

# Soil Nitrogen Concentrations in a Restored Sedge Meadow Wetland as Affected by the Application of High C:N Amendments

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## ABSTRACT

Invasive perennial species are frequently a barrier to native plant establishment in fertile sedge meadow wetland restorations. Amending soils with high carbon-to-nitrogen (C:N) materials may deplete nitrogen (N) and limit the establishment of invasive species, although the effectiveness of such amendments at depleting soil N in restored wetlands is largely unknown. Therefore we incorporated four high C:N materials (cedar, white pine, and red oak sawdust, as well as sucrose) into the soils of nonvegetated plots in a restored sedge meadow wetland and measured soil ammonium-N and nitrate-N in relation to nonamended control plots over a 25-week period. All amendments depleted nitrate-N concentrations equivalently (67%–100%), although both the timing and the duration of this depletion varied among amendments. White pine sawdust was most effective, depleting nitrate-N for the entire 25 weeks. Sawdust from red oak and cedar depleted nitrate-N after 5 and 13 weeks, respectively. Lowered nitrate-N concentrations then persisted for the remaining 20 weeks in the red oak treatments, while the effects of cedar were short-lived (8 weeks). Sucrose depleted nitrate-N for 13 weeks. No amendment depleted ammonium-N concentrations, although cedar sawdust caused initial ammonium-N concentrations to increase by 300%–700%. Based on our findings, white pine sawdust will be more likely to deplete N effectively during the establishment of sedge meadow wetlands. It is unknown, however, if this depletion will suppress invasive perennials long enough to allow desired native species to establish a closed canopy and take up nitrogen, thereby limiting the chances of future invasions.

**Keywords:** high C:N amendments, sedge meadow restorations, soil-nitrate depletion

Researchers have suggested incorporating high carbon-to-nitrogen (C:N) amendments into the soils of wetland restoration projects prior to seeding native species as a strategy to deplete nitrogen (N) and thereby limit the establishment of invasive plants (Perry et al. 2004). These amendments deplete N by triggering soil microbes to immobilize N that would otherwise be available for plant uptake (Tisdale et al. 1985, 122–125). To the best of our knowledge, however, no studies have evaluated the potential of different types of high C:N amendments to deplete soil N in wetland restoration projects. Lowering resource levels,

which can be high in restored ecosystems (e.g., Adams and Galatowitsch 2005, Orr and Stanley 2006), may aid the establishment of desired native plant species by reducing the establishment of invasive plants (Averett et al. 2004, Hovick and Reinartz 2007). Depleting soil N in restored wetlands may be particularly beneficial, since high N can promote the establishment of aggressive plants in these ecosystems (Verhoeven et al. 2006).

While many studies have tested the effectiveness of high C:N amendments at decreasing both N and the establishment of invasive plants in other ecosystems (e.g., Blumenthal et al. 2003, Eschen et al. 2007), less information is available on how high C:N amendments affect N availability and invasion in restored wetlands. Iannone and Galatowitsch (2008) and

Iannone and others (2008) conducted a study in a restored sedge meadow wetland that tested the effects of one such high C:N amendment, sawdust from cedar (*Thuja* sp.), on both N availability and competition between a native seed mix and seeds of the invasive species reed canarygrass (*Phalaris arundinacea*). They found that cedar sawdust depleted soil N for less than nine weeks and then later increased ammonium-N. Nonetheless, native species were able to outcompete reed canarygrass in cedar-amended plots. Sawdust from cedar has a low C:N ratio and a high ammonium-N concentration compared to sawdust from other species, possibly explaining both its short-lived effects on N availability and the increase in ammonium-N that it caused (Iannone and Galatowitsch 2008, Iannone et al. 2008).

The greater the duration of N depletion caused by incorporating high C:N amendments, the more likely C:N amendments will be able to enhance the establishment of desired native plants. Establishment and growth of invasive perennials such as reed canarygrass need to be restricted long enough to allow native seedlings to establish. Depleting soil N can disadvantage the growth of invasive wetland species relative to native species (Perry et al. 2004), allowing native species to establish. Once native species establish they can decrease available light by forming a canopy and deplete soil nutrients such as N, thereby reducing the likelihood of future invasions (Lindig-Cisneros and Zedler 2002, Iannone et al. 2008).

