Supplemental Information: Explanation, testing and implementation of Michaelis-Menten model from Speir et al. (2017) used to estimate contribution of denitrification during simulated runoff events from:

Vegetated Ditch Habitats Provide Net Nitrogen Sink and Phosphorus Storage Capacity in Agricultural Drainage Networks Despite Senescent Plant Leaching

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SI. 1. Michaelis-Menten Model Parameterization

Previously, we developed seasonal Michaelis-Menten models to predict net N_2 fluxes across NO_3^--N concentration using the same cutgrass mesocosms employed in the current study [1]. Briefly, we used non-linear mixed effects models based on the Michaelis-Menten equation to estimate V_{max} and K seasonally. We estimated V_{max} and K with potential N_2 fluxes as our input data, back-correcting the final models to reflect measured N_2 fluxes. In the final model, we included a random effect in the seasonal models to account for nested sampling in the original experiment ($\sim V_{max} \mid$ time; [2]) and an exponential variance structure (varExp) to improve heterogeneity of residuals. We evaluated Akaike information criterion (AIC) scores for all model iterations to assess model improvement [3]. We developed the Michaelis-Menten models using the *nlme* package [4] in R [5].

SI. 2. Application and Testing of Michaelis-Menten Models with Mesocosm Data

To assess the utility of the Michaelis-Menten models developed by Speir et al. [1], we applied the models to data from a simulated runoff event conducted during early June 2014 in similar cutgrass mesocosms [6]. While Michaelis-Menten models were developed across seasons [1], here we specifically used the spring model (Eq. S1) as temperature conditions in both studies were comparable:

$$N_2\ Flux = \frac{19.94 * [NO_3\ ]}{1.44 + [NO_3]} - 6.68$$

Equation S1

where $[NO_3]$ is water column NO_3^--N concentration (in mg L^{-1}) and N_2 flux is the resultant denitrification rate (in mg m^{-2} h^{-1}). During the simulated runoff event, both NO_3^--N concentrations and N_2 fluxes were measured at varying intervals over 168 hours [6]. At each sampling point, we used the measured NO_3^--N concentration to estimate N_2 flux with the spring Michaelis-Menten model. We then plotted N_2 fluxes over time (t= 0 to 48 hours) and integrated the area under the resulting curve to determine the total N_2 produced during the simulated runoff event (Figure S1 A-B). We used estimates from the first 48 hours of the experiment only to compare to estimates of N_2 flux derived via a mass balance approach from Taylor et al. [6].

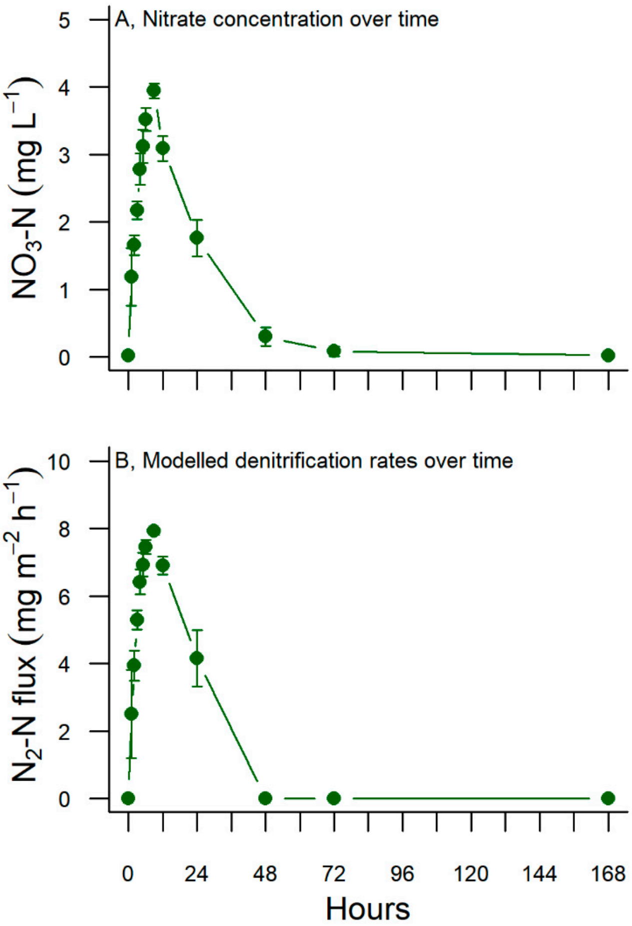

Figure S1. Measured NO_3-N concentrations over time during simulated runoff events from Taylor et al. 2015 (A). Estimated N_2-N flux rates over time calculated using equation 1 (B).

