Ecological Indicators 45 (2014) 68–74

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

# Plant tissue nutrients as a descriptor of plant productivity of created mitigation wetlands

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ARTICLE INFO

*Article history:*
Received 29 August 2013
Received in revised form 19 March 2014
Accepted 21 March 2014

*Keywords:*
Created wetlands
Plant tissue nutrients
Soil condition
Peak-biomass
Plant productivity
N:P ratios
Wetland soils

ABSTRACT

The study investigated vegetative nutrient levels and ratios, net primary productivity, and soil physicochemical conditions in four created mitigation wetlands, ranging in age from 3 to 10 years, all created in the Virginia Piedmont. Plant tissue nutrients from peak above-ground biomass samples included macronutrients (i.e., C, N, P, K, Ca, Mg, S) and micronutrients (i.e., Mn, Fe, Cu, B, Zn, Al). Ratios of major elements were calculated including C:N, N:P, K:P, and N:K. Four soil condition (SC) groups were developed based on soil organic matter (SOM), gravimetric soil moisture (GSM), pH, and bulk density ($D_b$), where study plots were grouped across the wetland sites based on common attribute levels (i.e., SC1 > SC2 > SC3 > SC4, trended more to less successionally developed). There was a lack of wetland site based differences in plant tissue macronutrient (i.e., N, P, K, and S) levels and ratios (i.e., C:N, N:P, N:K, K:P), but differences were seen for all micronutrients ($P < 0.005$). When plant tissue macronutrient levels were compared between SC groups, greater SOM, lower $D_b$, more circumneutral pH, and higher GSM, all indicative of wetland soil maturation, were associated with higher tissue macronutrient levels for C, N, K, Ca, and Mg ($P < 0.005$), and higher micronutrient levels for Fe, Cu, B, and Al ($P < 0.005$) in plant tissues. A significant predictive relationship was found between plant productivity (i.e., peak AGB) and plant tissue boron and aluminum (i.e., $AGB = -0.54B + 0.2Al - 0.38$, $F_{4,83} = 24.7$, $P < 0.001$, $R^2 = 0.47$). The results of this study show that plant tissue concentrations of macro- and micronutrients are associated with the physicochemical maturity of soils and can be used to estimate functional development (e.g., plant biomass production) in created mitigation wetlands.



## 1. Introduction

Wetland vegetation diversity and productivity are principally determined by hydrology, soil physicochemistry, and light regime (Ballantine and Schneider, 2009; Boutin and Keddy, 1993; Dee and Ahn, 2012; Ehrenfeld et al., 2005; Olde Venterink et al., 2003). Soil attributes including bulk density, porosity, organic matter, pH, texture, and moisture content affect chemical and microbial decomposition processes, which are the principle means by which nutrients essential to the development of wetland vegetation are made available (Angeloni et al., 2006; Cronk and Fennessy, 2001; Ehrenfeld et al., 2005; Yu and Ehrenfeld, 2010). Decomposition of organic matter replenishes bioavailable nutrients that are often lacking in created wetlands compared to their natural counterparts (Bayley and Guimond, 2009; Dee and Ahn, 2012; Hogan and Walbridge, 2007; Wolf et al., 2011). Created and restored wetlands are born from construction practices that cause high bulk densities and reduced porosities, which leads to multi-decadal soil development timelines (Ballantine and Schneider, 2009). Created wetland maturation is often a spatially heterogeneous process with variation across a site affected by topographical and hydrologic design features (Ahn and Dee, 2011; Bruland and Richardson, 2005; Dee and Ahn, 2012; Moser et al., 2009). Extended and highly variable soil development in created wetlands limits early development of diverse plant communities (Hossler and Bouchard, 2010; Hossler et al., 2011; Matthews et al., 2009; Dee and Ahn, 2012), thus delaying realization of functionality equivalent to that of natural wetlands.

A dearth of plant available nutrients in created wetlands, when compared to natural wetlands, can result in the lack of sufficient nutrient cycling functionality for carbon, nitrogen, and phosphorus (Fennessy et al., 2008; Hossler et al., 2011; Wolf et al., 2011). Wetland plants have evolved unique adaptations to address low oxygen, decomposition-limiting conditions in the root zone (i.e., saturated or inundated soils), including symbiotic or mutualistic relationships with nitrogen-fixing bacteria and

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http://dx.doi.org/10.1016/j.ecolind.2014.03.016
1470-160X/

fungal mycorrhiza, solubilizing phosphate root exudates, and oxygenated rhizospheres, in addition to root depth, density, and size adaptations (Angeloni et al., 2006; Cronk and Fennessy, 2001; Deng et al., 2009; Snowden and Wheeler, 1995; Verhoeven et al., 1996). More diverse wetlands tend to be lower in productivity (i.e., above ground biomass levels < 400 g $m^{-2}$), which has been linked to interspecific competition for low levels of plant available nutrients leading to populations of species with special adaptations to deal with specific limitations (Bedford et al., 1999; Svengsouk and Mitsch, 2000; Verhoeven et al., 1996). Highly productive herbaceous wetlands (i.e., above ground biomass > 1000 g $m^{-2}$) tend to be monotypic, in most case consisting of interstitial reed and clonal species, which has been attributed more to light limitation and to a lesser degree elevated nutrient levels (Angeloni et al., 2006; Bedford et al., 1999; Farrar and Goldberg, 2009; Verhoeven et al., 1996; Olde Venterink et al., 2003).