Based on the results of past research that used sawdust to deplete N in sedge meadow soils (Perry et al. 2004, Iannone and Galatowitsch 2008, Iannone et al. 2008), we hypothesized that sawdust with higher C:N ratios and lower ammonium-N concentrations will deplete N availability for longer periods than sawdust with low C:N ratios and high ammonium-N concentrations (e.g., cedar). Further, studies investigating high C:N amendments as restoration tools in other ecosystems have used sucrose (e.g., Prober et al. 2005, Vinton and Goergen 2006). We hypothesized, therefore, that sucrose can also reduce N availability in restored sedge meadows. The overall objective of this study was to detect and measure variations in the magnitude and the longevity of N depletion caused by incorporating four different high C:N amendments (sawdust from three tree species and sucrose) into sedge meadow soils. We assessed these differences to both determine the usefulness of particular high C:N amendments as N-depleting tools, and to identify which amendments will likely provide the greatest restoration benefits.

## Methods

### *Study Site and Experimental Setup*

We conducted a randomized complete-block experiment in one of four experimental wetland basins located at the University of Minnesota Horticultural Research Center in Carver County, Minnesota, lat 44°51'45" N, long 93°36'00" W. The soil at the site is Glencoe clay loam (Cumulic Endoaquoll) (USDA 1968). The site was historically a drained depressional wetland used for agriculture. In 1984, it was set aside for wetland restoration research and divided by earthen dikes into four approximately 0.20-ha basins. Each basin is equipped with both an adjustable drainage tile and a water inlet to allow for precise hydrological control. We raised the drainage tile and filled the basin that we used for this study with water during May 2005. We then used a small portion of the basin to study the effectiveness of different seed mixes at establishing desired plant communities. The plant community in the remainder of the basin (including the area used for this study) established via natural processes, resulting in a community dominated by weedy annuals and short-lived perennials.

In May 2006, we laid out a row of twenty 1-m<sup>2</sup> plots and hand-pulled the vegetation from each. We continued to weed plots each time we sampled soil (see below) so that plants did not interfere with soil-microbial processes triggered by our high C:N amendments. We placed all plots 3 m from where standing water occurred in the center of the basin and surveyed all plots to determine their elevation relative to this "standing-water line." Throughout the study, we maintained the standing-water line at 5 cm below the lowest plot in order to isolate the effects of the different high C:N amendments on N availability from the effects of fluctuating hydrology. Plot elevation averaged 10 ± 0.5 cm (mean ± SE) and ranged from 5 to 16 cm above the standing-water line.

Throughout the study, soil moisture in our plots averaged 38 ± 1% (mean ± SE) and ranged from 28% to 64% (dry-weight basis). To account for any environmental variation, we divided the row of plots into four blocks of five plots. We then randomly assigned one of five possible high C:N amendment treatments to each plot within each block (i.e., four replications). The treatments were cedar (*Thuja* sp.), red oak (*Quercus rubra*), and white pine (*Pinus strobus*) sawdust, as well as sucrose (white granulated cane sugar) and a control (no amendment).

The materials used in past studies, the comparisons we wished to make, and material availability all dictated our decisions about what high C:N amendments to use. While Perry and others (2004) used pine sawdust in a greenhouse study, no study had ever tested the effects of pine on N availability in an actual wetland. Since cedar had already been used under similar conditions (Iannone and Galatowitsch 2008, Iannone et al. 2008), we decided to include cedar in this study so we could compare its effects on N availability to those of amendments with both higher C:N ratios and lower ammonium-N concentrations. In order to make comparisons between sawdust from hardwood and gymnosperm species as well as between sucrose and sawdust, we decided to include red oak sawdust and sucrose. Ultimately, we chose the specific sawdust types to use based on their availability from local suppliers. Serv-a-Dock (Victoria MN) provided the cedar sawdust, and Larson's Sawmill (Mora MN) donated the red oak and white pine sawdust. We purchased sucrose from an area supermarket.

