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Effect of Soil Transplantation to Abandoned Paddy Field on the Conservation of Threatened Hydrophyte Species

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Abstract

Threatened or near threatened hydrophytes, *Ottelia alismoides*, *Monochoria korsakowii*, *Najas graminea*, *Najas minor* and *Chara braunii*, appeared in an inundated paddy field after the 2011 Tohoku-oki Tsunami in Japan. Due to the reconstruction of roads and agricultural restoration efforts implemented following the disaster, the top soil of the paddy field was transplanted to another abandoned paddy field in 2014 to avoid extirpation of the aforementioned species. We then conducted vegetation surveys in July and September from 2014 to 2016. *Monochoria korsakowii* appeared at the transplantation site from 2014 to 2016, forming a large community in 2016. The volume of this species was significantly higher than that in July 2014 and 2015. Although *Ottelia alismoides* and *Chara braunii* appeared in 2014, they were not observed in 2015. *Najas graminea* and *Najas minor* were not observed during the vegetation survey, and *Salvinia natans* and *Alisma plantago-aquatica* newly appeared at the transplantation site. Our findings suggest that transplantation of surface soil and the seed bank therein to an abandoned paddy field is well suited for the conservation of hygrophytes such as *Monochoria korsakowii*, *Ottelia alismoides* and *Chara braunii*. Preventing disturbances that suppress the growth of herbaceous perennial plants is considered necessary for maintaining the habitats of threatened plant species.

Keywords

Threatened Hydrophytes, Donor Soil, Soil Disturbance, Abandoned Paddy Field, Tsunami

1. Introduction

On 11 March 2011, a devastating earthquake and tsunami struck the northern

Pacific coast of Japan, devastating the coastal areas of Iwate, Miyagi and Fukushima prefectures. Although the tsunamis have long-lasting impacts on vegetation [1], several threatened plant species appeared in many of the areas that were inundated by the 2011 Tohoku-oki Tsunami in Miyagi Prefecture, Japan [2] [3] [4]. For example, threatened or near threatened hygrophyte species, such as *Ottelia alismoides*, *Monochoria korsakowii*, *Najas graminea*, *Najas minor* and *Chara braunii* appeared along coastal areas of Miyagi Prefecture in August 2013 [5]. Of these species, *Monochoria korsakowii* was widely distributed in swampy paddy fields from Iwate to Miyagi prefectures after the tsunami [6] [7] [8]. This increase in the distribution of these species was promoted by ground subsidence, which inundated paddy fields, and subsequent disturbance of surface soils containing buried seeds, which enhanced the germination of hygrophytes and promoted the establishment of hygrophyte communities after the tsunami. Many of these hygrophyte species are classified as near threatened or vulnerable in the Red Data Book of Plants published by the Ministry of Environment, Japan [9]. However, the areas in which these hygrophytes became newly established were redeveloped when the roads and paddy fields in the affected areas were repaired and reclaimed, respectively. Soil including buried seeds contribute greatly to vegetation restoration [10] [11]. Transplantation of surface soil containing seeds to wetlands is considered to be effective for the conservation and reestablishment of hygrophyte communities [12] [13]. We therefore transplanted surface soil from these hygrophyte communities to an abandoned paddy field to conserve these threatened and near threatened plant species. Although previous studies have investigated plant succession in original habitat after tsunami [1] [14] [15], plant succession in abandoned paddy fields areas using soil transplanted from original habitat has not yet been clarified. This study therefore provides information on how threatened and near threatened species conservation can be effectively combined with using abandoned paddy fields following a natural disaster, like a tsunami.

We hypothesized that the transplantation of donor soil from original habitat to an abandoned paddy field can be effective for the conservation of threatened or near threatened hygrophyte species. The present study aims to investigate whether threatened or near threatened species emerge and grow in abandoned paddy fields, and whether methods could be developed for managing abandoned paddy fields to conserve hygrophyte species.

