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# LIMITATIONS ON NITROGEN REMOVAL BY TREATMENT WETLANDS UNDER MARITIME CLIMATIC CONDITIONS

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A Thesis

Presented to the

Faculty of the

Division of Science and Environmental Policy

California State University Monterey Bay

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In Partial Fulfillment

of the Requirements for the Degree

Master of Science

in

Coastal and Watershed Science and Policy

_____

by

Gwen Miller

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**CALIFORNIA STATE UNIVERSITY MONTEREY BAY**

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**Limitations on nitrogen removal by treatment wetlands under maritime climatic conditions**

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Abstract

The use of fertilizer has in part elevated nitrogen concentrations within surface and groundwater. High nitrate concentration can negatively impact human health as well as lead to algal blooms. Treatment wetlands can reduce the negative impacts of nitrate runoff, but the drivers of nitrate removal within maritime climates maybe limited by the cool winter temperatures despite a year-around growing season. We sought to understand the key limitations on nitrate removal within an experimental treatment wetland located within in California's Central Coast region. We collected water samples and analyzed them for nitrate, ammonium, and dissolved organic carbon, temperature, pH, salinity and dissolved oxygen. We modeled outlet nitrate concentrations using inlet nitrate, temperature and dissolved organic carbon as a priori predictor variables, using a Tobit distribution to account for the positive zero-truncated distribution of water quality data. We compared models using Akaike's Information Criterion (AIC). The best-supported model included all *a priori* predictors. There was decisive evidence for a dependence of nitrate removal on high inlet nitrate concentrations and high temperatures, and some evidence for a dependence on high dissolved carbon concentrations. Nitrate removal was limited in winter, despite source waters containing elevated nitrate concentrations year-round. To better optimize nitrate removal, wetland design should include ways to increase water temperature and available carbon.

Keywords: Treatment Wetland, Temperature, Akaike Information Criterion, AIC, Carbon, Denitrification, Model

## 1.0 Introduction

Nitrogen runoff from agriculture negatively affects water quality in many regions. Nitrogen is an essential plant nutrient and is a limiting factor for growth; hence there is intensive nitrogen fertilizer use in agriculture (Tilman et al., 2002). Worldwide nitrogen loading has more than doubled since the pre-industrial era, and fertilizer is the largest source of anthropogenic nitrogen inputs (Galloway et al., 2004; Sobota et al., 2013). Nitrogen often enters into waterways and causes several environmentally harmful events such as algal blooms and eutrophication (Anderson et al., 2002; Heisler et al., 2008). Algal blooms can be comprised of toxic algae, cyanobacteria or protists; the blooms can also decrease dissolved oxygen (Heisler et al., 2008). Furthermore, human health is negatively impacted when exposed to high nitrate concentrations in drinking water, which can lead to increased cases of cancer and methemoglobinemia (Weyer et al., 2001). Treatment wetlands are one tool that can assist in remediating and improving water quality. In anoxic environments, denitrifying bacteria convert nitrate to dinitrogen gas or nitrous oxides in a process called denitrification (Burgoon, 2001; Songliu et al., 2009; Díaz et al., 2012).

Within maritime climatic regions, there is a knowledge gap with respect to understanding limitations on nitrate removal within treatment wetlands. Treatment wetlands have the potential to help improve a variety of water quality impairment issues (Kovacic et al., 2000; Kadlec and Wallace, 2008; García-García et al., 2013; Krone-Davis et al., 2013), but their functionality can vary based on climatic location. The cool winter temperatures within maritime climates are warm enough to grow crops year-round but we are unaware of any studies that have determined if these temperatures are sufficiently warm for rapid year-round denitrification. Several studies have analyzed seasonal changes of nitrate removal

within treatment wetlands (Picard et al., 2005; Sirivedhin and Gray, 2006; García-Lledó et al., 2011) but we were unable to find results from temperate maritime climates – those with cool but not cold winters.

Microbial activity is influenced by temperature and carbon supply. Given that temperature influences microbial activity, denitrification within wetlands is similarly controlled by seasonal temperature fluctuations (Bachand and Horne, 2000; Poe et al., 2003; Hernandez and Mitsch, 2007) . When temperatures are between 20 °C and 25 °C denitrification rates are maximized, with some studies indicating that temperatures above 25 °C can lead to even higher nitrate removal rates, while denitrification rates are decreased at temperatures below 10 °C (Sutton et al., 1975; Elefsiniotis and Li, 2006).

California's Central Coast region (CC) experience mild winters and cool summers, which poses challenges for using wetlands to treat agricultural nitrate runoff. Mean monthly temperatures in the coldest month (January) dip below 10 °C, which is well below the optimal temperature for denitrification (Sutton et al., 1975; Spieles and Mitsch, 2000; Poe et al., 2003; WRCC 2014), but still high enough for year-round crop growth (Krone-Davis et al., 2013). We use the term 'maritime' to characterize this important 'cool but not cold' winter climatic condition, to reflect the fact that it is ultimately due to the moderating influence of the nearby marine environment on air temperatures. It contrasts with 'continental' climate, where crops are only grown in summer, such that there is less potential for nitrate pollution to occur during periods when wetland denitrifying capacity may be limited.

In addition, many studies have analyzed how nitrate removal in wetlands is influenced by plant species composition, carbon augmentations, and increasing carbon to

nitrate ratios, but the limiting relationship between low carbon concentrations found naturally within a system and nitrate removal is not well known (Bachand and Horne, 2000; Burgoon, 2001; Coleman et al., 2001; Songliu et al., 2009; Playchoom and Pungrasmi, 2011; Zhu et al., 2014). Since denitrifying bacteria utilize carbon as an energy source, a wetland is often carbon limited in situations with high concentrations of nitrate (Kadlec and Wallace, 2008; Tao et al., 2013). Denitrification rates are maximized when C: N ratios are between 4:1 to 10:1; the range in ratios is due to differing plant carbon composition (Hume et al., 2002).

Another knowledge gap in treatment wetland research is that there is relatively little exploration using contemporary statistical methods of approaches for discerning what controls denitrification in field situations where multiple potential influences are simultaneously varying. Sirivedhin and Gray (2006) performed a variety of laboratory experiments assessing how varying temperature, nitrate and carbon influence denitrification. Though their study was similar to ours, their experiment was highly controlled and unrepresentative of the simultaneous variation in multiple influences that occurs in field settings. Similarly, Bastviken et al. (2009) analyzed how manipulating residence time and vegetation type influences nitrate removal. They did break their study up by season but did not thoroughly asses how temperature may be directly impacting denitrification. Furthermore, both of these studies did not apply statistical models to their findings. Several studies used a mass transfer coefficient approach to model nitrate removal (Appelboom et al., 2010; Etheridge et al., 2014), an approach initially proposed by Kelly (1987), while many other studies used a dynamic catchment nitrogen model to estimate nitrogen transport and denitrification (Arheimer and Wittgren, 2002; O'Shea and Wade,

2009). Both of these approaches are simplified denitrification models that typically only include initial nitrate concentrations and temperature to estimate nitrate removal. Spieles and Mitsch (2000) created a more encompassing nitrate removal model including temperature, water depth and retention time as predictor variables, but left out other chemical parameters in their model such as carbon. There is a need for predictive models based on formal statistical analysis involving multiple simultaneous influences.