The study began on 30 May 2006 (week 1) when we incorporated the amendments. To incorporate sawdust, we used methods similar to Perry et al. (2004). We removed approximately 7 cm of soil from plots that were assigned sawdust treatments and replaced the missing volume with the appropriate species of sawdust. We then hand-tilled the sawdust to a depth of 20 cm

**Table 1. Ammonium-N and nitrate-N concentrations, percent C and N, C:N ratios, and the dry weight added to plots of the four high C:N amendments. We added these amendments to the soil of an experimental wetland basin located at the University of Minnesota's Horticultural Research Center in Carver County, Minnesota.**

High C:N amendment	NH <sub>4</sub> -N (mg/kg)	NO <sub>3</sub> -N (mg/kg)	% C	% N	C:N	Dry wt/plot (kg /m <sup>2</sup> )
Cedar ( <i>Thuja</i> sp.)	161	4.5	48	1.40	34	8.4 ± 0.1
White pine ( <i>Pinus strobus</i> )	29	2.9	50	0.22	227	8.4 ± 0.1
Red Oak ( <i>Quercus rubra</i> )	36	1.8	48	0.15	320	13.7 ± 0.1
Sucrose	13	3.2	43	0.01	4300	0.5 ± 0.0

(2:1 soil-to-sawdust ratio by volume; for estimated dry weights see Table 1). Removing soil was necessary to assure that all plots had a similar volume of soil. To control for any effects that tilling may have had on soil nitrate-N and soil ammonium-N concentrations, we hand-tilled plots assigned to control or sucrose to a depth of 20 cm. We broadcasted sucrose over the appropriate plots at a rate of 0.5 kg/m<sup>2</sup>.

### Data Collection and Analysis

We determined the nitrate-N and ammonium-N concentrations, total C and N (percent), C:N ratios, and the approximate dry weight added to each plot for each high C:N amendment (Table 1). We dried the sawdust at 70°C for 48 h and later ground the tissue to pass through a 40-mesh screen (≈ 0.6 mm) using a Wiley mill. The University of Minnesota Research Analytical Laboratory (St. Paul MN) then extracted inorganic-N from the ground sawdust and sucrose using 30 ml of 2 M KCl, and measured nitrate-N and ammonium-N concentrations of these extracts colorimetrically using an Alpkem Rapid Flow Analyzer at 660 nm (Astoria-Pacific International, College Station TX) (Keeney and Nelson 1982). To calculate C:N ratios, we measured total C and N on three 15-mg subsamples of the ground-up sawdust and sucrose using a Vario EL III CNS elemental analyzer (Elementar Americas, Mt. Laurel NJ) (Kirsten 1983). After drying the sawdust and prior to grinding, we weighed a specific volume of sawdust three times, took the average of these weights, and extrapolated to the volume at which we added the sawdust to each plot

to determine the approximate dry weight.

To determine the effects of the different high C:N amendments on N availability, we measured nitrate-N and ammonium-N concentrations in the soil of all plots at weeks 1, 3, 5, 9, 13, 17, 21, and 25. At these times, we collected five 1.5-cm × 20-cm soil cores from random points and homogenized the cores. We then extracted inorganic N from a moist 8.0-g subsample using 25 ml of a 2 M KCl solution (Mulvaney 1996) and determined nitrate-N and ammonium-N concentrations in the extract using a Wescan N Analyzer (Wescan Instruments, Deerfield IL) (Carlson 1986). In order to adjust soil ammonium-N and nitrate-N concentrations to a dry weight basis, we first determined the percentage of moisture of all the soil that we sampled. We did this by weighing a moist subsample in a 42-mL weighing tin and drying the sample at 70°C until the sample reached a constant weight. We then reweighed the sample and divided the difference between its wet weight and its dry weight by its dry weight.

To determine treatment effects on soil N, we analyzed our nitrate-N and ammonium-N data using a random mixed-effects model. In the model we included block (replication) as a random effect, plot elevation as a covariate, high C:N amendment treatment type as a fixed effect, and time as a continuous variable. We determined differences among and between treatment levels using a Tukey HSD test. To meet the model's assumptions, we Box-Cox transformed our data before analyses. We conducted our analyses in "R" (R Development Core

Team 2008) and considered  $p < 0.05$  significant.

### Results and Discussion

In this study we found that each type of high C:N amendment depleted nitrate-N by about the same amount relative to the control (67%–100%), although both the timing and the longevity of this depletion varied among amendments ( $F = 6.81$ ,  $df = 4,145$ ,  $p < 0.0001$ ; week × high C:N amendment) (Figure 1). In contrast, no amendments depleted ammonium-N concentrations. In fact, cedar-amended plots had ammonium-N concentrations three to seven times higher than control plots during the first three weeks of the study ( $F = 6.52$ ,  $df = 4,145$ ,  $p = 0.0001$ ; week × high C:N amendment) (Figure 2). The high ammonium-N concentration of cedar sawdust likely caused this increase (Table 1). Both the lack of effects from the other amendments on ammonium-N concentrations and the short-lived ammonium-N spike observed in cedar-amended plots suggest that nitrification was occurring at a sufficient rate to deplete ammonium-N. Nitrification rates tend to be highest in soils that are moist yet unsaturated (Booth et al. 2005), such as the soil at our study site. The results of this study will have consequences for the use of high C:N amendments as N-depleting strategies in restored sedge meadows.