Macronutrients (i.e., carbon, nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur) are responsible for plant development and growth through control of enzyme activation for the synthesis of proteins, nucleotides, and chlorophyll (Cronk and Fennessy, 2001; Uno et al., 2001). Plant tissues macronutrient ratios (i.e., N:P, N:K, and K:P) below critical levels are symptomatic of nutrient limitation or co-limitation, which can affect wetland productivity and richness-productivity patterns (Bedford et al., 1999; Koerselman and Meuleman, 1996; Olde Venterink et al., 2003; Verhoeven et al.,1996). Critical N:P ratios in wetland vegetation and soils have been extensively studied by Koerselman and Meuleman (1996) with ratios below about 14 indicative of nitrogen limitation, ratios above 16 indicative of phosphorus limitation, and ratios between 14 and 16 being N and P co-limited. Olde Venterink et al. (2003) further refined critical nutrient ratios based on European wetland and grassland fertilization studies to include potassium (i.e., N:K and K:P) as a factor in determination of N, P, or K limitation or co-limitation.

Micronutrients (i.e., manganese, zinc, copper, iron, and boron) are only needed by plants in trace amounts (i.e., 0.01% or less) with zinc and boron instrumental in pollen and pollen tube formation, manganese in photosynthetic electron transfer, copper in plastid pigments and lignin, and iron in chlorophyll production (Cronk and Fennessy, 2001; Uno et al., 2001). Micronutrients, especially the reduced form of iron ($Fe^{2+}$) and manganese ($Mn^{2+}$), can be present at toxic levels in saturated wetland soils, but rhizospheric oxygenation and the ability to store excess micronutrients in vacuoles and senesced material allow wetland plants to adjust their metabolisms (Deng et al., 2009; Mitsch and Gosselink, 2007; Snowden and Wheeler, 1995). Rhizospheric oxygenation (radial oxygen loss – ROL) causes the formation of ferric oxide or ferric phosphate root precipitates, with monocots (i.e., rushes and sedges) being more tolerant to high iron concentrations than dicots (Snowden and Wheeler, 1995). Wetland monocots are often used in treatment wetlands for uptake of excess nutrients and phytoremediation of heavy metals including micronutrients, aluminum, lead, and cadmium (Aslam et al., 2007; Debing et al., 2009; Deng et al., 2009; Lai et al., 2011). Toxic levels of heavy metals has been shown to reduce overall levels of tissue macronutrients depending on tolerance as a function of the spatial pattern of ROL along the root, thus can negatively affect growth and development (Deng et al., 2009; Snowden and Wheeler, 1995).

This study investigated above ground plant tissue nutrient levels and soil conditions in four created mitigation wetlands in the northern Virginia piedmont. The wetlands ranged in age from 3 to 10 years. Vegetation tissue macro- and micro-nutrient levels were examined by both wetland site and soil condition (SC). Soil conditions developed in our previous work on vegetation community development were leveraged for this assessment (Dee and Ahn, 2012). The study focused on the following research questions:

(1) Do vegetation tissue macro- and micronutrient concentrations differ significantly by wetland site and soil condition?
(2) Do macronutrient ratios vary significantly by wetland site and soil condition?
(3) Can plant tissue nutrient levels be used as indicators of plant biomass in created wetlands?

## 2. Methods

### 2.1. Site descriptions

The study sites consisted of four created mitigation wetlands located in the northern Virginia Piedmont physiographic province that is part of the Potomac River watershed in either in Prince William or Loudoun counties. Loudoun County Mitigation Bank (LC) is a 12.9 ha wetland and upland buffer complex, constructed by Wetland Studies and Solutions, Inc. (WSSI) in the summer of 2006 (3 years old during study year) in Loudoun County, Virginia (39°1′ N, 77°36′ W). Bull Run Wetland Bank (BR, 38°51′13″ N, 77°32.6′59″ W) is a 20.2 ha wetland and upland buffer complex, constructed by WSSI in 2002 (7 years old during study year) in Prince William County, Virginia (38°51′ N, 77°32′ W). North Fork Wetlands Bank (NF) is a 50.6 ha wetland, constructed by WSSI in 1999 (10 years old during study year) in Prince William County, Virginia (38°49′ N, 77°40′ W). Manassas Wetland Compensation Site is a 16.2 ha wetland, located where Broad Run and Cannon Branch converge east of the Manassas Regional Airport (MW, 38°43.3′ N, 77°30.2′ E), that was created by Parsons Transportation Group (PTG) in 2000 under a Virginia Department of Transportation (VDOT) permit (HDR, 2009). More site details can be found in Dee and Ahn (2012).

### 2.2. Field work

A total of 22 study plots (10 m × 10 m), representative of site hydrology, soil, and vegetation, were selected for sampling across the four sites (i.e., LC $n = 8$, BR $n = 5$, MW $n = 4$, NF $n = 5$) (Ahn and Peralta, 2009; Ahn and Dee, 2011; Wolf et al., 2011). Sampling occurred in August and September 2009 including vegetative species identification, species percent cover, peak above-ground biomass (AGB), and soil. A nested quadrat approach was used to collect four matched vegetation and soil samples per plot (i.e., $n = 88$ total per attribute for 16 different measured or calculated attributes) using a square meter quadrat for vegetation identification and percent cover, a 0.25 $m^2$ quadrat for AGB and a beveled soil auger with a removable aluminum liner (diameter = 4.7 cm, length = 10 cm) for soil samples. Percent cover of AGB samples was 100% or greater and all live plants had normal healthy turgidity. Collection method details can be found in Dee and Ahn (2012).

