A mesocosm experiment to test the effects of *Typha x glauca* invasion

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Introduction

Invasive species are a major problem in the Great Lakes region, with over 162 exotic plants and animals established to date (Angeloni *et al.* 2006, Galatowitsch *et al.* 1999). Wetlands are especially susceptible to invasion due to their position as watershed sinks, subject to inputs of nutrients and other pollutants resulting in frequent disturbance (Zedler and Kercher 2004). According to the Millennium Ecosystem Assessment (2005), invasive species are one of the five most important drivers of ecosystem change.

There are four nuisance wetland emergent plants that particularly plague Great Lakes region wetlands. They include *Phragmites australis, Lythrum salicaria* (purple loosestrife), *Typha x glauca* and *Phalaris arundinacea* (reed canary grass) (Galatowitsch *et al.* 1999, Zedler and Kercher 2004). These invasive plants share many physical characteristics and tend to alter habitats in similar ways. They are large in stature with high above ground biomass, have high productivity, grow earlier in the season and have faster growth rates than many native species (Ehrenfeld 2003). The soil associated with invasive species tends to have higher concentrations of soluble inorganic nitrogen and altered cycling of elements such as carbon (C) and nitrogen (N) (Ehrenfeld 2003). These invasive plant species are usually composed of low quality material (high C:N), often containing large amounts of airspace to facilitate air and nutrient exchange with water-logged roots (Zedler and Kercher 2004, Ehrenfeld 2003).

Many of these changes can be found at Cheboygan Marsh as it has been subject to *Typha x glauca (Typha)* invasion for the last 30-40 years. The University of Michigan Biological Station (UMBS) Biology of Birds class noticed the shift in plants and associated birds during this time through their frequent fieldtrips to the marsh. Since then, the *Typha* has expanded at rates as high as 4m each year. Currently, the marsh is more than 80% occupied by invasive *Typha* (N. Tuchman, pers. commun.). *Typha x glauca* is an very aggressive, invasive hybrid species between the native broad-leaved cattail *Typha latifolia* and the introduced narrow-leaved cattail from Europe, *Typha angustifolia (*Galatowitsch *et al.* 1999*).*

Cheboygan Marsh is divided into three zones of vegetation. The first is the *Typha* zone where the *Typha* has created a monoculture and the landscape has become increasingly terrestrial. The second is the transition zone where the *Typha* has been present for just one or two seasons and has had only a minimal effect. And last, the native zone where there is no *Typha* present and the native community is intact. As shown in Table 1, substantial changes associated with *Tyhpa* have occurred in both the plant community and in abiotic parameters in Cheboygan Marsh. Some of the most important traits that differ between the native and *Typha* zones are aboveground biomass (3x increase), litter mass (14x increase), species diversity (0.68 decrease) and total soil inorganic nitrogen (1000x increase) (Angeloni *et al.* 2006).

Table 1. Cheboygan Marsh characteristics across the three vegetative zones

	Native	Transition	Typha
Typha density (shoots/m^2)	0	12.5	37.5
Above ground biomass (g/m^2)	**290.0**	354.8	**849.9**
Litter mass (g/m^2)	**177.2**	571.2	**2470.4**
Soil organic matter (%)	1.89	8.20	36.71
Species diversity (index)	**0.82**	1.25	**0.14**
Water depth (cm)	30.3	18.2	9.2
Soil TIN (ug N/g AFDM)	**0.013**	1.833	**12.003**
T°C sediment-water interface	27.5	26.6	25.4

The goal of this study is to explain the mechanisms through which *Typha x glauca* comes to dominate the pant community. Based on previous research, it appears that the increase in above ground biomass, litter mass, total soil inorganic nitrogen and other factors, from the native to *Typha* zones may be the effects of *Typha* invasion. However, research at Cheboygan Marsh is still bound by confounding variables, limiting the ability to make conclusions about causation.