The variation in both timing and duration of nitrate-N depletion caused by the different amendments will help to determine when restorationists should incorporate these materials. Our results suggest that white pine

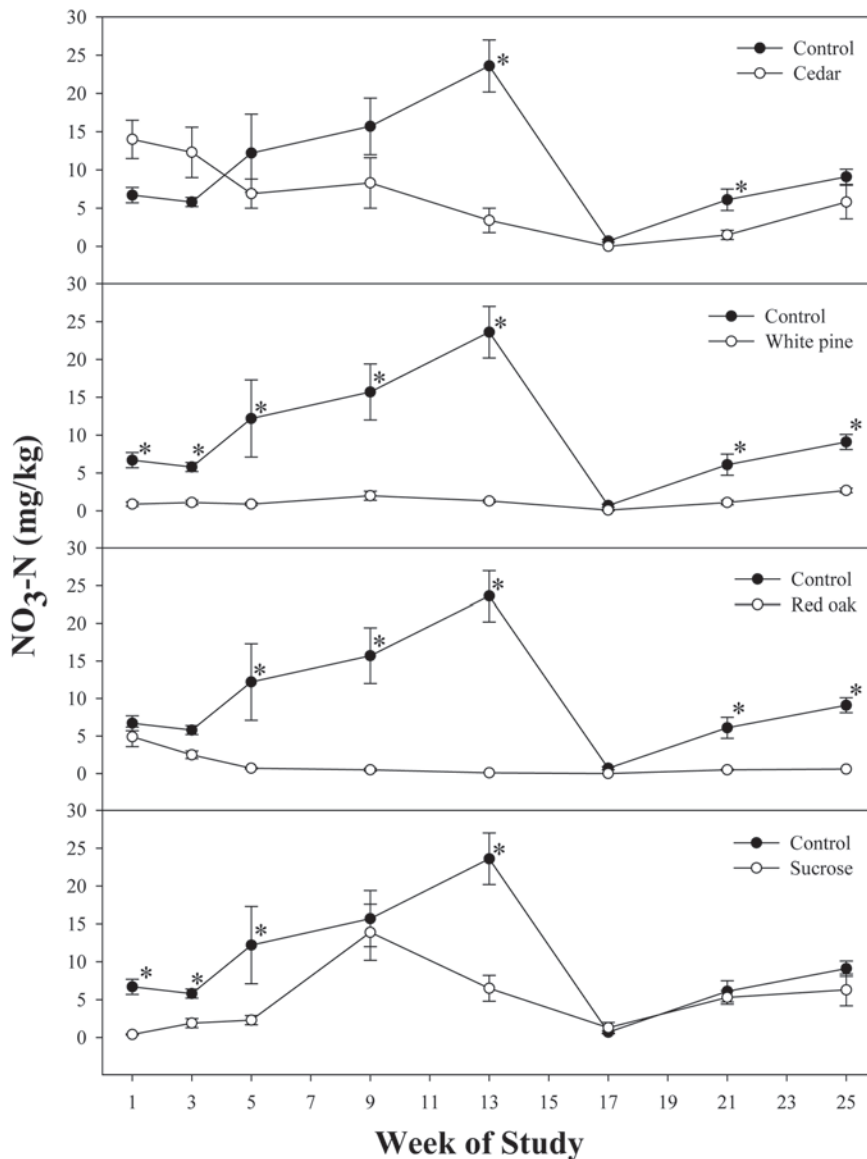


Figure 1. The effects (mean  $\pm$  SE) of four different high C:N amendments on soil nitrate-N concentrations in relation to soil nitrate-N concentrations of nonamended control plots. An asterisk signifies a significant difference between the values for that particular sampling period based on  $p < 0.05$ .