### 2.3. Lab work

AGB samples were dried at 48 °C (drying cabinet maximum temperature) until a constant mass was reached (i.e., <5 g difference). Dried live (i.e., not standing litter) plant matter including leaves, blades, and stems was proportionally selected from each AGB sample (i.e., based on a visual estimate of species percent in dry biomass) and ground using a Wiley Mill. Ground tissue and soil samples were sieved through a 2 mm mesh then placed in 5 mm vials for shipment to the Plant and Soil analysis lab at University of Delaware (UD). Plant tissue total elemental composition was accomplished using microwave digestion and Inductively Coupled Plasma (ICP) spectroscopy procedures for percent macronutrients (i.e., P, K, S, Ca, Mg, and S) and micronutrient (mg $kg^{-1}$) levels

(i.e., Mn, Zn, Cu, Fe, B, and Al) (Soil UD, 2008). Mehlich-3 extraction procedures were used for soil elemental analysis (i.e., P, K, S, Ca, Mg, S, Mn, Zn, Cu, Fe, B, and Al; mg $kg^{-1}$) (Soil UD, 2008). Total C and N were determined by dry combustion of ground tissue and soil sub-samples in a 2400 Series II CHN/O elemental analyzer (Perkin-Elmer, Waltham, Massachusetts). Lab processing details for AGB, soil bulk density ($D_b$), soil organic matter (SOM), soil pH, and gravimetric soil moisture (GSM) can be found in Dee and Ahn (2012).

### 2.4. Data analysis

Attribute data sets (i.e., $n = 88$ per attribute) were assessed for outliers, normality, and linearity. A combination of modifying outliers to mean plot values and base 10 logarithm transformations addressed all normality and linearity issues (Mertler and Vannatta, 2010). Bi-variate Pearson correlation coefficients were calculated to determine the degree of correlation between productivity and tissue nutrient attributes. Euclidean clustering with average linkage was used on standardized soil attributes (i.e., plot means, $n = 22$) to determine soil condition (SC) groups (i.e., plot combinations across sites with similar soil characteristics) (Zuur et al., 2007). Significant differences in vegetation tissue nutrient levels as affected by wetland site and SC groups were evaluated using General Linear Model (GLM) univariate Analysis of Variance (ANOVA) techniques (Mertler and Vannatta, 2010). Principal Component Analysis (PCA) using varimax rotation and Kaiser normalization was used to compute composite nutrient variables for use in multi-regression. Stepwise multi-regression was used to evaluate AGB prediction using principal components and variables of plant tissue nutrients and soil condition variables. All statistical analyses were conducted using IBM SPSS Statistics version 20 (SPSS, 2012).

## 3. Results

### 3.1. Characterization of plant communities

A companion study for the same wetland sites by the same authors (Dee and Ahn, 2012) found a total of 41 species were across the four sites, with hydrophytic vegetation representing 85% of the total species present. Plot level Importance Value (IV) was calculated as the sum of relative cover (RC), determined as the mean relative cover across all samples collected in a plot, and relative frequency (RF), the percentage of total samples containing a given species $I$ in a plot: $IV_i = RC_i + RF_i$ (Atkinson et al., 2005). Plot level species IVs illustrated that *Juncus effusus*, a monocot, was dominant in 36% of the plots, other tussock and matrix monocots were dominant in 23% of the plots, and the remainder of the plots were dominated by dicots (Table 1). 10 of the 22 plots displayed monotypic behavior with IVs close to or greater than 150 (Table 1).

**Table 1**
Plot-level species dominance and class based on importance value.

| Site | Plot | Dominant species[a] | IV[b] | Class |
|---|---|---|---|---|
| LC | A | ECHCRU | 138 | Dicot |
| | AA | BIDCER | 135 | Dicot |
| | B | CARFRA | 138 | Monocot |
| | BB | CARFRA | 160 | Monocot |
| | CC | ECHCRU | 102 | Dicot |
| | DD | JUNEFF | 200 | Monocot |
| | E | ECHCRU | 89 | Dicot |
| | F | JUNEFF | 172 | Monocot |
| BR | BR1 | LEEORY | 111 | Monocot |
| | BR3 | POLHYD | 154 | Dicot |
| | BR4 | POLHYD | 124 | Dicot |
| | BR5 | JUNEFF | 146 | Monocot |
| | BR6 | JUNEFF | 165 | Monocot |
| MW | MW1 | TYPANG | 149 | Monocot |
| | MW11 | SYMERI | 148 | Dicot |
| | MW14 | JUNEFF | 199 | Monocot |
| | MW15 | JUNEFF | 128 | Monocot |
| NF | NF4 | CARVUL | 123 | Monocot |
| | NF6 | JUNEFF | 101 | Monocot |
| | NF14 | BIDARI | 163 | Dicot |
| | NF15 | ARTHIS | 120 | Dicot |
| | NF40 | JUNEFF | 127 | Monocot |

[a] ECHCRU (*Echinochloa crusgalli* L.), BIDCER(*Bidens cernua* L.), CARFRA (*Carex frankii* Kunth.), JUNEFF (*Juncus effusus* L.), LEEORY (*Leersia oryzoides* L.), POLHYD (*Polygonum hydropiper* L.), TYPANG (*Typha angustifolia* L.), SYMERI (*Symphyotrichum ericoides* L.), CARVU (*Carex vulpinoidea* Michx.), BIDARI (*Bidens aristosa* Michx.), ARTHIS (*Arthraxon hispidus* Thunb.).
[b] Based on Dee and Ahn (2012).