Agricultural pollution negatively influences water quality along the CC region. The CC is a extensively farmed region, and Monterey County is the top vegetable producing area within the United States (MPCC, 2012). There are over sixty nutrient impaired waterbodies listed under the United States Clean Water Act within the CC, many of these are impaired by excess nitrate originating from agricultural sources (SWRCB, 2010). To combat nitrate runoff, the Central Coast Regional Water Quality Control Board (CCRWQCB) established a Conditional Waiver of Waste Discharge Requirements for Discharges from Irrigated Lands (known as the "Ag Waiver"). Some of the requirements include taking individual or cooperative measurements of receiving water quality, and participation in management practices that protect water quality (CCRWQCB, 2012).

Our goal was to analyze the limitation of temperature, carbon and nitrate supply on nitrate removal by treatment wetlands in a maritime climatic region using a predictive model-based approach. We examined the functioning of a specific experimental treatment wetland, and postulated that the wetland would remove more nitrate from the system when water temperatures were the highest. We also postulated that carbon availability would limit nitrate removal; low dissolved organic carbon concentrations in the water would lead to reduced nitrate removal. We aimed to develop a predictive capability to illustrate if

nitrate removal follows a zero-order decay, first-order decay or a combination of the two, which could inform design questions on establishing optimal residence times within wetlands. Growers and regulators can use information from this study to achieve a realistic expectations of the performance of treatment wetland design within maritime climates. If naturally occurring carbon limits nitrate removal, future design considerations could include carbon augmentation. If seasonal variations in temperature drive nitrate removal, consideration could be given to increasing water temperatures by either warming the water before it enters the wetland or implementing insulation techniques to maximize heat retention.

## 2.0 Methods

### *2.1 Site description*

The study location was within California's Central Coast region at a constructed wetland known as the Molera wetland, located roughly 1 kilometer from the ocean (Fig.1). Highly-productive row-crop agricultural land comprises 36% of the 380 $km^2$ watershed in which the wetland resides. The wetland was constructed on a 4856 $m^2$ parcel and is located adjacent to impaired waterbodies listed under the United States Clean Water Act, the Tembladero Slough and the Old Salinas River (Krone-Davis et al., 2013; Daniels et al., 2014).

Water was pumped from the Tembladero Slough, into the upper portion of the wetland, which has a volume of approximately 555 $m^2$. The water flowed via gravity from the inlet to the outlet through a 285 meter long, 6.5 meter wide, and 0.3 meter deep sinuous channel. The berms along the channel were vegetated predominantly with *Schoenoplectus californicus* (California Bulrush; Hogan et al., 2012). Over a period of 22 months, for 7.5

hours each day, we pumped water into the wetland at a rate of 0.36 $m^3$/min, which amounted to a mean inflow rate of 162 $m^3$/day. The water then drained under gravity from the upper portion of the wetland into a marsh-like area before flowing back into the slough (Fig.1).

*2.2 Residence time*

We manipulated the residence time by changing the pump runtime with a programmable timer. Knowing the residence time within the wetland allowed for a parcel of water collected at the outlet of the wetland to correspond as much as possible with water collected beforehand at the inlet of the wetland. We established a 3.5-day residence time within the wetland to reduce the frequency at which outlet nitrate concentrations would reach zero, which would have made it difficult to observe seasonal variability. A 3.5-day residence was logistically convenient, being one-half of the length of a week. We established and verified the residence time using Rhodamine dye tracer tests.

*2.3 Water Quality analysis*

Using standard procedures, we collected water samples every 3.5-days at the inlet and outlet of the wetland starting June 2012 and continuing through March 2014 (American Water Works Association, 2012). Samples were kept on ice then filtered using 0.45 µm filters in the laboratory. Samples were either frozen or refrigerated depending on standard procedures and analyzed within 28 days (American Water Works Association, 2012). We analyzed water samples for total nitrogen (TN) and dissolved organic carbon (DOC) using a Shimadzu TOC Analyzer (Tokyo, Japan) and we analyzed water samples for nitrate plus nitrite ($NO_3$-N) and ammonium using a Lachat Instruments 8500 Flow Injection Analyzer (FIA; Loveland, CO, USA). We used QuikChem Method 10-107-04-1-A to measure

nitrate, QuikChem Method 10-107-06-1-B for ammonium, and EPA method 415.1 for DOC and TN analysis. For quality control, we ran solutions with a known nutrient concentration every 15 samples. For data to be valid, standards had to be between 80 and 100 % of their known concentration. We recorded salinity, pH, temperature, and dissolved oxygen at both sample sites using a Hydrolab DS5x water quality sonde (Loveland, CO, USA).

*2.5 Statistical Analysis*

We examined our postulates by hypothesizing that the following model for mean nitrate outlet concentrations ($\mu_{out}$), or a subset of it, could predict the distribution of outlet nitrate concentrations ($N_{out}$):

$$H_{ZFTC}: \mu_{out} = N_{in,lag} + \beta_0 + \beta_N N_{in,lag} + \beta_T T + \beta_C C$$

where $N_{in,lag}$ is nitrate concentration at the inlet, lagged by 3.5-days, $T$ is temperature (°C), DOC is dissolved organic carbon concentration (mg/L), $\beta_0$ is a fitted parameter representing a constant reduction rate, and $\beta_N$, $\beta_T$, and $\beta_C$ are fitted coefficients representing the degree of reduction that is related to inlet nitrate concentration, temperature, and carbon, respectively. We named the full model and its subsets according to various subscripts. Subscript Z denotes a zero-order (i.e. constant) reduction rate between inlet and outlet (Kadlec and Wallace, 2008). Subscript F denotes a first-order (i.e. concentration-dependent) reduction rate. Subscripts T and C denote dependency of reduction rate on temperature and carbon, respectively. The full model above includes both zero-order and first-order nitrate decay processes; the theory of which we explain in the Appendix. We expressed strictly zero-order subsets of this model by excluding the inlet nitrate term; and

we expressed a strictly first-order subset excluding all terms except the inlet nitrate term (see Appendix). On occasions when we did not collect samples 3.5-days apart, we linearly interpolated lagged inlet concentration measurements between the two closest matches to the prescribed 3.5-day lag time. We assumed the actual outlet nitrate concentration, $N_{out}$, was distributed about its expected value, $\mu_{out}$, according to a left-censored Tobit-normal distribution as follows:

$$N_{out} \sim \textit{Tobit-normal}\ (\mu_{out}, \lambda, \infty)$$

where $\lambda$ has specified left-censoring threshold of 0.2 mg/L and $\infty$ indicates the omission of right-censoring. We used a 0.2 mg/L threshold because this is the nitrate detection limit of the method used with the FIA. A Tobit distribution was necessary because we assumed that zero or near-zero nitrate concentration would be an absorbing boundary condition.

The above model and all possible subsets were fit to our observational data, and all models were compared using $\log_{10}$ evidence ratios (LERs) derived from Akaike's Information Criterion (AIC; Burnham and Anderson 2002). We began with an *a priori* balanced model comparison in order to be able to calculate relative importance (RIs) values and LERs for each predictor. To interpret LERs, the terms "decisive", "strong", "substantial", or "minimal" were used when $\log_{10}$ evidence ratios reached positive or negative thresholds of 2, 1, 0.5, and 0 respectively (Kass and Raftery, 1995). We also computed a *post hoc* analysis and included various other predictors such as pH and salinity to the winning model, resulting in 26 models. Statistical analyses were conducted using the *tobit* function in the *AER* (Applied Econometrics with R) package within R (R Core Development Team, 2013). We interpreted model coefficients as indicators of positive or

negative effects of predictor variables on nitrate reduction. To determine the magnitude of the effect each predictor variable had within the best model, we obtained standardized coefficients from models fit to standardized covariates.

