EFFECTS OF NITROGEN ADDITIONS ON SOIL SEED BANK OF A FRESHWATER MARSH IN SANJIANG PLAIN, NORTHEASTERN CHINA: A SHORT-TERM STUDY

Guodong Wang[1*], Ming Wang[2], Ming Jiang[1]

1. Key Laboratory of Wetland Ecology and Environment, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun, Jilin, 130102, China;
2. School of Geographical Sciences, Northeast Normal University, Changchun, Jilin, 130024, China;

ABSTRACT

Over the past five decades, the natural wetlands in Sanjiang Plain, Northeast China, have been extensively reclaimed for agriculture with a total loss of nearly 80% of the surface area and the un-drained ones have received a large amount of exogenous N input from the adjacent agricultural land because of fertilization. In the present study, the effects of nitrogen additions on seed germination and seedling biomass of *Calamagrostis angustifolia* freshwater marsh were tested in a greenhouse study. Seed bank soil was exposed to different N additions (0, 5, 10, 20 and 40 g/m^2) under non-flooded water regime. Results revealed that, low level of N additions (less than 10 g/m^2) did not significantly affect the species richness and seedling density, while the seedling biomass at 5 g/m^2 of N addition was higher than other nutrient conditions. But species richness, seedling emergence and biomass decreased significantly at high level of N additions (20-40 g/m^2). The responses were species-specific. High level of N additions had negative impacts on seed germination, seedling growth and biomass of dominant species *Eleocharis ovata*, *Calamagrostis angustifolia*, *Juncus effusus* in the seed bank. To protect and restore the wetland vegetation community in the Sanjiang plain, fertilization, irrigation and land management strategies will need to be implemented to reduce the nutrient input from the agricultural land to the wetlands.

KEYWORDS:
Nitrogen addition, freshwater marsh, seed bank, seed germination, seedling biomass, *Calamagrostis angustifolia*

INTRODUCTION

Increased input of nitrogen and phosphorus from agricultural, urban or atmospheric sources has caused the alteration of wetland plant community structure and composition, which are known to alter ecosystem function, such as primary production and nutrient cycling [1, 2], in a variety of habitats worldwide. Nutrient enrichment has also been shown to cause the expansion of dominant species and loss of community diversity in different types of wetlands [3-8]. Although wetland vegetation is often maintained by vegetative expansion of dominant species, moderate disturbance can create gaps allowing germination of the seeds, spores, and vegetative bodies present in the soil. If the seed bank composition is significantly different from standing vegetation, shifts in community composition following disturbance may be seen [9].

In areas of frequent disturbance due to fires or fluctuating water level such as in seasonally drying wetlands, seed bank composition and germination characteristics are necessary for modeling wetland vegetation composition over time [10]. Environmental factors such as water level, nutrient levels or temperature can affect which species are contributing to the seed bank, longevity of seeds, germination success, and seedling recruitment from the seed bank [10, 11]. These factors may be quite different than those that drive the composition of the standing vegetation [12].

Nitrogen (N) is commonly a limited nutrient in wetland ecosystems, and it directly affects the species composition and the productivity of the wetland [13]. Yet we still know little about how the exogenous nitrogen affect seed germination from soil seed banks, and further influence the composition and productivity in the freshwater wetlands. Ket et al. [14] found that nitrogen additions could promote seed germination and seedling growth rate of the *Zizaniopsis miliacea* and further increase the primary production in a tidal freshwater marsh, Gerritsen and Greening [15] found that N addition could inhibit the germination of *Nymphaea*, while it has no significantly influence on other wetland species in the Okefenokee Swamp. The specific response may depend on the plant species, hydrology, soil nutrient status [15-17].