### 3.2. Plant tissue nutrient differences by wetland site

Tissue macronutrient level variation included carbon 41.2–43.2%, nitrogen 1.22–1.51%, phosphorus 0.12–0.16%, potassium 1.29–1.52%, calcium 0.30–0.56%, magnesium 0.15–0.32%, and sulfur 0.16–0.23% (Table 2). MW carbon levels were highest (MW 42.9–43.1%; other sites 41.2–42.1%; $P < 0.005$) and magnesium levels were lowest (MW 0.15–0.17%; other sites 0.20–0.32%; $P < 0.005$), while calcium levels were marginally different between sites ($P < 0.05$) (Table 2). Remaining macronutrient levels were not significantly different between sites (Table 2). Tissue micronutrient and aluminum levels were between 185 and 935 mg $kg^{-1}$ for manganese, 85–311 mg $kg^{-1}$ for iron, 5.7–10.2 mg $kg^{-1}$ for copper, 7.5–174.9 mg $kg^{-1}$ for boron, 23–39 mg $kg^{-1}$ for zinc, and 34–279 mg $kg^{-1}$ for aluminum (Table 2). There were significant differences between sites for all micronutrients with manganese highest at MW (MW 737–935 mg $kg^{-1}$; other sites 185–617 mg $kg^{-1}$; $P < 0.005$) and aluminum lowest at MW (MW 34–40 mg $kg^{-1}$; other sites 68–279 mg $kg^{-1}$; $P < 0.005$), boron (NF 15.4–19.2 mg $kg^{-1}$; other sites 7.5–13.7 mg $kg^{-1}$; $P < 0.005$) and copper (NF 9.9–10.2 mg $kg^{-1}$; other sites 5.7–8.9 mg $kg^{-1}$; $P < 0.05$) highest at NF, iron (BR 277–311 mg $kg^{-1}$, other sites 85–178 mg $kg^{-1}$; $P < 0.005$) and aluminum highest at BR (BR 229–279 mg $kg^{-1}$; other sites 34–116 mg $kg^{-1}$, $P < 0.005$), and zinc higher at BR and NF ($P < 0.05$, Table 2). Macronutrient ratios were not significantly different between sites with C:N ranging from 28.9 to 35.8 ($P = 0.273$), N:P from 9.1 to 13.3 ($P = 0.341$), N:K from 0.84 to 1.10 ($P = 0.121$), and K:P from 10 to 14 ($P = 0.758$, Table 2).

### 3.3. Plant tissue nutrient differences by soil condition (SC) groups across wetland sites

SOM, pH, $D_b$, and GSM variables were used to assess similarities at the plot-level resulting in four SC groups, resulted from cluster analysis at 60% dissimilarity applied, across the wetland sites (see Dee and Ahn, 2012). SC plot groups trended from more to less developed from SC1 to SC4 (e.g., greater SOM, higher, GSM, more circumneutral pH, lower Db) with at least three different significance levels between SCs ($P < 0.001$) for each attribute (Dee and Ahn, 2012). More detailed SC group results and discussion can be found in Dee and Ahn (2012).

Plant tissue macronutrients showed more significant differences when analyzed by SC group than by wetland site. Six of seven macronutrient attributes were different by SC (Table 3), as opposed to only three that were different by site (Tables 2 and 3). SC3 and/or SC4 tissues contained significantly lower nitrogen (SC4 1.11–1.19%, SC1–SC3 1.31–1.64%, $P < 0.005$), potassium (SC3–SC4 1.27–1.39%, SC1–SC2 1.48–.171, $P < 0.005$), calcium (SC3–SC4

**Table 2**
Wetland site-based differences for tissue nutrient attributes (mean ± standard error).

| | LC | BR | MW | NF | $F_{3,84}$ | $P^{a,b}$ |
|---|---|---|---|---|---|---|
| *Macronutrients* | | | | | | |
| C (%) | 41.6 ± 0.2b | 41.9 ± 0.3b | 43.0 ± 0.1a | 41.6 ± 0.4b | 6.5 | ** |
| N (%) | 1.31 ± 0.04a | 1.25 ± 0.03a | 1.35 ± 0.08a | 1.43 ± 0.08a | 1.2 | NS |
| P (%) | 0.15 ± 0.01a | 0.14 ± 0.01a | 0.13 ± 0.01a | 0.14 ± 0.02a | 0.4 | NS |
| K (%) | 1.49 ± 0.03a | 1.47 ± 0.05ab | 1.34 ± 0.05b | 1.47 ± 0.04a | 2.4 | NS |
| Ca (%) | 0.33 ± 0.03b | 0.42 ± 0.04ab | 0.40 ± 0.05ab | 0.50 ± 0.06a | 3.3 | * |
| Mg (%) | 0.22 ± 0.02b | 0.29 ± 0.03a | 0.16 ± 0.01c | 0.26 ± 0.02ab | 6.2 | ** |
| S (%) | 0.22 ± 0.01a | 0.19 ± 0.01a | 0.17 ± 0.01a | 0.21 ± 0.02a | 1.6 | NS |
| *Micronutrients* | | | | | | |
| Mn (mg kg$^{-1}$) | 508 ± 55b | 569 ± 48b | 866 ± 69a | 217 ± 32c | 22.7 | ** |
| Fe (mg kg$^{-1}$) | 160 ± 18b | 284 ± 27a | 94 ± 9c | 132 ± 21bc | 8.1 | ** |
| Cu (mg kg$^{-1}$) | 8.3 ± 0.6b | 6.5 ± 0.3c | 6.4 ± 0.7bc | 10.1 ± 0.1a | 5.3 | ** |
| B (mg kg$^{-1}$) | 8.8 ± 1.3b | 10.9 ± 1.5b | 11.5 ± 2.2b | 17.3 ± 1.9a | 7.9 | ** |
| Zn (mg kg$^{-1}$) | 29 ± 2b | 36 ± 2a | 25 ± 2b | 35 ± 4ab | 3.8 | * |
| Al (mg kg$^{-1}$) | 102 ± 14b | 254 ± 25a | 37 ± 3c | 84 ± 16b | 24.7 | ** |
| C:N | 32.9 ± 1.1a | 33.9 ± 0.9a | 33.7 ± 2.1a | 30.7 ± 1.8a | 1.3 | NS |
| N:P | 9.7 ± 0.6a | 9.9 ± 0.6a | 10.4 ± 0.6a | 12.1 ± 1.2a | 1.1 | NS |
| N:K | 0.89 ± 0.03a | 0.87 ± 0.03a | 1.03 ± 0.07a | 0.98 ± 0.05a | 1.9 | NS |
| K:P | 11.1 ± 0.6a | 11.5 ± 0.6a | 10.5 ± 0.5a | 12.7 ± 1.3a | 0.4 | NS |
| AGB (g m$^{-2}$) | 1520 ± 100a | 1640 ± 120a | 1830 ± 140a | 770 ± 120b | 16.338 | ** |

