



# Richness, biomass, and nutrient content of a wetland macrophyte community affect soil nitrogen cycling in a diversity-ecosystem functioning experiment



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## ABSTRACT

The development of soil nitrogen (N) cycling in created wetlands promotes the maturation of multiple biogeochemical cycles necessary for ecosystem functioning. This development proceeds from gradual changes in soil physicochemical properties and influential characteristics of the plant community, such as competitive behavior, phenology, productivity, and nutrient composition. In the context of a 2-year diversity experiment in freshwater mesocosms (0, 1, 2, 3, or 4 richness levels), we assessed the direct and indirect impacts of three plant community characteristics – species richness, total biomass, and tissue N concentration – on three processes in the soil N cycle – soil net ammonification, net nitrification, and denitrification potentials. Species richness had a positive effect on net ammonification potential (NAP) through higher redox potentials and likely faster microbial respiration. All NAP rates were negative, however, due to immobilization and high rates of ammonium removal. Net nitrification was inhibited at higher species richness without mediation from the measured soil properties. Higher species richness also inhibited denitrification potential through increased redox potential and decreased nitrification. Both lower biomass and/or higher tissue ratios of carbon to nitrogen, characteristics indicative of the two annual plants, were shown to have stimulatory effects on all three soil N processes. The two mediating physicochemical links between the young macrophyte community and microbial N processes were soil redox potential and temperature. Our results suggest that early-successional annual plant communities play an important role in the development of ecosystem N multifunctionality in newly created wetland soils.

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## 1. Introduction

Wetlands promote biogeochemical pathways within the nitrogen cycle through a diversity of physicochemical properties and biological controls. Mature wetland soils feature a build-up of organic nutrient stores, specialized interactions between plants and microorganisms, and internal feedbacks buffering environmental disturbances which maximize the potential for N transformations (Mitsch et al., 2012; Ballantine and Schneider et al., 2009; Tang et al., 2011). In newly created wetlands, cycling occurs in more homogenous soils and nitrogen cycling is under greater influence from the external environment (Anderson et al., 2005;

Bruland and Richardson, 2005). Plants exert a strong influence in these young soils by providing carbon subsidies, modifying soil structure, conserving N, and creating habitat for microbial communities (Morgan et al., 2008; Ruiz-Rueda et al., 2009; Forshay and Dodson, 2011). The promotion of N biogeochemistry in created wetlands, such as the important soil processes of ammonification, nitrification and denitrification, requires greater understanding of how attributes of early plant communities affect multiple aspects of the soil N cycle.

Macrophytes are generally a positive long-term structuring force on soil N cycling, but the effect over shorter time-scales depends on both species individual traits and community diversity (Balvanera et al., 2006; Gutknecht et al., 2006). No clearly positive or negative relationships have been established between species diversity and N processing in wetlands in part for this reason, as well as because of the inherent complexity of feedbacks

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between multiple trophic levels (e.g., plants and microorganisms). Specifically for plant effects on soil N transformations, “noise” from conflicting indirect and direct effect mechanisms could contribute to the obscurity of diversity-ecosystem function relationships (Balvanera et al., 2006). For instance, macrophytes indirectly affect microbial N processing by regulating chemical conditions of the mediating environment (i.e., soil N, carbon, oxygen, pH, and temperature) that adds variability to plant-microbe interactions (Booth et al., 2005; Laughlin, 2011; Sutton-Grier et al., 2011). At the same time, plant species directly and differentially interact with soil microorganisms through resource competition and by hosting a variety of multi-functioning microbial communities in their rhizosphere (Månsson et al., 2009; Hu et al., 2014; Schlatter et al., 2015). Some of these effects are more immediately influential than others. To counter soil anoxia that creates strongly N-limiting conditions in wetlands, macrophytes release oxygen from their roots that has been shown to indirectly enable organic N mineralization, the aerobic production of nitrate, and the coupled anaerobic process of denitrification in adjacent soil zones (Reddy et al., 1989).

Two theories help explain how plant communities affect ecosystem function. The theory of species diversity indicates that multiple species promote ecosystem efficiency through greater non-additive complementary use of niche space and greater total impacts on biogeochemical cycling (Tilman et al., 1996; von Felten et al., 2009). The second theory, or the “mass ratio hypothesis”, asserts that other community functions are more important such that positive effects on ecosystem functioning are proportional to dominant species’ investments in primary production and are relatively insensitive to subordinate species impacts (Grime, 1998). In the case of a diversity-ecosystem function experiment, employed for this study, these theorized mechanisms affecting ecosystem N functioning should co-occur in plant communities (Hooper et al., 2005; Mokany et al., 2008) and can be studied with investigations of manipulated plant richness alongside additional plant community attributes.

Plant N use, as an important fate of soil N, is a less well-studied plant trait in diversity-ecosystem function experiments. Tissue concentrations of N vary by species, reflecting differences in nutrient acquisition and physiological use, and have been shown to change along a species richness gradient (van Ruijven and Berendse, 2005). Plants are thought to facilitate competitive coexistence through differences in N uptake, which intensifies nutrient extraction and soil N depression (Kahmen et al., 2006; von Felten et al., 2009). Both N extraction and the quality of senesced plant material (i.e., carbon:nitrogen [C:N] ratio) have important consequences on N processing in soils, particularly young developing soils.

In contrast, researchers have commonly reproduced positive plant diversity-productivity relationships in controlled experiments and much research has been devoted to the effects of community-level productivity on ecosystem functioning (Cardinale et al., 2007; Cong et al., 2014). Supported by empirical findings of higher rates of N processing in older created wetlands exhibiting greater organic carbon stores (Hossler et al., 2011; Wolf et al., 2011; Ballantine et al., 2014), some theorize that plant diversity positively drives N cycling from a combination of higher productivity, faster decomposition, and greater nutrient and matter turnover (Knops et al., 2002). In newly created wetlands, this carbon-flow pathway may be most readily observed in early-successional communities with highly productive annual plants with quick life cycles and unique physiology (Matthews and Endress, 2010). Other studies suggest more specific mechanisms for the observed effect of high productivity on soil N cycling. DeMeester and Richter (2010) demonstrated that an aggressive non-native wetland annual with the habit of forming almost monotypic communities promoted soil denitrification from

lowered redox potentials in its root zone (DeMeester and Richter, 2010). High productivity may also be linked to greater soil carbon resources, though not from biomass production but from root exudates (Zhai et al., 2013).

To determine how plant or planting characteristics – richness (as a measure of diversity), together with resulting biomass production (as a measure of productivity) and tissue nutrient ratios – influence ecosystem N multifunctionality through the microbial N processes of soil ammonification, nitrification, and denitrification, we conducted a mesocosm experiment with freshwater wetland annual and perennial macrophytes over two growing seasons. We hypothesized that the most species-rich mesocosms, in association with high biomass production and N concentrations in plant tissue would provide greater carbon availability and quality of N substrate to the soil and would be positively linked to heterotrophic and autotrophic N processes. Because richness effects on ecosystem functions are often explained in terms of the long-term effects of productivity, we investigated whether additional direct and indirect mechanisms stimulated N cycling in the short-term. Specifically, we asked the following research questions: (1) Does species richness impact soil N cycling through mechanisms relating to biomass quantity (biomass), quality (tissue C:N mass ratio), or modifications of the soil environment? (2) Does plant richness influence soil N cycling more strongly than other plant community attributes (such as biomass quantity or quality)? and (3) which are the most important soil physicochemical properties linking plant community attributes to microbial N functioning? We expected to identify important synergistic and antagonistic mechanisms comprising the overall compound effect between plant community attributes and soil N processes that would illuminate details of soil N development in young created wetlands.

## 2. Mesocosm experiment

The study was conducted in 40 aboveground mesocosms, 568 L Rubbermaid tubs with a 1.11 m<sup>2</sup> surface area each (long dimension = 147 cm, narrow dimension = 99 cm), located in the Ahn Wetland Mesocosm Compound of George Mason University, Fairfax Campus. In March 2012, mesocosms were filled with 20 cm of sand on top of river pea gravel, and topped with 30 cm of locally-produced topsoil commonly used in wetland creation in the Virginia Piedmont Physiographic Region. Water levels were influenced by precipitation events and were periodically supplemented with de-chlorinated tap-water in the hottest weeks of summer to maintain a minimum of 5 cm standing water above the soil surface (N loading rate was 1.3 µg N/m<sup>2</sup> yr in 2012 and 0.4 µg N/m<sup>2</sup> yr in 2013).