In recent decades, reclamation of natural wetlands has been one of the major land use changes in Northeast China. Sanjiang Plain, the largest freshwater marshland in China, has experienced intensive and extensive cultivation over the past 50 years [18]. An increasing number of marshes have been drained for conversion into agricultural lands, and the un-drained ones have received a large amount of exogenous N input from the adjacent agricultural land because of fertilization [19, 20]. Yet, there is no information about the response of soil seed bank to N enrichment in freshwater marsh ecosystems of Northeast China. So we conducted a germination experiment in the greenhouse to evaluate the effects of different N additions on the seed bank of *Calamagrostis angustifolia*-dominated freshwater marsh, which is a major component of wetlands in the Sanjiang Plain [18]. Results from this study shall provide important insight into the responses of wetland vegetation in mid-high latitudes to environmental disturbances such as increasing input of nitrogen from agricultural land. This information is important to the protection of aquatic plants and the restoration of impaired wetlands.

MATERIALS AND METHODS

Study site. The study site is located in a *C. angustifolia*-dominated freshwater marsh near the Sanjiang Mire Wetland Experimental Station (47°35′N, 133°31′E, 56 m a.s.l) in the Sanjiang Plain, Heilongjiang Province, northeastern China. *C. angustifolia*-dominated seasonally inundated wetland is one of the main wetland types in the Sanjiang Plain, as this ecosystem type accounts about 31% for the wetland area in this region [18]. The study site has a temperate continental monsoon climate with a mean annual (1990-2010) temperature of 2.53℃ (month range -20.4 to 21.6℃), a mean annual precipitation of 566 mm (approximately 60% fall in July and August) with growing season duration of 125 days per year. During the dry season marsh fires occur regularly. The soil type in the study site is meadow marsh soil and the soil properties are as below: the concentration of total organic C (TOC) and total N (TN) is 23.60 and 2.18 mg g^{-1}, respectively. Soil bulk density is 0.88 g cm^{-3}, and pH (H_2O) is 5.58.

The study sites are dominated by freshwater marsh with shallow and intermittent water levels, varying from no standing water to an average depth of approximately 12 cm. The flora mainly consists of *Calamagrostis angustifolia* Kom., with less dominant, but common species such as *Glyceria spiculosa* (Fr. Schmidt.) Rosh., *Phragmites australis* (Clav.) Trin., *Eleocharis ovata* (Roth.) Röem. et Schult., *Menyanthes trifoliate* L. and *Carex humida* Y.L.Chang.et Y.L.Yang.. We chose one 50 m×50 m area of *C. angustifolia* community as the sampling site to do soil samplings.

Seed bank collection. Sampling was conducted right after snow melt (6–8 May, 2012) to assess the seed bank present at the time of spring germination including both transient and persistent types of seeds. Note that transient seed banks are from seed produced in the previous year, which do not live until a second growing season. Persistent seed banks are comprised of seeds one or more year old [21, 22]. Soil samples from 20 replicate plots (25 cm×25 cm×5 cm) in natural site were taken and placed into soil bags. All samples were taken back to the greenhouse. In the laboratory, each soil sample was sieved to remove stones and plant fragments, and mixed thoroughly.

Seedling germination assays. Seed banks from the natural site were studied with one treatment factor: N addition. Five levels of N addition: 0, 5, 10, 20 and 40 g/m^2 were used. Nine replicates were used for each level of N addition, resulting in a total of 45 replicates.

The seedling germination assays were conducted in the greenhouse during 9-10 May, 2012. The greenhouse had a glass roof that did not significantly attenuate of disrupt visible or near-infrared radiation. It was also well ventilated to maintain an inside temperature comparable to that of the outside. Monthly air temperature in the greenhouse during the study period ranged from 16.4℃ in May to 23.8℃ in July. Soil samples were divided into 45 equal parts. And then each part was spread as an even layer, 2 cm thick, in experimental pots (14 cm diameter and 11 cm depth) previously filled with washed vermiculite to an 8 cm depth, a procedure similar to that described by van der Valk and Rosburg [23] and Middleton [24]. All the pots were placed in a big tank randomly assigned to non-flooded (moist soil) water treatment. The tank was filled with distilled (DI) water from the bottom to keep the non-flooded water treatment, and water depths were maintained throughout the experiment with DI water. The total amount of N added was based on the amount applied in a year at a rate of 5, 10, 20 and 40 g m^{-2} $year^{-1}$, Nitrogen was added a total of six times in 20 day' intervals by injecting into the soil 10 mL of 0.25, 0.5, 1, 2 g N L^{-1} solution at five points in each pot in each treatment respectively. Nitrogen was added as ammonium nitrate (NH_4NO_3). Treatments without N were injected with DI water to control for the mechanical disturbance caused by injection. Pots were rotated weekly within the tank. Newly emerged seedlings were identified and counted each week. The seedling germination assays continued until no additional seedlings emerged. The germination assay lasted approximately 3 months. Nomenclature follows Yi et al. [25].