2. Materials and Methods

2.1. Study Area and Transplantation of Soil to Abandoned Paddy Field

The original hygrophyte community was located in Hadenya, Minamisanriku-machi, in Miyagi Prefecture, Japan (38˚38'37.9"N, 141˚28'10.5"E). Hygrophyte communities became established at three sites, each of which was dominated by *Monochoria korsakowii* (patch size approx. 3.5 × 6.5 m), *Ottelia alis-*

moides (5.5 × 3.5 m) and *Bolboschoenus maritimus* (8.5 × 6.5 m). We obtained the donor soil from these three locations, hereafter referred to as the donor soil site. Each community included threatened or near threatened plant species, such as *Monochoria korsakowii* and *Najas minor* in the *Monochoria korsakowii* community, and *Bolboschoenus maritimus*, *Ottelia alismoides*, *Najas minor*, *Najas graminea* and *Chara braunii* in the *Ottelia alismoides* community. The surface soil at each of the three communities was transported to an abandoned paddy field in Terahama, Minamisanriku-machi, hereafter referred to as the soil transplant site (38˚37'59.9''N, 141˚31'04.6''E) (**Figure 1**). The area of the abandoned paddy field was 18 m × 15 m and *Phragmites australis* and *Typha* spp. were the dominant species at the site. The abandoned paddy field was located in a valley bottom and was continuously inundated by spring water originating from a stand of secondary forest that surrounded the site before the earthquake.

Transplantation of donor soil was conducted on April 9 and 26, 2014. We collected the surface soil into piles using shovels at the donor soil site. Approximately 1.1 m^3 (3.5 × 6 × 0.05 m), 1.0 m^3 (5.5 × 3.5 × 0.05 m) and 1.1 m^3 (6.5 × 3.5 × 0.05 m) of surface soil was collected from the *Monochoria korsakowii*, *Ottelia alismoides* and *Bolboschoenus maritimus* communities, respectively. This donor soil was stored in a plastic container containing water. After collecting the soil, we removed the plant roots and pebbles and spread the soil over an area of 15 × 9 m at the soil transplant site.

2.2. Paddy Field Maintenance

Suppressing the proliferation of other perennial plants, which were not included in the original transplanted plant community, is important for hydrophyte restoration [16] [17]. We therefore regularly removed dominant species such as *Phragmites australis*, *Typha* spp. and *Bidens frondosa* to facilitate the recovery of threatened and near-threatened hygrophytes during 2014-2016. The time required for two people to weed these plants was 6 hours in 2014 and 2015. In addition, topsoil disturbance (5 cm depth) was performed by humans on 9 and 26 April in 2014 and 20 April in 2015. Nonetheless, tall herbaceous perennial plants, such as *Phragmites australis* and *Typha* spp. increased in paddy fields. Thus, the soil surface (20 cm depth) was tilled using an agricultural tractor to suppress the growth of tall, perennial, herbaceous plants on 27 and 28 April in 2016. Water was supplied regularly by the landowner of the abandoned paddy field to maintain a water depth of 5 cm from April to September. The water source for irrigating the paddy field was derived from a small reservoir.

2.3. Vegetation Surveys

Twelve plots (3 m× 3 m) of vegetation were surveyed at the soil transplant site in 2014 and 2015. We spread the donor soil over nine plots and three plots were used as controls to identify the plants that emerged naturally from the abandoned paddy field. Vegetation surveys were conducted using a 4 m^2 sampling