A richness gradient with four functionally distinct wetland macrophytes was established and maintained by weeding in a substitutive experimental design that varied species not plant density. Wetland macrophytes were classifiable within either a ruderal (i.e., annual) or interstitial (i.e., perennial) functional group: *Eleocharis obtusa* (Willd.) Shult. (obligate annual), *Mimulus ringens* L. (facultative annual), *Juncus effusus* L. (interstitial reed) and *Carex vulpinoidea* Michx. (a sedge, herein classified as an interstitial tussock) (Boutin and Keddy, 1993). In early May 2012, a combination of four similar-sized plugs were planted in a linear array in each mesocosm. Each of the four species was assigned two monocultures, for eight mesocosms at the lowest richness level; the second level had all unique two-species combinations once for six mesocosms total; the third level had all unique three-species combinations once with twelve mesocosms total; and the highest richness level had eight mesocosms with all species represented. Six mesocosms were left unplanted as an experiment control that provided baseline soil physicochemical conditions in the absence of macrophytes but in

the presence of other colonizing or adventive species (e.g., algae and invertebrates) and allochthonous litterfall. The shoots of the annuals died back completely over the winter between growing seasons, depositing all aboveground on the soil surface. Whereas the obligate annual is presumed to have demonstrated strict annual behavior and died completely, the facultative annual emerged early in the calendar year (approximately early March, before the official growing season in Virginia) which is taken as a sign of perennial behavior. The reed and fox sedge (*C. vulpinoidea*) remained partially green throughout the winter.

### 3. Methods

#### 3.1. Soil sampling and laboratory analyses

Soil net ammonification potential (NAP) and net nitrification potential (NNP) were measured using laboratory incubations (Binkley and Hart, 1989) following Hart et al. (1994). The procedure tracks the net change in mineralized N by measuring the initial and 28-day KCl-extractable ammonium and nitrate concentrations in soil cores. To more closely reflect field rates, longitudinal sections of intact soil cores (i.e. soils were not mixed) were incubated in complete darkness in flasks partially open to the atmosphere. Soil moisture of the cores was maintained at field conditions by adding one or more drops of water each week to the surface of cores to counter any mass loss due to evaporation. In August 2013, three replicate soil samples per mesocosm were systematically collected from different areas outside of the centralized planting area down to 10 cm depth using 30 mL disposable syringes (2 cm diameter) that created suction. The soils were processed within 1–2 days of collection. Two 10 g dry-weight equivalent wet subsamples from half of each core split length-wise were each placed in 125 mL flasks, one for pre- and post-incubation extractions, respectively. Thus, the three replicates per mesocosm were not mixed, and the soils were not broken apart, prior to incubation. Day-0 soils were processed immediately while day-28 soils were incubated in the dark at 21 °C. For both pre- and post-incubation extractions, soils were covered with 100 mL of 2 M KCl and mixed with the extraction solution on a reciprocal shaker for an hour. The supernatant was syringe filtered to <0.45 μm and quantified for ammonium and nitrate (quantified as nitrate + nitrite) by colorimetric analysis on an Astoria-Pacific segmented-flow autoanalyzer. Net N mineralization potential (NMP) was calculated as the post incubation quantity of combined mineralized N (i.e. ammonium + nitrate + nitrite) less the initial quantity; the changes in ammonium and nitrate concentrations were used to calculate NAP and NNP separately.

Soil denitrification was measured as denitrification potential (DP) using the denitrification enzyme assay (Smith and Tiedje, 1979; Tiedje et al., 1989) following Groffman et al. (1999). Three soil cores were collected in each mesocosm using the same sampling method as used for NAP/NNP in August 2013. Analysis of soils occurred no later than 3 days after sampling. Soils were homogenized per mesocosm and then assessed for DP in triplicate. Field-moist soil (~25 g ww) was weighed into 125 mL flasks and mixed to form slurries with 25 mL solutions of dextrose (1 g/L), potassium nitrate (1.01 g/L), and chloramphenicol (0.1 g/L) in deionized water. Acid-scrubbed acetylene (10 mL) was then injected into the flasks to inhibit the reduction of N<sub>2</sub>O at T<sub>0</sub>. Flasks were incubated in a reciprocal shaker and gas samples withdrawn at 45 and 105 min. Samples were determined for N<sub>2</sub>O (=N<sub>2</sub>O+N<sub>2</sub> production) by gas chromatography on a Shimadzu GC-8A equipped with a Supelco Hayesep Q 80/100 packed column (1 m × 1/8 in × 2.1 mm) and an electron-capture detector. Because leakage was detected in the gas-tight syringe used for sample injections towards the end of the gas chromatography runs, we only

quantified DP in a subset of mesocosms comprised of five controls (unplanted control), five monocultures (richness level 1), and a total of four, seven, and four mesocosms in richness treatments 2, 3, and 4, respectively.

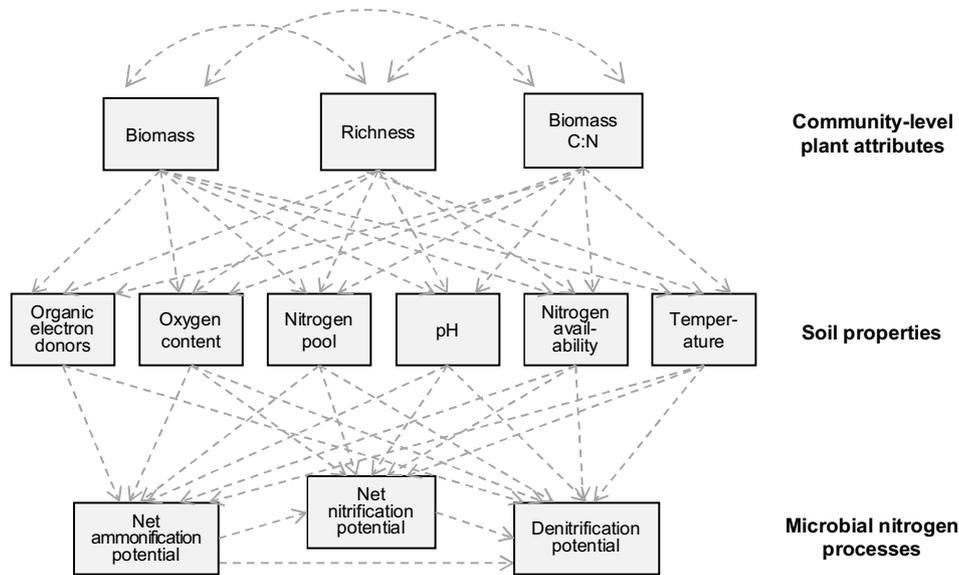
Physicochemical properties of the mesocosm soils that were possible correlates of N processes were measured in August 2013. Temperature readings were recorded continuously at 90 min intervals for the entire month using iButtons (Embedded Data Systems) inserted 5–10 cm in the mesocosm soils under the plant canopy. Redox potential was measured *in situ* to 5 cm in triplicate using a handheld ORP redox meter (Extech). Soil samples were taken to 10 cm depth for additional analyses. Soil pH was measured with a Hach pH electrode in the laboratory with ~10 g of dry soil in a 1:1 soil to water solution. Soil organic matter was measured by loss-on-ignition where soils dried to 105 °C were combusted in a furnace at 550 °C for 4 h. Percent total carbon and total N of soil, and the derived mass ratio of C:N, were measured in triplicate by dry combustion using soils dried to 105 °C in a Perkin Elmer 2400 Series II Element Analyzer. NAP, NNP, and DP were calculated on a dry mass basis (μg N/kg soil). Soil for bulk density (cored using 59 mL metal containers) and moisture, which were collected on the same day as soils collected for denitrification, were dried at 105 °C for 48 h.

#### 3.2. Biomass harvesting and tissue nutrient analysis

Peak total (above- and belowground) biomass was harvested in the second growing season in early September 2013 after all soil sampling was completed. All aboveground biomass (AGB) was cut at the soil surface and separated by species, while four samples per mesocosm of belowground biomass (BGB) were taken from the original location of each planted plug down to 30 cm using 7.62 cm diameter steel duct pipes. Soils for nitrogen processing were collected only to 10 cm depth because 94% of BGB was found in this zone. Plant biomass, which was dried to a constant weight between 48 and 60 °C, is presented as dry weight. Our sampling scheme provides species-specific information but may overstate the BGB estimates for the reed and sedge whose root densities were likely highest directly beneath their culm clusters (Korol and Ahn, 2016). Community biomass, from species data aggregated to the mesocosm level, was scaled to 1 m<sup>2</sup>. Based on the assumption that the most probable location of roots was under the canopy area of their shoots (i.e., where other species were not located), weighted multipliers derived from the relative aboveground percent cover were used to scale the four individual BGB values. A subsample of the dried AGB and BGB plant tissue was ground in a steel Thomas Wiley Mini Mill and then analyzed for percent mass of carbon and N by dry combustion on a Perkin Elmer 2400 Series II Element Analyzer.

#### 3.3. Conceptualizing macrophyte and soil ecosystem function links

Ecosystem functioning of the macrophyte and soil microbial communities are often linked without identification of the causal pathways. Our conceptual model (Fig. 1) displays the hypothesized hierarchical organization of plant-driven control on N cycling. Macrophytes are thought to affect N processing distally, primarily by influencing the proximate soil biogeochemical controls of microbially-mediated N mineralization and denitrification. Physicochemical factors regulating NAP, NNP, and DP overlap but differ in regards to the metabolic needs of microorganisms. We used soil carbon and organic matter to model the availability and types of organic electron donors, soil N to model the pool of N substrate, and the soil C:N ratio to model N availability. In accordance with the mass ratio hypothesis and diversity theories, we expect a “plant effect” of rooted macrophytes on the soil properties and processes of interest to primarily reflect a combination of species functional



**Fig. 1.** Conceptual model of hypothesized causal effects of three characteristics of the wetland macrophyte community on microbial nitrogen processing as mediated by soil physicochemical controls.

group richness, total (above- and belowground) plant biomass, and tissue concentrations of carbon and N. To maintain focus on the relationship between plant characteristics and N processes, interactions between soil properties were not assessed.

