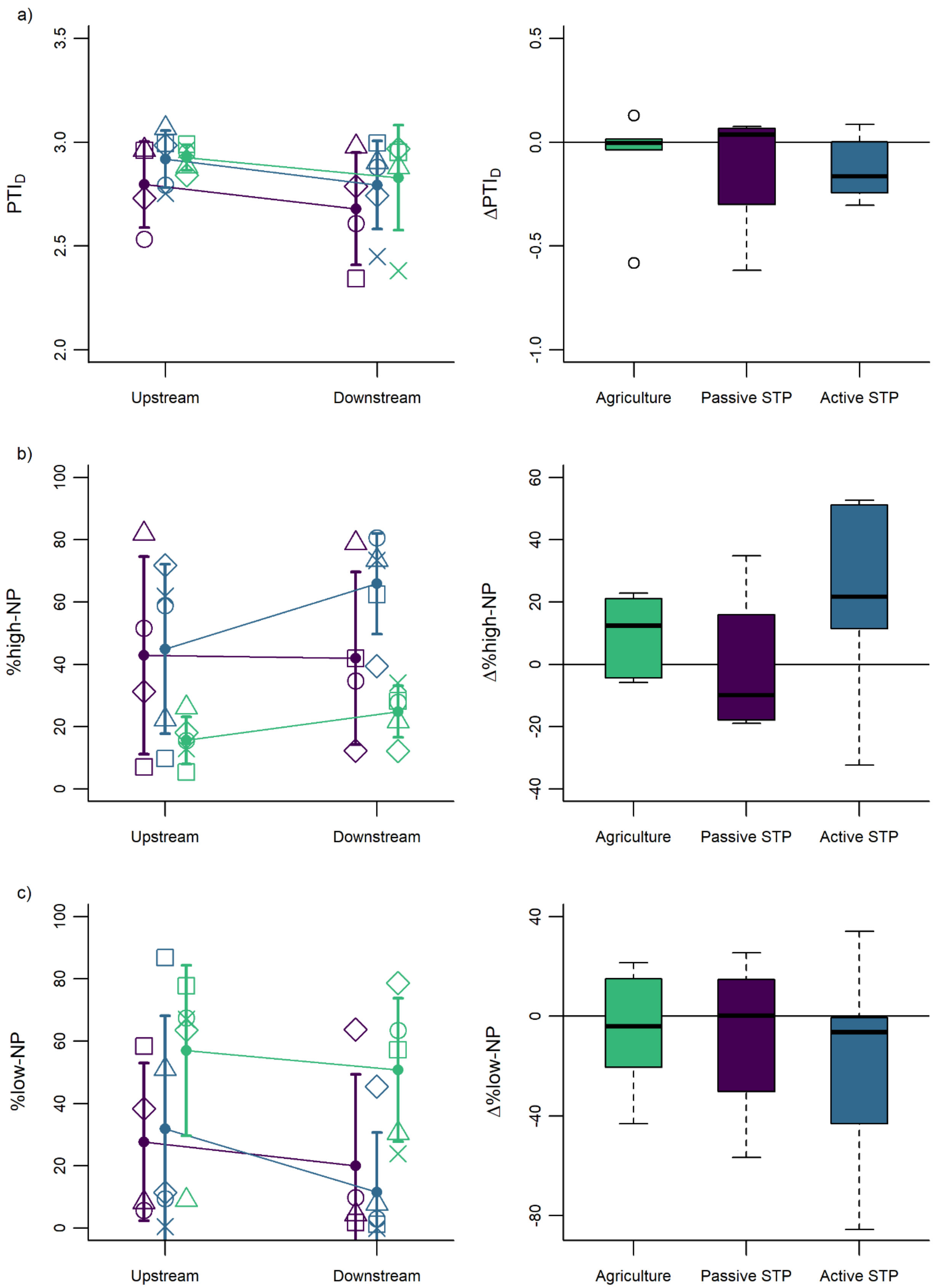
		Analysis of Variance	
Indicator	Predictor	3 Human Impact Levels	2 Human Impact Levels
Nutrients			
TP	Location	$F_{(1,11)}$ = 2.92, p = 0.116	$F_{(1,12)}$ = 3.00, p = 0.109
	Human Impact	$F_{(2,11)}$ = 1.12, p = 0.361	$F_{(1,12)}$ = 2.19, p = 0.165
	Location : Human Impact	$F_{(2,11)}$ = 1.03, p = 0.389	$F_{(1,12)}$ = 1.43, p = 0.255
TN	Location	**$F_{(1,11)}$ = 6.22, p = 0.030***	**$F_{(1,12)}$ = 5.61, p = 0.036***
	Human Impact	$F_{(2,11)}$ = 0.25, p = 0.785	$F_{(1,12)}$ = 0.40, p = 0.538
	Location : Human Impact	$F_{(2,11)}$ = 2.57, p = 0.121	$F_{(1,12)}$ = 2.56, p = 0.136
Diatoms			
IDEC	Location	**$F_{(1,11)}$ = 7.95, p = 0.017***	**$F_{(1,12)}$ = 8.57, p = 0.013***
	Human Impact	$F_{(2,11)}$ = 1.67, p = 0.233	***$F_{(1,12)}$ = 3.23, p = 0.098***
	Location : Human Impact	$F_{(2,11)}$ = 0.56, p = 0.587	$F_{(1,12)}$ = 1.06, p = 0.323
PTI$_D$	Location	$F_{(1,11)}$ = 2.58, p = 0.136	$F_{(1,12)}$ = 2.82, p = 0.119
	Human Impact	$F_{(2,11)}$ = 1.07, p = 0.377	$F_{(1,12)}$ = 0.74, p = 0.405
	Location : Human Impact	$F_{(2,11)}$ = 0.02, p = 0.984	$F_{(1,12)}$ = 0.03, p = 0.860
%high-NP	Location	$F_{(1,11)}$ = 2.27, p = 0.160	$F_{(1,12)}$ = 2.17, p = 0.167
	Human Impact	**$F_{(2,11)}$ = 5.71, p = 0.020***	**$F_{(1,12)}$ = 9.78, p = 0.009***
	Location : Human Impact	$F_{(2,11)}$ = 0.80, p = 0.475	$F_{(1,12)}$ = 0.02, p = 0.896
%low-NP	Location	$F_{(1,11)}$ = 1.43, p = 0.258	$F_{(1,12)}$ = 1.52, p = 0.241
	Human Impact	***$F_{(2,11)}$ = 3.84, p = 0.054***	**$F_{(1,12)}$ = 8.34, p = 0.014***
	Location : Human Impact	$F_{(2,11)}$ = 0.22, p = 0.808	$F_{(1,12)}$ = 0.18, p = 0.676
s$_D$	Location	$F_{(1,11)}$ = 0.00, p = 1.000	$F_{(1,12)}$ = 0.00, p = 1.000
	Human Impact	$F_{(2,11)}$ = 0.99, p = 0.402	$F_{(1,12)}$ = 1.90, p = 0.193
	Location : Human Impact	$F_{(2,11)}$ < 0.01, p = 0.991	$F_{(1,12)}$ = 0.01, p = 0.906
Dissimilarity$_D$	Location	–	–
	Human Impact	–	–
	Location : Human Impact	***$F_{(2,11)}$ = 3.34, p = 0.073***	**$F_{(1,12)}$ = 6.60, p = 0.025***
Benthic Macroinvertebrates			
FBI	Location	$F_{(1,11)}$ = 1.43, p = 0.258	$F_{(1,12)}$ = 1.52, p = 0.242
	Human Impact	$F_{(2,11)}$ = 0.63, p = 0.552	$F_{(1,12)}$ = 0.02, p = 0.896
	Location : Human Impact	$F_{(2,11)}$ = 0.73, p = 0.502	$F_{(1,12)}$ = 1.26, p = 0.283
PTI$_{BMI}$	Location	$F_{(1,11)}$ = 0.08, p = 0.787	$F_{(1,12)}$ = 1.12, p = 0.313
	Human Impact	$F_{(2,11)}$ = 0.33, p = 0.726	$F_{(1,12)}$ = 0.09, p = 0.776
	Location : Human Impact	$F_{(2,11)}$ = 0.11, p = 0.899	$F_{(1,12)}$ = 1.42, p = 0.257
%EPT	Location	$F_{(1,11)}$ = 0.02, p = 0.905	$F_{(1,12)}$ = 0.08, p = 0.777
	Human Impact	$F_{(2,11)}$ = 0.63, p = 0.552	$F_{(1,12)}$ = 0.03, p = 0.867
	Location : Human Impact	$F_{(2,11)}$ = 0.24, p = 0.789	$F_{(1,12)}$ = 0.20, p = 0.661
%Dipt.	Location	$F_{(1,11)}$ < 0.01, p = 0.979	$F_{(1,12)}$ < 0.01, p = 0.978
	Human Impact	$F_{(2,11)}$ = 0.05, p = 0.955	$F_{(1,12)}$ = 0.06, p = 0.817
	Location : Human Impact	$F_{(2,11)}$ = 0.23, p = 0.796	$F_{(1,12)}$ = 0.19, p = 0.668
s$_{BMI}$	Location	$F_{(1,11)}$ = 0.01, p = 0.908	$F_{(1,12)}$ = 0.01, p = 0.909
	Human Impact	$F_{(2,11)}$ = 0.43, p = 0.658	$F_{(1,12)}$ = 0.08, p = 0.388
	Location : Human Impact	***$F_{(2,11)}$ = 3.27, p = 0.077***	***$F_{(1,12)}$ = 3.90, p = 0.072***
Dissimilarity$_{BMI}$	Location	–	–
	Human Impact	–	–
	Location : Human Impact	$F_{(2,11)}$ = 0.06, p = 0.938	$F_{(1,12)}$ = 0.13, p = 0.726