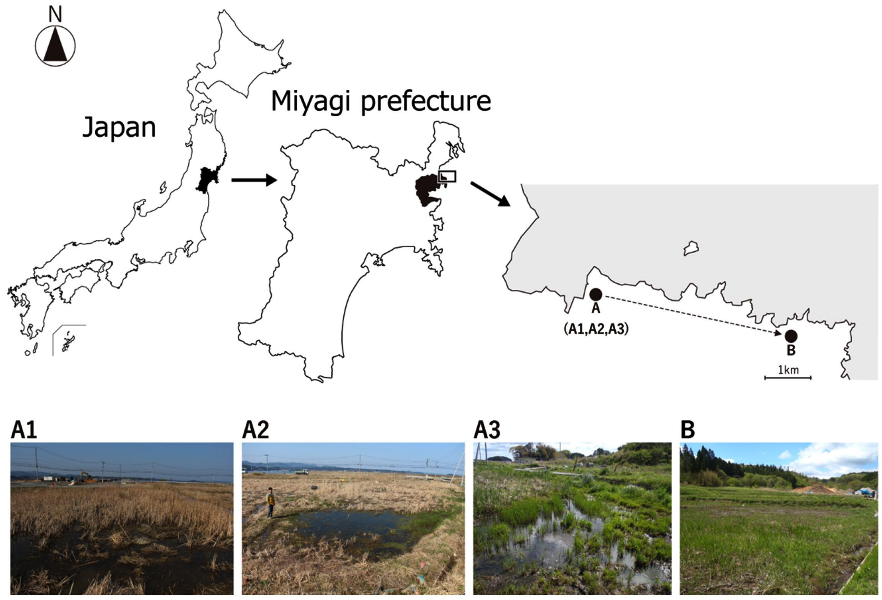

Figure 1. Threatened or near threatened plants at the donor soil site A (Hadenya) and the abandoned paddy field or soil transplant site B (Terahana). A1, *Monochoria korsakowii* (March 23, 2014); A2, *Ottelia alismoides* (March 23, 2014); A3, *Bolboschoenus maritimus* (March 23, 2014); B, soil transplant site before transplantation (April 9, 2014).

quadrat (1 m × 1 m × four places) in the center of each of the twelve plots. The species composition of the vegetation in each plot was surveyed using the method of [18]. The degree of each plant cover in a quadrate and plant height was measured in July and September of 2014-2015. The plant volume of each species was defined as the product of average cover degree and height. The index values for each cover degree class, "+" (<1%), "1" (1% - 5%), "2" (5% - 25%), "3" (25% - 50%), "4" (50% - 75%), and "5" (75% - 100%), were treated as 0.1%, 2.5%, 15%, 37.5%, 62.5%, and 87.5%, respectively [19].

The surface soil at the soil transplant site was tilled vigorously using an agricultural tractor on April 2016. The tilling was conducted on the entire abandoned paddy field, including the soil transplant site. In this way, twenty plots were established covering the whole soil transplant area in 2016. The vegetation surveys were conducted in the same way as in the previous two year.

Water temperature was recorded using a thermo-logger (Tidbit, Onset Computer Corp., Bourne, MA) at the center of each survey plot. The water temperature at the soil transplant site was measured from May to September during 2014-2016. The measurement of water temperature in the donor soil site was conducted from May to July in 2014 because infrastructure reconstruction projects at the study site continued to August 2014. The water depth was measured with ruler in the center of each survey plot. In addition, water quality measurements of pH, EC and DO were recorded using a water quality meter (WQC-24, TOA, Japan) near the drainage outlet of the paddy field. The water depth and water quality measurements were performed during the vegetation survey.

2.4. Data Analysis

To compare differences between years, a one-way ANOVA was performed to compare the volume of threatened or near threatened species, medium and tall herbaceous plants such as *Phragmites australis*, *Typha* spp., *Bidens frondosa* and *Echinochloa* spp., followed by multiple comparison tests (Tukey HSD post hoc test). We verified statistically whether period and tillage affect the succession and plant volume of threatened or near-threatened plant species and medium and tall herbaceous plants. All statistical analyses were performed using the R software package [20].

3. Results

3.1. The Emergence of Threatened and Near Threatened Plant Species

Monochoria korsakowii, *Ottelia alismoides* and *Chara braunii* all emerged at the soil transplant site. However, *Najas graminea* and *Najas minor* were not observed at the soil transplant site or in the control plots during 2014-2016 (**Table 1**). Although *Monochoria korsakowii* and *Chara braunii* were observed in one of the control plots in 2014, we considered that these plants were derived from the donor soil. Although *Salvinia natans* and *Alisma plantago-aquatica* were not observed at the donor soil site, these species were observed at the soil transplant site.

