Short title: RPAS-based *Phragmites* control

Suppression efficacy of Remotely-Piloted Aircraft Systems-based herbicide application on invasive *Phragmites australis* in wetlands

Grace K. Lew-Kowal[1], Derek T. Robinson[2], Janice M. Gilbert[3], and Rebecca C. Rooney[4]

[1]Grace Lew-Kowal Graduate Student (ORCID: 0009-0005-8277-8741), Biology, University of Waterloo, Waterloo, ON, Canada

[2]Derek Robinson Associate Professor (ORCID: 0000-0002-4293-1095), Geography and Environmental Management, University of Waterloo, Waterloo, ON, Canada

[3]Janice Gilbert Wetland Ecologist, Executive Director, Invasive *Phragmites* Control Centre, Langton, ON, Canada

[4]Rebecca Rooney Associate Professor (ORCID: 0000-0002-3956-7210), Biology, University of Waterloo, Waterloo, ON, Canada

Author for correspondence: Email: rrooney@uwaterloo.ca

Abstract

Invasive common reed, *Phragmites australis* ssp. *australis* ((Cav.) Trin. ex Steud.) has established and dominated Ontario wetlands for decades. The detrimental effects of *P. australis* invasions on wetland habitats have demanded intervention through aggressive suppression efforts. However, constraints in available control methods to suppress *P. australis* have led to persistent invasions. To improve *P. australis* management in wetlands, we investigated remotely-piloted aircraft systems (RPASs) as a precision tool for herbicide application. We applied an imazapyr-based herbicide (240 g active ingredient L^{-1}) with a spray-equipped RPAS at selected test sites, marking the first-ever application of its kind in Canada. We evaluated 1) the efficacy of RPAS-based herbicide application to *P. australis* and 2) examined the plant community changes one year after the initial herbicide application. We found a $> 99\%$ reduction in live *P. australis* stems, along with reductions in species richness (33%), Shannon-Weiner diversity (73%), Simpson's reciprocal diversity (50%), and Pielou's evenness (73%) in the year following herbicide application. Plant community changes varied by field site; one wetland underwent a secondary invasion by European Frog-bit (*Hydrocharis morsus-ranae* (L.)) while the other was dominated by the native Spotted Jewelweed (*Impatiens capensis* (Meerb.)), highlighting the complexities of plant community succession following herbicide application in biodiverse wetlands.

Keywords: European common reed, herbicide management, imazapyr, invasive species, remotely-piloted aircraft systems, wetland restoration

Management Implications

Managing *Phragmites australis* invasions with herbicides in wetlands poses significant challenges due to the presence of closely surrounding native vegetation and constraints in treating patches that are physically challenging to access on foot or with ground spray equipment. This study highlights the potential of remotely-piloted aircraft systems (RPAS) as a precision tool for herbicide application, offering a solution for targeting invasive species in difficult to access areas with reduced off-target impacts. RPAS technology affords smaller swath widths compared to helicopter spraying, reducing potential for herbicide drift and collateral damage to native vegetation, which is especially valuable in ecologically sensitive wetlands. The > 99% reduction in live *P. australis* stems observed with RPAS-based herbicide application demonstrates its capacity to effectively suppress the target species with values as high or higher than any imazapyr-based *P. australis* suppression study reported in the literature. As the first study to employ RPAS-based herbicide application to *P. australis* in Canada, this research demonstrates the potential for improved precision in aerial herbicide applications to *P. australis* in wetlands. Future research should focus on long-term native vegetation recovery and quantify the accuracy of RPAS-based herbicide applications to minimize off-target damage to native vegetation in wetlands.

Introduction

Non-native common reed [*Phragmites australis* ssp. *australis* ((Cav.) Trin. ex Steud.)] has invaded an estimated 13,000 ha of Great Lakes coastal wetland in Ontario alone (Bourgeau-Chavez et al. 2013; Bourgeau-Chavez et al. 2015). Despite extensive wetland management efforts (e.g., mowing, burning, grazing, and flooding of *P. australis* stems, as well as others summarized by Hazelton et al. 2014 and Martin and Blossey 2013), long-term eradication of *P. australis* in many wetlands has not been achieved (Lombard et al. 2012; Martin and Blossey 2013). As *P. australis* can rapidly colonize when left uncontrolled and can re-colonize controlled areas when left unmanaged (Farnsworth and Meyerson 1999), efforts to suppress invasions have required the integration of nocuous approaches, such as the use of herbicides.