**3.0 Results**

The wetland successfully removed nitrate as water flowed from the inlet to the outlet. Inlet nitrate ($NO_3$-N) concentrations ranged between 0.39 mg/L and 84.9 mg/L with a mean value of 21.6 mg/L. The range of outlet nitrate concentrations was below detection limit (0.2 mg/L) to 65.1 mg/L, with a mean of 13.9 mg/L. Typically nitrate concentrations were highest at the inlet and lowest at the outlet, but occasionally outlet nitrate concentrations exceeded inlet nitrate concentrations (Fig. 2). There was also more variability in nitrate concentrations at the inlet (SD = 16.69 mg/L), than there was at the outlet (SD = 8.52 mg/L). During the coolest winter month (January), and median carbon concentrations, the modeled average nitrate removal was 1.29 mg/L while during the warmest summer month (August) and median carbon concentrations, the modeled average nitrate removal was 7.91 mg/L.

Water temperature also fluctuated seasonally, with the warmest temperatures occurring between June and August and the coolest temperatures in January. During December 2012 through March 2013 as well as November 2013 and January 2014, water temperatures were below 10 °C. Water temperature was also the highest at the inlet, ranging between 3.9 and 24.5 °C and lowest at the outlet, ranging between 3.6 and 18.4 °C. During the winter, water temperature averaged 13.0°C at the inlet and 10.9 °C at the outlet. During the summer, water temperatures averaged 18.0°C at the inlet and 16.0°C at the outlet.

DOC peaked with a concentration of 261.2 mg/L and 242 mg/L at the inlet and outlet respectively. The median value at the inlet was 21.4 mg/L while the median at the outlet was 12.29 mg/L. In 2013 and 2014 DOC concentrations remained relatively low and there was no apparent seasonal pattern in DOC concentrations.

There was decisive evidence that nitrate removal was greatest at higher temperatures (LER = 2.82) and higher inlet nitrate concentrations (LER = 10.71), based on the *a priori* AIC analysis (Tables 1 & 2). Although the winning model included a carbon effect, the strength of evidence for this effect was less than substantial. Nitrate removal also most closely followed a combined zero/first order decay model; since the best-supported model contained both zero and first-order terms. Initial nitrate concentrations had the largest influence on nitrate removal, as indicated by the standardized model coefficient for the best model ($\beta_{N,std} = -10.120$), followed by temperature ($\beta_{T,std} = -2.594$), and then carbon ($\beta_{C,std} = -1.249$; Table 2). Results of the *post hoc* model comparison showed substantial evidence that pH and salinity also influenced nitrate removal, and no substantial evidence that DO and Ammonia had an effect (Table 1).

Using the coefficients derived from the model, we were able to predict outlet nitrate removal over time with varying inlet nitrate, carbon or temperature conditions (Fig. 3). The model predicted that outlet nitrate concentrations decreased most rapidly when nitrate input concentrations were high. The model predicted that outlet nitrate concentrations would increase within the wetland with low initial nitrate concentrations. Modeled nitrate values converged at 10 mg/L near day 9.5 when modeling outlet nitrate concentrations with varying initial nitrate concentrations. When modeled temperature was low, only minimal

nitrate removal occurred, while high temperatures led to the highest nitrate removal. Low and medium carbon concentrations led to roughly the same nitrate removal efficiencies while high carbon concentrations resulted in the highest modeled nitrate removal.

## 4.0 Discussion

Nitrate concentrations within failed to meet water quality objectives frequently and often by a long margin, although objectives were obtained at the outlet more frequently than at the inlet. Relevant objectives include the 10 mg/L United States drinking water standard, and dry- and wet-season objectives of 6.4 mg/L and 8.0 mg/L under the California state-approved Lower Salinas Watershed TMDL for Tembladero Slough (CCRWQCB, 2013).

The results supported our original postulates that nitrate removal would increase with warmer temperatures and higher carbon concentrations, though the support for carbon as a predictor variable was relatively weak. Seasonal variations of temperature leading to reduced nitrate removal during cooler months are consistent with previous research (Bachand and Horne, 2000; García-Lledó et al., 2011). During the warmest month within our study (August), which experienced an average temperture of 16.7 °C, the fitted model predicted nitrate concentrations would decrease from 19.1 mg/L to 11.2 mg/L (under the median carbon concentration of 12.3 mg/L). During the coolest month (January), which had an average temperture of 8.1 °C, the fitted model predicted nitrate concentrations would decrease from 19.1 mg/L to 17.8 mg/L. Median carbon concentrations observed within the study, 12.3 mg/L, and median inlet nitrate concentrations 19.1 mg/L were used as inputs into the fitted model.

In terms of nitrate load removed per unit of wetland water surface area per day, the above predictions equate to nitrate load reductions of 0.69 g $m^{-2}$ $day^{-1}$ during August and 0.11 g $m^{-2}$ $day^{-1}$ during January. These values are similar to other studies; a wetland in Girona, Spain experienced an average nitrate reduction between 0.2 and 1.7 g $m^{-2}$ $day^{-1}$, (García-Lledó et al., 2011) while a wetland located in Halmstad City, Sweden experienced an average reduction between 0.001 – 0.48 g $m^{-2}$ $day^{-1}$ (Fleischer et al., 1994).

These results can also be interpreted as fractions of the total watershed load. The mean daily nitrate load exported from the watershed is on the order of 860 kg/day (Harris et al., 2007 corroborated independently by Novak, 2011), and the agricultural portion of the watershed covers approximately 155 $km^2$ (CCRWQCB 2011). Given these values, and with no further augmentations of carbon or temperature, and no accounting for seasonal variability in watershed load, the wetland water surface area required to treat the watershed load would range from 124 to 765 hectares between summer and winter, or between 0.8% and 4.9% of the total agricultural land area.

Our finding of higher carbon content leading to higher nitrate removal was also consistent with previous research (Hume et al., 2002; Playchoom and Pungrasmi, 2011). Under median inlet nitrate, and median temperature (14.25 °C), we estimated that outlet nitrate would go to 5.4 mg/L (71% removal), 10.3 mg/L (46% removal), and 13.57 mg/L (29 % removal) with a C:N ratio of 10:1, 4:1 and 0:1 respectively. These values are comparable to other research which found nitrate removal increased 20% during the winter and 30% in the summer when augmenting a wetland with glucose (Songliu et al., 2009), while other researchers found nitrate removal increased from a 36% nitrate removal

efficiency to a 97% nitrate removal efficiency when C:N ratios increased from 0:1to 4:1 (Ding et al., 2012).

We found substantial evidence that higher salinity led to reduced nitrate removal within the study wetland. We observed a salinity range between 0.48 ppt and 11.59 ppt, and salinity was generally lower than 2 ppt (Appendix B). With median inlet nitrate concentrations, temperature, carbon and pH (7.31), we estimated that nitrate removal would decrease from 12.06 mg/L to 1.97 mg/L when salinity increased from 0.81 ppt to 9.3 ppt. These findings are in line with other researchers who found when salinity increased from 0 ppt to 10 ppt nitrate removal was significantly reduced within their study wetland (Rysgaard et al., 1999). However, there are conflicting results on the influence of salinity on nitrate removal within the literature. Wu et al. (2008) found nitrate removal significantly decreased when salinity went from 0 ppt to 30 ppt but there was no significant difference in nitrate removal when salinity went from 0 ppt to 15 ppt. Magalhães (2005) found that varying salinity (between 0.1 ppt to 26.8 ppt) had no effect on nitrate removal within systems that experience a natural fluctuation in salinity.