3.2. Plant Volumes of Threatened, Near-Threatened and Tall Herbaceous Perennial Herbs

The volume of the *Monochoria korsakowii* in July 2016 was greater than that in 2014 and 2015 ($df = 2$, $F = 7.84$, $p = 0.013$). In addition, the volume of the other threatened herb species was small compared to *Monochoria korsakowii*. For example, the volumes of *Ottelia alismoides* in September 2014 and 2016 were 0.004 m^3 and 0.001 m^3, respectively. In *Chara braunii*, volumes in July 2014 and 2016 and in September 2014, were 0.01, 0.005 m^3 and 0.0007 m^3, respectively. In *Alisma plantago-aquatica*, volumes in July 2014, 2015 and 2016 were 0.004, 0.009 and 0.0009 m^3, respectively. Conversely, the volume of middle and tall herbaceous perennial herbs decreased markedly in 2016. In September 2016, the volume of *Phragmites australis* was significantly lower than that in September 2014 and 2015 ($df = 2$, $F = 6.5008$, $p = 0.003$). Similarly, the volume of *Typha* spp. in 2016 was significantly lower than that in July ($df = 2$, $F = 5.4562$, $p = 0.007$) and September ($df = 2$, $F = 5.3475$, $p = 0.008$) 2015. In 2016, the volume of *Bidens frondosa* was significantly lower than that in July 2014 and 2015 ($df = 2$, $F = 33.971$, $p = 0.0001$), and September 2014 ($df = 2$, $F = 3.3443$, $p = 0.045$). However, the volume of *Echinochloa spp.* increased significantly in July and September in 2016 compared to that in July ($df = 2$, $F = 15.886$, $p = 0.0001$) and September ($df = 2$, $F = 7.61$, $p = 0.0015$) in 2014 and 2015 (**Figure 2**).