Table S2: Cohen's d effect size and 90% confidence interval (CI) for comparisons of stream specific differences (downstream – upstream) in each dependent variable between agricultural and sewage effluent receiving streams. Bolded values indicate effect sizes that have 90% confidence intervals that do not overlap zero.

Indicator	Predictor	Cohen's d [90% CI]
Nutrients		
TP	All STP	0.667 [-0.352, 1.686]
	Passive STP	0.855 [-0.472, 2.182]
	Active STP	0.796 [-0.426, 2.018]
TN	All STP	0.892 [-0.147, 1.930]
	Passive STP	0.572 [-0.725, 1.868]
	Active STP	**1.314 [0.017, 2.611]**
Diatoms		
IDEC	All STP	0.574 [-0.438, 1.587]
	Passive STP	0.533 [-0.760, 1.826]
	Active STP	0.610 [-0.593, 1.813]
PTI_D	All STP	0.100 [-1.116, 1.316]
	Passive STP	0.069 [-1.202, 1.340]
	Active STP	0.125 [-1.052, 1.302]
%high-NP	All STP	0.074 [-1.141, 1.290]
	Passive STP	0.529 [-0.764, 1.822]
	Active STP	0.443 [-0.747, 1.634]
%low-NP	All STP	0.239 [-0.980, 1.458]
	Passive STP	0.048 [-1.222, 1.320]
	Active STP	0.378 [-0.809, 1.564]
s_D	All STP	0.068 [-1.148, 1.283]
	Passive STP	0.034 [-1.237, 1.305]
	Active STP	0.132 [-1.045, 1.309]
$Dissimilarity_D$	All STP	**1.433 [0.082, 2.784]**
	Passive STP	1.174 [-0.201, 2.549]
	Active STP	**1.833 [0.432, 3.235]**
Benthic Macroinvertebrates		
FBI	All STP	0.627 [-0.615, 1.869]
	Passive STP	0.589 [-0.709, 1.886]
	Active STP	0.677 [-0.532, 1.886]
PTI_{BMI}	All STP	0.664 [-0.581, -1.910]
	Passive STP	0.703 [-0.606, 2.012]
	Active STP	0.649 [-0.558, 1.855]
%EPT	All STP	0.251 [-0.969, 1.470]
	Passive STP	0.215 [-1.060, 1.489]
	Active STP	0.261 [-0.920, 1.442]
%Dipt.	All STP	0.245 [-0.974, 1.465]
	Passive STP	0.600 [-0.698, 1.900]
	Active STP	0.068 [-1.108, 1.245]
s_{BMI}	All STP	1.034 [-0.253, 2.322]
	Passive STP	0.703 [-0.582, 2.043]
	Active STP	**1.451 [0.129, 2.773]**
$Dissimilarity_{BMI}$	All STP	0.200 [-1.418, 1.018]
	Passive STP	0.217 [-1.058, 1.491]
	Active STP	0.149 [-1.029, 1.327]

Table S3: Independent cumulative effects multiple regression models between normalized stream specific differences (downstream – upstream) nutrient concentrations (untransformed and inverse transformed) and measured ecological indicators of benthic diatom and macroinvertebrate assemblages. Bolded coefficients (Coef.) and p values correspond to significant models at α = 0.05 (*) and α = 0.10 (italic). SE = standard error; Adj. = adjusted.

