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Using environmental variables and soil processes to forecast denitrification potential and nitrous oxide fluxes in coastal plain wetlands across different land uses

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[1] We examined relationships between denitrification (DNF) and nitrous oxide (N_2O) fluxes and potentially important chemical and physical predictors to build a predictive understanding of gaseous N losses from coastal plain wetlands. We collected soil, gas, and pore water samples from 48 sampling locations across a large (440 ha) restored wetland, an adjacent drained agricultural field, and nearby forested wetlands every two months over two years. In summer and fall 2007, we measured soil DNF potential ($21.6\text{--}3560 \text{ mg N m}^{-3} \text{ d}^{-1}$) and N_2O efflux ($-4.36\text{--}8.81 \text{ mg N m}^{-2} \text{ d}^{-1}$), along with 17 predictor variables. We developed statistical models for the most comprehensive subset of the data set (fall 2007) and used another subset (summer 2007) for cross-validation. Soil pH and total soil nitrogen were the best predictors of DNF potential ($R^2_{\text{adj}} = 0.68$). A regression using carbon dioxide flux and soil temperature together with soil extractable NH_4^+ and DNF potential explained 85% of the variation in fall N_2O fluxes. The model for DNF performed reasonably well when cross-validated with summer data ($R^2 = 0.40$), while the N_2O model did not predict summer N_2O fluxes ($R^2 < 0.1$). Poor model performance was likely due to nonlinear responses to high temperatures and/or higher and more variable root respiration by plants during the growing season, leading to overprediction of N_2O flux. Our results suggest that soil DNF potential may be modeled fairly effectively from a small number of soil parameters, that DNF potential is uncorrelated with N_2O effluxes, and that successful estimation of wetland N_2O effluxes will require finer-scale models that incorporate seasonal dynamics.

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1. Introduction

[2] Nitrous oxide (N_2O), formed as a byproduct of microbial processes in soil and water, is a long-lived gas that destroys stratospheric ozone and contributes to the greenhouse effect [Bremner, 1997; Forster *et al.*, 2007]. Nitrous oxide is primarily produced as a byproduct of the microbial processes of nitrification (NF), which converts ammonium (NH_4^+) to nitrate (NO_3^-) under oxic conditions, and denitrification (DNF), which converts nitrate (NO_3^-) to inert atmospheric

dinitrogen (N_2) gas under anoxic conditions [Knowles, 1982]. DNF can be a source (production) of N_2O when DNF is incomplete due to high NO_3^- availability or incomplete anoxia, as well as a sink (consumption) for N_2O , particularly under highly anoxic conditions and low NO_3^- availability [Chapuis-Lardy *et al.*, 2007; Firestone and Davidson, 1989]. N_2O can be produced during NF (as a chemical byproduct of the ammonia oxidation step); it can also be produced or consumed by nitrifying bacteria under low oxygen (O_2) conditions which undergo nitrifier DNF [Poth and Focht, 1985; Wrage *et al.*, 2001].

[3] Treatment wetlands and many wetland restoration projects seek to remove reactive forms of N (NH_4^+ and NO_3^-) that degrade water quality and affect human health [Carpenter *et al.*, 1998; Townsend *et al.*, 2003] by promoting NF and DNF [Mitsch *et al.*, 2001; Zedler, 2003]. Yet because both processes can be large sources of N_2O , an integrative assessment of their complex biological and environmental controls is required to understand whether N_2O fluxes from NF and DNF represent a substantial offset to the water quality benefits of these processes [Schlesinger *et al.*, 2006; Verhoeven *et al.*, 2006].

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[4] N₂O fluxes are difficult to predict because they represent the net effect of multiple, interacting, and highly variable processes influenced by a variety of environmental factors. While several classifications and conceptual models of N₂O-controlling factors have been presented in the literature, the “hole-in-the-pipe” model of N gas emissions [Davidson *et al.*, 2000; Firestone and Davidson, 1989] is one of the best known. This model describes three ways in which environmental factors functionally affect the flux of N gases, including N₂O: (1) by controlling rates of NF and DNF; (2) by affecting the proportion of N₂O produced by either process (hereafter the N₂O yield); and (3) by controlling the flux of N₂O from soil and water to the atmosphere.

[5] Rate-controlling factors include temperature, the availability of substrates [bioavailable carbon (C), NH₄⁺ and NO₃⁻], and the presence of O₂ [Firestone and Davidson, 1989; Firestone *et al.*, 1980; Knowles, 1982]. Factors that control the N₂O yield of NF and DNF include soil pH, soil O₂, and the ratio of electron donors to acceptors [Firestone *et al.*, 1979; Gilmour, 1984; Šimek and Cooper, 2002; Weier *et al.*, 1993]. Particularly in the denitrification pathway, where the terminal reductive step (N₂O → N₂) has the lowest energy yield, the abundance of labile carbon relative to NO₃⁻ has been shown to have a strong influence on N₂O yield. When carbon availability is high relative to the supply of the electron donor NO₃⁻ (high C:NO₃⁻) DNF tends to yield more N₂, while low C:NO₃⁻ can result in more N₂O [Firestone *et al.*, 1980; Ullah and Moore, 2011]. For aerobic, chemoautotrophic nitrifiers, N₂O yield is more sensitive to O₂, with the highest N₂O yields from NF observed at intermediate O₂ availability or 40%–60% water filled pore space (WFPS). N₂O yield from DNF is typically higher from soils and sediments with incomplete anoxia or 60%–80% WFPS [Davidson *et al.*, 2000]. In addition to factors controlling N₂O production, physical features of soils and sediment can affect the exchange of N₂O between soil and the atmosphere. Soil particle size and drainage class can influence N₂O fluxes by controlling the rate of gas diffusion between soil, water, and the atmosphere [Castellano *et al.*, 2010; Davidson, 1991; Schindlbacher *et al.*, 2004].

[6] Controlling factors operate at different spatial (from organisms to ecosystems) and temporal (from hourly to interannual) scales [Brumme *et al.*, 1999; Groffman *et al.*, 1987; Philippot *et al.*, 2011; Wixon and Balser, 2009]. Proximal factors (e.g., availability of O₂, C, and NO₃⁻) operate at the organismal and microsite scale, while increasingly distal factors (e.g., water table depth, NF rates, soil type, and land use) affect DNF rates and N₂O flux at the field and landscape scale [Groffman, 1991; Groffman *et al.*, 1987]. Although soil parameters control fluxes of gases through soil pore spaces, they are generally measured at coarser scales and generally do not vary on annual timescales [Groffman, 1991]. Hydrologic parameters respond to weather events and climate trends, and can influence N₂O fluxes on rapid time scales (minutes to hours) as well as seasonally, with freeze/thaw cycles and changes in evapotranspiration [Wu *et al.*, 2010].