The height of each species in each pot was measured and then the above-ground biomass in each pot was sampled at the end of germination assay. Plant material was clipped above the soil surface of each pot, and was oven-dried at 80 °C until constant weight.

Data analysis. A one-way ANOVA and a subsequent Tukey's test were used to test the difference in mean number of species, the mean seedling density, number of seedlings of each species and the above-ground biomass among the N additions. Significance was determined at an alpha level of 0.05. Data of species richness, seedling density and biomass were transformed (log(x+1)) to satisfy the assumption of homogeneous variances. All statistics were conducted using SPSS version 16.0.

RESULTS

Effects of N addition on the species richness and seedling emergence. N addition significantly affected the number of species and seedlings germinated from seed banks (F=16.018, p<0.001; F=11.276, p<0.001). There was a weak increase in the number of species when the N addition increased from 0 to 10 g/m^2, and it decreased significantly when the N addition increased from 10

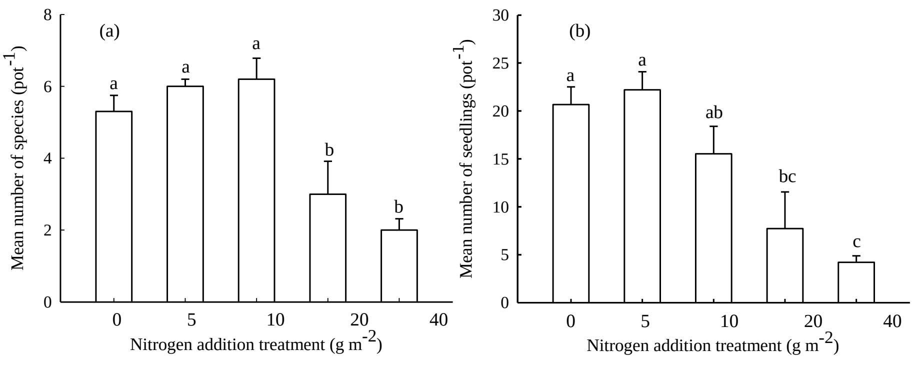

FIGURE 1

(a) Effects of N additions on the mean number of species emerging in the seed banks of the *Calamagrostis angustifolia* freshwater marsh in Sanjiang Plain. Values are means＋SE. Values followed by the same superscripted letter are not significantly different (Tukey's test; *p*>0.05; n=9 pots per treatment). (b) Effects of N additions on the mean number of seedlings emerging in the seed banks of the *Calamagrostis angustifolia* freshwater marsh in Sanjiang Plain. Values are means＋SE. Values followed by the same superscripted letter are not significantly different (Tukey's test; *p*>0.05; n=9 pots per treatment).

TABLE 1

Mean number of seedlings per pot in each N addition treatment in the *Calamagrostis angustifolia* community in Sanjiang Plain. Species are listed in order of decreasing abundance in the 0 g/m^2 of N addition treatment. Values followed by the same superscripted letter were not significantly different (Tukey's test; p>0.05; n=9 pots per treatment).