For four years, a fully crossed 2x2x2 factorial experimental design of 40 mesocosms has been maintained at the UMBS. The three controlled variables are as follows, +/- *Typha*, +/- *Typha* litter and high or low water level. The mesocosms were dug each to a dimension of 2m long x 1m wide x 1m deep, lined with pond liner material and gravel, then filled with uniformly mixed hydrosoil and sand. The 11 most commonly found native wetland plants at Cheboygan Marsh were planted in all mesocosms at the same densities as found in the marsh in 2004. The invasive *Typha* was planted in half the mesocosms during 2005. Each season after senescence, all the *Typha* litter from the live *Typha* only mesocosms is collected and evenly distributed to the 10 litter only treatment mesocosms. Water level is maintained through the growing season as low, where the soil is saturated 5 cm below the surface and as high, where the mesocosm has 5-10 cm standing water at all times. With these variables controlled and accounted for it becomes possible to separate out the effects of each factor. How do *Typha* and its litter affect the native plant community and soil nutrient dynamics? Do *Typha* and native plants differ in their nitrogen uptake rates? We conducted two experiments in the mesocosms to answer these questions.

Materials and methods

In order to address these questions, a stem count and species inventory was completed from July 20-24, 2007 on the mesocosms. Each mesocosm, with dimensions 2m long x 1m wide, was divided into ten 10cm wide transects across the width. Each stem, with record of species and height per stem was counted in four of the ten transects. Calculations of aboveground biomass were made for each species using regression equations generated from previous work done by the Tuchman lab strongly correlating biomass to stem height. Species diversity was analyzed using Simpson's diversity index (McCune and Grace 2002). Due to previous research suggesting greatly increased soil inorganic nitrogen associated with *Typha*, soil samples were taken at four random locations from each of the low water mesocosms and homogenized for a single measure of soil inorganic nitrogen and soil organic matter. Both nitrate (NO_3) and ammonia (NH_4) were measured for this using a Bran Lubber autoanalyzer 3 on KCl extracts. Soil organic matter was determined in a muffle furnace by loss on ignition (Robertson *et al.* 1999).

The second question, do *Typha* and natives differ in their nitrogen uptake rates, was addressed with a ^{15}N pulse nutrient uptake experiment conducted in the mesocosms. There are two stable nitrogen isotopes. The heavier isotope, ^{15}N is found in nature at an abundance of about 0.03%. Following the application of this heavier isotope in the form of a plant available nutrient solution, the investigator is able to analyze media for their heavy isotope ratio, $\delta^{15}N$ (Fry 2006). About 25g dissolved $^{15}NH_4Cl$ solution was added, with a watering can, to the soil surface of 10 mesocosms. Ten paired mesocosms received the same amount of unlabeled NH_4Cl. Following the application, the above ground vegetation was then rinsed to avoid contamination of the ^{15}N-enriched nutrient solution on the shoot samples. Eighteen-centimeter diameter soil cores along with plant above and below-ground biomass were taken after 2 days and 30 days. To be consistent, cores were selected from areas containing predominantly *Juncus balticus* and *Schoenoplectus acutus* in addition to *Typha x glauca* for live *Typha* treatment mesocosms. The samples were then taken back to the lab. The shoots were cut from the cores and divided based on the following species categories: *J. balticus, S. acutus, Typha* and other. The root samples were washed of their soil content and divided based on two factors. In this case obvious *Typha* rhizomes were separated from all other belowground biomass. All of these samples were then put in labeled paper bags and placed in a drying oven at 60°C for three days. The samples were then ground in a SPEX SamplePrep 8000d Mixer/Mill for 3 minutes per sample. Each of these samples were measured and weighed to a mass of approximately 2g and 5g for shoots and roots, respectively. They were then placed and wrapped in tin capsules for analysis in an elemental (CHNS) analyzer for a measure of their $\delta^{15}N$ isotope ratio. Careful attention was paid at all times to avoid contamination between control and ^{15}N labeled samples.

Results

Results from the mesocosm stem counts indicate significant effects of both the presence of live *Typha* (ANOVA: p=0.022) and of *Typha* litter (ANOVA: p=0.006) (Figure 1). There was a 50% reduction in total native community aboveground biomass in the *Typha* + litter treatment mesocosms for 2007 (Figure 2). Small native species such as *J. nodosus* and *J. alpinus* were completely eliminated by 2007 from both the litter only and *Typha* + litter treatment mesocosms (Figure 2). There was a decrease in native plant community diversity in the *Typha* + litter treatment mesocosms (Figure 2). Soil total inorganic nitrogen (TIN) was not significantly different across the treatments (ANOVA: NO_3: *Typha* p=0.059 Litter p=0.18 NH_4: *Typha* p=0.14 Litter p=0.78) (Figure 2). Though there is a visual appearance in the soil organic matter (SOM) enrichment, the increase through each treatment was not statistically significant (ANOVA: *Typha* p=0.34 Litter p=0.11) (Figure 2). Results from the ^{15}N nutrient pulse uptake experiment were not completed in time for inclusion in this paper. See Appendix A for all species biomass data.