sawdust is the best choice for depleting nitrate-N, since it did so longer than any other amendment and we observed this depletion within the first week of the study (Figure 1). Amending soils with red oak or cedar sawdust did not deplete nitrate-N until weeks 5 and 13, respectively (Figure 1). The nitrate-depleting effects of red oak then persisted throughout the remainder of the study, while those of cedar were short-lived (8 weeks). Because the nitrate-N depletion caused by both red oak and cedar was delayed, restorationists using these amendments as invasive-species control will

need to incorporate them in the fall so that nitrate-N depletion occurs in the spring when seedlings are emerging (Iannone and Galatowitsch 2008). The fact that cedar and red oak sawdust did not deplete initial nitrate-N concentrations, however, confirms that soil removal, during the set up of this study, did not alter inorganic-N concentrations. Sucrose-amended plots had depleted nitrate-N concentrations during the beginning of the study, but these effects did not persist beyond week 13 (Figure 1). Repeated applications of sucrose may be required to prolong the nitrate-depleting effects of

this amendment. Further, this study took place during a drier than average summer (NOAA 2006). We expect that under wetter conditions the water solubility of sucrose will greatly diminish the effectiveness of this treatment because it will wash away.

Since elevated N in wetlands can promote the establishment of aggressive plants (Verhoeven et al. 2006), increased ammonium-N concentrations caused by high C:N amendments may reduce the effectiveness of these amendments as invasive-species control. Although increased ammonium-N in cedar-amended plots did not persist beyond week 3 in this study (Figure 2), in a previous study Iannone and others (2008) observed a cedar-induced increase in ammonium-N that lasted two growing seasons. These increases, which likely resulted from the high ammonium-N content of cedar (Table 1), may be avoidable if restorationists use amendments that have lower ammonium-N concentrations (e.g., pine, red oak, sucrose), since these materials did not increase ammonium-N (Figure 2). Perry and others (2004), however, still noted increased ammonium-N concentrations in pine-amended soils of a greenhouse study despite pine having a much lower ammonium-N concentration than cedar (Table 1). They attributed this increase to reduced plant growth and subsequently reduced N uptake. Because of the potential for high C:N amendments to increase ammonium-N, restorationists using these amendments may want to consider how the invaders they wish to control will respond to possible spikes in ammonium-N prior to incorporation. Not all aggressive plant species, however, will respond positively to increased ammonium-N. For example, elevated ammonium-N concentrations in sedge meadows did not increase the establishment of the invasive species reed canarygrass (Iannone et al. 2008).

Although high C:N amendments did not deplete ammonium-N, by depleting nitrate-N during the early

stages of community establishment, they can help to achieve two goals of sedge meadow restorations—preventing invasion and establishing plant communities similar to those of natural sedge meadows. For example, Iannone and Galatowitsch (2008) observed a reduction in seedling establishment of reed canarygrass after one growing season in cedar-amended plots despite cedar's depleting of nitrate-N concentrations for less than nine weeks (Figure 3A). This reduction in reed canarygrass then persisted through a second growing season (Iannone et al. 2008). The short-lived period of nitrate-N depletion in cedar-amended plots also caused the community that established after two growing seasons to have a high abundance of species from the Cyperaceae family (bulrush and sedges) (Figure 3B); nonamended plots had a high abundance of forbs (Figure 3C) (Iannone et al. 2008). The Cyperaceae-rich community most resembles communities of natural sedge meadow wetlands (Galatowitsch and van der Valk 1996). Nitrate-N depletion caused by high C:N amendments can, therefore, have beneficial effects on restored communities that persist long after high C:N amendments stop depleting nitrate-N.

The benefits to be gained by using high C:N amendments may need to be evaluated with respect to the cost and challenges of incorporating these materials. The volume at which we applied sawdust to our plots is equivalent to about 670 m<sup>3</sup>/ha; transporting this volume of sawdust is a significant task, as is the logistics and labor of incorporation into large areas of moist wetland soils. This later concern may be less of an issue, however, if the wetland to be restored is still drained or has water-level control (e.g., Bohnen and Galatowitsch 2005). The amount of sucrose we applied to our plots is equivalent to 5,000 kg/ha. Purchasing this amount of sucrose is costly, especially considering that sucrose may only reduce nitrate-N short-term and may wash away.