	Nitrogen input (g/m^2)				
Species	0	5	10	20	40
Eleocharis ovata	7.22^a	8.89^a	3.44^b	2.0^c	1.67^c
Calamagrostis	3.78^a	3.44^a	3.67^a	1.67^b	0.44^c
Juncus effusus	1.89^a	3^a	2.44^a	0.22^b	0^b
Poa palustris	1.56^a	2.44^a	2.22^a	1.22^b	0^b
Typha latifolia	1.11^a	0.89^a	0.89^a	0.22^b	0^b
Stachys	0.89^a	0.33^a	0.56^a	0.84^a	0.33^a
Glyceria spiculosa	0.78^a	0.67^a	0.44^a	0.78^a	0.44^a
Echinochloa	0.67^a	0.22^a	0.22^a	0^a	0^a
Sagittaria trifolia	0.67	0.67^a	0^a	0.33^a	0.44^a
Lysimachia	0.56^a	0.67^a	0.22^a	0^a	0^a
Carex humida	0.33^a	0.22^a	0.22^a	0.11^a	0.22^a
Galium trifidum	0.33	0^a	0^a	0^a	0^a
Polygonum	0.22	0^a	0.22	0^a	0.44^a
Bidens bipinnata	0.22	0^a	0^a	0^a	0^a
Sium suave	0.11	0 .11^a	0^a	0^a	0 .11^a
Gnaphalium	0.11	0.22^a	0.44	0^a	0^a
Lycopus lucidus	0.11	0 .22^a	0^a	0 .11^a	0^a
Phragmites	0.11	0.44^a	0^a	0^a	0^a
Spiraea salicifolia	0^a	0^a	0.11	0^a	0^a
Saussurea	0^a	0.11^a	0.22	0.33^a	0.22^a
Rorippa palustris	0^a	0^a	0.22	0^a	0^a

to 40 g/m^2, the number of species was least in 40 g/m^2 of N addition (Fig. 1a).

There was a weak increase in the number of seedlings when the N addition increased from 0 to 5 g/m^2, and then it decreased significantly with increasing N addition. The number of seedlings in 20 and 40 g/m^2 was significantly lower than 0 g/m^2 of N addition, and it was least in 40 g/m^2 of N addition (Fig. 1b).

A total of 21 species germinated from the seed banks. Species responded differently to the addition of nutrient. The number of seedlings from *Eleocharis ovata* was high at 0-5 g/m^2 of N addition, and it decreased significantly at 10 g/m^2 of N addition, and it was lowest at 20-40 g/m^2 of N addition (Table 1). The number of seedlings from *Calamagrostis angustifolia*, *Juncus effuses*, *Poa palustris*, *Typha latifolia* was much lower at 20-40 g/m^2 of N addition than at 0-10 g/m^2 of N addition (Table 1). There was no significant difference among different N addition treatments for other species, as the numbers of seedling emergence were extremely low.

Effects of N addition on seedling biomass. N addition significantly affected the seedling biomass. It showed similar response to the N addition with the seedling density (Fig. 2). The seedling biomass was highest as 5 g/m^2 of N addition, and decreased significantly when the N addition reached 10 g/m^2 or greater (Fig. 2). The seedling biomass was different among different N addition mainly because that the individual seedling biomass of three dominant species *Eleocharis ovate*, *Calamagrostis angustifolia*, *Juncus effuses* responded to N addition. For *Eleocharis ovate*, higher seedling biomass was found at low N addition, and it decreased significantly when the nutrient addition increased to 10 g/m^2 or more. For *Calamagrostis angustifolia* and *Juncus effuses,* seedling biomasses were much lower at 20-40 g/m^2 of N addition than at 0-10 g/m^2 of N addition (Fig. 2).

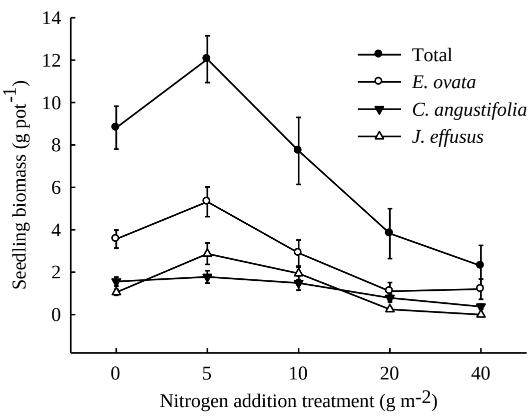

FIGURE 2
Effects of N additions on seedling biomass of the total species and three dominant species (*Eleocharis ovate, Calamagrostis angustifolia, Juncus effuses*) of the *Calamagrostis angustifolia* freshwater marsh in Sanjiang Plain. Values are means±SE. (n=9 pots per treatment).