Structural equation modeling (SEM) was used to test our conceptual model (Fig. 1) of the hierarchical causal network of macrophyte influence and soil physicochemical properties on soil N transformations. Direct effects were not included in the conceptual model but were necessary in constructing the final models (see section 3.4. Data Analysis). SEM, and specifically path modeling used herein, is a useful statistical technique for testing theoretical constructs of association between numerous predictor and response variables that conventional multiple regression is ill-equipped to handle without planned experimentation (Grace, 2006). SEM is well-suited for experimental studies of plant diversity on ecosystem function where the confounding influence of multiple feedbacks (e.g., microbial effects on plants) that weaken detection of macrophyte effects are minimized (Grace et al., 2007).

#### 3.4. Data analyses

Measured variables were assessed for the strength of linear relationships. All variables were linearly regressed against species richness levels, while plant and soil properties and processes were selectively assessed pair-wise for the strength of linear relationships using Pearson's correlation coefficient. Variables were screened for univariate linearity and residual normality and equal variance through visual assessment of residual scatterplots and q-q plots; Variables were transformed where necessary to improve these statistical assumptions. Univariate outliers ( $z$ -score  $>3.29$ ) were changed to be less deviant (Tabacknik and Fidell, 2013). For SEM, assumptions of multivariate linearity, normality, homoscedasticity were evaluated with scatterplots of standardized residuals and predicted values; Mahalanobis distances were calculated to identify multivariate outliers. Specifically for SEM, standardized residual covariances were screened for asymmetry and large values. Unplanted mesocosms were assessed qualitatively. Data screening and statistical tests were performed in SPSS v.18 software (SPSS, 2009).

We used our conceptual model to guide the construction of one or more structural equation models for NAP, NNP, and DP,

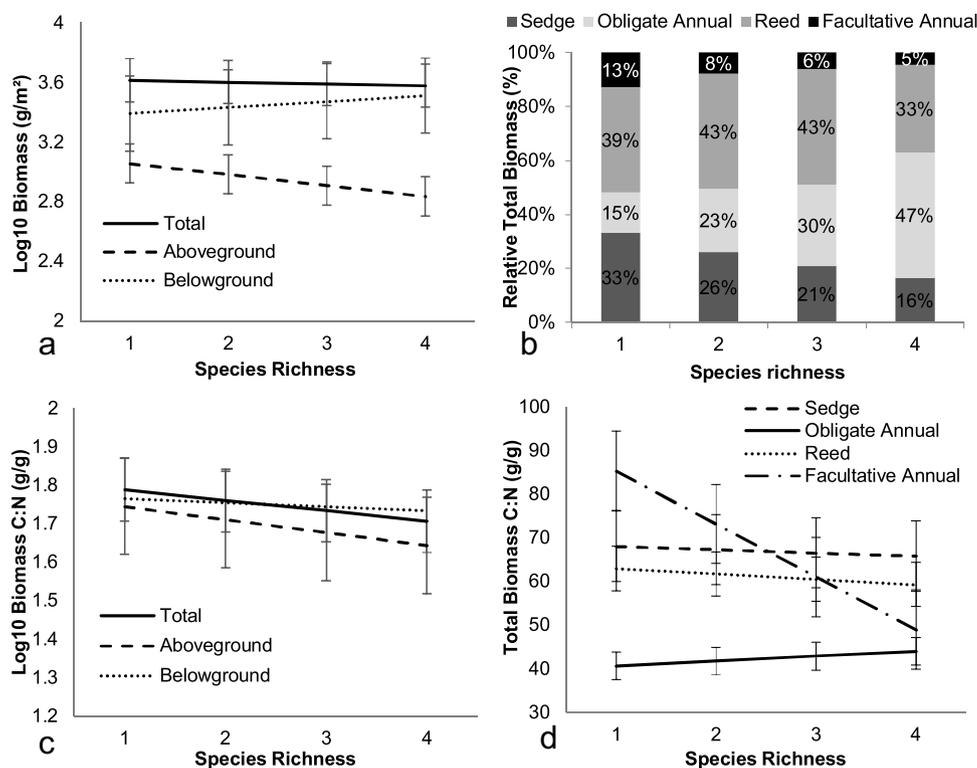
respectively. We followed a step-wise model generating procedure where functional attributes of the plant community and soil properties were first systematically assessed for statistical bivariate relationships with the N response variables; we then specified a multivariate model on the basis of theory and with the most significantly related variables where possible to produce a model most suitable to the observed data and with the highest explanatory power of NAP, NNP, and DP (Diaz et al., 2007). Model assessment of goodness-of-fit involved evaluation of the model  $\chi^2$  test, comparative fit index (CFI), and standardized root mean square residual (SRMSR) (Dimitrov, 2012). We had to modify our initial models to include direct paths from plant metrics to N response processes to improve model fit. Sample-size adjusted Bayesian Information Criterion ( $BIC_{adj}$ ) was referenced in selecting the final model. Structural equation modeling was performed with Mplus v. 7.11 software (Muthén and Muthén, 2013).

Missing-at-random data were present for DP, and three of the eight monoculture mesocosms (one each for *C. vulpinoidea*, *M. ringens*, and *J. effusus*) were not quantified. Due to the relatively high mean DP for the two *E. obtusa* monocultures, and the potential for bias considering the relatively lower and similar mean DP values for the other species in monoculture, missing data values for these three monocultures were estimated using multiple imputation (i.e. thus each species had mean DP values for two monocultures). Multiple imputation is considered the "gold standard" for estimating missing data with no subsequent loss of statistical power (Polit, 2010). Multiple imputation was performed in Mplus software using all the variables considered for use in SEM and any others with significant correlation. The new variable was more conservative and used in all statistical tests (i.e., correlation) and modeling (i.e., SEM). Model results generated with the original (raw) and new (imputed) variable were compared for consistency.

## 4. Results

### 4.1. Community and species performances

A linear relationship between plant species richness and total biomass was not found ( $r^2=0.008$ ,  $p=0.610$ ; Fig. 2a); however, community AGB was negatively related to species richness ( $r^2=0.288$ ,  $p=0.001$ , Fig. 2a). The reductions in AGB were not



**Fig. 2.** Linear regression slopes of biomass and biomass C:N for the community and of the sedge (*C. vulpinoidea*), obligate annual (*E. obtusa*), reed (*J. effusus*), and facultative annual (*M. ringens*) across richness levels of the planted mesocosms: (a) community biomass production; (b) species relative contribution to total (above- and belowground) biomass production; (c) community biomass C:N; and (d) species total (above- and belowground) biomass C:N. Statistical tests performed on transformed data as presented. Error bars represent  $\pm 1$  standard error of the regression slope.

reflected in total biomass in part because BGB remained consistent across species richness ( $r^2=0.031$ ,  $p=0.758$ ; Fig. 2a) and its variability around the mean was larger. Species performance by richness levels varied (Fig. 2b). Relative contributions from *C. vulpinoidea* (sedge) and *M. ringens* (facultative annual) to total biomass declined by an average of  $-59\%$  and  $-71\%$  from monocultures to the most diverse mixtures, respectively, while the total biomass of *J. effusus* (reed) was roughly independent of richness levels. *E. obtusa* (obligate annual) exhibited a counter trend with a 156% proportional increase in biomass at the high richness levels over monocultures (Fig. 2b). Total (i.e., above and belowground) biomass C:N was negatively related to species richness ( $r^2=0.123$ ,  $p=0.042$ ) (Fig. 2c), with no independent changes to the total pool of plant N ( $r^2=0.001$ ,  $p=0.856$ ) or carbon ( $r^2=0.019$ ,  $p=0.433$ ); aboveground ( $r^2=0.085$ ,  $p=0.094$ ) and belowground biomass ( $r^2=0.033$ ,  $p=0.301$ ) C:N ratios were not related to species richness. Only *M. ringens* demonstrated a change in total biomass C:N across richness treatments [ $r^2=0.627$ ,  $p<0.001$ ] (Fig. 2d).