[7] These factors interact at multiple spatial and temporal scales, collectively influencing NF and DNF rates and N₂O fluxes. In a wetland ecosystem, controls on N₂O flux are particularly complex due to significant potential for consumption by DNF. Identifying important predictors could inform modeling and monitoring efforts, even if such

predictors are likely to vary regionally or by ecosystem type [Groffman *et al.*, 2000]. In restored wetlands influenced by agricultural nutrient loading, many conditions exist that could promote high rates of NF and DNF (high inorganic N from legacy fertilizers), and high N₂O yields (low C relative to natural wetlands (low C:N), low pH, variable soil moisture). Alternatively, N₂O fluxes could be low if pH and O₂ availability or physical properties inhibit N cycling rates, thus limiting the supply of NO₃⁻ for DNF. If physical properties that are more stable or factors that are more temporally variable can be shown to be better predictors of N₂O fluxes or DNF potential, such information could be incorporated into larger scale models of N₂O flux and DNF and could be used to guide restoration practices and monitoring efforts.

[8] We conducted a multiyear study of gas fluxes from drained agricultural, recently restored, and forested wetlands of the NC coastal plain, finding differences among wetland types for soil characteristics such as pH, soil organic C, and N availability, as well as annual N₂O fluxes [Morse *et al.*, 2012]. For two comprehensive surveys, we measured a wide variety of potential predictors in an attempt to discover the most important and robust environmental correlates of N₂O fluxes and DNF potential across a wide range of soil conditions, under the hypothesis that mechanisms controlling these processes would function similarly across wetland types. There is great interest worldwide in using environmental variables to forecast landscape-scale N₂O fluxes to enable better understanding and regulation of greenhouse gas emissions [Olander and Haugen-Kozyra, 2011]. Our a priori prediction was that across all sites both DNF and N₂O fluxes would be highest in intermittently flooded sites where variation in soil oxygen content would provide ideal conditions for NF and DNF to occur and where a high N₂O yield from both processes would be expected. We anticipated that soil redox potential would be a good indicator of hydrologic and metabolic conditions leading to DNF and N₂O emissions. We expected that the high soil C:NO₃⁻ ratios of undisturbed wetlands would lead to low DNF and N₂O production, while the high NO₃⁻ and low soil C content of the active agricultural field and recently restored wetlands would result in higher rates of DNF and higher N₂O fluxes.

2. Methods

2.1. Site Descriptions

[9] This study focused on a 440 ha restored wetland (RW) and three reference sites (one agricultural field (Ag), and two forested wetlands (FW jointly; FW-dry and FW-wet)) in the Albemarle Peninsula in the Outer Coastal Plain of North Carolina. The Timberlake Restoration Project (TLRP) is a large, privately owned, compensatory mitigation site (1704 ha; 35°54'22"N, 76°09'25"W), hydrologically connected to the Little Alligator River and to Albemarle Sound. The RW site historically contained swamp forest and pocosin ecosystems prior to drainage and agricultural conversion in the 1970–1980s; it was planted with freshwater wetland tree species (e.g., *Taxodium distichum*) in 2004 and hydrologically restored in February 2007 [Needham, 2006]. The site has been described in more detail in [Ardón *et al.*, 2010] and [Morse *et al.*, 2012]. The main soil series within RW are mapped as Ponzer muck (loamy, mixed, dysic, thermic Terric Haplosaprist) and Hyde loam (fine-silty, mixed, active,

thermic Typic Umbraquult) [Natural Resources Conservation Service (NRCS), 2009].

[10] The three reference sites also are located on very poorly drained hydric soils. The drained agricultural site (Ag) adjacent to RW, currently planted in a corn and soybean rotation, is mainly comprised of Weeksville silt loam soils (coarse-silty, mixed, semiactive, thermic Typic Umbraquult) [NRCS, 2009]. One of the unmanaged forested wetlands is a preservation area (FW-dry) within TLRP in a minimally impacted mixed hardwood forest (oak-gum-cypress) [Needham, 2006] with soils mapped as Dorovan muck (dysic, thermic Typic Haplosaprist) [NRCS, 2009]. The other unmanaged forested wetland (FW-wet) is located 8 km away in the Palmetto Peartree Preserve, in a swamp hardwood stand of cypress and tupelo. Soils in FW-wet are mapped as Belhaven muck loamy, mixed, dysic, thermic Terric Haplosaprist [NRCS, 2009]. More complete descriptions can be found in [Morse et al., 2012].

2.2. Field Sampling

[11] Prior to hydrologic restoration in 2007, we established one transect within each of the two main soil types in RW ($n = 12$ sampling points in Hyde loam and 21 in Ponzer muck) along an elevation gradient, to capture the expected range of the flooding conditions. We set up five sampling points within each of the three reference sites (Ag, FW-dry, FW-wet). We collected soil samples (0–15 cm) and pore water samples from piezometers (15 cm depth) in June and October 2007 from all 48 sampling points across the four sites. We measured N₂O fluxes and related environmental variables every two months from July 2007 to June 2009. The methods and sampling approach for estimating gas fluxes are described extensively in [Morse et al., 2012]. Briefly, we applied the static chamber technique [Livingston and Hutchinson, 1995] to measure gas fluxes from soil and water to the atmosphere, from the accumulation rate of gas in the chamber headspace during incubations consisting of two 30–40 min intervals. Soil and air temperature were also measured during gas flux incubations. For other environmental variables, we also installed the following instruments at each of 48 sampling locations: one EC-5 soil moisture sensor (Decagon Devices, Pullman, Wash.) to measure volumetric water content, five platinum-tipped redox electrodes [Vepraskas and Faulkner, 2001] to measure soil redox potential with a voltmeter and calomel reference electrode (Fisher Scientific, Pittsburgh, Pa.), and one slotted PVC monitoring well with a water level recorder (Levellogger Gold or Silver, Solinst Instruments, Georgetown, Ontario, Canada) to record overlying water pressure and temperature every 15 min.

[12] This approach resulted in four groupings of predictor variables, based on the method of data collection and the timescales of variability: (1) soil properties; (2) soil microbial process variables; (3) soil nutrient pools; and (4) contemporaneously measured field-based variables (Table 1). Because summer and fall 2007 were abnormally dry [North Carolina Drought Management Advisory Council, 2008], the water table in many sampling points was below the depth of our monitoring wells and piezometers; we therefore had to exclude hydrologic variables related to water table depth and pore water chemistry altogether due to the number of missing observations.