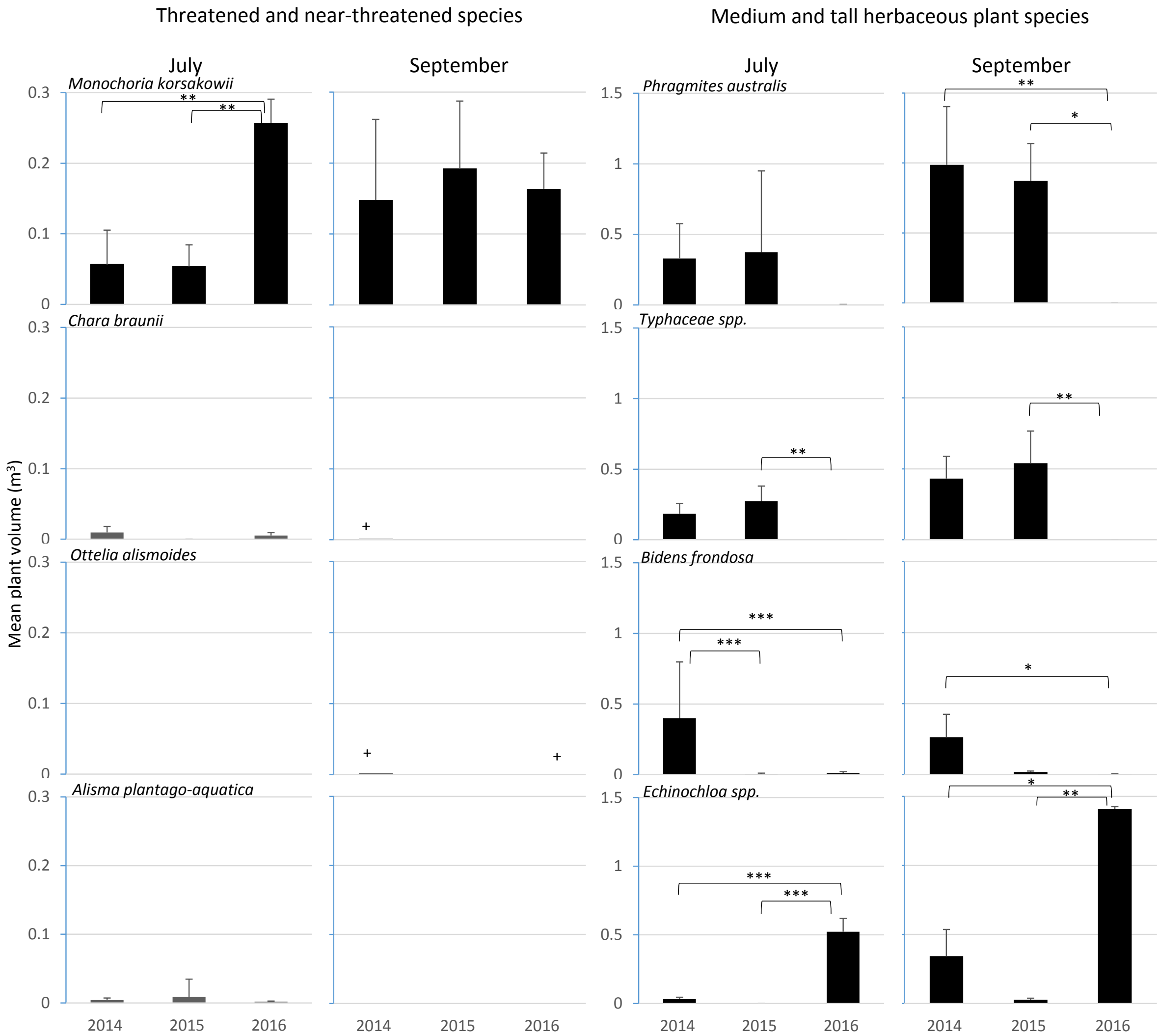

Figure 2. Plant volume of threatened or near threatened species and tall herbaceous plants. Vertical bars indicate standard error. Different characteristics indicate the significant difference by Tukey-HSD.

Table 1. Emergence of threatened or near threatened plant species at the soil transplant site.

Species	Year 2014 Transplant plot	2014 Control plot	2015 Transplant plot	2015 Control plot	2016	RDB of Japan	RDB of Miyagi Prefecture
Monochoria korsakowii	Observed	Not observed	Observed	Observed	Observed	NT	
Ottelia alismoides	Observed	Not observed	Not observed	Not observed	Observed	VU	NT
Chara braunii	Observed	Not observed	Not observed	Not observed	Observed	VU	
Najas graminea	Not observed	Not observed	Not observed	Not observed	Not observed		VU
Najas minor	Not observed	Not observed	Not observed	Not observed	Not observed	VU	CR+EN
Salvinia natans	Not observed	Not observed	Observed	Not observed	Not observed	VU	
Alisma plantago-aquatica	Observed	Not observed	Observed	Not observed	Observed		NT

VU, vulnerable; NT, near threatened; CR + EN, critically endangered or endangered.

3.3. Water Depth and Water Quality at the STUDY Site

The water depth in July 2016 (3.08 ± 0.32) was significantly higher than that in July 2014 (1.3 ± 0.2) and July 2015 (1.1 ± 0.3) ($df = 2$, $F = 11.58$, $p = 0.0001$). The water depth in September 2015 (4.0 ± 0.2) and September 2016 (3.7 ± 1.2) was significantly higher than that in September 2014 (1.5 ± 0.2) ($df = 2$, $F = 11.13$, $p = 0.0001$) (**Figure 3**).

No significant difference in water temperature between the donor soil site and the soil transplant site was observed in May and June (**Figure 4**). However, the water temperature at the soil transplant site in July during 2014-2016 was higher than that at the donor soil site in 2014 ($df = 3$, $F = 11.12$, $p = 0.0001$). No significant difference was observed in water temperatures in August for the period 2014-2016. However, the water temperature in September 2014 was higher than that in 2016 ($df = 2$, $F = 10.314$, $p = 0.0001$).

The pH, EC and DO measured at the transplantation site between July and September changed from 7.02 ± 0.2 to 7.61 ± 0.64, 15.08 ± 1.4 to 7.78 ± 0.64 mS/m, and 5.7 ± 0.9 to 7.8 ± 0.4 (mg/L), respectively, during 2014-2016. EC decreased significantly between July and September in all years (Welch test, $p = 0.018$).