[a] NS, not significant, *$P$ < 0.05, **$P$ < 0.005.
[b] Letters indicate significant differences between sites.

**Table 3**
Soil condition (SC) based differences in tissue nutrient attributes (mean ± standard error).

| | SC1 ($n$ = 2) | SC2 ($n$ = 12) | SC3 ($n$ = 3) | SC4 ($n$ = 5) | $F_{3,84}$ | $P^{a,b}$ |
|---|---|---|---|---|---|---|
| LC | | 5 plots | | 3 plots | | |
| BR | 1 plot | 3 plots | | 1 plot | | |
| MW | | | 3 plots | 1 plot | | |
| NF | 1 plot | 4 plots | | | | |
| *Macronutrients* | | | | | | |
| C (%) | 41.5 ± 0.6b | 41.4 ± 0.2b | 43.2 ± 0.1a | 42.7 ± 0.2a | 14.6 | ** |
| N (%) | 1.51 ± 0.13a | 1.35 ± 0.04a | 1.45 ± 0.1a | 1.15 ± 0.04b | 5.4 | ** |
| P (%) | 0.13 ± 0.01a | 0.15 ± 0.01a | 0.13 ± 0.01a | 0.13 ± 0.004a | 0.3 | NS |
| K (%) | 1.62 ± 0.09a | 1.50 ± 0.02a | 1.33 ± 0.06b | 1.33 ± 0.04b | 9.0 | ** |
| Ca (%) | 0.49 ± 0.07a | 0.45 ± 0.03a | 0.35 ± 0.05ab | 0.28 ± 0.03b | 5.0 | ** |
| Mg (%) | 0.34 ± 0.05a | 0.27 ± 0.01a | 0.16 ± 0.02b | 0.15 ± 0.01b | 23.7 | ** |
| S (%) | 0.24 ± 0.03a | 0.21 ± 0.01a | 0.17 ± 0.01b | 0.18 ± 0.01b | 4.1 | * |
| *Micronutrients* | | | | | | |
| Mn (mg kg$^{-1}$) | 382 ± 39b | 339 ± 33b | 826 ± 88a | 831 ± 48a | 28.5 | ** |
| Fe (mg kg$^{-1}$) | 162 ± 52ab | 209 ± 17a | 75 ± 3c | 135 ± 21b | 8.1 | ** |
| Cu (mg kg$^{-1}$) | 7.6 ± 0.6ab | 9.2 ± 0.6a | 5.7 ± 0.8c | 6.6 ± 0.4bc | 7.3 | ** |
| B (mg kg$^{-1}$) | 15.2 ± 0.7a | 13.1 ± 1.3a | 7.1 ± 0.8b | 9.8 ± 1.9b | 4.5 | ** |
| Zn (mg kg$^{-1}$) | 34 ± 4a | 33 ± 2a | 24 ± 3b | 31 ± 2a | 2.7 | * |
| Al (mg kg$^{-1}$) | 133 ± 51ab | 158 ± 15a | 35 ± 4c | 79 ± 20b | 11.4 | ** |
| C:N | 28.6 ± 2.0b | 31.7 ± 0.9b | 31.5 ± 2.4b | 37.8 ± 1.2a | 6.3 | ** |
| N:P | 12.6 ± 2.3ab | 10.4 ± 0.6ab | 11.5 ± 0.5a | 9.0 ± 0.3b | 1.9 | NS |
| N:K | 0.97 ± 0.12ab | 0.90 ± 0.03b | 1.11 ± 0.09a | 0.87 ± 0.03b | 2.8 | * |
| K:P | 12.6 ± 1.2a | 11.8 ± 0.7a | 10.8 ± 0.7a | 10.4 ± 0.4a | 0.6 | NS |
| AGB (g m$^{-2}$) | 1240 ± 200bc | 1180 ± 80c | 2120 ± 170a | 1700 ± 130ab | 10.20 | ** |

[a] NS, not significant, *$P$ < 0.05, **$P$ < 0.005.
[b] Letters indicate significant differences between soil condition groups.