We found substantial evidence that higher pH led to reduced nitrate removal. We estimated that at median inlet nitrate, temperature, dissolved organic carbon and salinity, outlet nitrate concentrations nitrate removal would be 9.3 mg/L at a pH of 6.68 and 3.7 mg/L at a pH of 7.87. In reviews of denitrifying enzyme activity, optimal pH values were between 7.0 and 7.5 for *Pseudomonas* species and enzyme activity was higher at pH values of 7 and 8 than a pH of 6 for Pseudomonas mandelii (Thomas et al., 1994; Saleh-Lakha et al., 2009). We found pH values ranging between 5.61 and 8.96 with an average pH of 7.37,

which indicates that pH occasionally was within a range that decreased denitrifying enzyme activity.

Future design considerations could potentially overcome cool-temperature limitatation of wetland function during winter by reducing heat loss. Adding a varietly of insulation materials or enhancing insulative materials such as increased emergent vegetation, Reflectix, and an ice or soil layer can reduce heat loss (Wittgren and Mæhlum, 1997; Picard et al., 2005). For example, a past study found through the use of Reflectix© winter water temperatures decreased to 0.7 °C while exposed water decreased to − 8.9 °C (Picard et al., 2005). Cameron and Schipper (2011) significantly increased water temperture within their study wetland by 3.4° C through the use of a passive solar system, but they did not detect an increase in nitrate removal, arguing that the increased temperatures led to thermal stratification, short-cruiting, and reduced residence times. Their minimum temperatures were also higher and thus less limiting than ours. Combining our results with those Cameron and Schipper suggests that substantial solar-driven increases in nitrate removal could be realized with an appropriate engineering approach to maintaining vertical mixing within the wetland

Reducing pumping rates and increasing the residence time within the study wetland might lead to the water quality meeting nitrate regulatory standards; but at the expense of reduced overall throughput and increased land requirements. Typically wetlands with longer residence time have resulted in higher nitrate removal within a parcel of water, since microbes have additional time to utilize available nitrate within the water (Sutton et al., 1975; Phipps and Crumpton, 1994; Ishida et al., 2006). The drawback to this approach is a

reduction of the total load of nitrate removal since this will decrease the total volume of water that flows through the system.

The mean observed carbon, dissolved organic carbon (mg/L), to nitrogen, nitrate (mg/L), ratio at the study site was 2.2:1 indicating that in general the carbon supply was too low to maximize denitrification (Hume et al., 2002; Zhu et al., 2014). The ideal carbon to nitrogen (C:N) ratio for maximizing nitrate removal is between 4:1 and 10:1 depending on the type of carbon present (Hume et al., 2002), and we found that the C:N ratio only exceeded 4:1 12 times. It is likely that there is both an internal and an external DOC supply within the study wetland; mean carbon concentrations at the inlet and outlet of the wetland were similar. The sources of carbon within the wetland are unknown, but typical carbon sources within wetlands include soils, sediments and biomass (Kadlec and Wallace, 2008). The study wetland was vegetated predominately with *S. californium*, and the decaying *S. californicus* was a likely internal source of DOC. Algae such as Lemna minor and *Ulva intestinalis* may additionally have been a source for DOC within the wetland and within the inlet water. Agricultural runoff can also be a source of DOC within waterways (Oh et al., 2013), and was a potential source of DOC within the inlet water of the study wetland.

Wetland performance may be enhanced by increasing carbon availability, for example, through the addition of molasses (Songliu et al., 2009). Alternate plant species, or planting multiple species can also lead to increase nitrate removal. *Schoeneoplectus* (the dominant wetland plant at the study site) is one of the least efficient wetland plants in regards to nitrate removal (Bachand and Horne, 2000; Coleman et al., 2001). *Schoeneoplectus* has lower acid-soluble carbon content (i.e. cellulose, sugars, starches and hemicelluloses) than other wetland plants such as cattails. Plants with a higher fraction of

acid soluble carbon, such as cattails, better facilitate denitrification since this fraction of carbon is most readably available for denitrifying bacteria (Hamersley and Howes, 2002; Hume et al., 2002). Furthermore, studies have shown that mixed wetland beds of *Scirpus* and *Typha* lead to increased nitrate removal as opposed to wetlands vegetated with just one wetland plant sepecies (Bachand and Horne, 2000). There are limited studies analyzing why polycultures better facilitate nitrate removal but it has been suggested that temporal and spatial root partitioning allow for maximized nitrate removal (Hammer, 1989).

## 5.0 Conclusions

Treatment wetlands are an effective tool for reducing nitrate concentrations within waterways. In cool climates with year-round growing seasons, temperature can be a major limitation in wetland function, and maximization of wetland temperature at the design stage should be considered. Lack of naturally avilable carbon can also limit nitrate removal, and wetland design should select plants known to yield maximum available carbon for dentrification, or involve an addition of external carbon sources such as sugar or straw. The decision to manipulate temperature or carbon should also take into account cost, and potential impacts on the role of wetlands as habitat, where applicable.

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## Appendix A. Theory of estimating pollutant decay dynamics from single-residence time data

(Developed by F. Watson)

We postulated that removal of nitrate (*N*) over time (*t*) occurs according to one or more of several different processes including: zero decay (0), zero-order decay (Z), first-order decay (F), and dependency on variables such as temperature (T). Each of these postulates and various combinations of them can be written as partial differential equations (PDEs) for nitrate concentration with respect to time as follows:

$P_0$: $\frac{\partial N}{\partial t} = 0$

$P_Z$: $\frac{\partial N}{\partial t} = \alpha_0$

$P_F$: $\frac{\partial N}{\partial t} = \alpha_N N$

$P_{ZF}$: $\frac{\partial N}{\partial t} = \alpha_0 + \alpha_N N$

$P_{ZFT}$: $\frac{\partial N}{\partial t} = \alpha_0 + \alpha_N N + \alpha_T T$

where $\alpha_0$ is a zero-order decay constant, $\alpha_N$ is a first-order decay coefficient, and $\alpha_T$ is a decay coefficient for temperature-dependency. Note that zero-order and first-order decay processes can act in combination where by the zero-order decay coefficient is positive (i.e. a constant increase in nitrate over time) and the first-order decay-coefficient is negative (a nitrate-dependent decrease in nitrate over time), leading to nitrate reaching an equilibrium at $N = -\alpha_0/\alpha_N$.