Discussion

Typha invasion at Cheboygan Marsh has clearly changed both plant community diversity and soil characteristics. These data motivated the construction of the experimental mesocosms at the UMBS. Separating the effects of *Typha, Typha* litter and water level in the mesocosms allows more definite conclusions to be made about the changes seen at Cheboygan Marsh and the effects of *Typha* invasion.

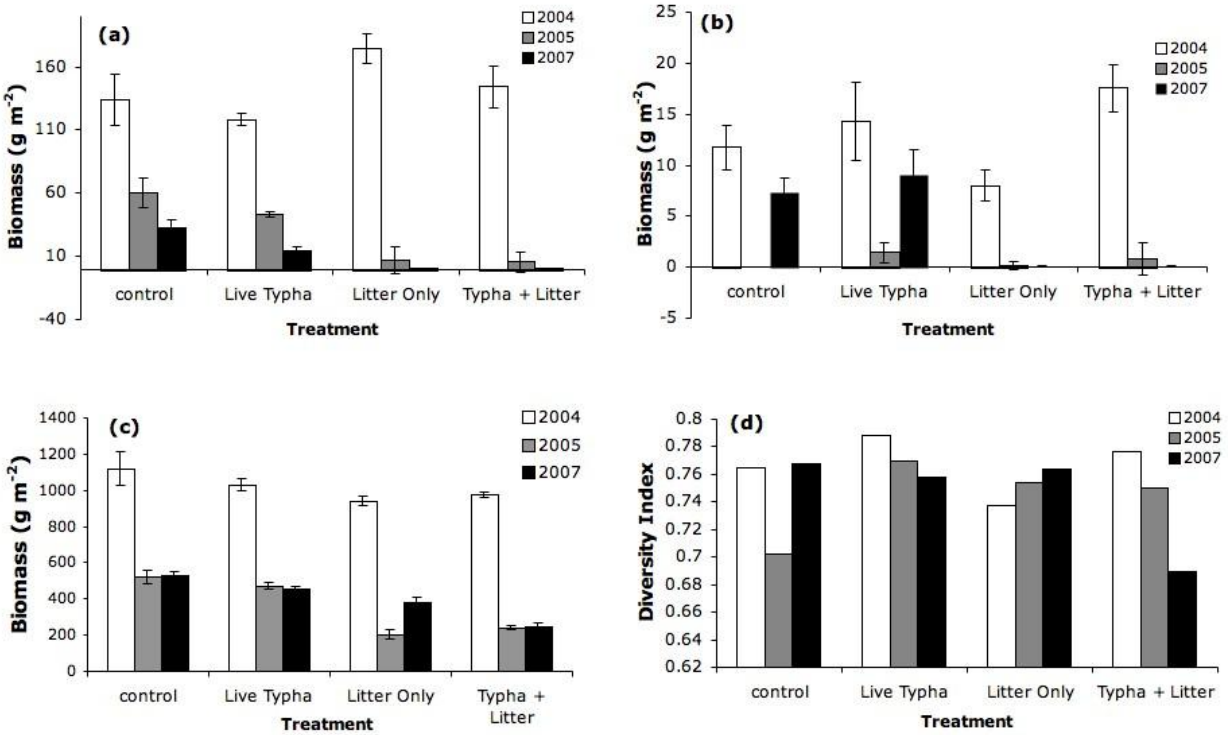

Figure 2. Plots (a) and (b) show the elimination of both *J. alpinus* and *J. nodosus* in the treatments containing litter. Total native species biomass (c) shows 50% native species biomass reduction for the *Typha* + litter treatment. Simpson's species diversity index (d) shows a decrease in species diversity for the *Typha* + litter treatment from 2004 to 2007.