Broad-spectrum, systemic herbicides (e.g., glyphosate- and imazapyr-based herbicides) are the most widely used chemicals in *P. australis* control (Hazelton et al. 2014) and have achieved high *P. australis* suppression efficacy (> 90% reduction in live biomass) across a range of application times (vegetative, flowering, and seed filling stages; Knezevic et al. 2013). While glyphosate- and imazapyr-based herbicides (e.g., Roundup® and Arsenal®) are the top herbicide choices for wetland managers, there are differences in their efficacy on *P. australis* (Kay 1995; Back and Holomuzki 2008; Derr 2008; Mozdzer et al. 2008). For example, using a pressurized backpack sprayer in 1 m^2 plots, imazapyr was 16% more effective (at reducing stem density, canopy height, and percent cover of *P. australis*) than glyphosate after one and two years of follow-up monitoring (Mozdzer et al. 2008). Similarly, a reduction in *P. australis* stem density was 11% greater using an imazapyr-based herbicide when treated in June, and 8% greater when treated in September, compared to a glyphosate-based herbicide in field trials using spray-to-wet application with a pressurized backpack sprayer (Derr 2008). Using a wipe-on application method, imazapyr reduced live stems by 75% compared to 33% with the same concentration of glyphosate (Kay 1995).

Although imazapyr may be more effective, it is a more costly product and it has greater persistence in soils than glyphosate (Breckels and Kilgour 2018). In Canada, the differences in efficacy and persistence between imazapyr and glyphosate are moot where *P. australis* grows in standing water, as only imazapyr is approved for use over open water on invasive *P. australis* in the formula Habitat® Aqua (240 g ai L^{-1}, BASF Canada Inc., 5025 Creekbank Road,

Mississauga, Ontario, Canada L4W 5R2, Pesticide Registration Number 32374; Proposed Registration Decision PRD2020-17). While differences in efficacy between glyphosate- and imazapyr-based herbicides have been reported, variations in efficacy based on application method are less studied but provide important context to support wetland management goals (Lombard et al. 2012).

Herbicide use to suppress *P. australis* presents considerable challenges in conservation efforts, particularly in wetland environments, where physical access can be severely limited or extensive coverage is required. For example, herbicide backpack spraying can be highly precise, but this labor-intensive approach is not always feasible when *P. australis* invasions are difficult to access and extensive. Similarly, heavy ground-application equipment may be damaging to wetland herptiles. Overcoming the spatial constraints and accessibility issues of in-person manual application, helicopter-based herbicide application can be used (e.g., MNRF 2017); however, the boom and swath widths used can result in collateral damage to surrounding native plants (Hogg 2018, unpublished data). Currently, the Canadian Pest Management and Regulatory Agency has not approved an herbicide application method that makes it feasible to treat inaccessible, expansive invasions of *P. australis* without extensive off-target effects, rendering long-term suppression of *P. australis* in many wetlands challenging.

Remotely-piloted aircraft systems (RPASs) have emerged as a promising tool for pesticide application in the precision agriculture sector, showcasing significant advancements in crop management practices. Studies of RPAS-based pesticide applications have displayed higher efficacy of harvest-aid in cotton plants (characterized by defoliation rate, boll opening rate, and final yield) than ground-based applications (Cavalaris et al. 2022) and of insecticides on alfalfa (characterized by control of pests) than fixed-wing, pilot-on-board aerial applications (Li et al. 2021). However, existing literature predominantly focuses on RPAS use in agricultural fields where crops present a near uniform height, density, and plant morphology. Little information is available on how this technology performs in natural settings such as wetlands (but see Takekawa et al. 2023), where the spatial distribution of the target species resides in a complex mosaic comprising vegetation of different heights (e.g., trees, grasses), native species, and open water. It is unclear whether RPAS-based herbicide application would provide adequate plant coverage and canopy penetration to suppress *P. australis* in a field setting. Further, it is not well-

understood to what extent off-target effects on native plants growing interspersed within *P. australis* or immediately adjacent to it will occur with RPAS application.