DISCUSSION

Boreal mire ecosystems are generally nutrient limited, and extra nitrogen could influence the community composition and plant production [13, 19]. Over the past five decades, the natural wetlands in Sanjiang Plain have been extensively reclaimed for agriculture with a total loss of nearly 80% of the surface area [26]. More and more marshes are being drained for conversion to agricultural production, whereas the still un-drained marshes often receive some leaching nitrogen during the agricultural activities (the total about 5.8 g N m^{-2}) or atmospheric deposition (wet deposition is about 0.8 g N m^{-2} yr^{-1}) and other inputs [19, 20]. This study tested seed germination and seedling responses to different N addition treatments in the freshwater marshes.

Nutrient addition significantly reduced the number of seedlings and species germinating from the seed banks, and the response is species-specific [15, 16, 27, 28]. In our study, Low level of nitrogen addition (less than 10 g/m^2) had no significant effects on the species richness and seedling density. But high level of nutrient addition (20-40 g/m^2) significantly decreased both the species richness and seedling density. High level of N additions suppressed the germination of the dominant species *Eleocharis ovate*, *Calamagrostis angustifolia*, *Juncus effuses*, *Poa palustris*, *Typha latifolia* from the seed bank, while it seemed to have no significant effect on other wetland species. Our findings are consistent with other studies. In other examples of nutrient enrichment effects on germination, N additions suppressed germination of *Nymphaea odorata* from Okfenokee seed banks [15]. Both Tilman [27] and Foster and Gross [28] have found negative correlations between N availability and seed germination. Nutrient addition reduced species richness by effectively preventing the seedling establishment and reduced the recruitment of *Andropogon gerardi* by inhibiting both germination and survival [28]. In freshwater herbaceous wetlands in Northern Belize, Nutrient addition reduced the number of seedlings of *Eleocharis Mimutissima* and *Chara* sp. from the seed bank [16]. But other study found that N addition did not have significant effects on the species richness and seedling density of wetland species from seed banks [29]. So nitrogen addition decreased the species richness and seedling density from the seed bank in our study, and level of nutrient addition may lead to different results.

Nitrogen is commonly a limited nutrient in wetland ecosystems, extra nitrogen is often very rapidly taken up by the plant, so it directly affects the productivity of the wetland [13]. Zhang et al. [19] found that nitrogen addition significantly affected the growth of *C. angustifoila* in the freshwater marshes. The above-ground biomass and plant height are both significantly enhanced after low level of nitrogen addition. Other studies also found that Nitrogen appears to limit marsh plant growth and additions of nitrogen fertilizer increased plant production [14, 15, 28]. In our study, low level of N addition (5 g/m^2) increased the above-ground biomass by enhancing both the seed germination and seedling growth, especially for the *Eleocharis ovata* and *Juncus effuses*, low level of N addition increased the seedling density, seedling height (data not showed) and individual seedling mass, and thus the above-ground biomass were higher than other nutrient conditions. Rejmánková et al. [29] also found that *Eleocharis* sp. showed a similar response, low level of nutrient addition increased stem density and height and thus the overall above-ground biomass was much higher. High level of N addition (20-40 g/m^2), however, significantly decreased the above-ground biomass in our study. This is mainly because high level of nutrient addition suppressed seed germination and seedling growth, so the species richness and seedling density were lower than at low level of nutrient addition, which led to the lower above-ground biomass. Liu et al. [30] also found that high level of nutrient input significantly decreased the number of seedlings of some wetland species, and thus decreased the above-ground biomass in marshes in Xingkai Lake, China.

CONCLUSION

Our study revealed the negative impacts on seed germination, seedling growth and biomass by high level of N additions while low level of N addition had slight positive effects on the seed bank germination in Sanjiang Plain, Northeast China. And the responses to the nutrient additions were species-specific. Knowledge gained from this and similar studies will provide important insights into fertilizer use, irrigation and land management that should be paid more attention to sustain wetlands located in the agricultural lands.

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CORRESPONDING AUTHOR

Guodong Wang,
Key Laboratory of Wetland Ecology and Environment, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun, 130102, China.

Email: wanggd@neigae.ac.cn