		Untransformed					Inverse Transformed				
Indicator	Predictor	Coef. (± SE)	p-value	$F_{(2,11)}$	*P*	Adj. R^2	Coef. (± SE)	p-value	$F_{(2,11)}$	*P*	Adj. R^2
Diatoms											
IDEC	TP	-3.18 (±2.24)	0.183	1.01	0.394	0.002	**-8.36 (±1.57)**	**< 0.001***	**14.47**	**< 0.001**	**0.675***
	TN	2.06 (±2.94)	0.497				1.81 (±1.97)	0.378			
PTI_D	TP	**-0.08 (±0.03)**	**0.008***	**5.35**	**0.024**	**0.400***	-0.06 (±0.04)	0.167	1.23	0.330	0.034
	TN	0.04 (±0.03)	0.325				-0.00 (±0.05)	0.942			
%high-NP	TP	2.98 (±3.56)	0.421	1.08	0.373	0.012	***7.86 (±3.61)***	***0.052***	**4.12**	**0.046**	**0.324***
	TN	3.43 (±4.68)	0.479				5.30 (±4.54)	0.268			
%low-NP	TP	-5.40 (±4.66)	0.271	1.42	0.282	0.061	-9.46 (±5.38)	0.107	2.27	0.149	0.164
	TN	-3.72 (±6.11)	0.555				-4.42 (±6.77)	0.527			
s_D	TP	***1.17 (±0.57)***	***0.065***	2.14	0.164	0.150	0.63 (±0.80)	0.449	0.34	0.719	-0.113
	TN	-0.47 (±0.75)	0.541				0.01 (±1.00)	0.989			
$Dissimilarity_D$	TP	***0.03 (±0.01)***	***0.071***	**4.48**	**0.038**	**0.349***	**0.04 (±0.02)**	**0.024**	**6.02**	**0.017**	**0.436***
	TN	0.02 (±0.02)	0.275				0.03 (±0.02)	0.181			
Macroinvertebrates											
FBI	TP	-0.03 (±0.13)	0.845	2.70	0.111	0.207	***-0.31 (±0.14)***	***0.056***	**4.65**	**0.034**	**0.360***
	TN	***0.37 (±0.17)***	***0.052***				**0.49 (±0.18)**	**0.021***			
PTI_{BMI}	TP	0.02 (±0.06)	0.754	1.88	0.198	0.119	-0.10 (±0.07)	0.186	***3.09***	***0.086***	***0.244***
	TN	0.13 (±0.08)	0.141				**0.21 (±0.09)**	**0.037***			
%EPT	TP	1.42 (±3.47)	0.691	0.80	0.474	-0.032	***7.52 (±3.45)***	***0.052***	**4.01**	**0.049**	**0.316***
	TN	-5.71 (±4.10)	0.235				**-10.25 (±4.34)**	**0.038***			
%Dipt.	TP	2.58 (±2.98)	0.327	2.24	0.153	0.160	-4.83 (±3.45)	0.189	***3.06***	***0.088***	***0.241***
	TN	5.44 (±3.91)	0.245				**10.23 (±2.36)**	**0.038***			
s_{BMI}	TP	-0.32 (±1.20)	0.794	**4.42**	**0.039**	**0.345***	0.61 (±1.48)	0.687	**4.34**	**0.041**	**0.339***
	TN	**-4.04 (±1.58)**	**0.027***				**-5.42 (±1.86)**	**0.014***			
$Dissimilarity_{BMI}$	TP	-0.01 (±0.01)	0.399	0.04	0.679	-0.102	-0.01 (±0.02)	0.624	0.128	0.881	-0.155
	TN	< 0.01 (±0.02)	0.836				< 0.01 (±0.02)	0.859			



a)
PTI_D
ΔPTI_D
b)
%high-NP
Δ%high-NP
c)
%low-NP
Δ%low-NP
Upstream
Downstream
Agriculture
Passive STP
Active STP

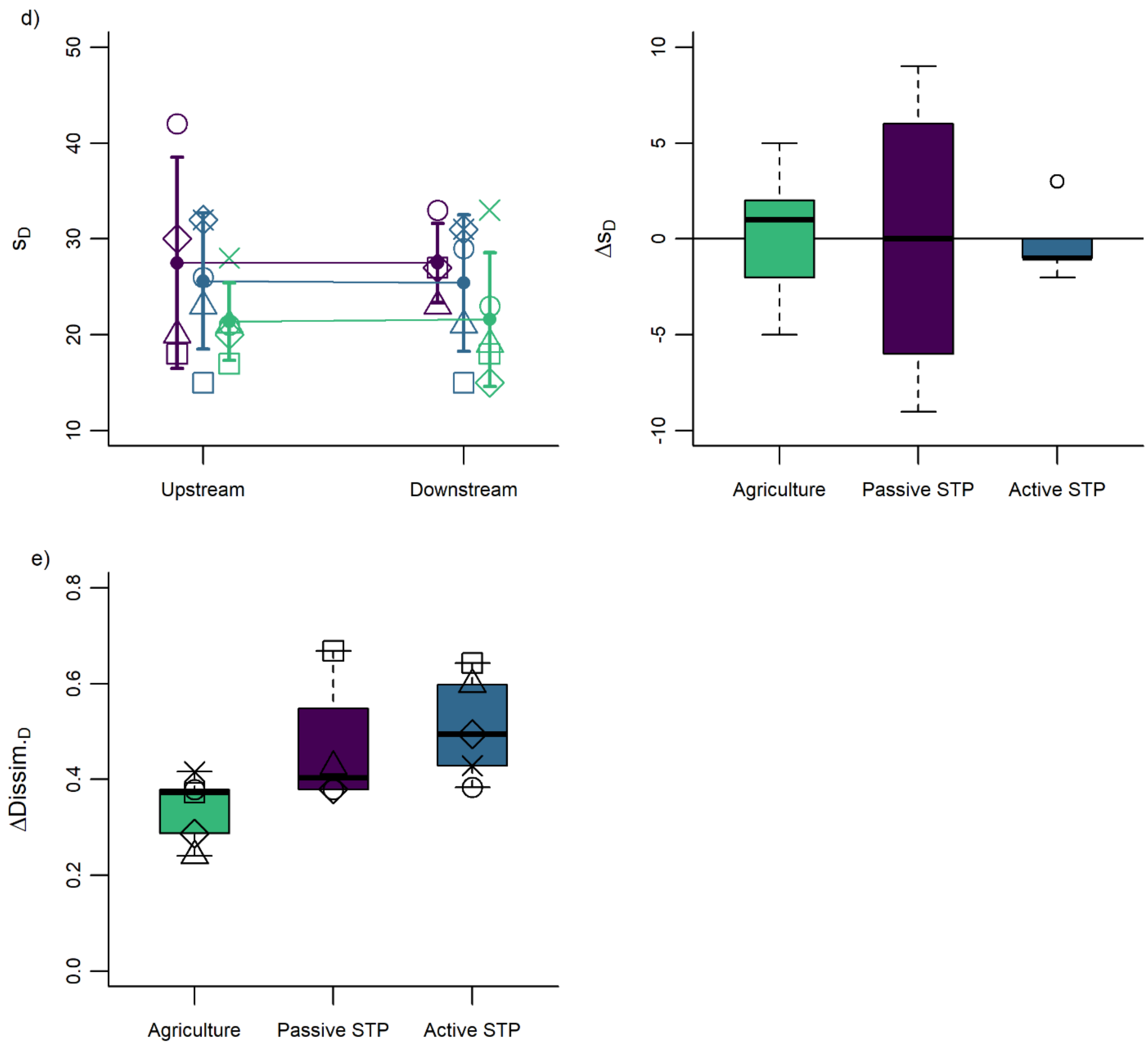
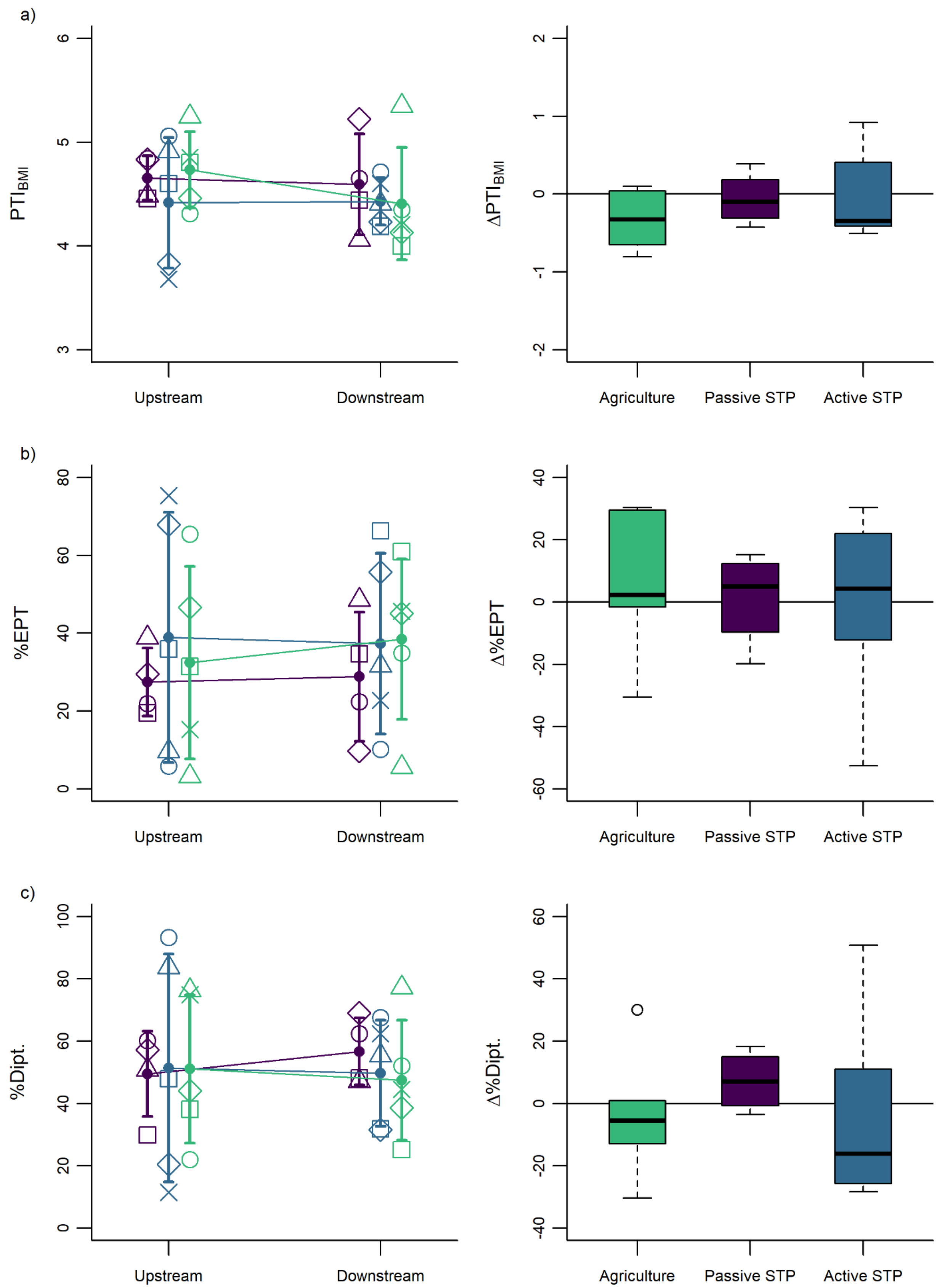