Achieving high herbicide efficacy is crucial to minimize regrowth of *P. australis* in the following growing seasons. Sub-lethal dosing of herbicide during treatment not only risks re-establishment of *P. australis* networks (Elsey-Quirk and Leck 2021) but may also lead to herbicide resistance (Government of Ontario 2001), further increasing the difficulty of long-term suppression efforts. Even when herbicide treatments achieve high levels of suppression (>90% reduction in live biomass) in the initial treatment, follow-up treatments are typically required (e.g., Kettenring and Adams 2011; Lombard et al. 2012; Knezevic et al. 2013; Quirion et al. 2018; Zimmerman et al. 2018). Follow-up treatments are often complex as surviving *P. australis* patches need to be targeted. However, as native vegetation grows back, broadly re-treating the original *P. australis* area can lead to unnecessary vegetation damage (Figure 1). Since the cumulative effects of repeated herbicide applications on wetlands are not well-understood (Crowe et al. 2011), evaluating herbicide and application-method efficacy is valuable to minimize the number of reapplications and the amount of herbicide applied. For example, glyphosate has been shown to accumulate in soils (Robichaud and Rooney 2021a), biofilm (Beecraft and Rooney 2021), litter (Sesin et al. 2022), and groundwater (Crowe et al. 2011). The potential of imazapyr-based herbicides to accumulate in the environment is not as well-studied, but cumulative effects from repeated applications present an equivalent risk (Breckels and Kilgour 2018).

To assess the *P. australis* suppression efficacy of RPAS-based herbicide application in provincially significant wetlands in southern Ontario, we conducted the first herbicide application of this kind to control invasive *P. australis* in Canada. Our objectives were: 1) to evaluate the suppression efficacy of RPAS-based herbicide application, quantified as the difference in live *P. australis* stem density and canopy height between treated and adjoining untreated *P. australis* patches. 2) to characterize the extent of native plant community succession one year after herbicide treatment, comparing native plant diversity and community composition in the treated and untreated *P. australis* patches.

Materials and Methods

Study area

Baie du Doré and Rondeau Provincial Park wetlands (Figure 2) are listed as Provincially Significant Coastal Wetlands with ecological values that are designated as Areas of Natural and Scientific Interest (ANSIs) and Environmentally Sensitive Policy Areas (EPSAs). ANSIs are regions that have significant features containing value worthy of protection, scientific study, or education (Government of Ontario 2014). ESPAs are designated to protect regions with rare species and valuable ecological functions from nearby development (ECCC 2007).

Baie du Doré (44.34ºN, 81.54ºW) is a coastal fen situated on the eastern shore of Lake Huron, adjacent to the Bruce Power nuclear power plant owned by Ontario Power Generation. This wetland supports significant biodiversity (Ball et al. 2003). The *P. australis* invasion at Baie du Doré is characterized by extremely high stem density (>70 live stems m^{-2}) distributed around at least 15 ha of the fen's coastline. While management of *P. australis* along Lake Huron's shoreline was proposed in 2013 by the Municipality of Kincardine (Gilbert and Alexander 2013), Baie du Doré warrants special consideration for RPAS-based herbicide application due to the presence of at-risk turtles (Ball et al. 2003). In this wetland, at-risk turtles could be injured or killed by heavy machinery or boats and have their habitat degraded.

The Rondeau Provincial Park wetland (42.26ºN, 81.86ºW) is part of an extensive swamp-marsh complex located at the southern end of Rondeau Provincial Park in Morpeth, Ontario. The wetland complex supports provincially rare vegetation and serves as critical habitat for migratory marsh birds and a diverse array of species at risk (MNRF 2015). Additionally, it is a popular tourism destination for outdoor recreation, adding considerable economic value to the region (MNRF 2015). The specific study area within Rondeau Provincial Park targeted for testing RPAS-based herbicide application is a small swamp slough (~50 ha), surrounded by old growth Carolinian forest. These trees prevent access by amphibious vehicles and the many downed trees and shrubs impede access by backpack sprayers. Furthermore, the narrow width (~50 m) of the slough and the risk of drift and overspray affecting the adjacent trees makes it unsuitable for treatment by large aircraft such as helicopters or planes.

Field methods

In August 2022, we conducted baseline vegetation surveys at Baie du Doré and Rondeau Provincial Park wetlands prior to the herbicide application. These surveys aimed to assess the initial state of the *P. australis* invasions and determine overall species composition within the

sites. We divided each site into control and treatment areas in a manner optimizing the comparability of control and treatment areas given the specific distribution of *P. australis* in each wetland. At Baie du Doré, we selected two *P. australis* patches of comparable size (~0.03 ha) and live stem density (~75 live stems m^{-2}) at similar water depths for surveying because the *P. australis* was distributed in smaller patches interspersing the open water (Figure 3). At Rondeau Provincial Park, where the *P. australis* grew in a swamp-marsh complex, we targeted a ~4 ha linear slough where *P. australis* live stem density was ~35 live stems m^{-2}. We divided the slough medially from north-east to south-west (Figure 4). Which area was designated for treatment and which for control was decided randomly.