If viewed over a fixed time interval between $t = 0$ and $t = t_{ref}$, these equations can be converted into linear models of final nitrate concentration as follows. Firstly, the PDEs are solved and $N = N_0$ is substituted at $t = 0$, leading to:

$P_0$: $N = N_0$

$P_Z$: $N = N_0 + \alpha_0 t$

$P_F$: $N = N_0 \exp(\alpha_N t)$

$P_{ZF}$:
$$\begin{aligned} N &= \left(N_0 + \frac{\alpha_0}{\alpha_N}\right)\exp(\alpha_N t) - \frac{\alpha_0}{\alpha_N} \\ &= \frac{\alpha_0}{\alpha_N}(\exp(\alpha_N t) - 1) + N_0 \exp(\alpha_N t) \end{aligned}$$

$P_{ZFT}$:
$$\begin{aligned} N &= \left(N_0 + \frac{\alpha_0 + \alpha_T T}{\alpha_N}\right)\exp(\alpha_N t) - \frac{\alpha_0 + \alpha_T T}{\alpha_N} \\ &= \frac{\alpha_0 + \alpha_T T}{\alpha_N}(\exp(\alpha_N t) - 1) + N_0 \exp(\alpha_N t) \end{aligned}$$

Secondly, $t$ is viewed as a constant ($t_{ref}$), enabling the equations to be written in linear form:

$P_0$: $N = N_0$

$P_Z$: $N = N_0 + \beta_0$

$P_F$: $N = N_0 + \beta_N N_0 = (1 + \beta_N) N_0$

$P_{ZF}$: $N = N_0 + \beta_0 + \beta_N N_0 = \beta_0 + (1 + \beta_N) N_0$

$P_{ZFT}$: $N = N_0 + \beta_0 + \beta_N N_0 + \beta_T T = \beta_0 + (1 + \beta_N) N_0 + \beta_T T$

where $\beta_0$, $\beta_N$, and $\beta_T$ are constants:

$P_0$: no constants

$P_Z$: $\beta_0 = \alpha_0 t_{ref}.$

$P_F$: $\beta_N = \exp(\alpha_N t_{ref}) - 1$

$P_{ZF}$: $\beta_N = \exp(\alpha_N t_{ref}) - 1$

$\beta_0 = \frac{\alpha_0}{\alpha_N}(\exp(\alpha_N t_{ref}) - 1),$

$P_{ZFT}$: $\beta_N = \exp(\alpha_N t_{ref}) - 1$

$\beta_0 + \beta_T T = \frac{\alpha_0 + \alpha_T T}{\alpha_N}(\exp(\alpha_N t_{ref}) - 1)$

Note that $\beta_0$ and $\beta_T$ are not identified individually, but as shown below, this is of no consequence for predictive purposes.

In combination with a suitable probability distribution, the linear equations form the basis of linear regression models:

$Y_N \sim \text{Tobit-normal}(\mu_N, \lambda, \infty)$

$P_0$: $\mu_N = N_0$

$P_Z$: $\mu_N = N_0 + \beta_0$

$P_F$: $\mu_N = N_0 + \beta_N N_0$

$P_{ZF}$: $\mu_N = N_0 + \beta_0 + \beta_N N_0$

$P_{ZFT}$: $\mu_N = N_0 + \beta_0 + \beta_N N_0 + \beta_T T$

which are the same as those reported in the main text, after renaming $\mu_N$ to $\mu_{out}$, $Y_N$ to $N_{out}$, and $N_0$ to $N_{in,lag}$ both to match the context and recognize that in a homogenous, elongate, flow-through wetland under steady flow conditions, time and distance are equivalent, and thus the time lapse between $t$ and $t_{ref}$ corresponds to the distance between the locations of measurement of $N_{in}$ and $N_{out}$. Note that each of these equations includes $N_0$ as an initial constant; this is intended to promote symmetry while reflecting the nature of the problem (removal of nitrate from an initial starting point at $N = N_0$), and can be accommodated within regression software by the use of an 'offset' term (see code below).

Once an appropriate regression procedure has been used to obtain estimates of $\beta_0$, $\beta_N$, and $\beta_T$, the corresponding values of $\alpha_0$, $\alpha_N$, and $\alpha_T$, can be obtained by inverting the above equations as:

$P_0$: no constants

$P_Z$: $\alpha_0 = \frac{\beta_0}{t_{ref}}$.

$P_F$: $\alpha_N = \frac{\ln(1+\beta_N)}{t_{ref}}$.

$P_{ZF}$: $\alpha_N = \frac{\ln(1+\beta_N)}{t_{ref}}$

$\alpha_0 = \frac{\beta_0 \ln(1+\beta_N)}{\beta_N t_{ref}}$

(obtained from $\frac{\alpha_0}{\alpha_N}(\exp(\alpha_N t) - 1) = \beta_0$, after substituting for $\alpha_N$)

$P_{ZFT}$: $\alpha_N = \frac{\ln(1+\beta_N)}{t_{ref}}$

$$\alpha_0 + \alpha_T T = \frac{(\beta_0+\beta_T T)\ln(1+\beta_N)}{\beta_N t_{ref}}$$

(obtained in similar manner to $\alpha_0$ under $P_{ZF}$)

Substitution back into the original PDE solutions then gives a final predictive capability i.e. time-varying models, derived from regression coefficients that were fitted to time-invariant data:

$P_0$: $N = N_0$

$P_Z$: $N = \frac{\beta_0}{t_{ref}} t + N_0$

$P_F$: $N = \exp\left(\frac{\ln(1+\beta_N)}{t_{ref}} t\right) N_0$

$P_{ZF}$: $N = \frac{\beta_0 \ln(1+\beta_N)}{\beta_N t_{ref}} + \exp\left(\frac{\ln(1+\beta_N)}{t_{ref}} t\right) N_0$

$P_{ZFT}$: $N = \frac{(\beta_0+\beta_T T)\ln(1+\beta_N)}{\beta_N t_{ref}} + \exp\left(\frac{\ln(1+\beta_N)}{t_{ref}} t\right) N_0$

Dependency on additional variables, such as carbon, can be incorporated exactly as for the temperature dependency.

R code corresponding to the above steps is:

```
dat = read.csv( filename )
library("AER")
LowerLim=0.2
tob = function(formula)
  tobit( formula, left=LowerLim, right=Inf, dist="gaussian",
data=dat )
m0    = tob( Nout ~ offset(Ninlag)                  )
mZ    = tob( Nout ~ offset(Ninlag) + 1              )
mF    = tob( Nout ~ offset(Ninlag) + 0 + Ninlag     )
mZF   = tob( Nout ~ offset(Ninlag) + 1 + Ninlag     )
mZFT  = tob( Nout ~ offset(Ninlag) + 1 + Ninlag + T )
mU    = tob( Nout ~                  + 1            )
# Simple model comparison:
AIC(m0,mZ,mF,mZF,mZFT,mU)
# Predictive code just for model mZFT (other models are simpler):
b0=mZFT$coef[1]; bN=mZFT$coef[2]; bT=mZFT$coef[3]
tref = 3.5 # Residence time (days).
```

```
T = 20 # Constant temperature (Celsius) for predictive purposes.
N0 = 50 # Initial nitrate (mg/L) for predictive purposes.
a0_aT = ((b0+(bT*T))*log(1+bN))/(bN*tref)
aN = log(1+bN)/tref
t=seq(0,20,0.1) # Sequence of times at which predictions are
desired.
plot(t,pmax(0,(N0+(a0_aT/aN))*exp(aN*t)-(a0_aT/aN)),type="line")
```

Note: An extra model, `mU`, is included in the above code for completeness. It represents the possibility that outlet concentration is uniform and unrelated (U) to the inlet concentration.

## Appendix B

Additional physical and chemical observed data within the Molera wetland or encompassing watershed are summarized in Figure B1.