2.3. Laboratory Analyses

[13] Concentrations of CO₂, N₂O, and CH₄ in field-collected 9 mL glass vials were measured on a Shimadzu 17A gas chromatograph with electron capture and flame ionization detectors, retrofitted with 6-port valves and a methanizer in series with the flame ionization detector to measure the three gases from the same sample. Soil properties [pH, bulk density (BD), %C, %N, and C:N] were determined using standard methods. Soil chemical variables were measured on subsamples of fresh soil sieved with a 2-mm mesh. We extracted inorganic N from duplicate 2.5 g soil samples with 25 mL 2M KCl, and analyzed the extracts for NH₄⁺ and NO₃⁻ on a Lachat QuickChem 8000 automated system using the phenate method for NH₄⁺ and the hydrazine reduction method for NO₂⁻ + NO₃⁻ (Lachat Instruments, Milwaukee, Wisc.). To determine soil dissolved organic C (DOC) and total dissolved N (TDN), we extracted duplicate 1 g (dry weight equivalent) subsamples of fresh soil with 30 mL of nanopure water for 15 min on a shaker table, centrifuged the samples at 3400 rpm for 10 min, and analyzed the filtered supernatant (Whatman GF/F filter) using a Shimadzu TOC-V total carbon analyzer with a TNM-1 nitrogen module (Shimadzu Scientific Instruments, Columbia, Md.).

[14] Soil microbial processes were likewise determined on fresh soils and included DNF potential, net nitrification (NF), and active microbial biomass. Denitrification potential was measured as DNF enzyme activity (DEA) [Groffman et al., 1999; Tiedje et al., 1989]. In this anoxic assay, 5 g of soil in a slurry with excess NO₃⁻ and labile C were incubated with acetylene, which blocks the activity of the nitrous oxide reductase enzyme, allowing the denitrification rate to be estimated through the accumulation of headspace N₂O. Net NF was estimated using a nitrapyrin-inhibition assay [Kemp and Dodds, 2002], in which parallel 5 g soil samples (with and without nitrapyrin, which blocks NH₄⁺ oxidation) were incubated in centrifuge tubes for 7 days in the dark on a shaker table at 150 rpm (aerated daily); accumulation of NH₄⁺ due to blocked NF was measured on the Lachat QuickChem 8000. A protocol for estimating active microbial biomass by substrate-induced respiration (SIR) using autolyzed yeast as the substrate was modified from [West and Sparling, 1986] and [Bradford et al., 2008].

2.4. Data Analysis and Modeling

[15] We used data from fall 2007 (October 2007 soil sampling and November 2007 gas sampling) for model development and validation because it was the most comprehensive, with 48 sampling points and more ancillary information on soil and environmental variables available. We used the smaller summer 2007 data set (soil sampling from June and gas sampling in July 2007 at 21 sampling points) for model cross-validation. To put this subset of gas analyses into context with our previous work on biogeochemical differences by land use in these sites [Morse et al., 2012], we applied a site classification based on mean water level within the restored wetland (RW), yielding three subgroups with 5 sampling sites in each, for balanced comparisons to the reference sites: rarely flooded (water table within 0.1 m of the surface 5% of the time; RW-dry), intermittently flooded (water table within 0.1 m of the surface 24% of the time; RW-int), and mostly flooded (water table within 0.1 m

Table 1. Summary Statistics, Including Mean and Root Mean Square (RMS), for 17 Explanatory Variables^a

Type (time scale)	Variable	Units	n	Mean	Range	RMS	Model Development (Fall 2007)						Model Cross-Validation (Summer 2007)					
							Relation to N ₂ O Flux			Relation to DNF Potential			Relation to N ₂ O Flux			Relation to DNF Potential		
							±	r ²	P	±	r ²	P	±	r ²	P	±	r ²	P
Soil properties (annual or longer)	N ₂ O Flux	mg N·m ⁻² d ⁻¹	36	.53	-4.36–8.81	2.81												
	BD	kg/m ³	36	683	60.5–1390	798	–	0.007	0.62	+	0.28	0.03	+	0.024	0.54	+	0.44	0.003
	%N	% mass	36	0.86	0.13–3.09	1.29	+	0.079	0.10	–	0.12	0.17	–	0.062	0.33	–	0.33	0.013
	%C	% mass	36	19.2	2.47–50.8	25.1	+	0.036	0.28	–	0.11	0.20	–	0.065	0.32	–	0.38	0.006
Microbial processes (days/weeks)	C:N	g C/gN	35	23.3	16.1–31.2	23.7	–	0.13	0.034	–	0.01	0.77	–	0.044	0.42	–	0.01	0.64
	pH	–log [H ⁺]	36	4.36	3.16–7.00	4.41	+	0.039	0.25	+	0.52	0.001	+	0.073	0.28	+	0.11	0.17
	DNF pot.	mg N·m ⁻³ d ⁻¹	36	816	67.4–3560	1110	–	0.011	0.54	+	0.01	0.75	–	0.000	0.96	–	0.025	0.53
	NF pot.	mg N·m ⁻³ d ⁻¹	36	–2.39	–90.5–112	41.8	–	0.002	0.79	+	0.17	0.10	–	0.117	0.16	–	0.19	0.073
Soil nutrient pools (hours/days)	SIR	g C·m ⁻³ d ⁻¹	36	15.2	0.154–45.2	20.6	–	0.011	0.54	+	0.07	0.28	–	0.077	0.26	–		
	DOC [#]	g C/m ³	31	71.7	0.383–398	103	+	0.005	0.71	+	0.64	<0.001	–	0.223	0.048	+	0.01	0.73
	TDN [#]	g N/m ³	31	10.9	0.0787–60.1	19.9	+	0.077	0.13	+	0.02	0.59	–	0.086	0.24	+	0.002	0.86
	NH ₄ ⁺	g N/m ³	36	4.34	0.585–19.8	6.60	+	0.001	0.84	–	0.81	<0.001	+	0.171	0.088			
Field-based variables (hours)	NO ₃ ⁻	g N/m ³	36	8.84	0.340–60.9	16.1	+	0.091	0.74	+			–	0.030	0.50			
	Redox	mV	36	135	–318–823	330	+	0.020	0.41				–	0.003	0.83			
	CH ₄ flux	mg C·m ⁻² d ⁻¹	33	4.61	–16.0–95.2	18.0	+	0.006	0.8				–	0.071	0.29			
	CO ₂ flux	g C·m ⁻² d ⁻¹	30	1.86	0.513–5.75	2.25	+	0.58	<0.001				+	0.003	0.83			
Soil moisture	Soil temperature	°C	36	13.8	10.0–19.5	14.0	+	0.19	0.008				+	0.071	0.29			
	Soil moisture	%	31	22.4	1.59–57.8	28.5	–	0.034	0.32				–	0.028	0.51			

^aVariables include bulk density (BD), percent nitrogen (%N), percent carbon (%C), nitrification potential (NF pot.), substrate inducible respiration (SIR), dissolved organic C (DOC), total dissolved N (TDN), ammonium (NH₄⁺), nitrate (NO₃⁻) and N₂O flux in fall and summer 2007, and the relation to N₂O flux and denitrification potential (DNF pot.), as applicable, in simple linear regression models. Variable groups are ordered from distal/slowly changing to proximal/dynamic controls. Field-based variables were not tested to explain DNF potential. Soil properties were not measured in summer 2007. Values are underlined when $P \leq 0.10$.