The summer before herbicide application, we completed quadrat surveys (1-m^2) (N=16 at Baie du Doré, Figure 3; n=20 at Rondeau Park, Figure 4) within the selected control and treatment areas. Plots were deployed in a semi-random manner with half established in the control areas and the other half in the treatment areas, such that they were paired by water depth to ensure that control and treatment plots spanned a similar range of water depths (0-43 cm) and plots were spread across each area (Figure 3 and 4). By comparing control and treatment plots, both before and after herbicide treatment, we accounted for year over year variation in vegetation communities that is not attributable to the herbicide treatment.

During the pre- and post-treatment vegetation surveys of each plot, we measured water depth, density of live and dead *P. australis* stems, and plant-canopy height. Vascular plants in the plots were identified to species, wherever possible, following the *Field Manual of Michigan Flora* (Voss and Reznicek 2013) and the relative cover of each plant was estimated to document the current vegetation community present in each plot. Additionally, we recorded GPS coordinates with sub-meter accuracy (SXPro GNSS, Geneq Inc., 10700 Rue Secant, Montreal, QC, Canada, H1J 1S5) at the center of each plot and placed bamboo stakes with flagged labels at the four corners of each plot to facilitate identifying plot location and orientation the following year. In August 2023, we conducted follow-up vegetation surveys at both wetlands following the same sampling methods to assess the post-treatment state of the plots.

RPAS-based herbicide application

In September 2022, imazapyr as an isopropylamine salt (Habitat® Aqua, 240 g ai L^{-1}, BASF Canada Inc., 5025 Creekbank Road, Mississauga, Ontario, Canada L4W 5R2, Pesticide

Registration Number 32374; Proposed Registration Decision PRD2020-17), was applied to the designated treatment areas by an RPAS under the Research Authorization 0009-RA-22. The herbicide was applied at a rate of 4.68L ha^{-1} and total spray volume of 100L ha^{-1} (includes 0.25% v/v Aquasurf® non-ionic spray adjuvant; Norac Concepts Inc., 27 Monarch Road Unit 1a, Guelph, Ontario, Canada, N1K 1N4, Pesticide Registration Number 32152), by a licensed drone pilot and pesticide applicator (Adrian Rivard, Drone Spray Canada, L-208-8129866762). The RPA used for application was a Hylio Inc. AG-110 multi-rotor (Hylio Inc., 1020 Agnes Road, Richmond, Texas, USA, 77469) carrying 2.5 gallon (9.5 litres) of herbicide. The AG-110 was equipped with eight AIXR110015 spray nozzles (two per rotor) and sprayed with a medium droplet size (236 to 340 µm; ASABE). The RPA was flown at an average height of 3.0 m above ground level at plot locations with an average speed of 1.7 m s^{-1}. Each flight was composed of an automated pattern of parallel flight lines with an average distance of 3 m between each flight line followed by a semi-assisted manually piloted flight around the perimeter of the application area (e.g., Figure 5). In both automated and semi-assisted flight modes, sprayers were turned off when the RPA was turning or stopped to minimize collateral damage.

RPAS herbicide application procedures adhered to the maximum wind speed constraint provided by the Pest Management and Regulatory Agency permit of 8 km h^{-1}, Habitat® Aqua's label recommendations for reducing spray drift, and weed specific instructions for *P. australis* (BASF Canada Inc.). In the field, treatment areas were delineated either using the RPA camera in first-person-view mode and establishing way points around the *P. australis* patch perimeter or through a combination of the first-person-view approach and ground observations of the aircraft position. Ground observations were made by walking out into the wetland in waders and using radio communication with the pilot to identify and confirm perimeter waypoints for flight plans. Following treatment, visual observations in the field showed no evidence of spray drift due to wind gusts as the rotors of the RPAS primarily pushed the herbicide droplets downwards and wind speed was very low (Table S1). We observed *P. australis* leaves to be dry within minutes of the herbicide application. Secondary treatment such as burning, rolling, or cutting of standing dead biomass was not undertaken after herbicide application.