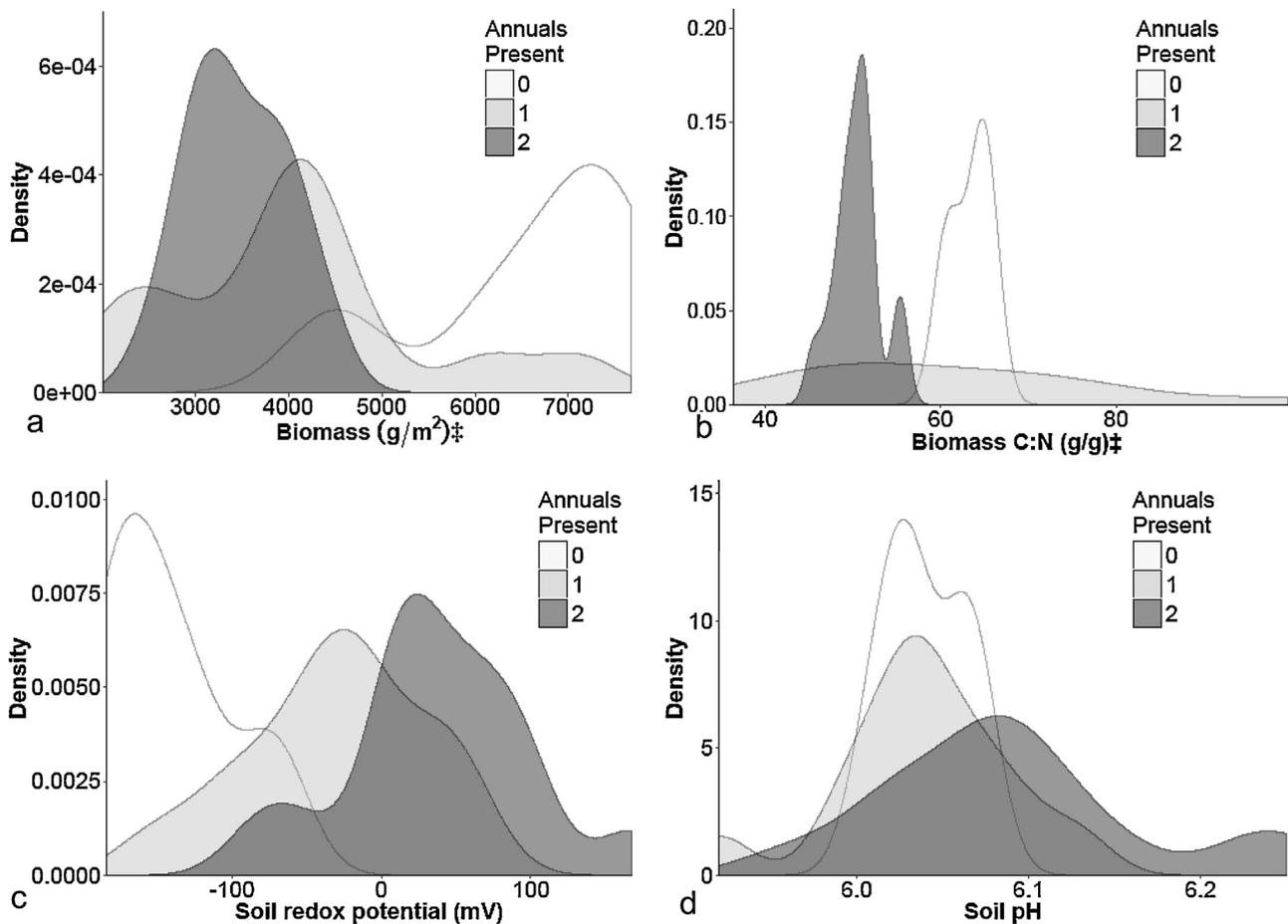
The annuals markedly differed from interstitial perennials in their functional performances, which had a large influence on community functioning. From visual observation, all stems from the obligate and facultative annual, compared to portions of AGB from the reed and sedge, died back over the winter and were deposited on the soil surface. Second, the average monoculture total biomass in stands of *M. ringens* ( $2299\text{ g/m}^2$ ) and *E. obtusa* ( $2693\text{ g/m}^2$ ) were less than half that of *C. vulpinoidea* ( $5905\text{ g/m}^2$ ) and *J. effusus* ( $6940\text{ g/m}^2$ ). Total mesocosm biomass was thus negatively related to the number of annual species present ( $r^2=0.334$ ,  $p<0.001$ ; Fig. 3a). Mesocosm biomass C:N also was influenced by the presence of annual species ( $r^2=0.199$ ,  $p=0.008$ ; Fig. 3b) and even more strongly by the presence of *E. obtusa* ( $r^2=0.677$ ,  $p<0.001$ ) because of the low C:N of *E. obtusa* and the stoichiometric plasticity of the *M. ringens* (Fig. 2d).

#### 4.2. Soil physicochemical properties

The soils of planted mesocosms had higher redox potentials than the unplanted controls due to plant root oxygenation (determined using iron sulfide (FeS) agar probes in June 2013; unpublished data). The facultative and obligate annuals had higher soil redox potentials on average (2 and  $-6\text{ mV}$ , respectively) than the reed and sedge ( $-98$  and  $-170$ , respectively) in monocultures, which explained part of the positive correlation between soil redox potential and planted species richness (Fig. 4a). The higher soil redox is also likely due to the greater spatial spread of roots by the more spatially dispersed and numerous annual plants within the mesocosm. Soil pH of the planted mesocosms was also positively associated with species richness (Fig. 4b), and both redox ( $r=0.723$ ,  $p<0.001$ ) and pH ( $r=0.334$ ,  $p=0.049$ ) were segregated on the basis of annual species (Fig. 3c,d). In contrast, soil temperature (Fig. 4c), organic matter (range: 5.2–5.9%;  $r=-0.109$ ,  $p=0.540$ ), carbon (range: 1.1–1.5%;  $r=-0.010$ ,  $p=0.955$ ), N (range: 0.09–0.12%;  $r=0.035$ ,  $p=0.845$ ), and C:N (Fig. 4d) were not affected by the planted richness level, though temperatures were higher by  $0.5\text{ }^\circ\text{C}$  in mesocosms with the obligate annual ( $r=0.498$ ,  $p=0.005$ ). Mesocosm biomass was negatively correlated with redox potential while biomass C:N was negatively correlated with pH and temperature (Table 1).

#### 4.3. Net ammonification (NAP), net nitrification (NNP), and denitrification potential (DP)

All NAP values were negative, with unplanted mesocosms an order of magnitude more negative than the negative rates for the planted mesocosms (Fig. 5a). NNP was largely positive, with roughly similar ranges of rates between unplanted and planted mesocosms (Fig. 5b). As the sum of NAP and NNP, NMP was nega-



**Fig. 3.** Density plots of mesocosm plant characteristics and soil properties partitioned by the number (0–2) of annual species (*E. obtusa* and *M. ringens*) present. Linear regression statistics not shown in figures. ‡Variable was log10 transformed prior to statistical analysis.

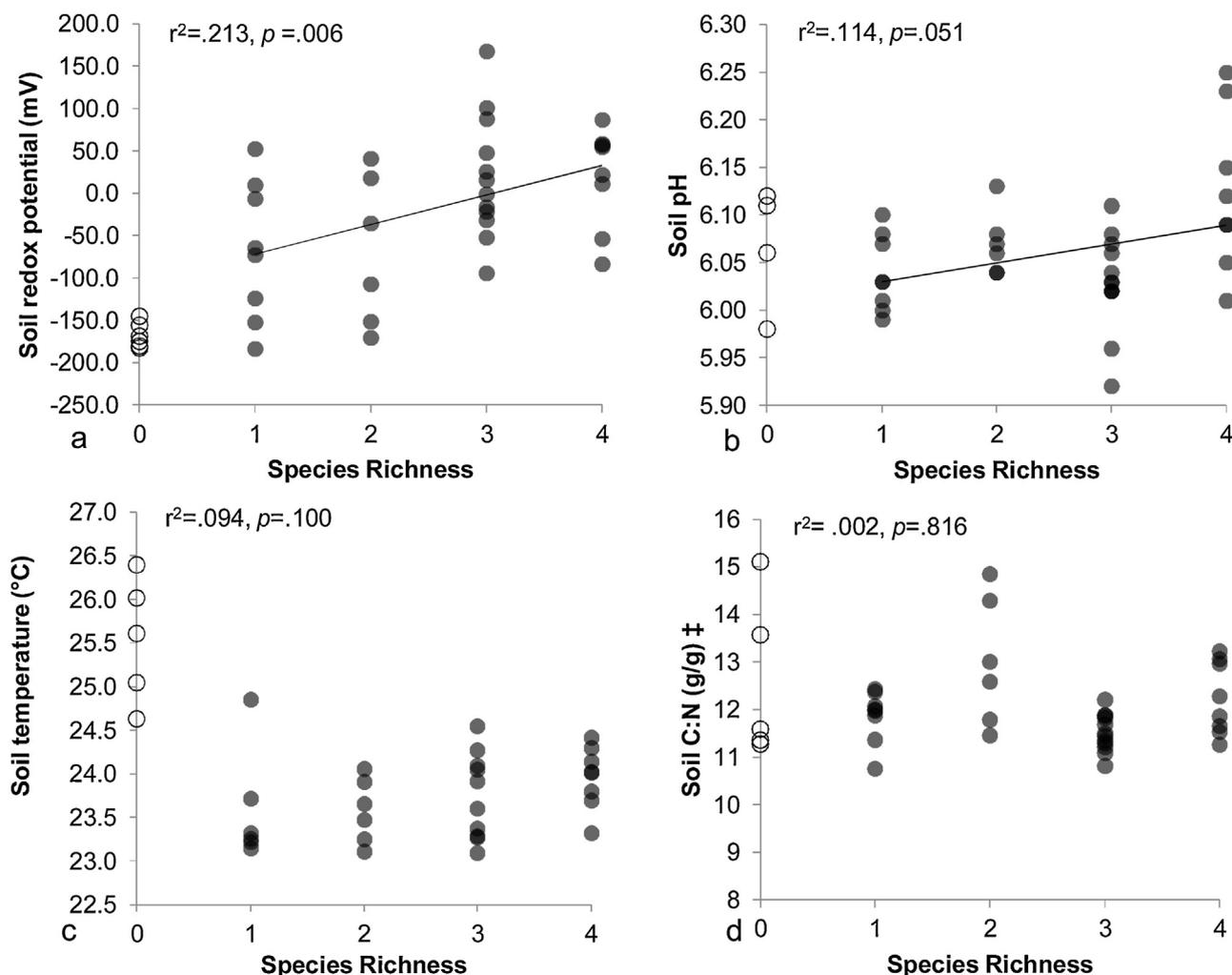
tive in the unplanted mesocosms and bridged negative and positive rates in the planted mesocosms without a significant trend by richness (Fig. 5c). Because a majority of NMP rates were negative (median =  $-13.2 \mu\text{g N/kg soil d}$ ), either not all ammonium loss could be due to nitrification, suggesting some uptake of ammonium into microbial biomass, or some nitrate was concurrently lost to denitrification. Higher species richness had a positive effect on NAP, reducing the intensity of ammonium immobilization, and a negative effect on NNP, reducing the net production of nitrate (Fig. 5a,b). Plants had a positive effect on DP relative to the unplanted mesocosms, but DP was not correlated with planted species richness (Fig. 5d).