Figure S1-1: Mixed model analysis of variance for modified before-after (upstream-downstream) control-impact (agriculture-sewage effluent) assessment for ecological indicators of diatom assemblages: (a) pollution tolerance index (PTI_D), (b) percent abundance of high nutrient taxon (%high-NP), (c) percent abundance of low nutrient taxon (%low-NP), (d) taxon richness (s_D), and (e) Bray-Curtis dissimilarity (dissimilarity$_D$). Green, purple, and blue colours correspond to streams exposed to agriculture, agriculture + passively treated lagoon sewage effluent, and agriculture + actively treated mechanical sewage effluent, respectively. Open symbols correspond to indicator values for individual study streams and filled points represent the mean ± one standard deviation within each human impact category. Longitudinal differences (downstream – upstream) in ecological indicators are summarized by boxplots (min, 25th percentile, 50th percentile, 75th percentile, max) for each human impact category.


a)
PTI_{BMI}
ΔPTI_{BMI}
b)
%EPT
Δ%EPT
c)
%Dipt.
Δ%Dipt.
Upstream
Downstream
Agriculture
Passive STP
Active STP

840

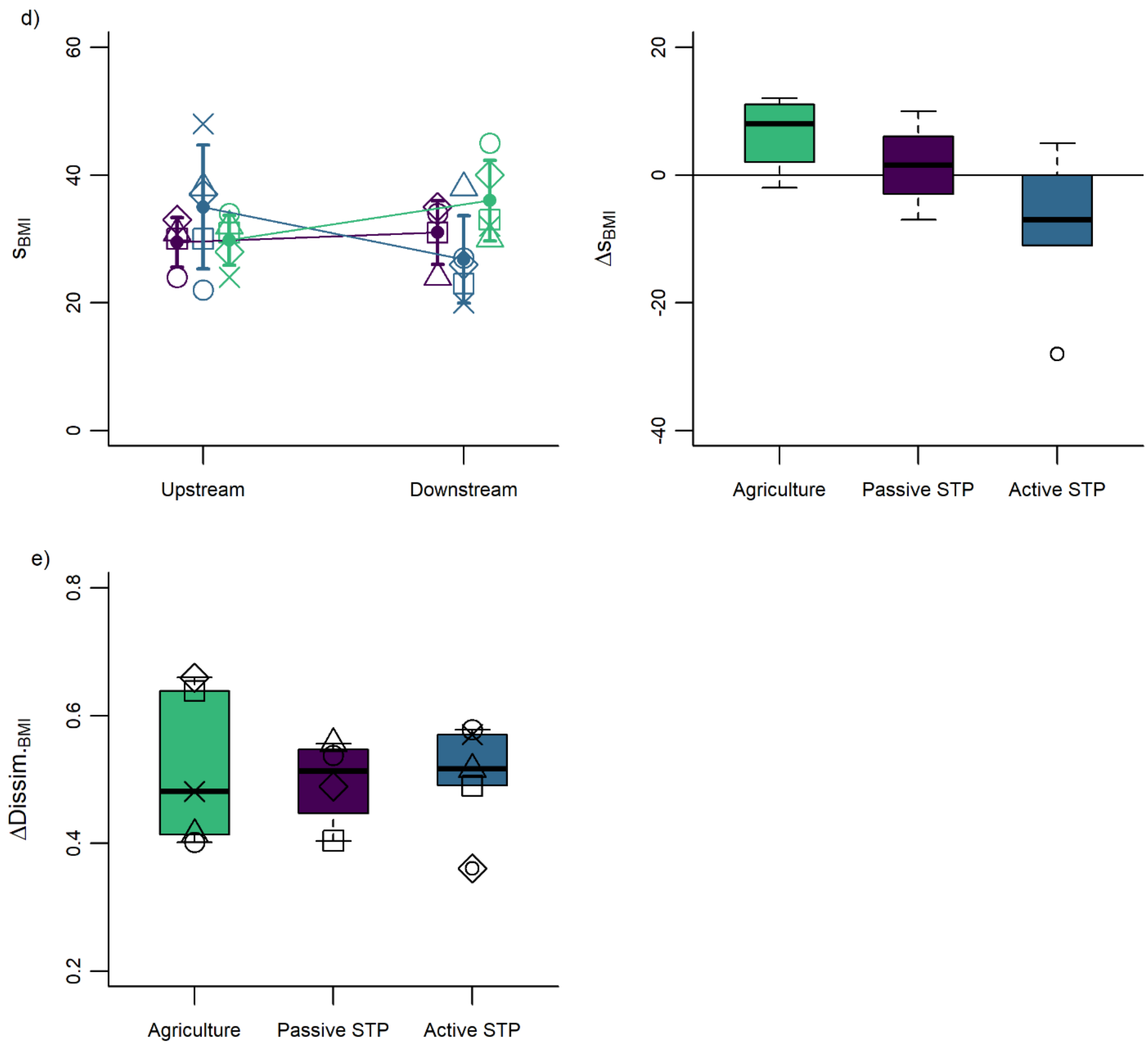
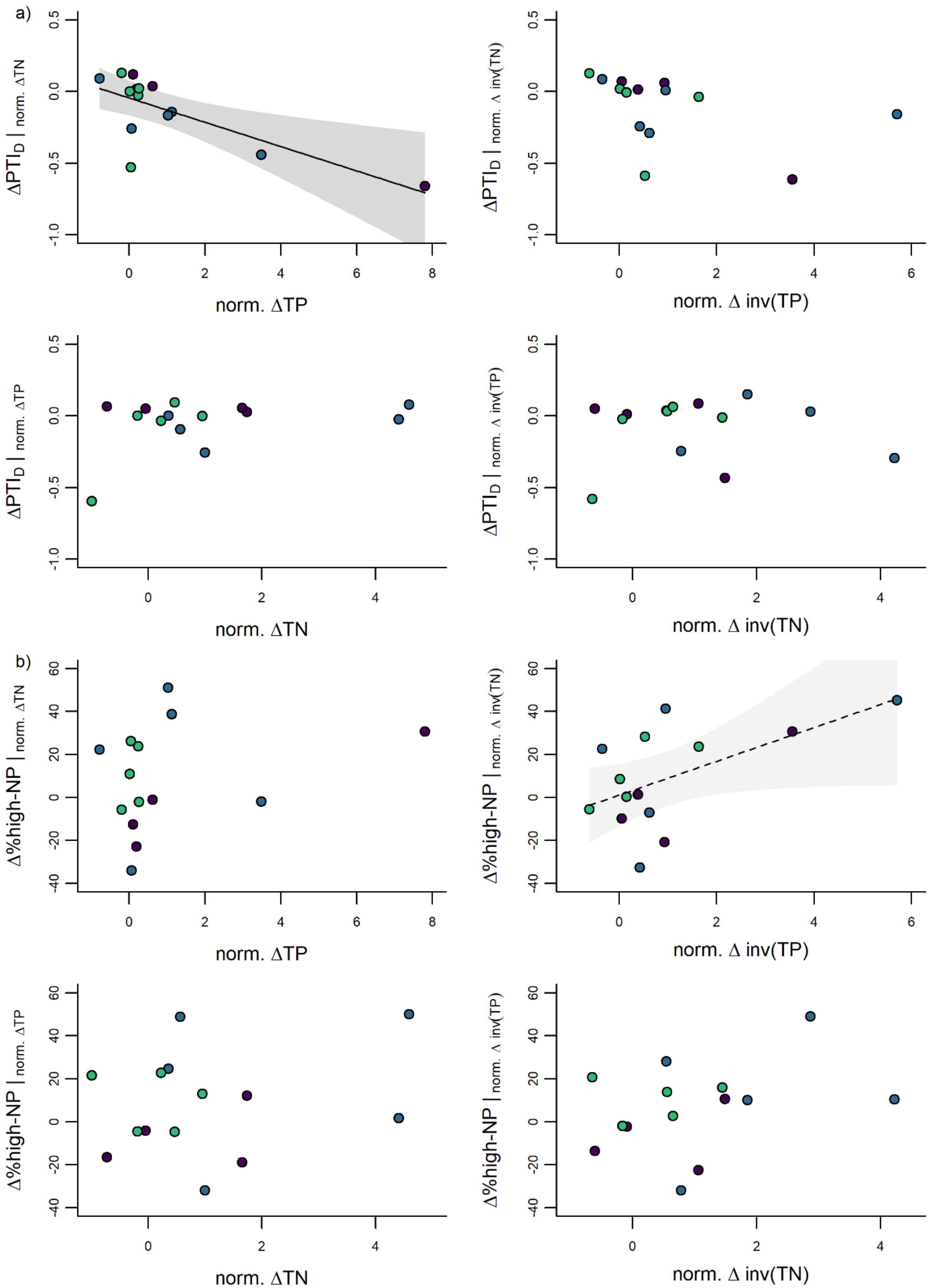
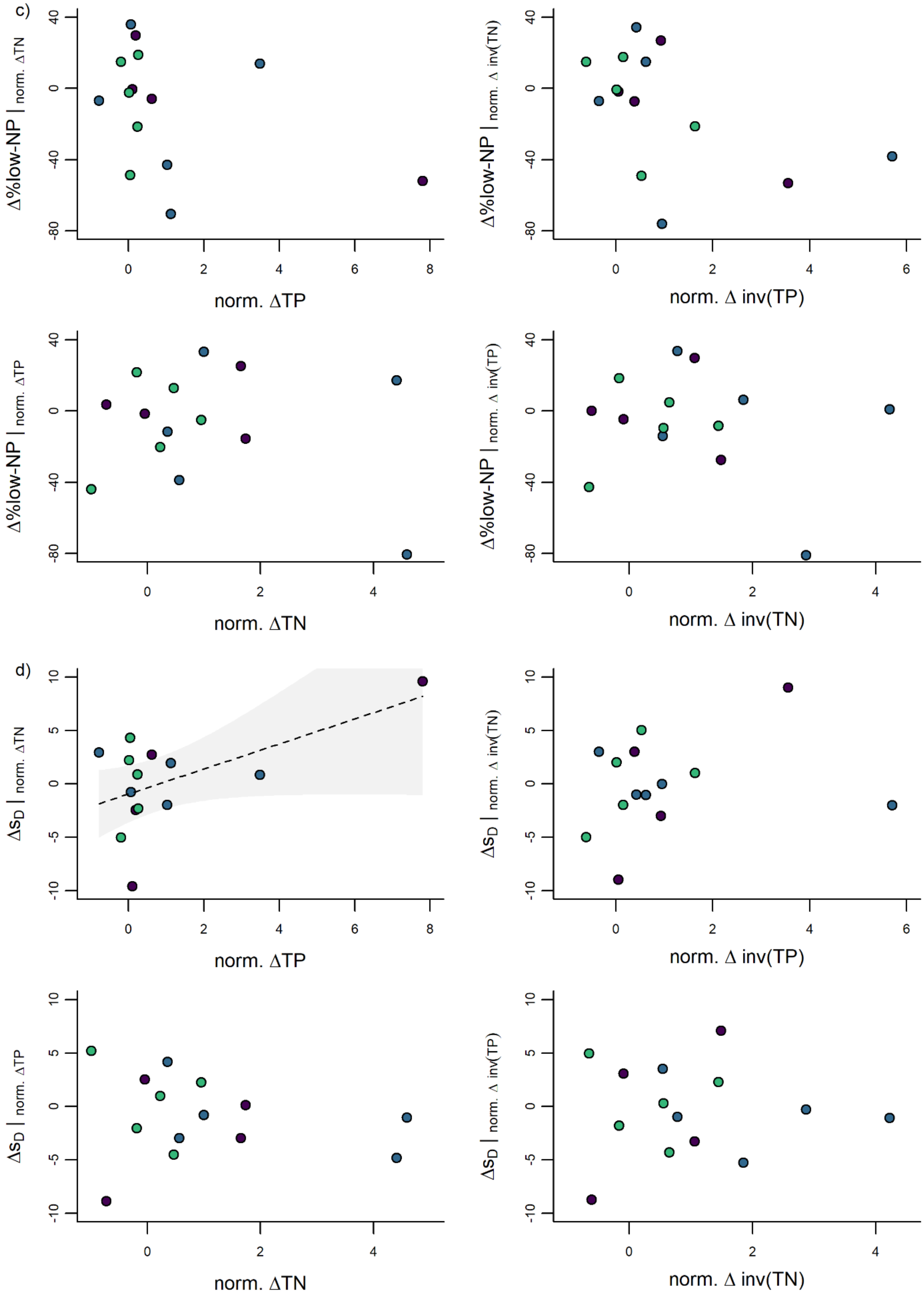