Figure 1. N₂O fluxes and soil redox potential in (a) summer and (b) fall 2007. N₂O flux results (means $\pm$ SE) are shown against 95% confidence interval of redox potential (right y axis), displayed by site: agricultural field (Ag), restored wetland (RW) with three hydrologic classes (dry, intermittent, wet), and two forested wetland sites (FW-dry, FW-wet). Different letters indicate differences at $P < 0.10$ in N₂O flux among sites.

of the surface 82% of the time; RW-wet) [Morse *et al.*, 2012]. For both summer and fall 2007 data sets, we tested group differences in DNF potential (as DEA) using permutation one-way tests with adjusted P -values for multiple comparisons between groups (oneway_test and Nemenyi-Damico-Wolfe-Dunn test, R coin package) [Hothorn *et al.*, 2008].

[16] We compiled soil and environmental variables from fall 2007 from 48 sampling points into a data set and excluded observations that had missing values for any predictor variables. We achieved a data set of 20 observations (out of a possible 48) that had no missing values for all potential predictors of N₂O flux (18 predictors) and DNF potential (11 predictors). While hydrologic variables such as water level are undoubtedly important for understanding DNF, missing values forced us to exclude water level and variables related to pore water chemistry. We used principal components analysis (PCA; princomp; R package stats) [Venables and Ripley, 2002] to identify correlations between predictor variables and to see how observations clustered together according to land use in parameter space.

[17] We developed multiple linear regression models for N₂O fluxes and DNF potential, but not for net nitrification; NF assays proved to be uninformative, with very low values across most observations, but those results were included in the subsequent data analysis as predictors of N₂O fluxes and DNF potential. With nearly as many predictor variables as observations, we grouped predictor variables into four groups according to the method of measurement and estimated time-scale of variability—soil properties, soil microbial processes, soil nutrient pools, and field-based variables—to avoid overfitting the model. We then built regression models using each of four variable categories (submodels) to predict N₂O flux and three of the variable groups (excluding field-based variables) to model potential DNF. While theoretical models might suggest nonlinear relationships between N₂O flux and variables that control oxygen availability, such as soil moisture or water level [Davidson *et al.*, 2000], we decided to use linear models as a simpler starting point and in accordance with similar studies [Davidson and Swank, 1986; Ullah and Moore, 2011].

[18] We used a stepwise method based on Akaike's Information Criterion (AIC) with forward and backward selection (stepAIC; R package MASS) [Venables and Ripley, 2002] to identify the combination of variables within each category that best fit the N₂O flux data. We then combined the four best fitting submodels into one model and repeated the stepwise AIC model selection procedure to identify the overall models that best fit the N₂O flux data. Final model comparisons were based on AIC_c to account for small sample size [McQuarrie and Tsai, 1998]. We repeated this multiple regression process to develop a model for DNF potential using soil properties, soil microbial processes, and soil nutrient pools. Again, we built submodels for each category of variables, used stepwise AIC to identify the best submodels, and then combined the submodels to identify the two models that best fit the DNF potential data over all groups of variables. After building models for N₂O flux and DNF potential using the fall 2007 data set ($n = 20$) with values for all candidate predictor variables, we tested model performance with observations from the same period that had been excluded from model building due to a missing value for one or more predictors ($n = 10$). We performed model cross-validation with input data from summer 2007 ($n = 18$), to test model performance across seasons.

3. Results

3.1. DNF Potential and N₂O Flux Patterns by Land Use Type

[19] Measured N₂O fluxes from summer and fall 2007 surveys encompassed much of the variation in N₂O efflux observed throughout our two year study period, except in the agricultural field where N₂O fluxes were substantially lower than the long-term mean in that site (Figure 1) [Morse *et al.*, 2012]. Consistent with our findings from the longer study, N₂O fluxes in the fall 2007 sampling period were significantly higher in the Ag and FW-dry sites than in the FW-wet and RW-wet sites ($P < 0.10$; Figure 1b); in the summer survey, the highest N₂O fluxes were measured in the drier portion of the restored wetland (RW-dry) and again in FW-dry (Figure 1a). DNF potential in laboratory assays varied

Figure 2. Denitrification potential and redox potential for soils collected in (a) summer and (b) fall 2007. DNF potential results (means $\pm$ SE) are shown against 95% confidence interval of redox potential (right y axis), displayed by site: agricultural field (Ag), restored wetland (RW) with three hydrologic classes (dry, intermittent, wet), and two forested wetland sites (FW-dry, FW-wet). Different letters indicate differences at $P < 0.10$ in DNF potential among sites.

between 21.6 and 3570 mg N m⁻³ d⁻¹, but was only significantly and consistently different between the Ag and FW-wet sites in both summer and fall 2007 (Figure 2). Within the intensive survey data sets, neither N₂O fluxes nor DNF potential showed a clear relationship with redox potential, and N₂O flux patterns were not correlated with variation in DNF potential across sites (Figure 3).

3.2. Distributions of and Correlations Between Predictor Variables

[20] Prior to building multiple regression models for the fall 2007 data set, we examined the distributions of predictor variables and their relationships to N₂O flux and DNF potential (Table 1). The highest variability across sampling points was seen in variables related to soil microbial processes, with intermediate variability found in soil solute and field-based variables, while the smallest range was seen in soil properties. We explored correlations of variables through PCA (Figure 4), with the first two components of the PCA together explaining 60% of the variation (41% and 19%, respectively) in environmental variables across sites. We found that many predictor variables were correlated (SIR, BD, redox, DOC, and NF at one end of axis 1, with NH₄⁺, %C and %N at the opposite end), while the orthogonal axis grouped NO₃⁻, DNF potential, CO₂ flux, pH, TDN, and

soil temperature (Figure 4a). Additionally, PCA revealed three clusters by land use, such that: (1) Ag sites were clearly separated from all other sites; (2) the forested wetland sites and three relatively wet sites within the restored wetland clustered together due to similarities; and (3) the remaining points within the restored wetland formed a third group (Figure 4b). Observations from the Ag site aligned with variables associated with soil fertilization, liming, and tillage (e.g., high pH, NO₃⁻, and soil temperature), while observations from the forested wetlands and wetter sites within the RW aligned with variables that were associated with high organic matter (high soil C and N content, NH₄⁺, and soil moisture; Figure 4). The third grouping of variables, along which the drier RW sites were aligned, was directly opposite the organic matter gradient, and included variables such as SIR, DOC, redox potential, and bulk density (Figure 4). CO₂ flux was aligned closely with N₂O flux; these two variables were the only ones with a significant correlation to a principal component, PC2 in this case ($r = 0.37$ and 0.39 , respectively; $P < 0.10$; Figure 4a).