Table 1. AIC analysis of the hypothesized Tobit regression models of the influence of *a priori* and *post hoc* predictor models on denitrification within the Molera treatment wetland. Predictor variables are denoted by subscripts: C for dissolved organic carbon, T for temperature, S for salinity, Am for ammonia, pH for pH. The initial nitrate predictor is designated by way of the designation of zero- and first-order models. Subscript 0 denotes lack of decay; subscript Z denotes zero-order decay models; subscript F denotes first-order decay models; subscript ZF denotes combined zero/first-order decay models, and subscript U denotes a model assuming constant outlet nitrate regardless of inlet nitrate. Temperature and carbon predictors were measured at the outlet location, and nitrate predictors were measured at the inlet location, lagged by 3.5-days. The winning models for the *a priori* and *post hoc* analyses are in bold.

| **Model** | **Multiple** | | | | |
|---|---|---|---|---|---|
| | | ***A priori*** | | ***Post hoc*** | |
| | K | ΔAIC | $AIC_w$ | ΔAIC | $AIC_w$ |
| **$H_{ZFTC}$** | 5 | **0.00** | **0.666** | 4.57 | 0.041 |
| $H_{ZFT}$ | 4 | 1.39 | 0.333 | 7.21 | 0.011 |
| $H_{ZFC}$ | 4 | 12.61 | 0.001 | 17.62 | 0.000 |
| $H_{ZF}$ | 3 | 15.56 | 0.000 | 21.73 | 0.000 |
| $H_{FTC}$ | 3 | 36.81 | 0.000 | 41.56 | 0.000 |
| $H_{FT}$ | 3 | 50.94 | 0.000 | 42.42 | 0.000 |
| $H_{F}$ | 3 | 36.94 | 0.000 | 54.18 | 0.000 |
| $H_{FC}$ | 4 | 50.57 | 0.000 | 56.14 | 0.000 |
| $H_{0TC}$ | 3 | 128.52 | 0.000 | 126.87 | 0.000 |
| $H_{ZTC}$ | 4 | 128.91 | 0.000 | 126.07 | 0.000 |
| $H_{0T}$ | 2 | 129.80 | 0.000 | 128.52 | 0.000 |
| $H_{ZT}$ | 3 | 130.42 | 0.000 | 128.08 | 0.000 |
| $H_{ZC}$ | 3 | 133.69 | 0.000 | 131.85 | 0.000 |
| $H_{0}$ | 2 | 136.47 | 0.000 | 135.25 | 0.000 |
| $H_{Z}$ | 2 | 136.47 | 0.000 | 135.25 | 0.000 |
| $H_{0C}$ | 2 | 146.45 | 0.000 | 141.16 | 0.000 |
| **$H_{ZFTCpHS}$** | 7 | NA | NA | **0.00** | **0.400** |
| $H_{ZFTCpH}$ | 6 | NA | NA | 1.80 | 0.163 |
| $H_{ZFTC1LpHS}$ | 7 | NA | NA | 2.48 | 0.116 |
| $H_{ZFTCS}$ | 6 | NA | NA | 3.05 | 0.087 |
| $H_{ZFTCpHS}$ | 6 | NA | NA | 3.57 | 0.067 |
| $H_{ZFTCpHSDO}$ | 7 | NA | NA | 5.28 | 0.029 |
| $H_{ZFTpHSAm}$ | 7 | NA | NA | 5.29 | 0.028 |
| $H_{ZFTC1L}$ | 5 | NA | NA | 5.82 | 0.022 |
| $H_{ZFTCDO}$ | 6 | NA | NA | 6.75 | 0.014 |
| $H_{ZFTCAm}$ | 6 | NA | NA | 6.77 | 0.014 |
| $H_{ZFTCpHSDOAm}$ | 8 | NA | NA | 7.55 | 0.009 |
| $H_{U}$ | 2 | NA | NA | 41.9 | 0.000 |

Table 2. $Log_{10}$ evidence ratio (LER) values for temperature, carbon, zero order and first order decay based on a balanced AIC model comparison. The LER for each parameter is the $log_{10}$ sum of the AIC weight divided by one minus that sum over a set of models containing all possible combinations of the predictor variables. This gives a standardized LER for each model parameter giving inferences on the certainty that the predictor has an effect.

| | **Overall Models** | | **Best Model** | |
|---|---|---|---|---|
| **Predictor** | Relative Importance | LER | $\beta$ | $\beta_{std}$ |
| (Intercept) | NA | NA | 19.017 | -7.371 |
| Zero-order | 1.000 | 7.88 | NA | NA |
| First-order (Nitrate) | 1.000 | 10.71 | -0.710 | -10.120 |
| Water Temperature | 0.999 | 2.82 | -0.771 | -2.594 |
| Carbon | 0.668 | 0.30 | -0.043 | -1.249 |

**Captions for Figures**

Figure 1. The two dots represent the inlet and outlet locations at the Molera treatment wetland. The water flow path is depicted by the grey line and the underground piping is shown in black. The black square indicates the pump house which houses the pump to draw water from the Tembladero slough into the wetland.

Figure 2. Measured physical and chemical characteristics at the inlet and outlet of the Molera treatment wetland observed between June 2012 and March 2014: (A) nitrate at inlet and outlet; (B) estimated nitrate removal (outlet minus 3.5-day-lagged inlet); (C) dissolved organic carbon; (D) temperature; (E) ammonium; (F) pH; and (G) water elevation above sea-level. Additional variables are plotted in Appendix B.

Figure 3. Modeled nitrate removal under the best-supported *a priori* model, including inlet nitrate, temperature and carbon as predictor variables. The grey vertical line indicates day 3.5, which was the mean residence time of water flowing through the wetland.

Figure B1. Additional physical chemical variables observed between June 2012 and January 2014: (A) flow measured within the source water body at a location of roughly 12 km upstream of the study wetland (USGS 11152650 Reclamation Ditch NR Salinas CA); (B) salinity; (C) C dissolved oxygen; and (D) total nitrogen.

Figure 1.