0.025–0.040%, SC1–SC2 0.042–0.056%, $P$ < 0.005), magnesium (SC3–SC4 0.014–0.018%, SC1–SC2 0.026–0.039%, $P$ < 0.005), and sulfur (SC3–SC4 0.016–0.019%, SC1–SC2 0.020–0.027%, $P$ < 0.05) nutrient levels compared to SC1 and SC2 (Table 3). Tissue phosphorus levels were not significantly different between SC groups (0.012–0.016%, $P$ = 0.856). All tissue micronutrient and aluminum levels displayed significant differences between SC groups (Table 3). Manganese (SC3–SC4 738–914 mg kg$^{-1}$, SC1–SC2 306–421 mg kg$^{-1}$, $P$ < 0.005) was higher in SC3 and SC4, while copper (SC3–SC4 4.9–7.0 mg kg$^{-1}$, SC1–SC2 7.0–9.8 mg kg$^{-1}$, $P$ < 0.005), and boron (SC3–SC4 6.3–11.7 mg kg$^{-1}$, SC1–SC2 11.8–15.9 mg kg$^{-1}$, $P$ < 0.005) were higher in SC1 and SC2 (Table 2). Iron ($P$ < 0.005), zinc ($P$ < 0.05), and aluminum ($P$ < 0.05) were lower in SC3 than the other groups (Table 3). Macronutrient ratios did show some differences based on soil condition with C:N ($P$ < 0.005) higher in SC4 and N:K ($P$ < 0.05) slightly higher in SC3, but both N:P ($P$ = 0.137) and K:P ($P$ = 0.638) were not different (Table 3).

### 3.4. Correlation between productivity and plant tissue nutrient attributes

Eight of the thirteen tissue nutrient attributes had significant correlations with AGB (Table 3). AGB was negatively correlated with the macronutrients potassium ($P$ < 0.05), calcium, magnesium, and sulfur ($P$ < 0.01), but positively correlated with carbon ($P$ < 0.01; Table 4). AGB was negatively correlated with the micronutrients copper and boron, but positively correlated with manganese ($P$ < 0.01, Table 4). There was no correlation between nutrient ratios and AGB (Table 4).

**Table 4**
Pearson bi-variate correlation coefficient matrix between above-ground biomass and tissue nutrient attributes.

| C | N | P | K | Ca | Mg | S | Mn | Fe | Cu | B | Zn | Al | C:N | N:P | N:K | K:P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AGB ($g\,m^{-2}$) | **0.445** | −0.140 | −0.166 | −0.234 | **−0.444** | **−0.398** | **−0.317** | **0.532** | −0.145 | **−0.388** | **−0.599** | −0.174 | −0.076 | 0.201 | −0.107 | 0.009 | −0.111 |

**Bolded**: $p < 0.01$, Underlined: $p < 0.05$.

### 3.5. Plant productivity and its relationship with tissue nutrients and soil condition

Multi-regression productivity (i.e., peak AGB) predictions, using macronutrients only, micronutrients and aluminum only, and a combination of all plant tissue nutrients, both with and without soil condition variables (i.e., SOM, GSM, pH, and Db), were assessed. Principal component analysis (PCA) was used to produce continuous variables (i.e., components PC1 and PC2) that reflected linear combinations of the selected nutrients as regression variable for the nutrient only analysis. The best nutrients only predictive model used the first two principal components calculated from the micronutrients and aluminum (AGB = 0.04 PC1 − 0.66 PC2, $F_{2,85} = 32.0$, $P < 0.001$, $R^2 = 0.43$), and explained 43% of the variation in AGB (Table 5). For micronutrients, Fe (0.969) and Al (0.959) drove PC1, and B and Cu drove PC2. When SC variables were included in the regression, the best model used tissue boron and manganese as predictors (AGB = −0.49 B + 0.41 Mn, $F_{2,85} = 44.9$, $P < 0.001$, $R^2 = 0.51$), but aluminum, boron, and pH variables also supported highly significant predictions (AGB −0.54 B + 0.2 Al–0.38 pH, $F_{2,85} = 24.7$, $P < 0.001$, $R^2 = 0.47$) (Table 5).

## 4. Discussion

### 4.1. Plant tissue macronutrient differences between site and SC

We observed limited differences in macronutrient tissue levels and no differences for nutrient ratios between wetland sites (Table 2). Macronutrient level and ratio differences were more pronounced as a function of soil condition (i.e., 8 versus 3; $P < 0.05$) than between sites (Tables 2 and 3). Previously, we found significant spatial heterogeneity in soil conditions within each site, and that more developed soils were related to better plant quality and diversity and lower biomass (Dee and Ahn, 2012). In this study, most tissue macronutrients trended from higher levels in the more developed SC1 and SC2 plots (i.e., higher N, K, Ca, Mg, and S) to lower levels in the less developed SC3 and SC4 plots (Table 3). Higher levels of C and lower levels of N in the less developed SC4 plot tissues led to a higher C:N ratio, while N:K ratio differences did not appear to be related to soil maturity (Table 3). Higher levels of plant tissue K, Ca, Mg, and S in the mature SC1 and SC2 plots were correlated with lower AGB (Table 4). Likewise, the more developed SC group soils had higher levels of macronutrients ($P < 0.005$), which may have contributed to greater macronutrient vegetation uptake (Cronk and Fennessy, 2001; Mitsch and Gosselink, 2007, Table 3). Hossler et al. (2011) found that litter C, N, and P content were more than 80% lower in created versus reference wetlands in Ohio, while similarly, Fennessy et al. (2008) observed lower decomposition rates in created wetlands, thus bioavailable macronutrient levels can be reduced by degraded retention and mineralization processes. Previous wetland plant studies have explored the variability of tissue macronutrient (i.e., N and P) levels as a function of fertilization and taxa, and have found that absolute N and P availability is not as important as the ratio between N and P in addition to "life history" (Koerselman and Meuleman, 1996; McJannet et al., 1995). SC groups better differentiated tissue macronutrients than wetland site in this study (Dee and Ahn, 2012, Table 3).