3.3. Multiple Regression Models for N₂O Flux

[21] Stepwise multiple regression with automated variable selection by AIC yielded submodels for N₂O fluxes for each group of candidate variables (Table 2). Of the soil

Figure 3. N₂O fluxes (July 2007–June 2009) across all sites plotted against (a) redox potential and (b) denitrification potential (summer and fall 2007). Note log-scale on y axis.

Figure 4. Biplots of principal components analysis showing (a) loadings of variables on the two principal components; and (b) scores on the two principal components for sampling points according to land use type (Ag: agricultural; RW: restored wetland; FW: forested wetland).

properties, %C alone was the best predictor but only explained 5% of the variance in N₂O flux ($P > 0.05$). The microbial process submodel was composed of DNF potential and NF potential ($R_{\text{adj}}^2 = 0.19$; $P = 0.087$), while the soil nutrient pools submodel selected KCl-extractable NO₃⁻ and NH₄⁺ ($R_{\text{adj}}^2 = 0.39$; $P = 0.0086$). The best model from field-based contemporaneously measured variables found that soil CO₂ flux and soil temperature explained 61% of the variance in N₂O fluxes (Table 2). The four submodels were combined into one model with seven parameters, which was refined again using stepwise AIC variable selection. This process yielded the final N₂O model based on four parameters: two field-based variables (soil CO₂ flux and soil temperature), one soil nutrient pool variable (NH₄⁺), and one microbial process variable (DNF potential), with R_{adj}^2 of 0.85. Bivariate plots of these individual predictors revealed significant positive linear relationships between N₂O flux and the first three predictors (soil CO₂ flux, temperature, and NH₄⁺

availability; $P < 0.006$), while DNF potential alone had no significant relationship to N₂O flux (Figure 5).

[22] The AIC values were corrected to AIC_c to account for small sample size [McQuarrie and Tsai, 1998], and the model with the smallest AIC_c was deemed to best fit the data given the competing models. The w_i column in Table 2 represents the relative weight of each model given the competing models, allowing comparisons between models. The overall model for N₂O flux had a weight of over 0.99, meaning that there was very strong support for this model. In comparing only the submodels to one another, we found that the field-based model (soil CO₂ flux and soil temperature) had a weight of 0.98, vastly outperforming the other submodels when the overall best model was removed from the comparison (not shown).

3.4. Model Fits and Model Validation for N₂O Flux

[23] The best fitting N₂O model accounted for 85% of the variance in N₂O flux, with soil CO₂ flux as the most

Table 2. Model Selection Results for N₂O Fluxes and Denitrification Potential, With Data From Fall 2007 as the Input Data Set ($n = 20$)^a

	AIC - Selected Variables	R_{adj}^2	K	AIC	AIC _c	w_i	Rejected Variables
N ₂ O flux model	CO ₂ .flux + soil.temp + NH ₄ ⁺ – DEA	0.85	4	-1.91	0.757	0.999	
<i>Submodels</i>							
Soil properties	%C	0.05	1	34.4	34.6	0	BD, C:N, pH, %N
Microbial processes	DEA – NF	0.08	2	33.6	34.3	0	SIR
Soil solutes	NO ₃ ⁻ + NH ₄ ⁺	0.39	2	25.4	26.1	0	DOC, TDN
Field-based	CO ₂ .flux + soil.temp	0.61	2	16.2	16.1	0.001	CH ₄ , flux, redox, soil moisture
DNF potential model	pH ² – pH + NO ₃ ⁻	0.68	3	255	256	0.59	
<i>Submodels</i>							
Soil properties	pH ² – pH – %N	0.65	3	257	258	0.22	BD, %C, C:N
Microbial processes	SIR	0.07	1	278	278	0	NF
Soil solutes	NO ₃ ⁻ + NH ₄ ⁺	0.61	2	259	259	0.20	DOC, TDN

^aFor each model and submodel, the predictor variables selected and rejected are shown, as well as the adjusted R^2 , number of parameters (K), Akaike's Information Criterion (AIC) and corrected AIC (AIC_c), and the relative weight (w_i) of each model given the other models. Italicized values identify the model that best fits the data.

Figure 5. Bivariate relationships between N₂O flux and individual predictor variables identified for best fit model: (a) soil CO₂ flux; (b) soil temperature; (c) KCl-extractable ammonium; and (d) DNF potential. Regression lines not shown when $r^2 \leq 0.10$.

important predictor in the model, followed by soil temperature and NH₄⁺ ($R^2 = 0.89$; $R^2_{\text{adj}} = 0.85$; Tables 2 and 3). In the second best fitting N₂O model, soil CO₂ flux was over three times as important as soil temperature in explaining N₂O flux (relative importance = 0.50 and 0.16, respectively). Soil temperature, CO₂ flux, and NH₄⁺ were each positively related to N₂O flux, while DNF potential had a negative relationship. We plotted observed against fitted values for each model (Figure 6a) to see how the models captured the range of values. Both models appeared to perform well at intermediate and high values, but did not predict negative flux values well. When the N₂O models were validated with data from the same time period but excluded from model building (because these data points lacked one or more of the 18 potential predictor variables but had results for the limited set of predictors included in the final model), they had similar fits (model 1 $R^2 = 0.73$ and model $R^2 = 0.67$; Figure 6b). Both models when cross-validated with predictor data collected in summer 2007 performed poorly, with little predictive power ($R^2 \leq 0.10$), and vastly overestimated observed N₂O fluxes (Figure 6c).