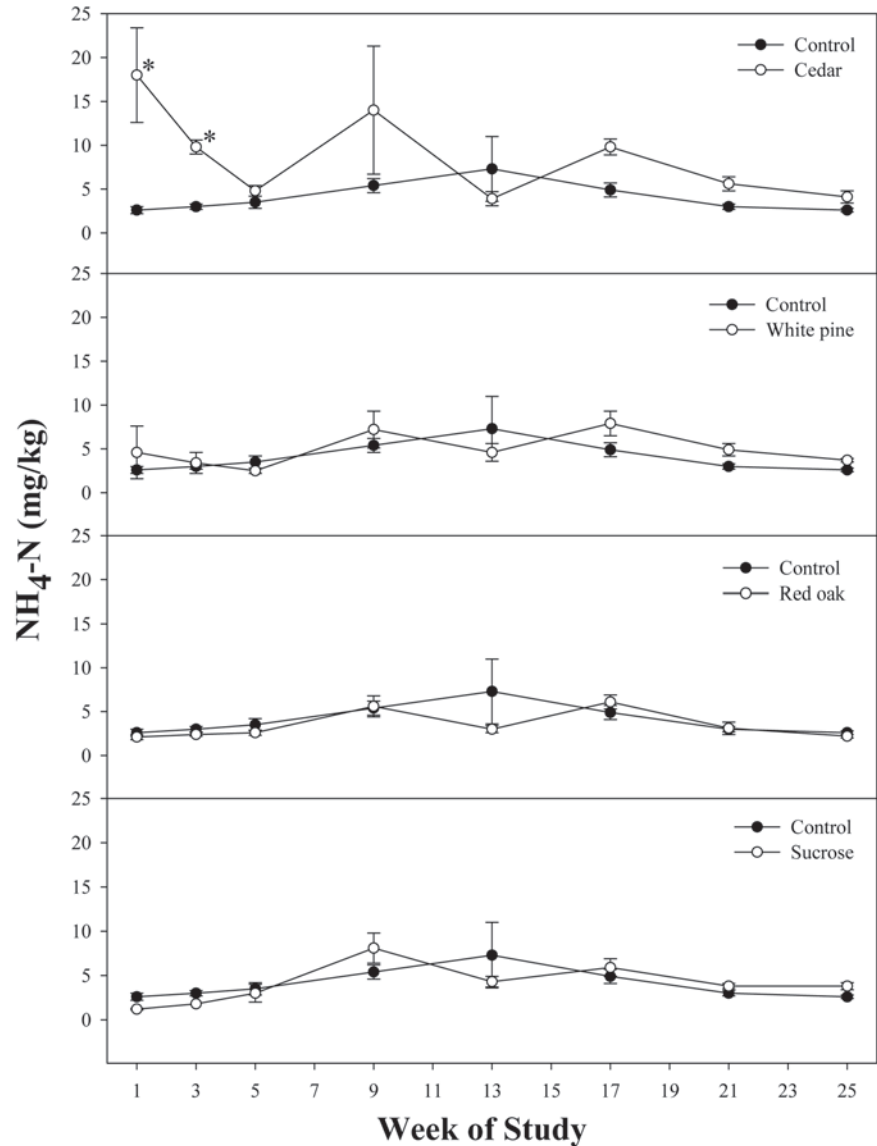


Figure 2. The effects (mean  $\pm$  SE) of four different high C:N amendments on soil ammonium-N concentrations in relation to soil ammonium-N concentrations of nonamended control plots. An asterisk signifies a significant difference between the values for that particular sampling period based on  $p < 0.05$ .

Despite these initial challenges and cost, incorporating high C:N amendments may result in long-term benefits such as the reduction of time and resources spent on follow-up invasive species control, which can be considerable (Bohnen and Galatowitsch 2005). Additionally, studies are needed to determine just how much of a particular amendment is actually needed to achieve N depletion long enough to suppress invasive species establishment.

The greatly depleted nitrate-N concentrations observed under all

treatments at week 17 (Figure 1) offers insight into how soil drainage and precipitation can affect the ability of high C:N amendments to deplete N as well as what types of wetland plants will benefit from the incorporation of such amendments. A large amount of rain that fell before sampling at week 17 may have created anoxic soil conditions that led to denitrification, depleting nitrate-N concentrations. The facts that soil nitrate-N concentrations were equivalent across all treatments after heavy rains and that amendments did not deplete soil