Statistical methods

To address objective 1 and measure the efficacy of the RPAS-based herbicide application in suppressing invasive *P. australis* , we used a Kruskal-Wallis test (Kruskal and Wallis 1952) with an alpha = 0.05, looking for a significant difference in live *P. australis* stem density, total *P. australis* stem density, and canopy height. We used this test because the response variables violated the assumptions of a one- or two-way ANOVA (normality of the residuals, that could not be corrected by transformation, and homogeneity of variance of the residuals), we opted for the nonparametric equivalent that does not hold the same assumptions about the distribution of residuals. If the null hypothesis was rejected by the Kruskal-Wallis test, we used a Dunn's test for multiple planned comparisons of median differences (Dunn 1964) with a Bonferroni correction to adjust the *p*-value in accordance with the number of pairwise comparisons. All statistical analyses were conducted in R v. 4.3.2 (R Core Team 2023). We used the same test for the same reasons to address objective 2 and characterize differences in plant community diversity and floristic quality in the year following *P. australis* suppression, including species richness (*S*), Shannon-Weiner diversity (*H'*), Simpson's reciprocal diversity index (*1/D*), Pielou's evenness (*J*), and mean coefficient of conservatism (mean CC_s) (Table S2). The Shannon-Weiner diversity and Simpson's reciprocal diversity indices were calculated with the R package *vegan* (Oksanen et al. 2013).

Coefficients of conservatism are values assigned to plant species by expert botanists, ranging from 0 to 10. These values represent the likelihood of a given plant species occurring in the assessed region as well as its ability to tolerate disturbance. Low values represent species that can withstand greater environmental and anthropogenic disturbances while high values represent plant species of high conservation value that have lower tolerance to disturbance; non-native species are given a value of zero. Coefficients of conservatism for each identified plant species were obtained from *Floristic Quality Assessment System for Southern Ontario* (Oldham et al. 1995). Mean coefficients of conservatism were calculated following the recommended equation for CC_s by Kutcher and Forrester (2018).

Results and Discussion

Phragmites australis *suppression efficacy*

We assessed theefficacy of RPAS-based herbicide application on *P. australis* in wetlands through response variables including live *P. australis* stem density and canopy height. Live *P.*

australis stem density and canopy height reduced dramatically one year after the herbicide treatment of Habitat® Aqua with the RPAS (Figure 6). Specifically, live *P. australis* stem density in the herbicide-treated plots decreased by > 99% across both sites (Kruskal-Wallis: $H_3 =$ 40.6, p<0.001), with only one of the 18 treatment plots surveyed after the RPAS-based herbicide treatment having live *P. australis* stems. Total *P. australis* stem density (including standing dead stems) did not change significantly after the herbicide treatment (Kruskal-Wallis: $H_3 = 0.44$, p = 0.931).

Median canopy height did not differ among 2022 control, 2022 treatment, and 2023 control plots, but did decrease significantly by ~94% in the herbicide-treated plots, (Kruskal-Wallis: $H_3 = 40.4$, p<0.001). We used a Dunn's post-hoc test for planned comparisons to support our interpretation of the Kruskal-Wallis results. These planned comparisons firstly contrast the 2022 (pre-) treatment and control plots to evaluate the pre-treatment similarity between treatment and controls; secondly, contrast the control plots in 2022 and 2023 to assess any interannual differences; and thirdly, contrast the 2023 (post-) control plots and RPAS-treated plots, as well as the 2022 (pre-) treatment plots and RPAS-treated plots to understand the nature of any significant effect of treatment. Our results (Table 1) corroborate others who found backpack spraying with imazapyr to reduce live *P. australis* stem density by 100% (using a rate of 560 g ai ha^{-1}, Knezevic et al. 2013) and 95% (5% concentration in 10 m^2 macro plots, Mozdzer et al. 2008). When Habitat® Aqua was applied by helicopter with a 7% solution, *P. australis* abundance was reduced by ~90% (Whyte et al. 2009). Our results demonstrate that RPAS-based herbicide application is an equally effective application method for initial suppression of *P. australis* in wetlands.