We used the spline integration method in the *MESS* package in R (version 3.2.3; R Development Core Team, Vienna, Austria) to integrate estimated rates over time and calculate a total mass mg) of N that was denitrified. Estimated 48-hour total N_2 flux from mesocosms planted with rice cutgrass were not significantly different (between approaches relying on mass balance (285 ± 36 mg N; [6]) and applying our Michaelis-Menten model (311 ± 32 mg N; paired t-test, $t_2 = -1.29$, $p = 0.32$; Figure S2).

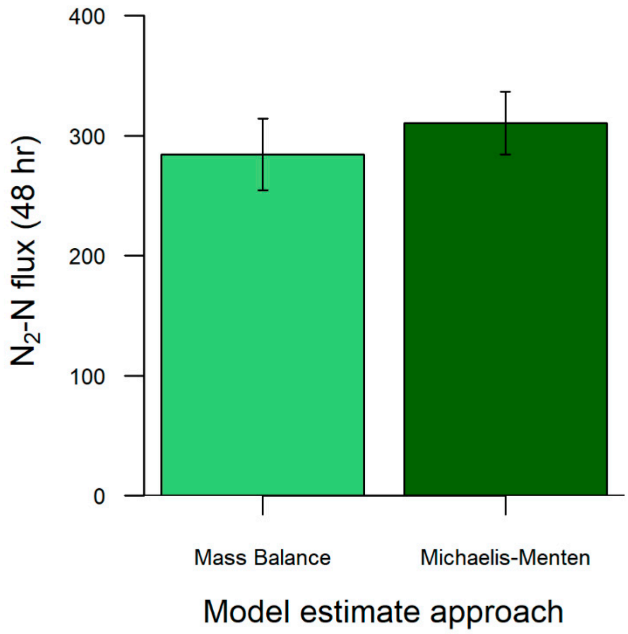

Figure S2. Comparison of 48-hr N_2-N flux estimates from mesocosms vegetated with rice cutgrass during simulated runoff events. Mass balance estimates are from Taylor et al. (2015) and Michaelis-Menten estimates are from application of models developed by Speir et al. (2017) to the same dataset.

SI. 3. Estimating contribution of denitrification to N retention in current study

After determining our modeled estimates of denitrification were similar to previously published mass balance estimates (Taylor et al. 2015), we used the model to estimate denitrification rates over time (t=0 to 72) based on changing NO_3^--N concentrations in our 20 mesocosms during simulated runoff events in June, July, and August (Figure S3). We then integrated N_2-N flux rates over time. Total contribution of denitrification to N retention was calculated for each mesocosm on each date and summarized statistically across P treatments and month (Figure 2 and Table S1). Contribution of the combined effects of denitrification across all 3 simulated runoff events are presented in Table 5 of the main text.

Table S1. Mean ± 1 SE N_2-N flux estimates (mg of N) for June, July and August simulated runoff events. Total N_2-N flux is also reported.

P treatment	June DNF	July DNF	August DNF	Total DNF
Control	257.8 ± 28.0	143.5 ± 23.5	218.6 ± 18.1	619.9 ± 50.7
Low	220.0 ± 45.9	161.0 ± 20.4	181.5 ± 53.2	563.5 ± 78.4
Medium	199.6 ± 35.7	190.8 ± 9.6	218.6 ± 15.6	608.9 ± 52.5
High	236.1 ± 65.3	156.4 ± 17.3	186.3 ± 19.3	578.8 ± 50.6