Figure S1-2: Mixed model analysis of variance for modified before-after (upstream-downstream) control-impact (agriculture-sewage effluent) assessment for ecological indicators of benthic macroinvertebrate assemblages: (a) pollution tolerance index (PTI_{BMI}), (b) percent Ephemeroptera, Plecoptera, and Tricoptera abundance (%EPT), (c) percent Diptera abundance (%Dipt.), (d) taxon richness (s_{BMI}), and (e) Bray-Curtis dissimilarity ($dissimilarity_{BMI}$). Green, purple, and blue colours correspond to streams exposed to agriculture, agriculture + passively treated lagoon sewage effluent, and agriculture + actively treated mechanical sewage effluent, respectively. Open symbols correspond to indicator values for individual study streams and filled points represent the mean ± one standard deviation within each human impact category. Longitudinal differences (downstream – upstream) in ecological indicators are summarized by boxplots (min, 25th percentile, 50th percentile, 75th percentile, max) for each human impact category.



a)
ΔPTI_D | norm. ΔTN
norm. ΔTP
ΔPTI_D | norm. Δ inv(TN)
norm. Δ inv(TP)
ΔPTI_D | norm. ΔTP
norm. ΔTN
ΔPTI_D | norm. Δ inv(TP)
norm. Δ inv(TN)
b)
Δ%high-NP | norm. ΔTN
norm. ΔTP
Δ%high-NP | norm. Δ inv(TN)
norm. Δ inv(TP)
Δ%high-NP | norm. ΔTP
norm. ΔTN
Δ%high-NP | norm. Δ inv(TP)
norm. Δ inv(TN)


c)
Δ%low-NP | norm. ΔTN
norm. ΔTP
Δ%low-NP | norm. Δ inv(TN)
norm. Δ inv(TP)
Δ%low-NP | norm. ΔTP
norm. ΔTN
Δ%low-NP | norm. Δ inv(TP)
norm. Δ inv(TN)
d)
ΔsD | norm. ΔTN
norm. ΔTP
ΔsD | norm. Δ inv(TN)
norm. Δ inv(TP)
ΔsD | norm. ΔTP
norm. ΔTN
ΔsD | norm. Δ inv(TP)
norm. Δ inv(TN)