### 4.2. Plant tissue macronutrient ratios and limitations

Wetlands are commonly N-limited due to extended anoxic conditions which result in loss of nitrogen through microbial reduction to nitrous oxide and dinitrogen gas (Bedford et al., 1999; Hossler et al., 2011; Olde Venterink et al., 2003; Mitsch and Gosselink, 2007). The study wetlands typically experience drawdown in the late summer followed by an extended saturated period again in the fall, so microbial oxidation processes can mineralize organic matter and potentially replenish bioavailable nitrogen supplies before dormancy (Ahn and Peralta, 2009; Dee and Ahn, 2012; Olde Venterink et al., 2003). Soil temperatures in the top 10 centimeters can stay above 5 °C well into January for the study plots, so microbial reduction activity could continue unabated into early winter, further depleting spring nitrogen stocks (Ahn Lab unpublished data 2009–2011; Rabenhorst, 2005). Over 90% of the study sample N:P ratios were below 14.5 and all N:K ratios were under 2.1, indicative of N-limitation for all wetlands (Koerselman and Meuleman, 1996; Olde Venterink et al., 2003, Fig. 1 and Tables 2 and 3).

**Table 5**
Multi-regression[b] models for above-ground biomass (AGB) using macro-, micro-, and combined plant tissue nutrients principal components[e], tissue nutrient variables and soil condition (SC) variables.

| Plant nutrients only | PC1 | PC2 | PC3 | $F_{2,85}$ | $P$[a] | $R^2$ |
|---|---|---|---|---|---|---|
| Macronutrients[c] | −0.37 | −0.22 | NA | 9.6 | ** | 0.184 |
| Micronutrients[d] | 0.04 | −0.66 | NA | 32.0 | ** | 0.431 |
| Combined | −0.41 | 0.14 | −0.48 | 19.9 | ** | 0.416 |

| SC[f] and plant nutrients | B | Mn | Ca | Al | Ph | $F_{2,85}$ | $P$ | $R^2$ |
|---|---|---|---|---|---|---|---|---|
| All variables | −0.49 | 0.41 | NA | NA | NA | 44.9 | ** | 0.514 |
| All soil/macro-nutrients | NA | NA | −0.35 | NA | −0.32 | 16.1 | ** | 0.275 |
| All soil/micro-nutrients | −0.49 | 0.41 | NA | NA | NA | 44.9 | ** | 0.514 |
| All soil/selected nutrients[g] | −0.54 | NA | NA | 0.2 | −0.38 | 24.7 | ** | 0.468 |

[a] NS, not significant, *$P < 0.05$, **$P < 0.001$.
[b] Regression on log transformed variables. Standardized coefficients.
[c] Macronutrients include total C, N, P, K, Ca, Mg, and S.
[d] Micronutrients include total Mn, Fe, B, Zn, Cu, and Al.
[e] PCA: varimax rotation with Kaiser normalization.
[f] Soil condition variables include soil organic matter, gravimetric soil moisture, pH, and bulk density.
[g] Tissue nutrients with combined PCA matrix components > 0.7 (B, Al, Fe, Zn, Cu, P, Ca).

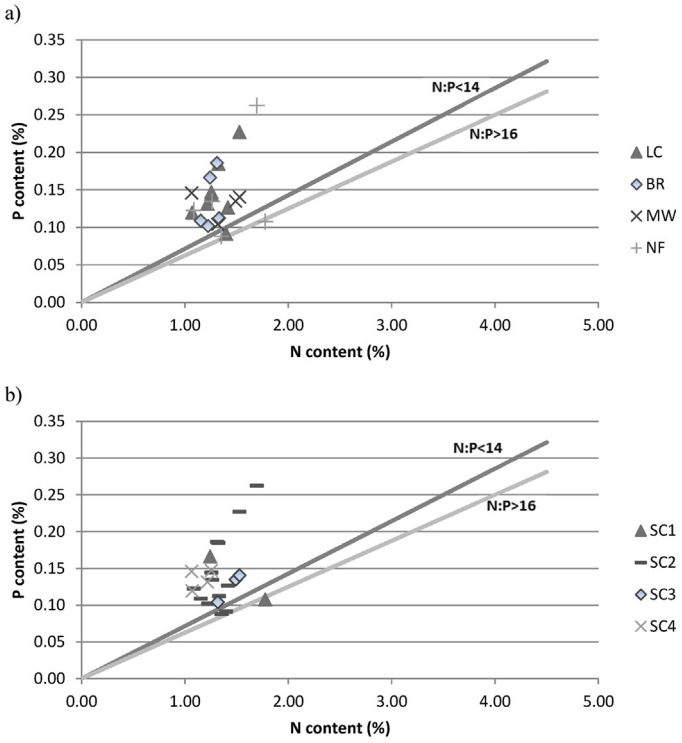


**Fig. 1.** Relationship between vegetation tissue N (%) and P (%) using mean values for wetland sites (a) and soil condition (b). Solid lines indicate N:P ratios (by weight) of 14 and 16, with N:P < 14 indicative of N-limitation, N:P > 16 indicative of P-limitation, and 14 < N:P < 16 indicative of co-limitation (Koerselman and Meuleman, 1996).