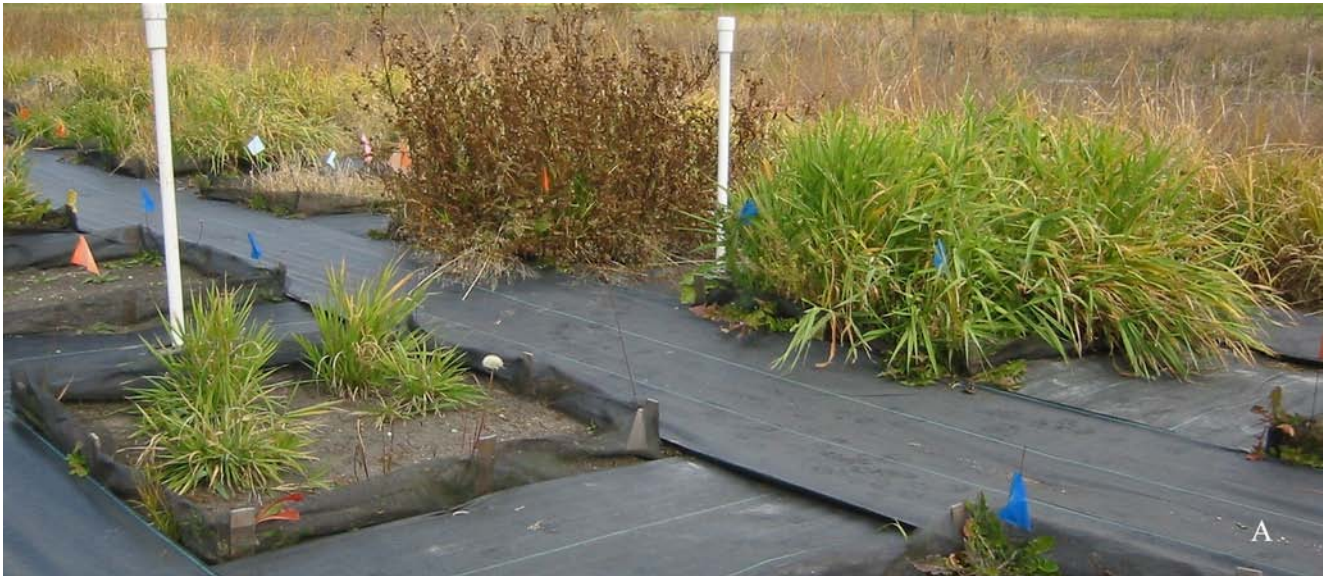


Figure 3. The benefits of incorporating cedar sawdust (high C:N amendments) into the soil of a restored sedge meadow wetland as observed in the field study that was reported in Iannone and Galatowitsch (2008) and Iannone and others (2008): A) reduced establishment of the invasive species reed canarygrass (*Phalaris arundinacea*) in the amended plot on the left compared to the nonamended plot on the right; B) establishment of a Cyperaceae-rich (bulrush and sedges) community typical of natural sedge meadows compared to C) the forb-rich community in nonamended plots. Photos by Basil V. Iannone III

ammonium-N suggest that high C:N amendments may have no effect on N availability under saturated soil conditions. If this is the case, then incorporating high C:N amendments to reduce invasion may only benefit sedge meadow and wet prairie species that typically grow where periods of flooding are brief.

## Conclusion

Amending soils of restored sedge meadows with sawdust or sucrose will be an effective strategy to deplete nitrate-N, but not ammonium-N. In general, restorationists should expect

that sawdust with high C:N ratios will deplete nitrate-N longer than sawdust with low C:N ratios. In order for native plants to outcompete invaders, the N depletion caused by high C:N amendments must occur in early spring while seedlings are emerging. Therefore, restorationists using high C:N amendments that cause delayed N depletion (e.g., red oak and cedar) will need to incorporate these materials in the fall. Restorationists using sucrose to deplete N need to consider that while sucrose is easy to apply, its nitrate-depleting effects will be short-lived and it may wash away under wet conditions. Restorationists

should use amendments with low ammonium-N concentrations to try to avoid increasing soil ammonium-N and any unknown effects that this increase may have on community invasibility. Because soils that stay saturated will already have low nitrate-N, high C:N amendments may not be a useful N-depleting strategy in these areas. Lastly, the long-term effects of high C:N amendments in restored sedge meadows are unknown. By conducting research over multiple years and sites and by monitoring soil N and plant communities in restored sedge meadows where high C:N amendments were incorporated,

scientists and restorationists can help to determine these long-term effects.

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