#### 4.4. Structural equation models

Two final SEM models were chosen, one for NAP and the other for NNP and DP, that maximized explained variance in the three N processes and minimized the  $\text{BIC}_{\text{adj}}$ . Regression coefficients remained significant ( $p < 0.05$ ) across the different models considered. Both models had good fit: for the NAP model, the  $\chi^2$  test of model fit (i.e., test of the degree of difference between observed and model implied covariance matrices) was not significant ( $\chi^2 = 5.77$ ,  $\text{df} = 6$ ,  $p = 0.45$ ),  $\text{CFI} = 1.00$  ( $\text{CFI} > 0.93$  is evidence of good fit), and  $\text{SRMR} = 0.093$  ( $\text{SRMR} > 0.10$  is a poor fit) (Fig. 6a); for the NNP and DP model, the  $\chi^2$  test of model fit was also not significant ( $\chi^2 = 0.721$ ,  $\text{df} = 3$ ,  $p = 0.87$ ),  $\text{CFI} = 1.00$ , and  $\text{SRMR} = 0.065$  (Fig. 6b). Three soil properties, redox potential, C:N, and temperature, were conserved across both models because of their correlation with plant metrics and their control over multiple N processes. Because of the

negative correlation between species richness and biomass C:N (equal to their bivariate correlation; Table 1), a single causal relationship is implied for these exogenous variables but their effects on soil processes were assessed independently. In both models, redox potential was higher in mesocosms with more species and in mesocosms with lower biomass, and soil temperature increased with decreasing biomass C:N (Fig. 6). While soil redox was well explained by both models ( $R^2 = 0.543$  for both models), soil C:N, temperature, and pH were not well-explained by either the NAP model ( $R^2 = 0.028$ ,  $R^2 = 0.280$ , and  $R^2 = 0.222$ , respectively) or the NNP-DP model ( $R^2 = 0.028$ ,  $R^2 = 0.285$ , and  $R^2 = 0.222$ , respectively). Soil total carbon, total N, and organic matter were either of low significance or insignificant as mediating soil physicochemical properties and were not modeled. No meaningful change in model fit ( $\chi^2 = 0.714$ ,  $\text{df} = 3$ ,  $p = 0.87$ ;  $\text{CFI} = 1.00$ , and  $\text{SRMR} = 0.065$ ) and no change in the identified significant pathways were found if the original DP variable was used in place of the imputed variable; only the strengths of the partial regression coefficients were marginally altered.

Net ammonification potential was directly related to soil C:N and redox, and was indirectly related to richness and biomass through soil redox, with 62% of its variation explained by the model (Fig. 6a, Table 2). Higher NAP was found at lower soil C:N ratios but higher soil redox potentials. The two compound pathways from species richness  $\rightarrow$  redox potential  $\rightarrow$  NAP ( $b = 0.242 \pm 0.097$ ,  $p = 0.013$ ) and biomass  $\rightarrow$  redox potential  $\rightarrow$  NAP ( $b = -0.349 \pm 0.115$ ,  $p = 0.002$ ) were statistically significant and contributed to the overall indirect effects of richness and biomass on NAP. Insignificant total effects of species richness and biomass C:N



**Fig. 4.** Scatter plots of mesocosm (a) soil redox potential, (b) soil pH, (c) soil temperature, and (d) the ratio of soil carbon to nitrogen across the planted species richness gradient (black circles) and the unplanted controls (empty circles). Statistical results pertain to the planted mesocosms only. Statistically significant linear regression slopes at  $\alpha=0.05$  are displayed. ‡Variable was  $\log_{10}$  transformed prior to statistical analysis.

on NAP in the model were inconsistent with significant bivariate relationships (Table 1) between the variables. Reciprocal suppression can occur when two explanatory variables are themselves related irrespective of the effects on a predicted variable. The loss of power in the model coefficients may derive from the shared variance between richness and biomass C:N as well as the mediating effect of redox potential on the richness-NAP relationship.

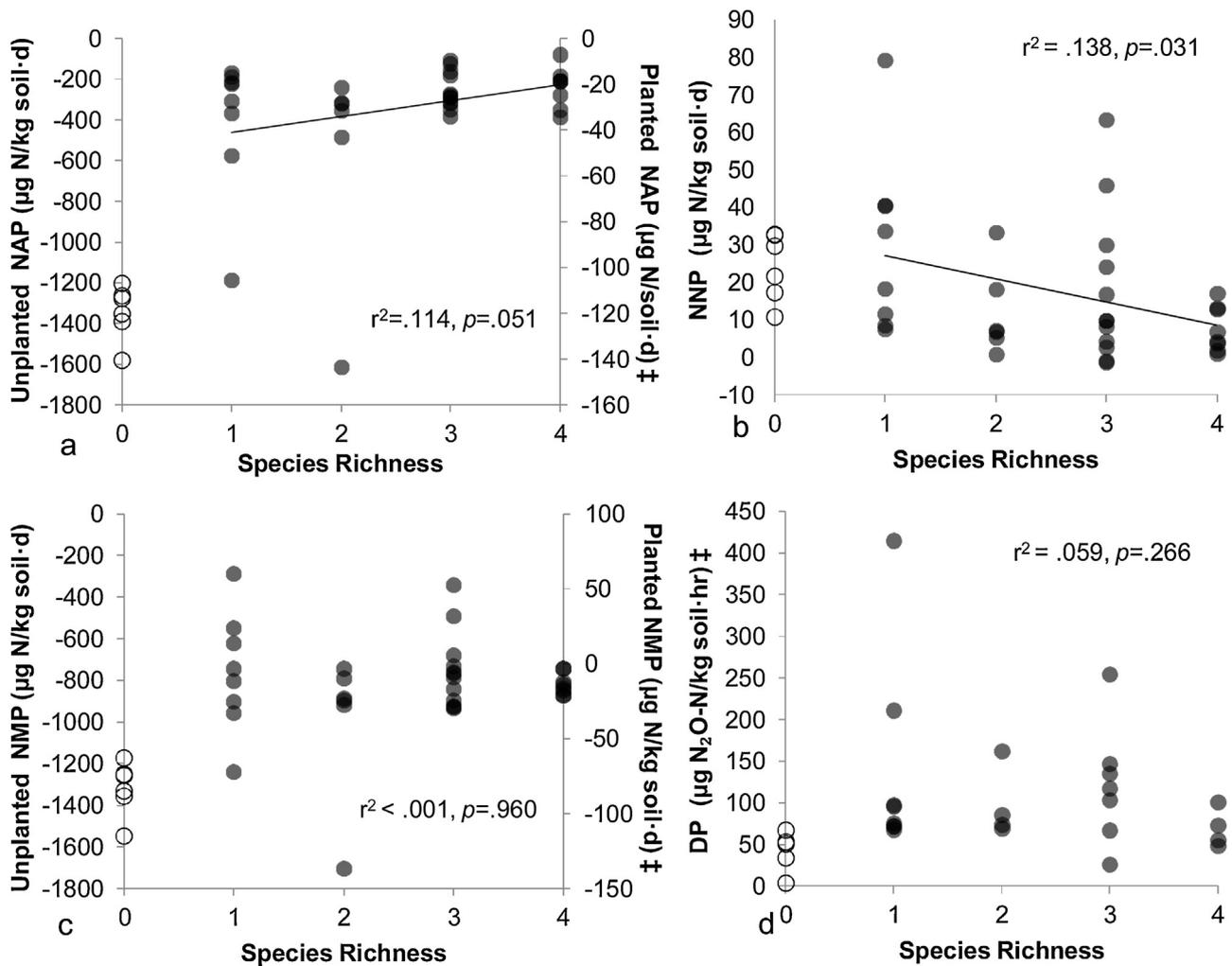
Net nitrification potential and denitrification potential were related to both soil properties and plant attributes (Fig. 6b, Table 2). NNP was positively correlated with soil temperature, while higher DP was correlated with higher NNP, lower soil C:N, and lower redox potential. Species richness had a moderately strong direct negative effect on NNP with a total negative effect consistent with the bivariate correlation coefficient between the variables (Table 1). Biomass C:N also had an indirect negative effect on NNP mainly through the compound pathway biomass C:N  $\rightarrow$  temperature  $\rightarrow$  NNP but also influenced by the compound pathway biomass C:N  $\rightarrow$  soil C:N  $\rightarrow$  NNP ( $b=-0.259$ ,  $p=0.035$ , for combined indirect effects). Denitrification potential was affected by all three plant community metrics either directly or indirectly through the cumulative effects on soil properties. As an extension of the negative effects on NNP, species richness had a significant negative indirect effect on DP through the cumulative effects of two compound pathways: richness  $\rightarrow$  NNP  $\rightarrow$  DP and

