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Effects of five years of nitrogen and phosphorus additions on a *Zizaniopsis miliacea* tidal freshwater marsh

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ABSTRACT

The purpose of this experiment was to determine if nitrogen (N) or phosphorus (P) acts as the limiting nutrient for tidal freshwater marsh vegetation. To answer this question, we added N, P, and N + P to a tidal freshwater marsh dominated by *Zizaniopsis miliacea* (Michx.) (giant cutgrass) in Georgia, USA, for five years to determine their effects on aboveground and belowground biomass and nutrient (N, P) uptake. Nitrogen and P were applied twice per year at an annual rate of 50 g m^{-2} $year^{-1}$ and 10 g m^{-2} $year^{-1}$, respectively. Aboveground biomass and leaf C, N, and P were sampled in August of each year. Belowground biomass and C, N, and P content were measured in August of year five. After two years, plots receiving N and N + P had significantly greater aboveground biomass than the control and P plots. This trend continued through the fifth year of the study and resulted in two to three times more aboveground biomass at the end of the fifth year in the N (1570 g m^{-2}) and N + P (1264 g m^{-2}) plots relative to P (710 g m^{-2}) and control (570 g m^{-2}) plots. After five years of nutrient additions, macro-organic matter (MOM), the living plus dead root and rhizome mat (0–10 cm), was significantly lower in the N (1457 g m^{-2}) and N + P (994 g m^{-2}) plots than the control (2189 g m^{-2}) plots. There was less live rhizome biomass in the N + P (23 g m^{-2}) plots than the control (1085 g m^{-2}) plots. We observed a 31–33% increase in the N content of *Z. miliacea* leaves in years three through five in the N and N + P plots relative to the control plots, but observed no P enrichment of leaves. In the N-treated plots, leaf C:N decreased 20–25% whereas N:P increased 21–64% in years three through five relative to the control and P plots. These findings collectively suggest that N, rather than P, limits productivity of tidal freshwater marsh vegetation. Reduced belowground biomass that accompanies N enrichment is of special concern as it may lead to increased erosion and reduced organic matter inputs to the soil, increasing their susceptibility to disturbances associated with wind, waves, river flooding and rising sea level.

1. Introduction

Tidal freshwater marshes occur in estuaries beyond the limits of salt water intrusion yet are still influenced by diurnal astronomical tides (Neubauer et al., 2005). Less is known about these ecosystems than other tidal wetlands such as salt marshes (Odum, 1984; Hopkinson, 1992). These freshwater marshes are known to have high plant productivity and aboveground biomass ranges from 1000 to 3000 g m^{-2} $year^{-1}$ (Whigham et al., 1978; Keefe, 1972; Sasser and Gosselink, 1984; Hopkinson, 1992). The productivity of these ecosystems is dependent upon the availability of limiting nutrients such as nitrogen (N) and phosphorus (P). In salt marshes, the limiting nutrient has been shown to be nitrogen (Valiela and Teal, 1974; Hopkinson and Schubauer, 1984; Kiehl et al., 1997; Visser and Sasser, 2006). The source (N versus P) of nutrient limitation in tidal freshwater marshes is less clear as some studies have suggested it is nitrogen (Morse et al., 2004; Frost et al., 2009) while others have suggested it is phosphorus (Paludan and Morris, 1999; Sundareshwar and Morris, 1999). Jordan et al. (2008) suggested that the observed shift from P limitation in freshwaters to N limitation in coastal marine waters is linked to P availability. In freshwaters, P precipitates with Fe-bearing minerals such as vivianite and other ferrous minerals that do not form in saline environments. Tidal freshwater marshes, located in the upper reaches of estuaries where saltwater seldom, but occasionally penetrates, are located where either N or P (or both) could be limiting, in the water column and possibly in the wetlands. The source of nutrient limitation is important as human activities have the ability to change natural nutrient loading in these ecosystems that may lead to changes in plant community structure (species diversity), function (productivity) and nutrient cycling.

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The ambiguity over nutrient limitation in these marshes is in part due to a lack of long-term nutrient enrichment experiments in these plant communities. Although nutrient enrichment manipulations in tidal freshwater marshes of short duration (i.e., one-year) have been conducted (Chambers and Fourqurean, 1991; Morse et al., 2004), such short-term studies may not allow for conclusive findings. For example, other researchers have shown it takes up to two years for plants to respond to nutrient additions (Craft et al., 1995). A more extended study in these marshes by Frost et al. (2009) examined nutrient limitation over two years. This study showed an increase in aboveground biomass with N fertilization and consistent leaf tissue N:P ratios <30, both of which suggested that N was the more limiting nutrient.

The current study was designed to continue the work of Frost et al. by tracking the effects of annual nutrient (N, P, N + P) additions over five years. We examined the response of marsh plant species to nutrient additions by measuring aboveground and belowground biomass, leaf C, N, P, C:N:P, and diversity. We also compared our results with published studies to determine whether our findings are consistent with other studies of N versus P limitation of tidal freshwater marsh vegetation.