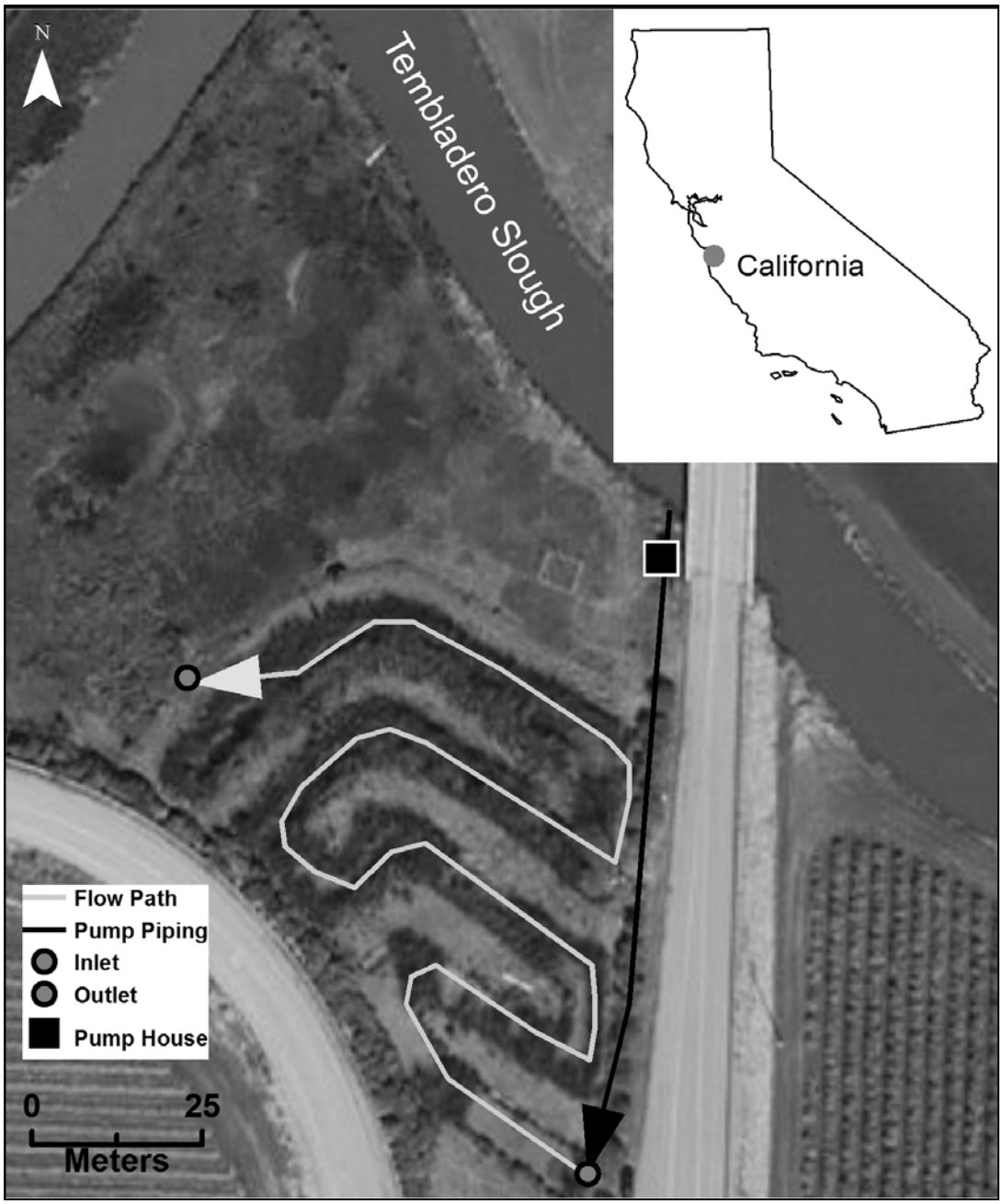

N
Tembladero Slough
California
Flow Path
Pump Piping
Inlet
Outlet
Pump House
0
25
Meters

Figure 2.

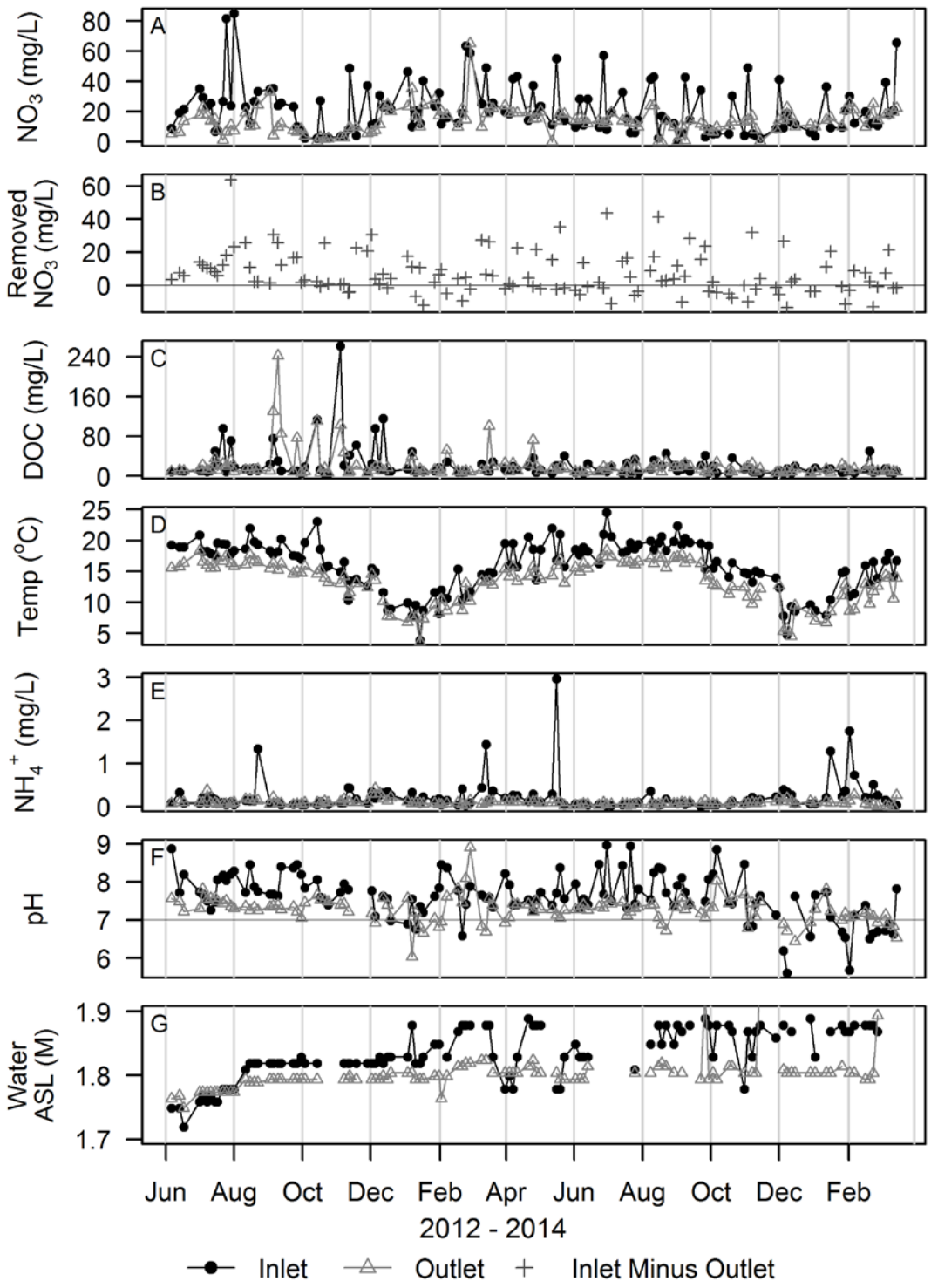

A
B
C
D
E
F
G
$NO_3$ (mg/L)
Removed $NO_3$ (mg/L)
DOC (mg/L)
Temp ($^oC$)
$NH_4^+$ (mg/L)
pH
Water ASL (M)
Jun
Aug
Oct
Dec
Feb
Apr
Jun
Aug
Oct
Dec
Feb
2012 - 2014
Inlet
Outlet
Inlet Minus Outlet

Figure 3.