richness  $\rightarrow$  redox  $\rightarrow$  DP ( $b=-0.386$ ,  $p=0.033$ , for combined indirect effects). Biomass, in contrast, had positive indirect effects on DP through the cumulative effects of redox and NNP, though this was accompanied by a direct negative relationship with DP: biomass  $\rightarrow$  redox  $\rightarrow$  DP and biomass  $\rightarrow$  NNP  $\rightarrow$  DP ( $b=0.369$ ,  $p=0.052$ , for combined indirect effects). Biomass C:N had a moderately strong direct and resulting overall negative effect on DP. The explanatory power of the model was higher for DP (59.2%) than for NNP (36.2%).

## 5. Discussion

### 5.1. Macrophyte performance in response to plant community richness

Species richness was an important determinant of plant community evenness and biomass quality. Complete occupancy of the most species-rich mesocosms was a result of the obligate annual's high abundance and the greater spatial spread of both obligate and facultative annuals. Higher biomass quality (lower C:N), which can positively influence litter decomposition (Espershützer et al., 2013), was promoted at higher richness levels because of greater annual plant contributions to biomass. In other terrestrial and wetland studies, diverse plant communities have often been found to



**Fig. 5.** Scatterplots of nitrogen processing in mesocosms across the species richness gradient (solid circles) and unplanted controls (open circles) for (a) net ammonification potential, (b) net nitrification potential, (c) net mineralization potential, and (d) denitrification potential. Statistical results pertain to the planted mesocosms only. Statistically significant linear regression slopes at  $\alpha = 0.05$  are displayed. ‡Variable was log<sub>10</sub> transformed prior to statistical analysis.

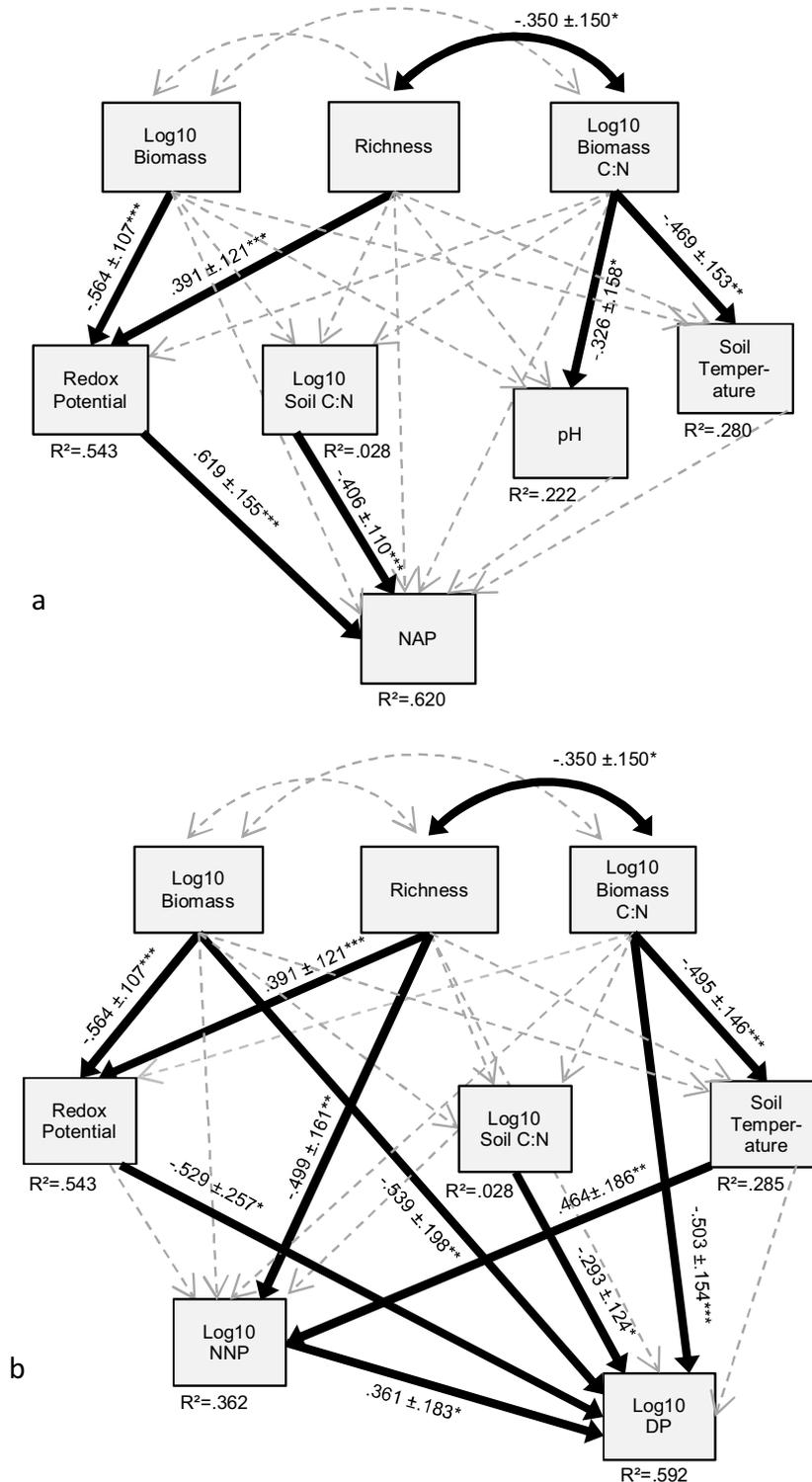
have lower biomass quality and higher N use efficiency than less diverse communities (Sullivan et al., 2007; Fornara and Tilman, 2009; Oelmann et al., 2007; Pasari et al., 2013; Fornara and Tilman, 2009; Oelmann et al., 2007; Pasari et al., 2013). Prolific growth has been attributed to a dilution effect in plant N biomass, where more productive individuals have higher C:N ratios because they theoretically invest in more structural, C-rich, tissue as they grow taller and compete for light (Ågren, 2008; Abbas et al., 2013). This hypothesis holds partly true for the facultative annual where it displayed highest biomass C:N at highest fitness levels, except this was found in monoculture and thus was not related to interspecific interactions. Because of the elemental plasticity of the facultative annual and the low biomass C:N concentrations of the obligate annual, our results do not support the conclusion that ruderal species have lower tissue N concentrations (McJannet et al., 1995) but rather that plant elemental compositions differ by species and respond variably to resource availability and community interactions (Güsewell and Koerselman, 2002; Novotny et al., 2007).

### 5.2. Macrophyte community effects on soil physicochemical properties

The presence and specific attributes of the macrophyte community affected the non-nutrient physicochemical properties of the young soils. As shown by the comparison between the planted and

unplanted soils, macrophytes raised redox potentials through root oxygenation and reduced temperatures through shading. Control of these properties in the planted mesocosms was further linked to the structure and characteristics of the plant community. Soil temperatures were lowest under communities with lower quality biomass, and namely those without the obligate annual, possibly from heavier shading. Soil redox levels and pH were highest in communities with greater species richness and communities of lower biomass (reflective of the annuals). Greater temperatures and redox levels have large effects on soil biogeochemical processing by promoting faster nutrient cycling, litter decomposition, and soil organic carbon mineralization, though greater temperatures also exacerbate oxygen or nutrient limitations. Many ion concentrations, states, and reactions are functions of soil pH. Because pH usually increases in flooded, anoxic soils, the opposite positive associations found between pH and both species richness (due to the presence of annuals) and redox potential likely resulted from greater organic matter decomposition and proton ( $H^+$ ) consumption in a weakly oxic environment (Reddy and DeLaune, 2008).