3.5. Multiple Regression Models for Denitrification Potential

[24] The DNF potential modeling effort identified submodels for each category of soil variables, with the soil

properties model and the soil nutrient pools model fitting the data well ($R^2_{\text{adj}} = 0.65$ and 0.61 , respectively; $P < 0.001$). The soil properties model included %N, pH, and (pH)², while the soil nutrient pools model was based on NO₃⁻ and NH₄⁺ (Table 2). With pH there was both a linear and quadratic relationship to DNF potential. Model selection for microbial process variables identified substrate-inducible respiration (SIR) as the best predictor of DNF potential, though it was not significant ($R^2_{\text{adj}} = 0.07$; $P = 0.27$). The three submodels were combined into an overall model with six parameters, from which NO₃⁻, pH, and (pH)² emerged as the best predictors of DNF potential by stepwise AIC model selection. Bivariate plots showed a significant quadratic relationship between DNF potential and pH ($r^2 = 0.69$; Figure 7a) and a significant positive linear relationship between DNF potential and NO₃⁻ availability ($r^2 = 0.62$; Figure 7b). This overall model fit the DNF potential results well ($R^2_{\text{adj}} = 0.68$; $P < 0.001$), and was the best fit to the data, with a relative weight of 0.59, followed by the soil properties submodel with a weight of 0.22 (Table 2).

3.6. Model Fits and Model Validation for Denitrification Potential

[25] The two best models of DNF potential data had similarly good fits to the data ($R^2_{\text{adj}} = 0.68$ and 0.65 , $R^2 = 0.74$ and 0.71 ; Tables 2 and 3). In the best fitting DNF potential

Table 3. Estimated Coefficients, Standard Errors, Relative Importance of Each Variable, and Proportion of Variance Explained (R^2) for the Two Best Models of N₂O Flux and Denitrification Potential

N ₂ O Flux					DNF Potential				
Variable Category	Variable	Estimated Coefficient	Standard Error	Relative Importance	Variable Category	Variable	Estimated Coefficient	Standard Error	Relative Importance
<i>Model 1</i>	(Int)	−10.5	1.69			(Int)	9.90×10^3	3.68×10^3	
Field-based	CO ₂ .flux	0.767	0.213	0.37	Soil property	pH ²	391	169	0.23
Field-based	soil.temp	0.777	0.132	0.27	Soil property	pH	-3.87×10^3	1.56×10^3	0.20
Solute pool	NH ₄ ⁺	0.432	0.0927	0.19	Solute pool	NO ₃ [−]	29.9	17.6	0.31
Microbial process	DNF pot.	-1.11×10^{-3}	2.91×10^{-4}	0.06					
				R ² = 0.87					
<i>Model 2</i>	(Int)	−3.62	1.85			(Int)	1.27×10^4	3.50×10^3	
Field-based	CO ₂ .flux	1.26	0.287	0.50	Soil property	pH ²	570	135	0.37
Field-based	soil.temp	0.230	0.139	0.16	Soil property	pH	-5.23×10^3	1.40×10^3	0.30
				R ² = 0.66	Soil property	%N	−150	164	0.04
					R ² = 0.71

Figure 6. Model fits to observed data and validation of N₂O models. (a) Fitted versus observed N₂O flux for modeled data set (model 1 slope = 0.93 and $R^2 = 0.89$; model 2 slope = 1.00 and $R^2 = 0.65$); (b) validation of N₂O models (model 1 slope = 0.43 and $R^2 = 0.73$; model 2 slope = 0.60 and $R^2 = 0.67$); and (c) cross-validation with data from summer 2007 (model 1 slope = -0.10 and $R^2 = 0.10$; model 2 slope = 0.035 and $R^2 < 0.01$). Regression lines not shown when $R^2 \leq 0.10$.

model, pH accounted for 43% of the variance, with NO₃[−] contributing 31% to the model. In the second best DNF potential model, pH again accounted for the bulk of the variance (67%), with %N representing only 4%. The pH and (pH)² coefficients were similar in both DNF potential models, with a U-shaped relationship and a minimum around pH 4.2 (Figure 7a). In the DNF potential models, DNF potential increased with increasing NO₃[−] in the first case and had a negative relationship to soil %N in the second case (Figures 7b–7c, Table 3). The fit of the two DNF potential models was similar, without major outliers in the data (Figure 8a). Validation of DNF potential models yielded very different results: Model 1 (including pH and NO₃[−]) fit the validation data set very poorly (slope = 0.28, $R^2 = 0.05$), but Model 2 (including pH and %N) fit the validation data set better (slope = 0.65, $R^2 = 0.30$, $P = 0.05$; Figure 8b). Cross-validation of the DNF potential models with data from summer 2007 showed similar results to the validation tests, with Model 2 again fitting the data much better than Model 1,

explaining 40% of the variance in DNF potential ($P = 0.003$; Figure 8c).

4. Discussion

4.1. Patterns in N₂O Flux, Denitrification Potential, and Predictor Variables by Wetland Type

[26] We expected N₂O fluxes to be highest in sites with intermediate or fluctuating hydrology, and DNF potential to be highest where redox potential was lowest, but patterns in N₂O fluxes and DNF potential across wetland land uses did not follow these expectations. Mean values and variability of N₂O flux, redox potential, and DNF potential were higher in fall than in summer data sets. In both summer and fall data sets, the lowest N₂O fluxes were measured in sites with lowest redox potentials (RW-wet) and one of the forested wetlands (FW-wet; fall only) (Figure 1). While this result could be consistent with complete DNF in these environments, DNF potential was not high in these sites. DNF

Figure 7. Bivariate relationships between DNF potential and individual predictor variables identified for best fit model: (a) soil pH; (b) KCl-extractable nitrate; and (c) soil N content. Regression lines not shown when $R^2 \leq 0.10$.

Figure 8. Model fits to observed data and validation of DNF potential models. (a) Fitted versus observed DNF potential for modeled data set (model 1 slope = 1.00 and $R^2 = 0.74$ and model 2 slope = 0.99 and $R^2 = 0.71$); (b) validation of DNF potential models 2 (model 1 slope = 0.28 and $R^2 = 0.05$ and model 2 slope = 0.65 and $R^2 = 0.30$); and (c) cross-validation with data from summer 2007 (model 1 slope = -0.005 and $R^2 < 0.01$; model 2 slope = 0.35 and $R^2 = 0.40$). Regression lines not shown when $R^2 \leq 0.10$.

potential was highest in both summer and fall in the Ag site, which also had the highest N₂O fluxes and redox potential in fall. The FW-dry site, with relatively high N₂O fluxes and intermediate redox potential, was the only site which aligned our expectations for N₂O flux, but DNF potential was low in this site in both sampling periods. These results suggest that DNF could be the source of N₂O in the Ag site, and that factors other than DNF were controlling N₂O fluxes in the FW-dry site, which is consistent with results from a related ¹⁵N tracer study [Morse, 2010].