2. Methods

2.1. Site description and nutrient addition

We established plots in a tidal freshwater marsh on Carrs Island on the northern bank of Hammersmith Creek near the Altamaha River (Georgia, USA) (31.334364°, −81.475900°), in spring 2004. Vegetation at the site is dominated by *Zizaniopsis miliacea* (Michx.) (giant cutgrass) along with small amounts of *Pontederia cordata* (L.) (pickerelweed) and *Sagittaria lancifolia* (L.) (bull tongue arrowhead). Sixteen 2 m × 2 m plots were established to provide four replicates of four treatments (nitrogen-N, phosphorus-P, N + P, and control). A 2 m buffer was established between each plot to minimize nutrient exchange between plots. Nitrogen and P were applied at an annual rate of 50 g m^{-2} year^{-1} and 10 g m^{-2} year^{-1}, respectively, by broadcasting it by hand twice a year during the growing season (March and May) in equal amounts. Fertilizer was broadcast at low tide when no water was present on the marsh surface to help ensure nutrient additions stayed within their specific experimental plot. Additionally, the fertilizer pellets we used were heavier than water and thus reduced concerns over it being removed during high tide. Triple Superphosphate (45% P_2O_5) was used as the P source. The nitrogen source in year one was ammonium chloride (NH_4Cl). In order to provide a longer lasting N source we switched to a polymer coated urea $CO(NH_2)_2$ (Polyon, Pursell Technologies Inc., Sylacauga, AL, USA) beginning in year two and continuing through the rest of the five year experiment.

2.2. Field and lab measurements

Aboveground biomass of emergent vegetation was nondestructively determined in August of each year by measuring the number and height of leaves in a 0.5 m × 0.5 m (0.25 m^2) subplot within each treatment plot. Height measurements were converted to mass using an allometric equation determined from the height and weight of 509 *Z. miliacea* leaves harvested outside of, but in close proximity, to the treatment plots (Frost et al., 2009). Beginning in 2007, we measured the number and height of *P. cordata* leaves present in the plots. An allometric equation (mass (g) = 0.000001(height (cm)^3); n = 77, r^2 = 0.85) was used to calculate leaf weight from height for *P. cordata*. The number and height of *S. lancifolia* also was measured in 2007 and 2008. This species was much less abundant in the plots than *P. cordata* (for example, in 2008, there were a total of seven leaves of *S. lancifolia* in the plots as compared to 105 leaves for *P. cordata*) so we used the same allometric equation for both species which have similar leaf height and shape.

Leaf C, N, and P was measured annually by selecting five random leaves of average height of *Z. miliacea* from each plot. In 2008, one leaf of *P. cordata* and, *S. lancifolia* was collected from each plot, where present, for C, N, and P analysis. Leaves were oven dried at 50 °C and ground using a Thomas Scientific Wiley Mill (Swedesboro, NJ, USA). Leaf C and N were measured using a Perkin-Elmer 2400 CHN analyzer with NIST 1515 Apple Leaves as the standard. We measured P content of leaves following the methods of Sommer and Nelson (1972) using NIST 1575a (pine needles) as the P standard. Recovery of NIST standards was 97% for N (n = 62) and 88% for P (n = 71).

Macro-organic matter mass, the living and dead root and rhizome mat (Gallagher and Plumbley, 1979), was sampled in year five by collecting an 11 cm diameter by 70 cm deep core from each plot. Macro-organic matter (MOM) cores were separated into 10 cm sections, washed, and sorted to separate live rhizomes from the remaining MOM following methods outlined in Hopkinson and Dunn (1984) and Schubauer and Hopkinson (1984). Belowground fractions were oven dried at 50 °C to a constant weight and ground using a Wiley Mill. Macro-organic matter and rhizomes were analysed for C, N, and P using the same procedures and standards as for leaves.

2.3. Statistical analyses

We used repeated measures analysis of variance (ANOVA) to determine the effect of nutrient additions on stem height, stem density, and aboveground biomass (SAS, 2002). ANOVA was also used to test the effects of treatment and year on leaf C, N, P, and C:N:P ratios (SAS, 2002). MOM and rhizome biomass and nutrient (C, N, P) concentrations were analysed using a 2-way ANOVA based on treatment and sampling depth (SAS, 2002). Means were separated using the Ryan–Einot–Gabriel–Welsch multiple range test (SAS, 2002). All tests of significance were conducted at α = 0.05 level.

3. Results

3.1. Aboveground and belowground biomass

Nitrogen additions resulted in a significant increase in aboveground biomass of *Z. miliacea*. Aboveground biomass was two times greater in the N and N + P plots than in the control and P plots beginning in year two and continuing through year five (Fig. 1a). Phosphorus additions had no effect on aboveground biomass of *Z. miliacea*. Increased aboveground biomass was attributed to increased leaf height (Fig. 1b) and to some extent the number of leaves. Leaf height in the N plots was greater than the control and P plots in years two to five. Leaf height in the N + P had varied results and was greater than the control and P plots only in years three and five. Over the five year duration of the study, the mean number of leaves per m^2 was significantly greater in the N (241 ± 15) and N + P (238 ± 20) plots than in the control (159 ± 16) plots.

There was no difference in species richness among the treatments. On average, there were 5 species present in the control plots, 4.75 species in the P plots, and 3.75 species in both the N and N + P plots. *Z. miliacea* was the dominant species in all treatments throughout the five-year experiment. In years four and five, it accounted for at least 84% of total aboveground biomass. There was a total of seven other plant species (*Rhynchospora corniculata*, *Polygonum hydropiperoides*, *Ludwigia linearis*, *Smilax tamnoides*, *P. cordata*, *S. lancifolia*, and an unidentifiable *Asteraceae*) present in the