Phosphorus is stored in wetlands in large unavailable quantities in organic matter, through sorption to clay, and via precipitation with ferric iron, calcium, and aluminum (Mitsch and Gosselink, 2007). When soils are saturated long enough for redox potentials to fall between ±100 mV, inorganic P can be released through reduction of ferric iron to soluble ferrous iron and thus made available for plant uptake (Mitsch and Gosselink, 2007). Moser et al. (2009) found that P availability in Virginia piedmont wetland soils (i.e., created and reference sites), including NF that was one of our sites was well below an agricultural optimum as defined by Sims et al. (2002) as total P < 50 mg kg$^{-1}$ and when considering iron and aluminum levels in the Mehlich-3 molar ratio where [P/(Al + Fe)] < 0.06. Our study soil P levels were under 30 mg kg$^{-1}$ and molar ratios were less than 0.04 aligned with previous findings (Moser et al., 2009). The N:K ratios ranged from 7.7 to 15.4, well above the 3.1 threshold found by Olde Venterink et al. (2003) for P or N + P co-limitation when coupled with N:P > 14.5 (Fig. 1, Tables 2 and 3). Almost half of the plots across the sites had N:P ratios close to 14, which Koerselman and Meuleman (1996) considered the N + P co-limitation threshold (i.e., 14 < N:P < 16), and three of the plots were P-limited with N:P > 16 (Fig. 1).

### 4.3. Plant tissue micronutrient differences between site and SC

All five plant tissue micronutrient and aluminum levels differed for both site and SC groups, and except for zinc and manganese ($P < 0.05$), the differences were highly significant ($P < 0.005$, Tables 2 and 3). Iron, aluminum, and zinc tissue levels were highest in the 7-year-old site, BR, and in the most developed SC groups, SC1 and SC2, which also tended to be the more connected to adjacent streams (Dee and Ahn, 2012, Tables 2 and 3). Boron and copper tissue levels were highest in the oldest site, NF, in SC1 and SC2 (Tables 2 and 3). Manganese tissue levels were highest in the most anthropogenically impacted site (i.e., adjacent to highway and airport-industrial complex so subject to more polluted stormwater run-off), MW, the SC3 group composed of three MW plots (Dee and Ahn, 2012, Table 3). Mean tissue and soil iron levels across all categories were below (i.e., tissue Fe < 300 mg kg$^{-1}$, soil Fe < 225 mg kg$^{-1}$) those seen in toxicity experiments (i.e., Fe supply concentrations 10–100 mg l$^{-1}$ in 10% Rorisol solutions at pH 5.5) by Snowden and Wheeler (1995), who saw monocots shoot iron levels between 400 and 1500 mg kg$^{-1}$, dicots levels between 900 and 3200 mg kg$^{-1}$, and levels causing 10% productivity reduction around 750 mg kg$^{-1}$ (Tables 2 and 3). Monocots, which were dominant in 59% of the study plots, are better adapted to restricting uptake of heavy metals to roots and translocation to shoots due to root structure (i.e., thicker versus fibrous roots) and exclusion mechanisms that allow exudation or external precipitation of heavy metal complexes (Lai et al., 2011; Snowden and Wheeler, 1995). In wetlands, where both Fe and P are made available under reducing conditions, Fe approaching toxic levels also acts as a control mechanism, reducing the ability to uptake other nutrients and even immobilizing P within the roots (Mitsch and Gosselink, 2007; Snowdon and Wheeler, 1995). Because study tissue Fe levels were well below those considered to be toxic, we did not see a resulting reduction in uptake. Overall, we saw a greater accumulation of micronutrients in the soil and plant tissues in the more developed SC1 and SC2 plots where 46% of the samples were dominated by dicots.

In this study, micronutrient only principal components predominantly reflecting iron, aluminum, boron, and copper levels proved significant predictors ($R^2 = 0.43$, $P < 0.001$) of productivity, increasing the explained variability over macronutrients alone by almost 25% (Table 5). When individual tissue nutrients and soil condition variables were used in the regression, pH, aluminum, and boron based predictions explained 4% more variation ($R^2 = 0.47$) than micronutrient principal components, yet tissue boron and manganese supported the best predictions ($R^2 = 0.51$) (Table 5).

## 5. Conclusions

This study investigated tissue nutrients, soil conditions, and their relationship to plant productivity as a surrogate for functional development in four mitigation wetlands created in the Piedmont region of Virginia. Soil condition reflecting spatially heterogeneous maturation within each site turned out better associated with more significant differences in tissue macronutrient levels and ratios. We also found micronutrients differences for both site and SC group variables, and that micronutrient levels in plant tissues were better predictors of plant productivity in the created wetlands. The significant relationship between plant tissue nutrients and productivity found in this study shows that plant tissue nutrient levels, a structural component indicative of wetland soil maturation, can be an indicator for tracking the progress of plant productivity of created mitigation wetlands.

## Acknowledgments

Research was supported by the Thomas F. and Kate Miller Jeffress Memorial Trust. For site access, thanks go to Wetland Studies and Solutions, Inc. and the Virginia Department of Transportation. Thanks go to Rita Peralta, Johnny Kim, Kyle, Gretchen, and Timothy Goeke Dee for their valuable assistance in the field and lab.

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