[27] In addition to the differences in N₂O flux and DNF potential we found among sites, the principal components analysis (PCA) of predictor variables provided more evidence of differences among wetland types, identifying three distinct groupings by land use: (1) agricultural, (2) forested wetland and relatively wet RW, and (3) drier RW sites (Figure 4b). N₂O fluxes and DNF potential were both positively related with PC2 (an axis of soil fertility and nitrogen availability), and not correlated at all to PC1 (related to organic matter and soil moisture), suggesting that N availability and pH status are more important drivers of N₂O fluxes than organic C and moisture across these sites, and that these variables would be good predictors of both N₂O flux and DNF potential, which is consistent with our regression models.

[28] We hypothesized initially that hydrologic variables would be primary controls on N₂O fluxes, with highest rates at intermediate soil moisture or redox potential, due to contributions from both NF and DNF. We did not find a simple relationship between N₂O fluxes and redox potential across the entire study period, or between N₂O fluxes and either DNF or NF potentials, indicators of the two biological sources of N₂O (Figure 2; NF results not shown). This was not surprising, given that multiple controls can interact to influence both NF and DNF rates, product ratios, and transport to the atmosphere [Brumme et al., 1999; Davidson, 1991; Wu et al., 2010]. In wetland sites with redox potentials that would be thermodynamically conducive to DNF, NO₃⁻ availability may be low enough that DNF rates are low, and any DNF activity may proceed through the terminal

reductive step [e.g., Yu et al., 2008]. We conclude that DNF potential as measured by the DNF enzyme assay was not a good indicator of N₂O flux patterns across land uses (Table 1). This finding is not surprising given that the DNF enzyme assay is meant to be a more stable indicator of DNF, while N₂O fluxes may exhibit very large spatiotemporal variability [Groffman et al., 2000, 2006; Yanai et al., 2003].

4.2. Predicting N₂O Flux

[29] Despite differences in N₂O fluxes and DNF potentials among wetland types, we identified robust multiple regression models that fit observed N₂O flux across agricultural soils, forested wetlands, and restored wetlands during fall 2007. We found that the best models for N₂O fluxes depended primarily on CO₂ flux and soil temperature, explaining 65% of the variance, with an additional 29% explained by NH₄⁺ availability and DNF potential (Table 3). The tight relationship between N₂O flux and CO₂ flux has been recently demonstrated in a synthesis of gas exchange studies in eight ecosystems throughout the world, which found a slope of 0.66 ($r^2 = 0.79$) in rice paddies, 0.19 ($r^2 = 0.66$) in temperate forests, and 0.16 ($r^2 = 0.79$) in dry croplands [Xu et al., 2008]. In our data set, in a model with soil CO₂ flux alone, the slope was 0.52 ($r^2 = 0.58$; $P < 0.001$; Table 1), placing our study sites closer to rice paddies than temperate forests or croplands. Soil CO₂ flux is used to represent C availability in some simulation models of N-gas products from DNF, such as DAYCENT [Del Grosso et al., 2000]. The temperature sensitivity of N₂O emissions has been shown to depend in part on N availability, with lower temperature responses in N-limited forests [Barnard et al., 2005; Grant and Pattey, 2008; Schindlbacher et al., 2004]. The correlation of N₂O fluxes with soil CO₂ fluxes and temperature shows that these environmental variables could be useful for predictive purposes because they integrate physical parameters, such as gas diffusivity and solubility, with biological factors such as respiration and enzyme activities that increase soil C and N availability and cycling.

[30] While robust models emerged for N₂O fluxes across all sampling points during fall 2007, when applied to data

from summer 2007, both models over-predicted N₂O flux, with poor fit to observed values (Figure 6c). The range of N₂O fluxes in summer 2007 was smaller over all sites, compared to the fall data set, which included more observations of negative N₂O flux (Table 1). The most likely explanation for poor model performance in predicting summer N₂O fluxes is that much higher root respiration by plants during the growing season would increase soil CO₂ flux without necessarily affecting N₂O production, leading to over-prediction of N₂O flux. For this to be true, root respiration would have to vary substantially across sites, rather than being a constant proportion of soil respiration, which can range from 30% to 50% [Schlesinger and Andrews, 2000]. While this is plausible, given that the vegetation cover varies across these sites from soybeans/corn crops to young forest with dense grassland understory to mature forest, it is not something we could estimate after the fact. Our model also did not include any estimate of plant N demand, which could limit N availability during the growing season. Temperatures were higher in July than November 2007, which could explain lack of fit due to temperature, if a nonlinear relationship between temperature and N₂O fluxes were more appropriate across seasons. These results suggest that simple models of N₂O flux based on soil respiration may be most applicable outside the growing season, when root respiration and plant N uptake are not major factors.

[31] The role of N availability and DNF in regulating N₂O fluxes in our models was not straightforward. We expected that high soil NO₃⁻ content would lead to high DNF and N₂O production, while high soil C would promote the production of N₂ over N₂O in DNF. Although NH₄⁺ and NO₃⁻ together helped to explain 39% of the variation in N₂O flux in the soil solute submodel (Table 2), the final model included NH₄⁺ rather than NO₃⁻ as a predictor, perhaps because NO₃⁻ was already strongly correlated with soil temperature and soil CO₂ flux (Figure 4). Since we found a positive relationship between NH₄⁺ and N₂O flux in the best fitting N₂O model, one explanation could be that N₂O fluxes are dominated by nitrification, or coupled nitrification-denitrification, driven by high NH₄⁺ availability in these wetlands. While we have no evidence for direct NF emissions of N₂O to explicitly support or refute this interpretation, our best fit model for N₂O flux does suggest that more N₂O is produced in soils with higher soil CO₂ flux, temperature, and NH₄⁺, and lower DNF potential. The simplest explanation is that NF, or coupled NF-DNF, was a major contributor to N₂O fluxes during the fall season. This interpretation is also supported by laboratory ¹⁵N stable isotope tracer experiments which demonstrated a higher ¹⁵N₂O yield from soil cores from these sites amended with ¹⁵NH₄⁺ than from cores amended with ¹⁵NO₃⁻ [Morse, 2010]. Alternatively, the “leaky pipe” model [Davidson, 1991] proposes that incomplete DNF would also yield high N₂O flux under conditions of excess N availability and low DNF potential, which is also consistent with the parameters in our multiple regression model (Table 2).

[32] Soil redox potential was not the integrator of hydrologic and metabolic conditions that we expected: redox potential alone was not correlated with N₂O flux (Figure 3), and did not contribute to the overall N₂O flux model. Potentially important finer-scale spatial and temporal dynamics, such as pulsed emissions of N₂O or lags between

controlling variables and N₂O fluxes, were not captured in our modeling approach. It has been shown that point measurements of redox potential in the soil may not adequately reflect the conditions in microsites within the soil where N₂O is produced [Yu et al., 2006]. Covariance between predictor variables could also perhaps explain why certain variables we expected to be important did not emerge as significant predictors in our analysis. Factors known to be important for DNF that surprisingly were not selected for the final models were variables related to C availability, soil pH, and active microbial biomass; however they were likely correlated with parameters in the models: carbon quality and availability were related to CO₂ flux, while soil pH was negatively correlated with NH₄⁺ (Figure 4a).