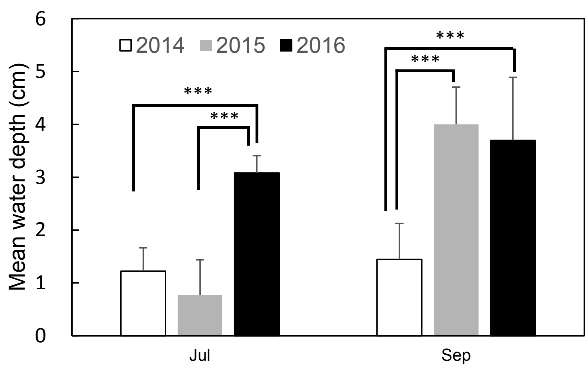

Figure 3. Mean water depth at the transplantation site. Vertical bars indicate standard error. Significant Significance was determined by Tukey-HSD (***: $p < 0.001$).

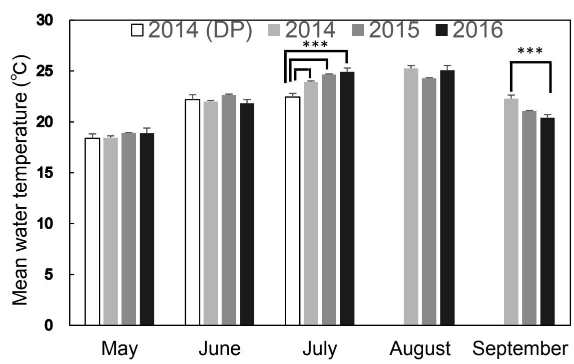

Figure 4. Mean water temperature at the transplantation site. DP indicates the water temperature at the donor soil site. Vertical bars indicate standard error. Significance was determined by Tukey-HSD (***: $p < 0.001$).

4. Discussion

Our results showed that the recovery of species such *Ottelia alismoides*, *Monochoria korsakowii* and *Chara braunii* can be achieved by transplanting donor soil to an abandoned paddy field. In addition, other threatened herb species, such as *Salvinia natans* and *Alisma plantago-aquatica* also appeared in the abandoned paddy field. Intense physical disturbance of sediments, such as by tilling with an agricultural tractor, appears to play a role in determining the emergence and growth of *Ottelia alismoides*, *Monochoria korsakowii* and *Chara braunii* because initial germination and subsequent growth of annual herbs are the primary driving forces underlying vegetation recovery. To promote the growth of annual herb species, site maintenance is required in order to suppress the germination and growth of herbaceous perennial herbs, such as *Phragmites australis* and *Typha* spp. *Monochoria korsakowii* is an emergent, summer annual, aquatic plant that occurs in pools, ditches, canals and rice fields in East Asia [21] [22]. Continuous growth in this species is promoted by tilling in original habitat [23] [24] [25] [26] [27]. For example, disturbance with bulldozers promotes germination and growth of *Monochoria korsakowii* and other threatened species in wetlands [28]. Our results suggest that intense disturbance caused by an agricultural tractor (depth: 20 cm) promoted the growth of *Monochoria korsakowi* in the abandoned paddy field. Consequently, the volume of *Monochoria korsakowii* was five-fold that observed in 2014 and 2015 (**Figure 2**). Conversely, perennial herb species, such as *Phragmites australis* and *Typha spp*. decreased significantly in 2016 compared to 2014 and 2015. These results are consistent with a previous study by [17], who reported that tilling in an abandoned paddy field promoted the growth of *Monochoria korsakowii* but suppressed the growth of *Phragmites australis* and *Typha spp*.

The water temperature at the soil transplant site was suitable for *Monochoria korsakowii* germination and growth. The germination rate of this species has been reported to increase at temperatures of 15˚C to 29˚C (Wan et al. 2004). In the present study, the water temperature at the soil transplant site from June to September was 20.4˚C to 25.1˚C (**Figure 4**). The reason for the water temperature being maintained at 20.4˚C - 25.1˚C might be due to the springs from the secondary forest surrounding the site and groundwater, as the site is in a valley bottom. The germination rate of *Monochoria korsakowii* has been shown to be 100% at water depths from 3 to 5 cm [24]. The water depth at the soil transplant site was approximately 1 cm in July 2015. Although the plant volume of *Monochoria korsakowii* was small at lower water depths, the population can expand when water depth exceeds 5 cm. *Ottelia alismoides* and *Chara braunii* were observed in 2014 and 2016, but not in 2015. Although the surface soil was disturbed to a depth of 5 cm by human activity in 2014 and 2015, *Phragmites australis* and *Typha spp*. flourished at the transplant site (**Figure 2**). The intensity of soil disturbance by plowing has been shown to be effective for the conservation of *Ottelia alismoides* in abandoned paddy fields [29] [30]. In addition, *Ottelia* *alismoides* needs sufficient light in order to germinate (May to July), which is why the growth of tall herbaceous plants needs to be managed [31].