In contrast, the macrophyte community did not alter the bulk soil nutrient pools of N, carbon, or organic matter, or the proportion of C:N. These findings were unexpected considering that the two annual species died back either partially or fully at the end of the first growing season. Additionally, relative growth rate, often higher in annual plants, has been found to be one positive indicator



**Fig. 6.** Structural equation models of the causal effects of plant community biomass, richness, and C:N mass content of biomass on (a) net ammonification potential (NAP) and (b) net nitrification potential (NNP) and denitrification potential (DP). Statistically significant ( $* p \leq 0.05$ ;  $** p \leq 0.01$ ;  $*** p \leq 0.001$ ) standardized partial regression coefficients  $\pm 1$  standard error are shown with solid lines and insignificant standardized partial regression coefficients are not shown but represented with dotted lines. Curved arrows are equal to the standardized bivariate correlations between variables.

of root exudates release of labile carbon (Zhai et al., 2013; Cantarel et al., 2015). On the other hand, this study did not examine changes in the make-up of organic material (e.g., water-extractable organic carbon) or concentrations of inorganic nitrogen which might be more responsive to variations in plant community type (Espershützel et al., 2013). Only the effect on pH and that the two heterotrophic

N processes – NAP and DP – tracked strongly with the presence of annuals (as measured by total biomass and total biomass C:N) provides some indication that changes in soil organic matter or N pools occurred on a finer scale than could be detected in this study. Empirical and theoretical evidence does suggest that attributes of the microbial community are primary controls on short-term organic

**Table 1**  
Selected Pearson correlation coefficients (r) for the relationships between macrophyte biomass attributes, soil properties, and nitrogen transformations for the planted mesocosms in the second growing season.

Nitrogen Processes		Macrophytes				Soil Properties				redox		temp	pH
NAP	NNP <sup>a</sup>	BM <sup>a</sup>	BMCN <sup>a</sup>	OM	C	N <sup>a</sup>	CN <sup>a</sup>						
NAP	-0.019 p = 0.913	0.171 p = 0.436	-0.315 p = 0.069	-0.399* p = 0.019	-0.315 p = 0.070	-0.055 p = 0.759	0.361* p = 0.036	-0.377* p = 0.028	0.631* p < 0.001	0.176 p = 0.361	0.280 p = 0.108		
NNP <sup>a</sup>		0.416* p = 0.048	0.129 p = 0.468	0.000 p = 0.999	-0.063 p = 0.722	-0.202 p = 0.251	-0.010 p = 0.957	-0.228 p = 0.194	-0.175 p = 0.322	0.130 p = 0.501	-0.199 p = 0.260		
			-0.232 p = 0.286	-0.459* p = 0.028	-0.138 p = 0.531	0.279 p = 0.198	0.236 p = 0.278	-0.421* p = 0.046	0.006 p = 0.980	-0.120 p = 0.626	-0.193 p = 0.378		
			BM <sup>a</sup>	0.218 p = 0.215	0.035 p = 0.843	-0.201 p = 0.254	-0.249 p = 0.156	-0.023 p = 0.899	-0.612* p < 0.001	0.016 p = 0.932	-0.166 p = 0.350		
			BMCN <sup>a</sup>		-0.086 p = 0.628	0.038 p = 0.831	-0.131 p = 0.460	0.156 p = 0.378	-0.316 p = 0.069	-0.417* p = 0.024	-0.419* p = 0.014		

\*Correlation significant at  $\alpha = 0.05$ . Abbreviations alphabetically: BM = total mesocosm biomass; BMCN = total mesocosm biomass C:N; C = carbon; CN = carbon:nitrogen mass ratio; DP = denitrification potential; NAP = net ammonification potential; NNP = net nitrification potential; OM = organic matter; redox = redox potential; temp = mean August soil temperature.  
<sup>a</sup> Variable was log10 transformed.

**Table 2**

Standardized effects from the two structural equation models. Total, direct, and indirect effects  $\pm 1$  standard error of the three plant explanatory characteristics, richness, biomass C:N, and biomass, on net ammonification potential, net nitrification potential, and denitrification potential.

	Richness	Biomass C:N	Biomass
Net Ammonification Potential			
Total Effects	0.216 $\pm$ 0.156 <sup>a</sup>	-0.264 $\pm$ 0.158 <sup>a</sup>	-0.231 $\pm$ 0.149
Total Direct	-0.043 $\pm$ 0.133	-0.163 $\pm$ 0.138	0.105 $\pm$ 0.142
Total Indirect	0.259 $\pm$ 0.128*	-0.101 $\pm$ 0.135	-0.336 $\pm$ 0.137*
Net Nitrification Potential			
Total Effects	-0.425 $\pm$ 0.153**	-0.177 $\pm$ 0.168	0.130 $\pm$ 0.158
Total Direct	-0.499 $\pm$ 0.161**	0.088 $\pm$ 0.179	0.195 $\pm$ 0.183
Total Indirect	0.074 $\pm$ 0.119	-0.265 $\pm$ 0.124*	-0.065 $\pm$ 0.142
Net Denitrification Potential			
Total Effects	-0.272 $\pm$ 0.194	-0.490 $\pm$ 0.164**	-0.171 $\pm$ 0.152
Total Direct	0.107 $\pm$ 0.275	-0.503 $\pm$ 0.154***	-0.539 $\pm$ 0.198**
Total Indirect	-0.379 $\pm$ 0.182*	0.013 $\pm$ 0.133	0.369 $\pm$ 0.190*

\*P value significant at  $\alpha = 0.05$ . \*\*P value significant at  $\alpha = 0.01$ . \*\*\*P value significant at  $\alpha = 0.001$ .

<sup>a</sup> Effects statistically inconsistent with bivariate correlation coefficients.

matter and N pools that decouple the influence of plants (Cole et al., 2001; Knops et al., 2002; Robertson and Groffman, 2015).

### 5.3. Macrophyte community effects on nitrogen processing

In our 2-year study, greater macrophyte richness and higher biomass quality, which were positively correlated, each uniquely promoted NAP, while greater species richness inhibited NNP. Because of the opposing trends, species richness had no overall effect on NMP. Our results suggest a degree of nitrogen limitation, where the average combined change in the mineralized N pool was negative, all NAP was negative, and the magnitude of rates for the most part were lower than those reported for freshwater wetlands (Dick and Gilliam, 2007; van Hoewyk et al., 2000; Hanson et al., 1994; Fellman and D'Amore, 2007). Net negative mineralization rates reflect low dissolved N availability resulting from a possible combination of microbial immobilization, slower respiration, or nitrate consumption. A study by Zhu et al. (2012) that used experimental treatment wetlands receiving domestic wastewater offers a counterpoint that may speak to the importance of nutrient availability. Across 16 species, the authors found that greater richness of wetland plants augmented soil N mineralization, nitrification, and ratio of net nitrate produced to the total pool of N mineralized (nitrate + ammonium) from in situ soil cores over four weeks. In our study, we infer that the predominant microbial uptake of ammonium was due in part to the consistently low redox potentials in all mesocosms, which possibly contributed to smaller initial dissolved ammonium pools. The markedly greater rates of immobilization in the unplanted mesocosm soils with the lowest redox potentials and lack of plant influence highlights how root oxygenation strongly promotes microbial respiration. Immobilization may also have been promoted by the abrupt alteration in oxygen availability to the previously anoxic soils: N demand grew in the incubations as microbial activity and microbial biomass N increased. Some oxygen limitation persisted in our whole-core incubations and nitrate consumption from denitrification would also have reduced rates of NNP and NMP.

Studies have identified multiple mechanisms underlying the positive relationships between species richness and N mineralization. Species complementarity in a diverse community can increase microbial biomass and N mineralization through greater productivity and faster decomposability of plant biomass (van der Krift and Berendse, 2001; Cong et al., 2014). Alternatively, certain species may “prime” N mineralization in their rhizosphere by releasing carbon compounds that increases the need for mineralized N (Finzi et al., 2015; Mueller et al., 2015). Here, the SEM model indicated

that higher redox potentials, which can increase rates of microbial respiration, directly regulated the positive NAP trend across species richness, a potentially important mechanism in wetlands for both N and carbon cycling. The higher biomass quality at higher species richness would have also increased ammonification as the greater proportion of nitrogen to carbon in organic matter stimulates net microbial release of mineralized ammonium.

Ammonium production and oxygen availability, both of which co-varied positively with species richness, are two primary facilitators of nitrification (Bodelier et al., 1996). Yet in our study, NNP was uncorrelated with NAP and was inhibited at higher species richness. The similarity between the monocultures and the unplanted mesocosms, both highest in NNP on average, would suggest that the trends in NNP relate to the build-up of ammonium substrate in the mesocosms of lower species richness and redox potentials. Under anoxic conditions, ammonium accumulates in soils from nitrification inhibition, lower diffusion and volatilization, lower microbial demand, and greater clay fixation (Reddy et al., 1984; Schneiders and Scherer, 1998). A lack of correlation between NAP and NNP was also found by Wolf et al. (2013) in young created wetlands. This outcome may reflect the consequence of measuring more veiled net flux rates, but incubating the soil cores whole might have contributed to preserving microsite complexity that spatially segregated ammonification and nitrification processes (Schimel and Bennett, 2004). Temperature, another primary facilitator of nitrification, positively mediated the effect of higher biomass quality on NNP (Forshay and Dodson, 2011; Laughlin, 2011). Higher temperatures are known to increase microbial activity and have been shown to stimulate surficial oxygen production from algal photosynthesis (Christensen and Sørensen, 1986).