To address objective 1, which looks at how *Typha* and its litter affect the native plant community and soil nutrient dynamics, the data show an elimination of several small native species as of the 2007 growing season including *J. nodosus, J. alpinus* and *Carex viridula* (Table 2). It is thought that the *Typha* litter has the effect of suppressing native speices growth by building up a relatively impenetrable layer through which the native species cannot grow. In addition to this reduction in species richness is a dramatic reduction in the biomass of several other native species, *S. validus, S. acutus, S. americanus* and *Eleocharis smallii.* In contrast to this biomass reduction were several species that have done quite well since being planted. First, *J. balticus* had an increase of 15% in biomass for the litter only treatment mesocosms. *J. balticus* has a sharp, stiff stem that may allow it to better penetrate the litter, possibly allowing it to counter the suppressive effect of the litter. It has been observed at Cheboygan Marsh deep in the transition zone, as one of the last remaining native species to grow along side *Typha*. Other species to experience an increase in biomass from 2004 to present are *C. aquatilis, C. hystericina* and *E. erythropoda.* The two *Carex* species from this group were both planted in relatively low abundance and have managed very well across the treatments. Their success may be due to their large stiff stalks aiding in litter penetration. The reduction in overall native biomass allows more light penetration to the surviving plants. The advantage of this, for the two *Carex* species especially due to their relatively large leaf area, is enhanced photosynthesis and an advantage in mesocosms with lower plant densities. It has also been observed that the *Typha* litter has a suppressive effect on *Typha* itself at Cheboygan Marsh. This is evident when *Typha* in the transition zone, not yet impeded by the litter build-up, are the first in the marsh to flower earlier than individual *Typha* in the heavily invaded areas.

Table 2. Percent change in biomass by species and treatment in the mesocosms from 2004 to 2007.

Species	Control	Live Typha	Litter Only	*Typha + Litter*
Juncus nodosus	-75.7%	-87.6%	-99.7%	-100.0%
Juncus balticus	37.2%	-28.7%	14.8%	-24.5%
Eleocharis smallii	15.6%	-12.1%	-31.9%	-73.0%
S. validus	-84.8%	-84.9%	-89.8%	-98.9%
S. acutus	-49.6%	-32.0%	-34.9%	-69.6%
Juncus alpinus	-38.3%	-37.4%	-99.0%	-99.6%
S. americanus	-21.1%	1.2%	-42.5%	-78.2%
Carex viridula	-100.0%	-100.0%	-100.0%	-100.0%
Carex aquatilis	128.7%	195.6%	2239.5%	322.3%
Carex hystericina	-64.8%	8.9%	2116.2%	578.1%
E. erythropoda	128.4%	139.6%	520.1%	-53.3%

There are several factors to consider when looking at competition between native plants and *Typha* in the absence of litter. Factors such as light, space and nutrient are among the most important. The live *Typha* treatment effect was significant, these results are shown. In the two treatments containing *Typha*, *J. balticus* biomass decreased but increased in those without *Typha*. From this, it may be that *J. balticus* is more affected by nutrient competition than by litter. Another interesting pattern to note is with the *C. aquatilis* and *E. erythropoda*; they were not nearly as successful as in the litter only treatment. This could be a nutrient competition issue but due to their small size could also be an issue of light availability.

Results from the ^{15}N pulse nutrient uptake experiment had yet to get back from the chemist at the time of writing this paper. Due to the high concentration of the heavy isotope, contamination was an issue. Aboveground vegetation needed to be washed after harvest and more thorough cleaning methods of equipment in between treatments may have been necessary. It was expected that *Typha* would take up more nitrogen than the native species due to its significantly larger biomass. A recent experiment tested the effect of nutrient addition to *Typha* and native wetland plant communities to find that while *Typha* experienced enhanced growth, the native community did not respond (Woo and Zedler 2002). Though the source of nitrogen to Cheboygan Marsh is not known, this research shows that *Typha* benefit while the native community does not. The continuation of this experiment after senescence in October and next spring in May will allow the full investigation of the nitrogen cycle. This future work will look at the application of the enriched ^{15}N litter to fresh, control mesocosms to investigate the decomposition and subsequent uptake of those enriched nutrients into new plant growth. A more complete understanding of *Typha* nitrogen cycling can be developed from this information.