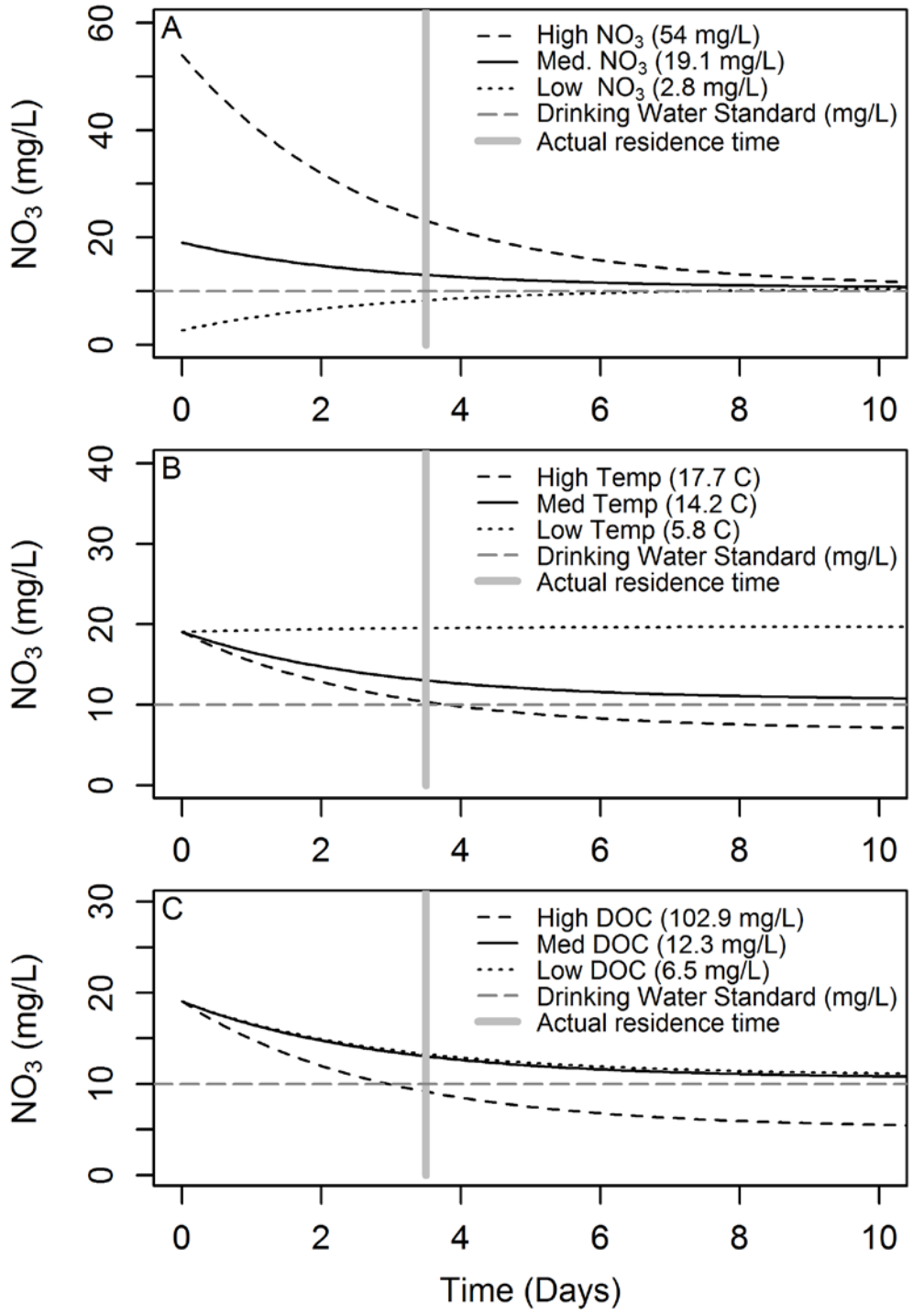
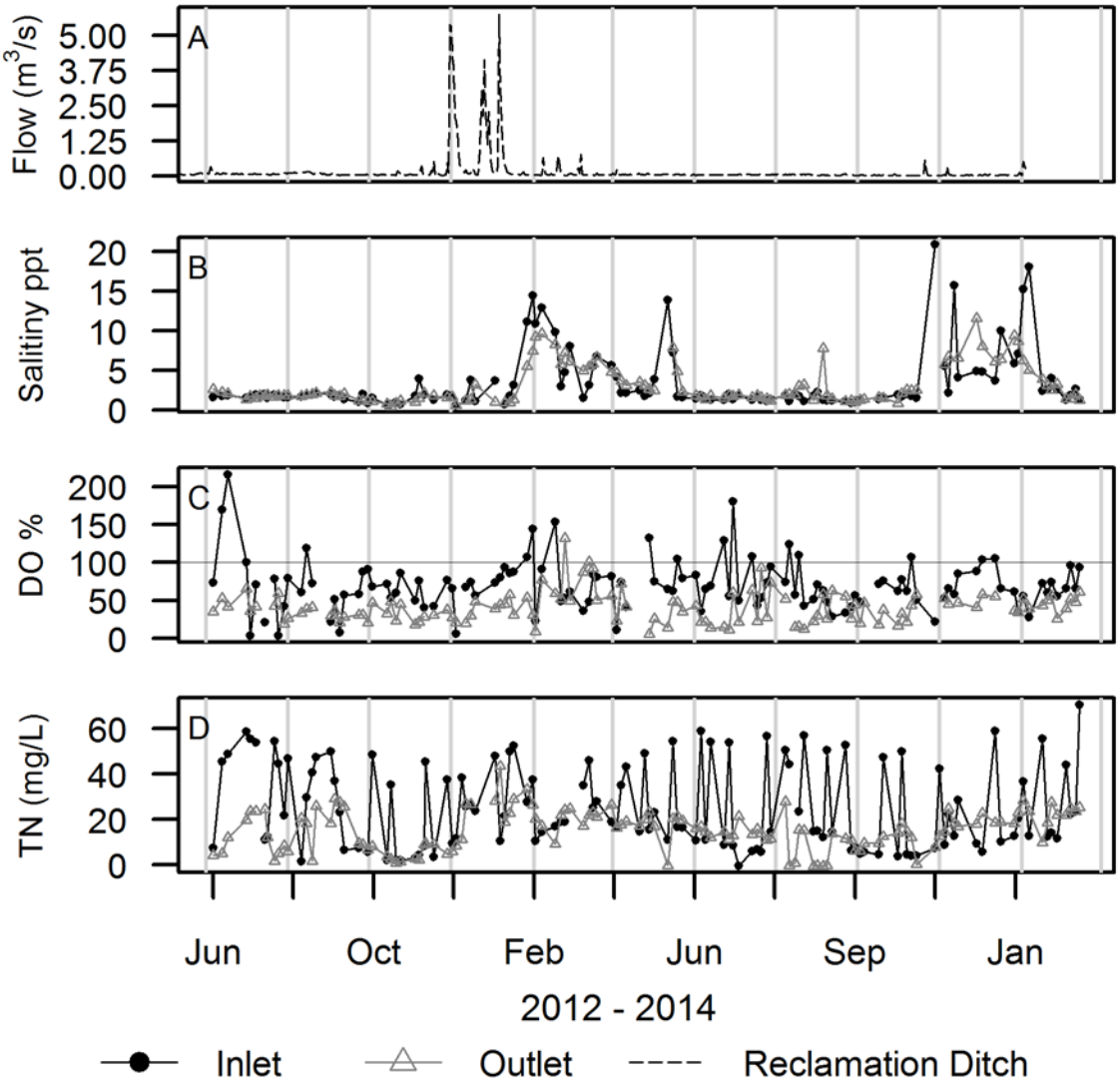

A
High $NO_3$ (54 mg/L)
Med. $NO_3$ (19.1 mg/L)
Low $NO_3$ (2.8 mg/L)
Drinking Water Standard (mg/L)
Actual residence time
$NO_3$ (mg/L)
0
20
40
60
0
2
4
6
8
10
B
High Temp (17.7 C)
Med Temp (14.2 C)
Low Temp (5.8 C)
Drinking Water Standard (mg/L)
Actual residence time
$NO_3$ (mg/L)
0
10
20
30
40
C
High DOC (102.9 mg/L)
Med DOC (12.3 mg/L)
Low DOC (6.5 mg/L)
Drinking Water Standard (mg/L)
Actual residence time
$NO_3$ (mg/L)
Time (Days)

Figure B1.


Flow (m3/s)
5.00
3.75
2.50
1.25
0.00
A
Salitiny ppt
20
15
10
5
0
B
DO %
200
150
100
50
0
C
TN (mg/L)
60
40
20
0
D
Jun
Oct
Feb
Jun
Sep
Jan
2012 - 2014
Inlet
Outlet
Reclamation Ditch