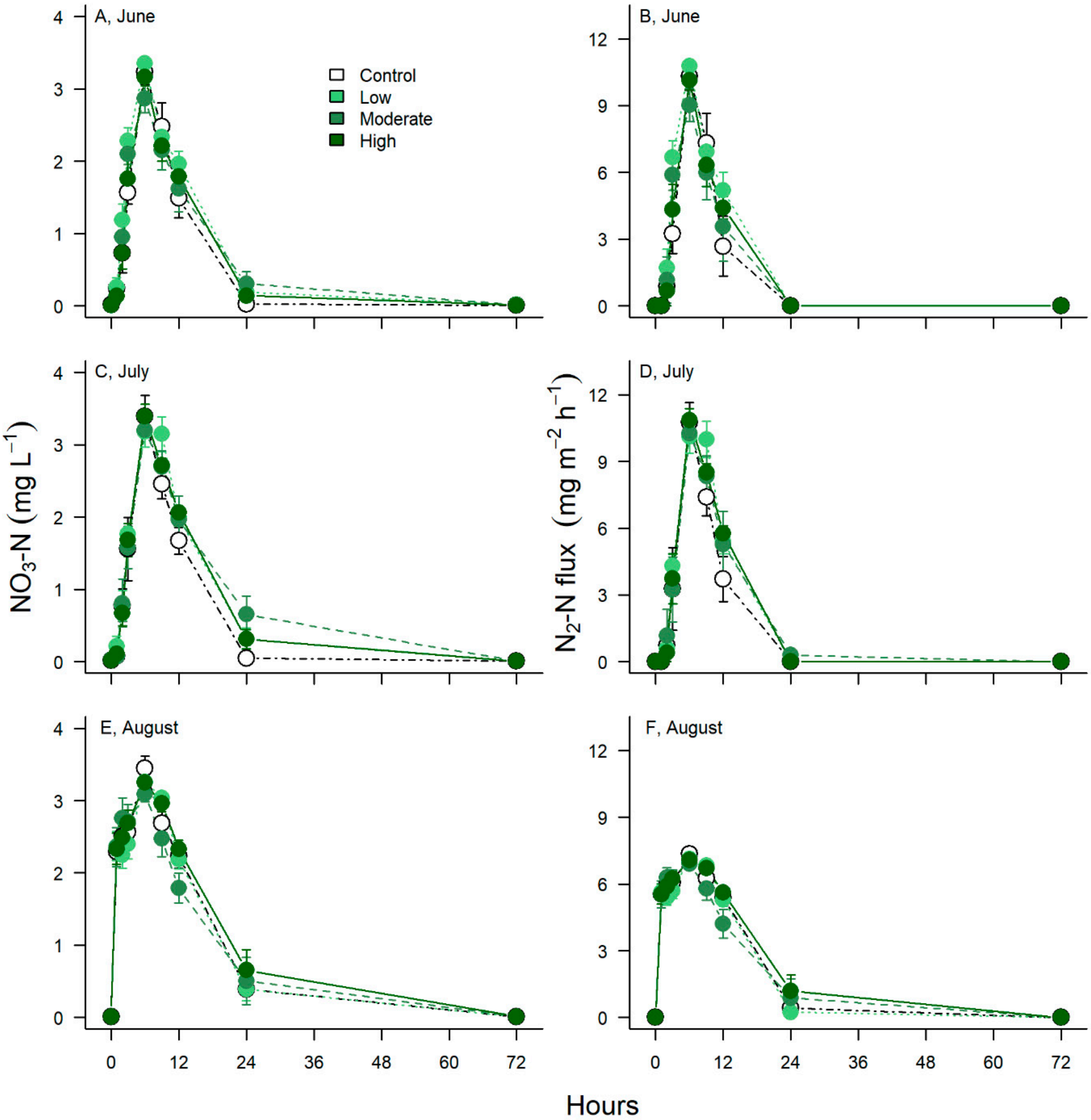

Figure S3. Measured NO_3-N concentrations over time during simulated runoff events in June (A), July (C), and August (E) and estimated N_2-N flux rates over time for June (B), July (D) and August (F) calculated by applying equation S1 to NO_3-N data.

References

1. Speir, S.L., Taylor, J.M., Scott, J.T., 2017. Seasonal Differences in Relationships between Nitrate Concentration and Denitrification Rates in Ditch Sediments Vegetated with Rice Cutgrass. J. Environ. Qual. 46, 1500–1509. https://doi.org/10.2134/jeq2016.11.0450
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