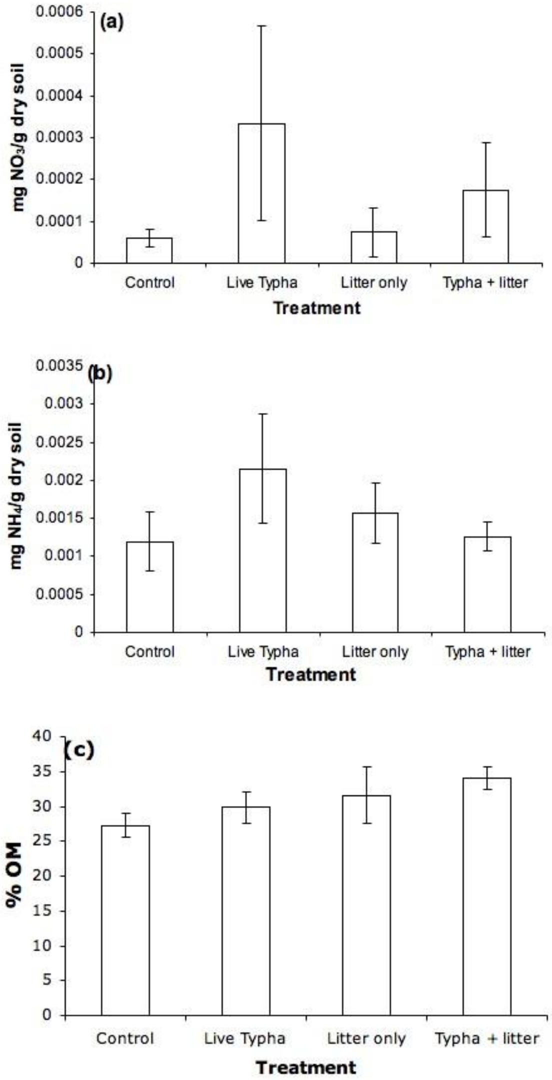

Figure 2. Soil inorganic nitrogen for both NO_3 (a) and NH_4 (b) for each treatment in the mesocosms. Soil organic matter for each treatment in the mesocosms. None of these results were significant.

Two other measures from the mesocosms were taken; total soil inorganic nitrogen and soil organic matter. The expectation from previous research at Cheboygan Marsh is that the inorganic nitrogen would be higher in treatment mesocosms compared to the control. The thought behind this is that there is a positive feedback mechanism associated with the large amount of organic matter added to the soil by the litter accumulation (Ehrenfeld 2003). This large carbon source serves as a fuel for microbes, including nitrogen-fixing bacteria (N. Tuchman pers. commun.). This trend should be seen in the live *Typha* treatment mesocosms as well, with the logic that large root masses are still left in the soil to add significantly to the SOM carbon pool. The soil characteristics do not yet differ enough to have statistical significance. With more time for the effects of *Typha* to be fully realized in the mesocosms, these differences may become greater and more solid conclusions will be possible. Soil organic matter was not significantly different either, although a clearer trend appears to be developing. The soil organic matter appears to be increasing through each treatment: control<live *Typha*<litter only<*Typha* + litter. This trend fits the description provided for TIN where there is root mass left behind in the live *Typha* mesocosms. In addition to this leftover root biomass of course is the litter which adds significantly to the organic matter pool (Ehrenfeld 2003). This measurement may become statistically significant with time as treatments progress.

Conclusion

The results of the mesocosm experiment suggest that the effects of *Typha x glauca* invasion are due to two main mechanisms: out competition for resources and suppression of native plant growth by *Typha* litter accumulation. These data provide valuable information about the individual effects of each mechanism, allowing us to untangle confounding variables observed at Cheboygan Marsh. Fundamental changes to the soil environment have occurred at the marsh. Should action be taken to restore the marsh to its original native community, this research will provide valuable information as to the correct mode of action. Removing the invasive species is only the first step to restoration. In order to address the soil legacy, further action such as fire and removal of the nutrient-rich soil will be necessary. Further research will continue on the ^{15}N pulse nutrient uptake experiment as well as annual stem counts on the mesocosms.

Acknowledgements

I would like to thank first Team *Typha* for all their help, Nancy Tuchman and Dan Larkin, Dave Karowe and Mary Anne Carroll, the University of Michigan Biological Station for supplying room and board, all my fellow REUs, especially those that helped out, and of course the National Science Foundation – Research Experiences for Undergraduates program for funding my work this summer.