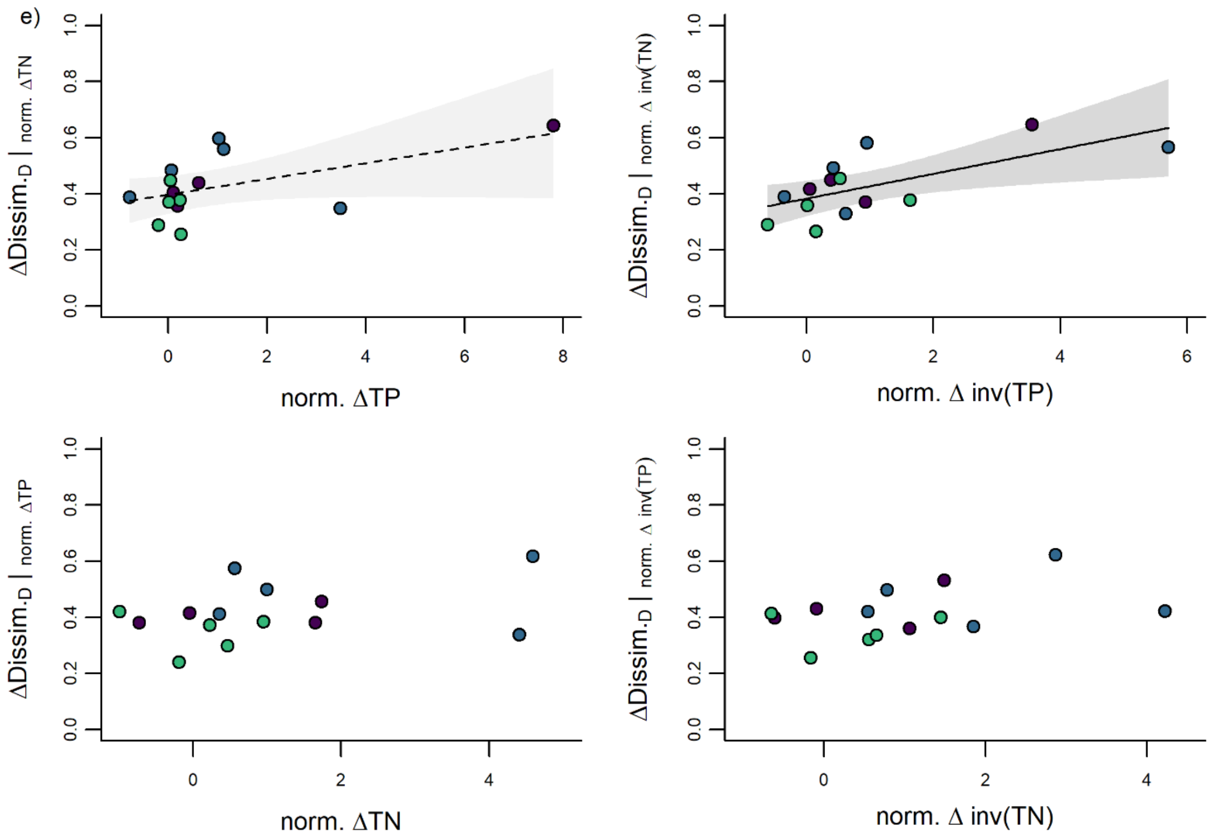
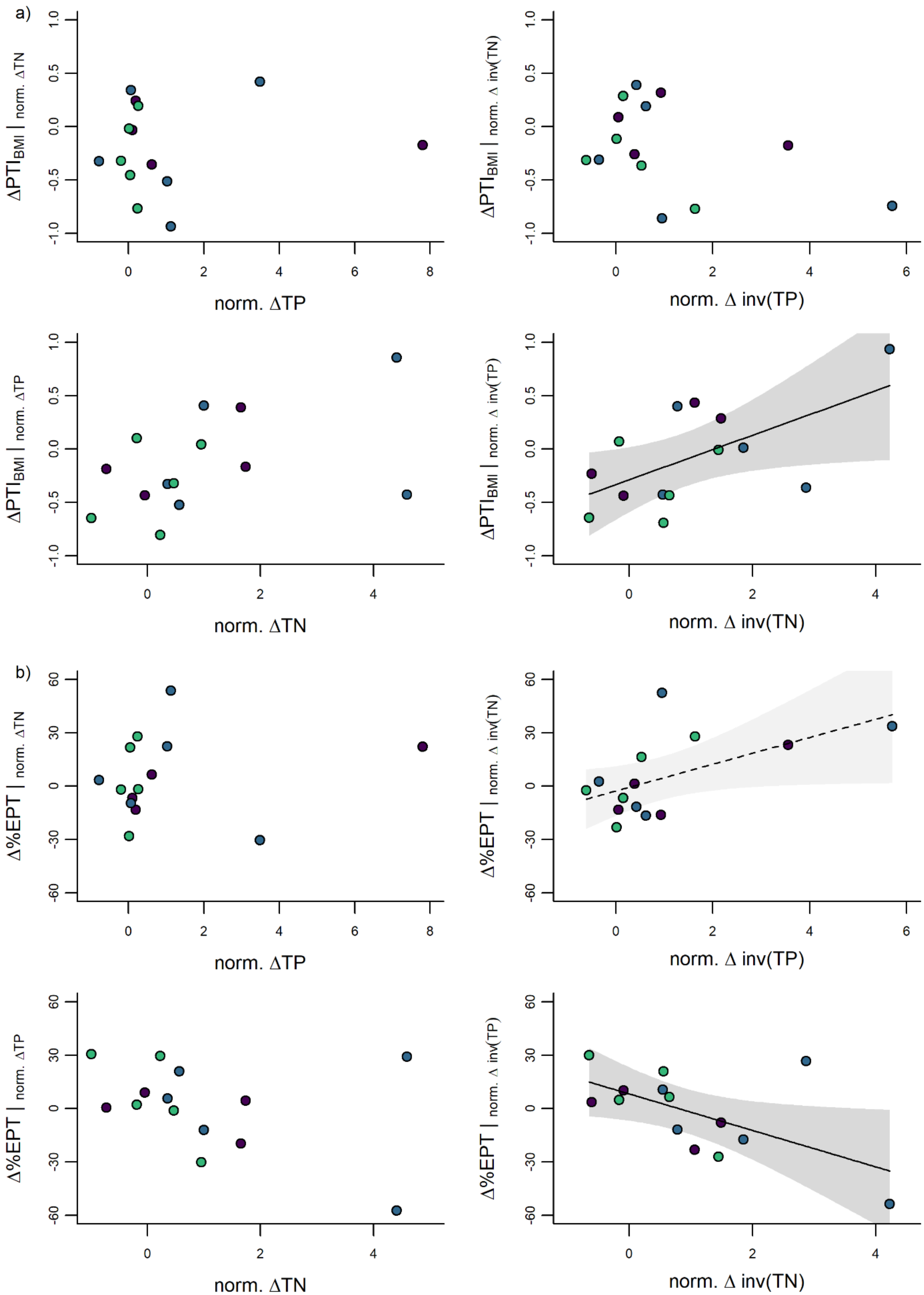
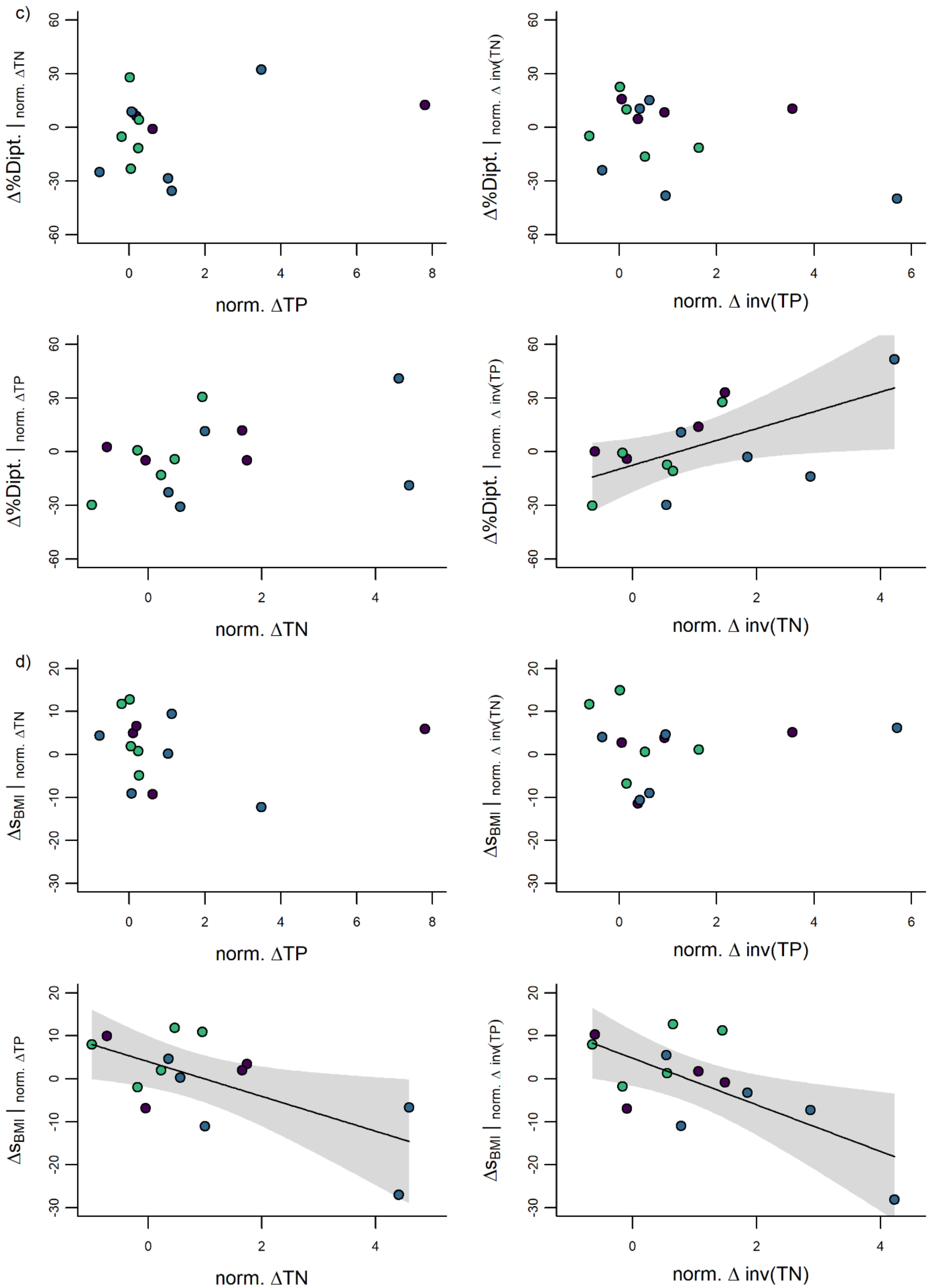