Due to the low explanatory power of our model for predicting NNP, the dominant regulatory factors of nitrification were not identified by this study. The invariance of NNP between the planted and unplanted mesocosms suggests that NNP was largely suppressed. Competition for ammonium from heterotrophic microbes and plant roots has been known to inhibit nitrifiers (Verhagen et al., 1994; Arth and Frenzel, 2000). The obligate annual was found to proliferate its roots at higher species richness levels (Korol and Ahn, 2016), a plant strategy used to increase nutrient uptake particularly under interspecific competition (Nacry et al., 2013), which would have increased the delivery of labile carbon exudates and uptake of soil nitrogen. Further, greater microbial respiration and readily decomposable litter (e.g., the obligate annual was a particularly good indicator of biomass C:N) could have intensified the soil oxygen demand along the richness gradient that limited the potential for nitrification during the incubations (Lee et al., 2009). The suppression of NNP was not present in a study of soil incubated under fully oxic conditions with added ammonium: NNP was higher in wetland soils with emergent vegetation than in bare sediment and NNP was strongly correlated with redox potential (Soana and Bartoli, 2014).

Plant community characteristics exerted the most control on DP, with the majority of variability explained by biomass and biomass C:N. Denitrification potential was directly stimulated in mesocosms with lower biomass, one indicator of the presence of annuals, and with higher qualities of biomass and soil organic matter, indicators of the availability of organic carbon and N. The concomitant soil additions of decomposable biomass with low C:N and potentially greater rhizodeposition from the annuals would have increased bioavailable organic carbon resources for heterotrophic denitrifiers. Ballantine et al. (2014) demonstrated that denitrification potential in restored freshwater wetlands was positively related to topsoil amendments with relatively low C:N content. Direct additions of glucose, the most labile form of organic carbon, has been shown to increase microbial N stores, decrease dissolved soil N, and decrease N content in plants (Schmidt et al., 1997).

While DP was directly promoted by lower biomass quantity and greater quality, lower biomass and greater richness concomitantly suppressed DP indirectly through higher redox potentials (i.e. root oxygenation). While some soil oxygenation facilitates coupled nitrification-denitrification (Burgin et al., 2010), high levels of root oxygenation suppresses the process. The inhibition of DP through this mechanism stands in contrast to the elevation of DP in planted mesocosms compared to unplanted mesocosms which lacked rhizosphere oxygenation and plant-derived carbon inputs. Because two of the three highest DP rates were found in the monocultures of the obligate annual, which also exhibited relatively high redox levels, rhizosphere oxygenation in the anoxic mesocosm soils was one important positive driver of DP. As the two annuals increased the redox potential in more species-rich mesocosms from greater root oxygen leakage, increased microbial respiration may have also promoted heterotrophic activity and a demand for ammonium that inhibited the autotrophic nitrifier community.

Support for a direct relationship between species richness (or diversity) and denitrification potential in wetlands is lacking (Bouchard et al., 2007; McGill et al., 2010). Sutton-Grier et al. (2011) found an insignificant direct relationship between functional diversity and DP but determined that background concentrations of N, organic matter, and soil moisture were statistically significant mediators to the relationship. Our study corroborates this finding of joint control of DP and identifies specific mechanisms for young wetland soils. Specifically, higher redox potentials suppressed DP and possibly diminished nitrate pools, while lower productivity but higher biomass quality associated with the annuals promoted carbon-coupled N cycling. Our denitrification rates were similar to other rates reported for freshwater marshes with mineral soils and for diversity-ecosystem function studies (McGill et al., 2010; Sutton-Grier et al., 2011; Wolf et al., 2011; Ballantine et al., 2014). An interesting avenue for future research would be to test the richness-DP relationship in wetlands under soil conditions (i.e., greater nitrogen, organic matter, or flood pulses) that could support higher DP rates (i.e., up to one order of magnitude) that have been reported for some mineral freshwater wetlands (Jordan et al., 2007).

#### 5.4. Model insights and interpretations

An overarching advantage of the SEM approach was illustrating the tested multiple hierarchical, direct and indirect relationships among variables that would be difficult in the absence of a visual model. Our results provide insights into the relative strength and mediating mechanisms of plant effects that have rarely been demonstrated in wetlands. Plant-soil feedbacks depend on these initial plant-induced soil changes and understanding these mechanisms are important for further research on the development of plant-driven N functionality in created wetlands. The modification of our conceptual model to incorporate direct effects indicated that certain mechanistic details of plant effects on soil N processes were not captured by this study. In particular, our analysis raises research questions about the structural controls on NNP in wetlands and on nutrient forms and concentrations in the rhizosphere. Since the conclusions of this diversity study were derived from four species, two annuals and two perennials, planted at moderate densities, similar research in wetlands is needed across different species composition, species density, and soil conditions.

Did higher species richness levels promote ecosystem multifunctionality within the N cycle? The negative trends in this study would suggest the opposite, seemingly contradictory to recent understanding that multiple species are more likely to maintain multiple ecosystem processes (Lefcheck et al., 2014). Here, greater species richness promoted one but not all three N functions measured in this study, and was not associated with biomass

production. In a meta-analysis of biodiversity studies conducted in grasslands, Isbell et al. (2011) concluded that pairs of mixtures comprised of more unique species could together promote ecosystem functions better than mixtures with fewer unique species. That we found richness to both promote soil oxidation and NAP but at the same time have inhibitory effects on NNP and DP suggests that more community composition heterogeneity in early wetlands may be better suited to maximize all three of these ecosystem N functions.

At the same time, the presence of annuals, which explained a main axis of variability in decreasing biomass quantity and increasing quality, was found to positively affect all three of the N processes. The various mechanisms of higher redox potential, temperature, carbon inputs from decomposable litter, and the quality of litter all suggest that the annuals promoted greater carbon processing. This finding provides evidence that species composition differentially regulates the effects of plant carbon on the microbial use of N in young ecosystems and finds support of a carbon-flow mechanism of macrophyte stimulation of N cycling. Allowing annual plants to establish in young created wetlands as a natural strategy to boost soil carbon and N development may be cost-effective and more reliable than planting a mix of early and late successional plants (*i.e.*, annual and perennial) that may maximize plant productivity but may not promote soil biogeochemical cycling (Mitsch et al., 2012; Schultz et al., 2012). As microbial N demand and release (*e.g.*, denitrification) increase in response to greater inputs of plant carbon, environmental conditions supporting natural N flux, such as hydrologic connectivity or pulses, would benefit soil N functionality in often N-poor created wetlands (Ballantine and Schneider, 2009; Wolf et al., 2013).

## 6. Conclusion

In a 2-year plant richness-wetland N functioning study, multiple characteristics of a planted macrophyte community regulated soil N cycling as measured by net N transformation potentials. Species richness positively impacted NAP, but directly and indirectly inhibited NNP and DP, respectively. Lower biomass quantity and tissue C:N, two characteristics that strongly related to the morphological and phenological traits of the annuals, more consistently stimulated N cycling with positive effects on all three N processes. Our structural equation model revealed that the community characteristics of lower tissue C:N and lower biomass, related to the potential for greater decomposable biomass inputs from the annuals, increased NAP via higher redox potential, increased NNP through higher temperature, and increased but also decreased DP through inhibition from higher redox potentials. Both redox and temperature were significant mediators of the plant community-soil N functioning relationships. Other soil physicochemical properties – pH, soil C:N, and soil N – that were associated with either the macrophyte community or N processing but not both, were possibly exhibiting slower mediating influences (*i.e.*, effects and responses) on ecosystem functionality that could grow more important with time. Localized plant effects in the rhizosphere on soil carbon or inorganic N may have been more pronounced than overall changes in bulk soil stocks in these young systems and suggests similar processes at created wetland sites. Our findings highlight the utility of *a priori* annual-perennial functional group classifications in predicting ecosystem responses to planting schemes in created wetlands. Permitting early successional, annual plant-dominated communities to establish through an active management approach (*e.g.*, mitigating the influence of volunteer species) may be a successful restoration strategy that could improve the development of soil biogeochemical cycles and important wetland services such as denitrification.

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## Glossary of Terms

**Ammonification:** The conversion of organic nitrogen to ammonium, an inorganic form, by oxidation-reduction reactions facilitated by heterotrophic microorganisms.

**Nitrification:** The production of nitrate by microorganisms in the presence of oxygen through electron transfer to ammonium.

**Nitrogen Mineralization:** The combined biological production of ammonium and nitrate.

**Denitrification:** The conversion of dissolved nitrate to dinitrogen gas for the respiration of heterotrophic bacteria under anoxic conditions through oxidation-reduction reactions.

**Soil Redox Potential:** A measure of the ratio of the available oxidized to reduced substances, with positive potentials indicating a net oxidizing ability of the soil and negative potentials indicating a net reducing ability of the soil.