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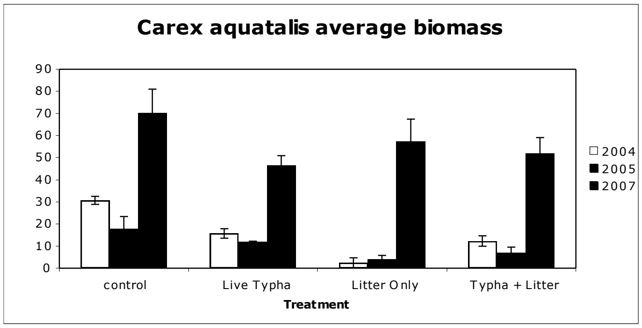
APPENDIX A


Carex aquatalis average biomass
0
10
20
30
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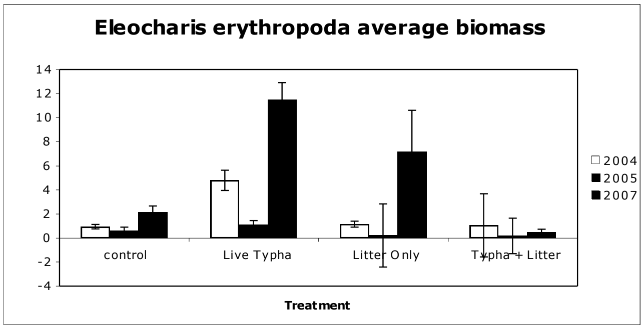
60
70
80
90
control
Live Typha
Litter Only
Typha + Litter
Treatment
□ 2004
■ 2005
■ 2007


Eleocharis erythropoda average biomass
-4
-2
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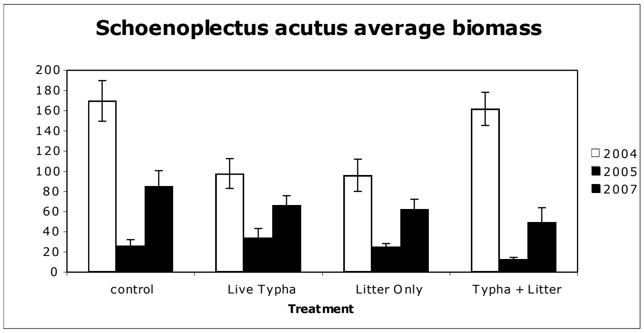
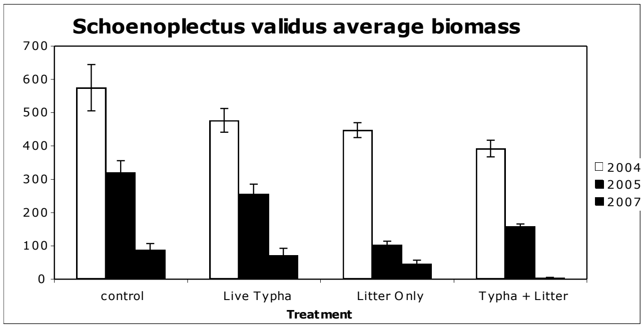
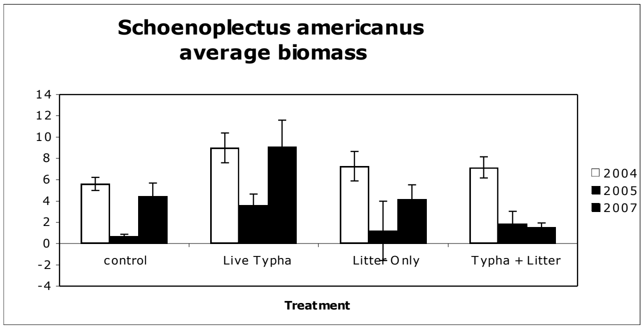
14
control
Live Typha
Litter Only
Typha + Litter
Treatment
□ 2004
■ 2005
■ 2007


Schoenoplectus acutus average biomass
0
20
40
60
80
100
120
140
160
180
200
control
Live Typha
Litter Only
Typha + Litter
Treatment
□ 2004
■ 2005
■ 2007