Figure S2-1: Multiple regression models of the association between normalized (norm.) longitudinal differences (downstream – upstream) in untransformed and inverse transformed (inv.) total phosphorus (TP) and total nitrogen (TN) concentrations and measures of ecological indicators of diatom assemblages: (a) pollution tolerance index (PTI_D), (b) percent abundance of high nutrient taxon (%high-NP), (c) percent abundance of low nutrient taxon (%low-NP), (d) taxon richness (s_D), and (e) Bray-Curtis dissimilarity ($dissimilarity_D$). Green, purple, and blue points correspond to streams exposed to agriculture, agriculture + passively treated lagoon sewage effluent, and agriculture + actively treated mechanical sewage effluent, respectively. Solid trend lines correspond to significant associations at $\alpha = 0.05$ and dashed trend lines correspond to significant associations at $\alpha = 0.10$.



a)
ΔPTI$_{BMI}$ | norm. ΔTN
norm. ΔTP
ΔPTI$_{BMI}$ | norm. Δ inv(TN)
norm. Δ inv(TP)
ΔPTI$_{BMI}$ | norm. ΔTP
norm. ΔTN
ΔPTI$_{BMI}$ | norm. Δ inv(TP)
norm. Δ inv(TN)
b)
Δ%EPT | norm. ΔTN
norm. ΔTP
Δ%EPT | norm. Δ inv(TN)
norm. Δ inv(TP)
Δ%EPT | norm. ΔTP
norm. ΔTN
Δ%EPT | norm. Δ inv(TP)
norm. Δ inv(TN)



c)
d)
Δ%Dipt. | norm. ΔTN
Δ%Dipt. | norm. Δ inv(TN)
Δ%Dipt. | norm. ΔTP
Δ%Dipt. | norm. Δ inv(TP)
ΔsBMI | norm. ΔTN
ΔsBMI | norm. Δ inv(TN)
ΔsBMI | norm. ΔTP
ΔsBMI | norm. Δ inv(TP)
norm. ΔTP
norm. Δ inv(TP)
norm. ΔTN
norm. Δ inv(TN)

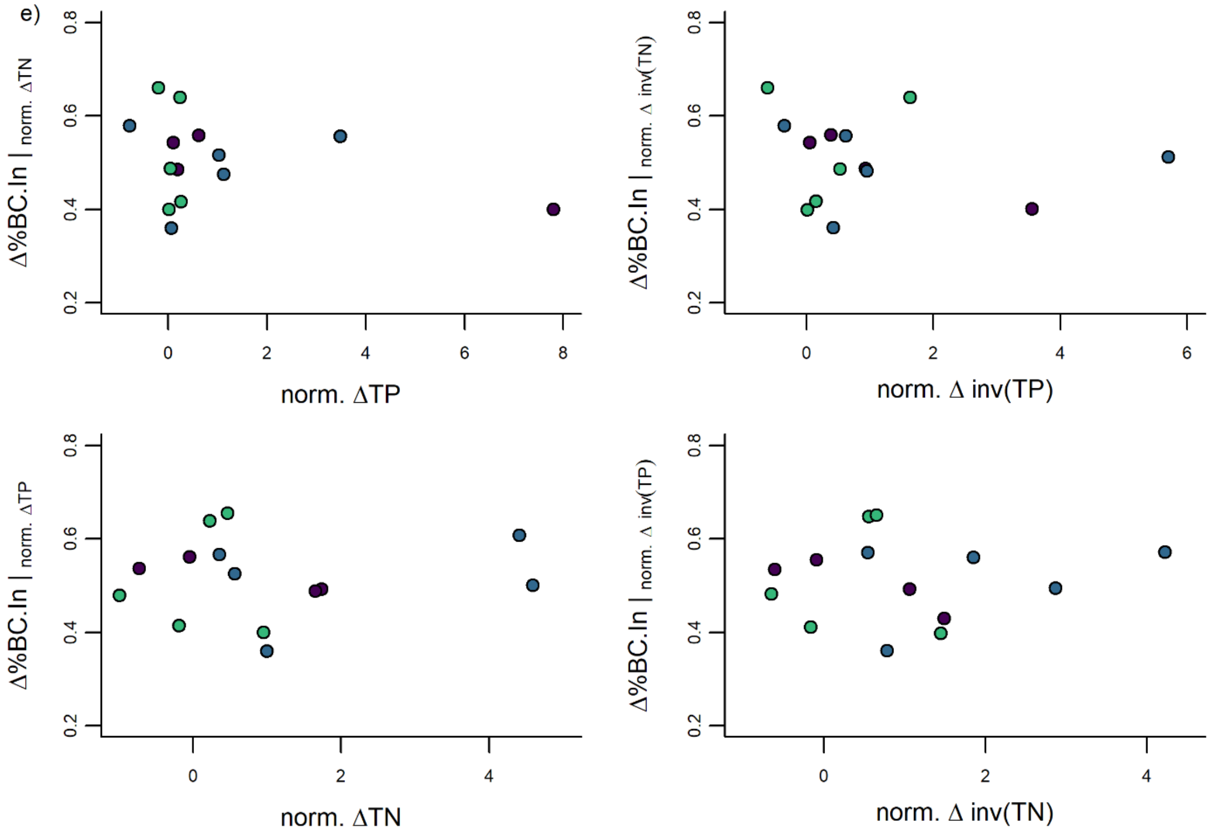


Figure S2-2: Multiple regression models of the association between normalized (norm.) longitudinal differences (downstream – upstream) in untransformed and inverse transformed (inv.) total phosphorus (TP) and total nitrogen (TN) concentrations and measures of ecological indicators of benthic macroinvertebrate assemblages: (a) pollution tolerance index (PTI_{BMI}), (b) percent Ephemeroptera, Plecoptera, and Tricoptera abundance (%EPT), (c) percent Diptera abundance (%Dipt.), (d) taxon richness (s_{BMI}), and (e) Bray-Curtis dissimilarity ($dissimilarity_{BMI}$). Green, purple, and blue points correspond to streams exposed to agriculture, agriculture + passively treated lagoon sewage effluent, and agriculture + actively treated mechanical sewage effluent, respectively. Solid trend lines correspond to significant associations at $\alpha = 0.05$ and dashed trend lines correspond to significant associations at $\alpha = 0.10$.