4.3. Multiple Regression Models for Denitrification Potential

[33] With respect to DNF potential, we found that pH was the single most important variable in the two best fitting models. The influence of ongoing liming in the Ag site, along with waning effects of past liming in RW has led to a wide range in soil pH across land use types (3.75 in RW-wet to 5.53 in Ag) [Morse et al., 2012]. Other studies have found that pH below 4 tends to inhibit DNF in laboratory settings [e.g., van den Heuvel et al., 2011]; however, in acid soils below pH 4, it appears that microbial communities can be adapted to and denitrify at low pH [Parkin et al., 1985; Šimek and Cooper, 2002; Weier and Gilliam, 1986]. Our survey showed DNF optima at the low and high ends of our pH gradient, such that the relationship between DNF and pH was U-shaped, with DNF potential minimized at pH 4.2. The other important factors in DNF potential models were related to N availability, with KCl-extractable NO₃⁻ and %N explaining 31% and 4% of the variation in DNF potential, respectively, in the two best fitting models (Table 3). Nitrate is one of the required substrates for denitrification, and thus the positive relationship to DNF potential was expected [Groffman, 1991]. In the second-best DNF potential model, soil N content was negatively related to DNF potential, which is not intuitive. In the PCA plot (Figure 4a), soil %N was positively correlated with %C and NH₄⁺ availability, factors that could indicate lower NF rates [Reddy and Patrick, 1975; Strauss and Lamberti, 2000], and lower NO₃⁻ supplies for DNF in these sites (Figure 4a). Model validation and cross-validation offered stronger support for the second-best model (with pH and %N as predictors), which fit observed DNF potential in both fall and summer 2007 ($R^2 = 0.30$ and 0.40 , respectively; Figure 6). This suggests that NO₃⁻ availability is more dynamic than DNF potential, and as such may not be useful for predicting values of an assay that integrates soil properties, microbial processes, and environmental variables over longer periods than measurements of soil N pools or in situ N cycling rates [Groffman et al., 1999; McGill et al., 2010].

4.4. Spatial and Temporal Aspects of Factors Controlling N₂O Flux and DNF Potential

[34] Soil biogeochemical processes, like DNF, are often thought to be controlled by factors at multiple levels in a complex system [Brumme et al., 1999; Wixom and Balser, 2009]. We aimed to identify relationships between N₂O flux and a suite of environmental variables that change over

different time scales. By comparing the relative influence of distal, slowly changing variables (e.g., soil properties) versus more proximal, rapidly changing variables (e.g., soil NO₃⁻ pools or soil temperature) we hoped to provide insights that could be useful for monitoring and modeling N₂O fluxes and denitrification more broadly [Groffman, 1991; Groffman *et al.*, 2009]. Organizing controlling factors into a hierarchy should ultimately allow us to construct more mechanistic and realistic models than classifying predictors as rate-controlling, yield-controlling, or flux-controlling variables as per Davidson [1991].

[35] We found that N₂O fluxes across all sampling points during one season were best predicted by either two contemporaneously field-measured variables (soil CO₂ flux and soil temperature) or with an additional microbial process variable (DNF potential) and one soil nutrient variable (NH₄⁺ availability). Some studies have shown N₂O fluxes to be predictable based on general soil characteristics such as soil C:N [Klemedtsson *et al.*, 2005], or multiple factors including bulk density, precipitation regime, and plant community [Brumme *et al.*, 1999]. However, the fact that more dynamic variables were most useful in predicting N₂O fluxes in this study, and that the influence of these factors over N₂O fluxes changes temporally, is not surprising [Groffman, 1991; Harms and Grimm, 2008], given that N₂O fluxes tend to have high variability in space and time [Harms and Grimm, 2012; Toma *et al.*, 2010; Yanai *et al.*, 2003]. Conversely, the DNF potential model based on two soil property variables (pH and %N) fit the validation and cross-validation data sets much better than the model based on one soil property variable (pH) and one soil nutrient variable (NO₃⁻ availability; Figure 7). This again suggests that NO₃⁻ availability may be too dynamic to predict DNF potential, even if it may control DNF rates. Further, DNF potential in summer 2007 was predicted reasonably well (R² = 0.42) by two soil properties, so it may be a robust parameter that likewise does not change very rapidly in these sites.

4.5. Denitrification and N₂O Fluxes in Restored Wetlands

[36] This study found that contemporaneously measured environmental variables were the best predictors of N₂O fluxes for a given sampling event across a range of wetland land uses, and that soil N availability and DNF potential can provide additional predictive ability for such models. Although there is much support in the literature for hydrologic controls over soil N₂O effluxes [Davidson *et al.*, 2000; Hernandez and Mitsch, 2006; Yu *et al.*, 2008], we did not find evidence for this during summer and fall 2007, which was a relatively dry period. For both N₂O flux and DNF potential, N-related variables were more informative than C availability in these organic, acidic soils. Given that many similar restored wetlands receive N-enriched agricultural effluent, such N inputs are likely to drive N₂O fluxes and DNF more than C availability. To determine whether a wetland restoration site is a significant source of N₂O fluxes, we recommend that both spatial and temporal variability at appropriate scales be addressed through measurement or models [Brumme *et al.*, 1999]. However, in this restored wetland, N₂O fluxes over two years were significantly lower than in adjacent agricultural fields and a nearby forested wetland [Morse *et al.*, 2012], while water quality benefits

attributed to DNF were substantial [Ardón *et al.*, 2010]. This suggests N₂O fluxes should not be considered an impediment to wetland restoration in similar coastal freshwater sites with agricultural N loading.

[37] We found the relative temporal stability of DNF potential encouraging since the assay was developed to facilitate cross-system comparisons and to give a broader view of DNF, given that actual DNF rates are difficult to measure and are highly variable [Groffman *et al.*, 2006]. On the other hand, these qualities make DEA assays a relatively blunt tool for assessing responses to seasonal changes or weather events, and suggest that DNF potential is only partially useful for predicting sensitive and rapidly changing variables such as field N₂O fluxes. Our analyses in this study could not distinguish between nitrification and denitrification as sources of N₂O in these wetlands, but model results suggest that NF-derived N₂O is likely to be a larger component of N₂O emissions than has previously been considered.

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