Oospores of *Charales* are abundant in the soil of submerged plants area [32] [33] [34]. Transplantation of soil oospore banks into other areas has been shown to be effective for regenerating stands of the endangered *Chara braunii* [35]. In the present study, transplantation of donor soil was shown to be effective for the conservation of *Chara braunii* in abandoned paddy fields. *Charales plantas* colonizes shallow to deep water and needs abundant sunlight and clear water in order to thrive [36]. The reasons why *Chara braunii* was not widespread in 2015 may have been because the tall herbaceous plants obstructed the sunlight and covered the available soil surface, preventing the germination of *Chara braunii* oospores. Therefore, continuous soil disturbance by agricultural tractors is considered essential for the maintenance of *Ottelia alismoides* and *Chara braunii* populations.

[37] reported that the optimal water temperature for anthesis and growth in *Ottelia alismoides* was 20˚C - 27˚C in a lotus paddy field. The water temperature at our study site from August to September was 20.4˚C to 25.3˚C, which is suitable for anthesis and growth in *Ottelia alismoides*.

Seeds of *Alisma plantago-aquatica* germinate and grow at water depths below 3 cm [38]. At our study site, *Alisma plantago-aquatica* was observed to have emerged in July at a water depth of 1.08 cm in 2015, 1.33 cm in 2014 and 3.80 cm in 2016 (**Figure 3**). Thus, the water depth at the soil transplant site was considered to be suitable for the germination and growth of *Alisma plantago-aquatica*.

Najas graminea and *Najas minor* were not found at the soil transplant site during 2014-2016. [39] reported that *Najas graminea* is sensitive to some herbicides and is therefore found frequently in paddy fields free of such herbicides. The field used for transplantation had been abandoned for 3 years (*i.e.* since 2011), and the EC was low. Therefore, *Najas graminea* growth is not directly affected by water quality. Water depth at sites colonized by *Najas graminea* and *Najas minor* at the donor soil site were approximately 25 cm. It is thus possible that *Najas graminea* and *Najas minor* may prefer deeper water than the 5 cm at the study site.

5. Conclusions

Our results describe how transplanting a donor soil can be applied to the recovery of *Ottelia alismoides*, *Monochoria korsakowii* and *Chara braunii* communities in an abandoned paddy field. Maintenance to regulate tall herbaceous perennial plants, such as *Phragmites australis and Typha* spp. is necessary to promote the germination and growth of threatened annual plant species. In the present study, the maintenance work was performed by hand in 2014 and 2015, and a tractor in 2016. The intensity of the disturbance achieved by a machine is sufficient to suppress the growth of *Phragmites australis* and *Typha spp*. Sustained and intense disturbance by tilling has been shown to be effective for suppressing tall perennial plants and, in so doing, for conserving threatened or near threatened species. However, continuous and intense disturbance is also capable of suppressing efficiently *Echinochloa spp*. In the present study, *Echinochloa spp*. biomass also increased after intense disturbance in 2016. In future, maintaining intense disturbance using a tractor is considered desirable in April. In addition, weeding of *Echinochloa spp*. by hand is necessary to conserve threatened plant species.

The water temperature at the site remained at 20.4˚C to 25.1˚C due to the study site being located in a swampy paddy field. It is considered desirable to use swampy or continuously inundated abandoned paddy fields for the transplantation of donor soil. Furthermore, the depth of the water in field should be increased to 25 cm to encourage colonization (and transplantation) by *Najas graminea* and *Najas minor*. Long-term field monitoring is needed to elucidate the frequency, intensity and period of suitable disturbance and water depth that is required in order to maintain threatened or near threatened hygrophytes.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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