Schoenoplectus validus average biomass
0
100
200
300
400
500
600
700
control
Live Typha
Litter Only
Typha + Litter
Treatment
□ 2004
■ 2005
■ 2007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-2
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8
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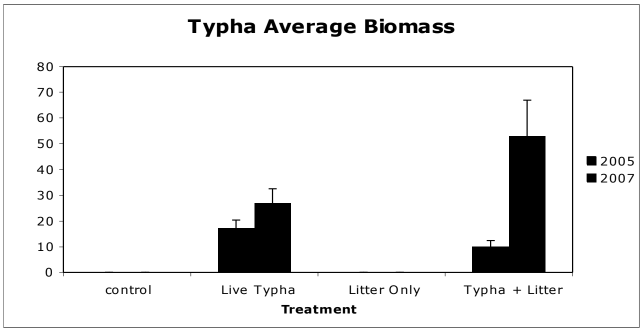
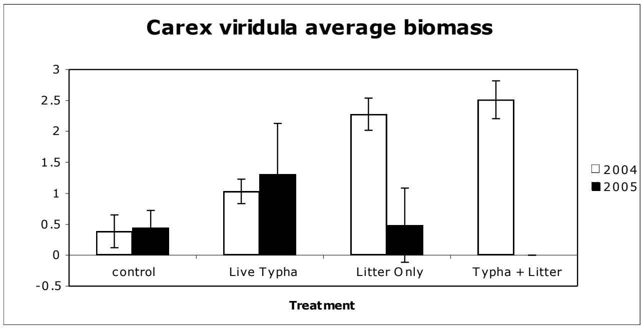
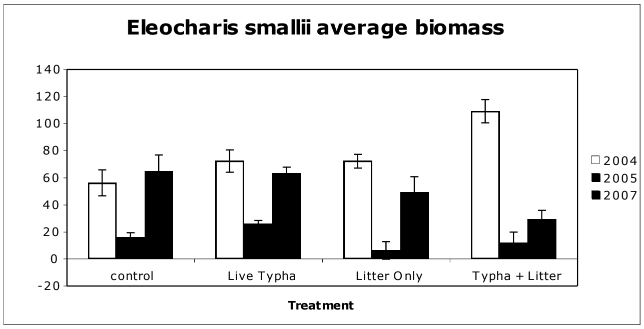
control
Live Typha
Litter Only
Typha + Litter
Treatment
□ 2004
■ 2005
■ 2007


Typha Average Biomass
0
10
20
30
40
50
60
70
80
control
Live Typha
Litter Only
Typha + Litter
Treatment
■ 2005
■ 2007


Carex viridula average biomass
-0.5
0
0.5
1
1.5
2
2.5
3
control
Live Typha
Litter Only
Typha + Litter
Treatment
□ 2004
■ 2005


Eleocharis smallii average biomass
-20
0
20
40
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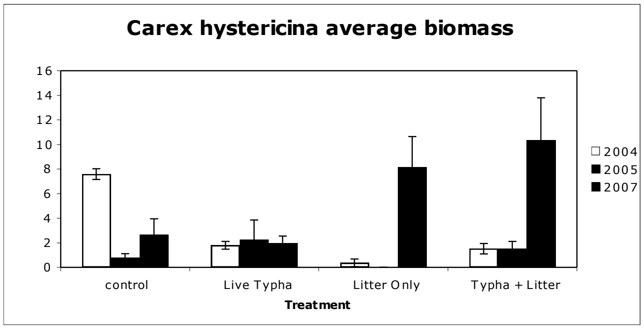
80
100
120
140
control
Live Typha
Litter Only
Typha + Litter
Treatment
□ 2004
■ 2005
■ 2007


Carex hystericina average biomass
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2
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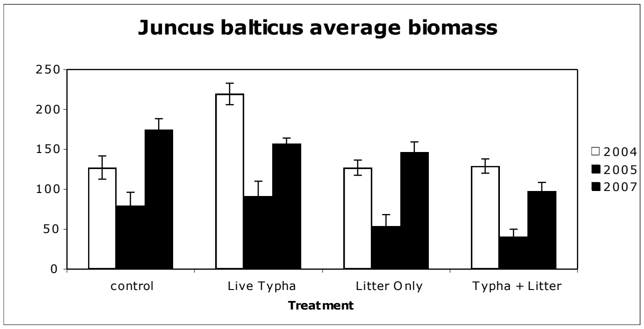
8
10
12
14
16
control
Live Typha
Litter Only
Typha + Litter
Treatment
□ 2004
■ 2005
■ 2007


Juncus balticus average biomass
0
50
100
150
200
250
control
Live Typha
Litter Only
Typha + Litter
Treatment
□ 2004
■ 2005
■ 2007