Plant diversity following **P. australis** *suppression*

In addition to the impact on *P. australis* abundance, there was also an anticipated significant reduction on plant diversity, one year after the herbicide application (Figure 7). Biodiversity was not different across the non-treated plots (2022 control, 2022 treatment, and 2023 control plots) as represented by all plant diversity metrics (Table 2). However, the median value for all plant diversity metrics reduced significantly following the RPAS-based herbicide treatment (Table 2). Species richness decreased by approximately 33% (Kruskal-Wallis: $H_3 =$ 18.8, p<0.001), the Shannon-Weiner diversity index decreased by nearly 73% (Kruskal-Wallis:

$H_3 = 23.7$, p<0.001), the Simpson's reciprocal diversity index decreased by 50% (Kruskal-Wallis: $H_3 = 23.1$, p<0.001), and Pielou's evenness decreased by 73% in herbicide treated plots (Kruskal-Wallis: $H_3 = 18.3$, p<0.001). The Kruskal-Wallis test revealed a significant difference among the control and treatment groups ($H_3 = 8.73$, p = 0.033) for the mean coefficient of conservatism. However, from the post-hoc Dunn's test, no significant differences were observed among control and treatment groups due to the strictness of the Bonferroni correction used for planned comparisons.

An initial reduction of these response variables has been reported by others (e.g., Zimmerman et al. 2018; Robichaud and Rooney 2021b; Jordan 2022). In similar study areas (Long Point and Rondeau Provincial Parks in Ontario, Canada), helicopter glyphosate treatments showed a 33% reduction in species richness, 69% reduction in Shannon-Weiner diversity, 9% reduction in Simpson's reciprocal diversity index, and 63% reduction in Pielou's evenness one year after the initial herbicide treatment (Robichaud and Rooney 2021b), exhibiting similar values to our plots that had reductions of 33%, 73%, 50%, and 73%, respectively. It has been shown in similarly biodiverse areas that it takes a minimum of three years after initial treatment before the plant community will begin to transition to predominantly native vegetation (Jordan 2022). Consequently, these declines in plant community diversity are not likely permanent, but reflect the early stages of community succession following the removal of the dominant invasive *P. australis*. These early stages of succession are important to report on, however, as priority effects are recognized as important in plant community outcomes of wetland restoration (e.g., Tarsa et al. 2022).

The level of hydrologic disturbance and health of the seedbank will largely dictate whether native plants will re-establish after herbicide suppression of *P. australis* (Rohal et al. 2019) and the plots surveyed in our studies represent biodiverse coastal wetlands, likely representing a best-case scenario for plant community recovery from a strong seedbank. While we expect the response variables in our plots to follow a similar plant recovery trajectory, continuous monitoring of the herbicide-managed wetlands is needed to document the short- and long-term plant community composition changes (Kettenring and Adams 2011; Bonello and Judd 2020; Robichaud and Rooney 2021b). The presence of standing dead biomass in the first year after treatment likely increased shading in the surveyed plots and may have hindered

germination of species from the seedbank that rely on high light interception through the canopy (Minchinton et al. 2006). However, standing dead biomass and light penetration of areas that did not undergo secondary treatment reached similar levels to areas that underwent burning, rolling, or cutting within a two-year timeframe (Robichaud and Rooney 2021b), suggesting that the standing dead biomass may not limit species richness, evenness, and diversity in the treated marsh permanently. Additionally, inclusion of secondary treatment can offset the benefits of RPAS-based herbicide application, since heavy machinery is needed to mechanically knock down the standing dead biomass and risks crushing sensitive species such as snakes and turtles in its path (Angoh et al. 2021).

Plant community composition changes in herbicide-treated plots

At Baie du Doré, a total of eight species were identified in the pre-treatment plots, while nine species were identified in the plots post treatment. At Rondeau Provincial Park, 15 species were identified in the pre-treatment plots, and 17 species were identified in the plots post treatment (Table S3). At Baie du Doré, only two plant species present in the plots before treatment with herbicide persisted in the herbicide-treated plots (*Lemna minor* and *P. australis*) the following year. After the RPAS-based herbicide treatment, five species including *Typha* x *glauca* were excluded from the herbicide-treated plots and seven species that were not found in the baseline vegetation surveys appeared in the herbicide-treated plots, including the native plant *Impatiens capensis*. At Rondeau Provincial Park, four plant species present before the herbicide treatment persisted in the herbicide-treated plots the following year (*Bidens tripartita*, *Cicuta bulbifera*, *Hydrocharis morsus-ranae*, and *Lemna minor*). After the RPAS-based herbicide treatment, six species including *P. australis* were excluded from the herbicide-treated plots and three species that were not found in the baseline vegetation surveys appeared in the herbicide-treated plots, including the native *Zizania aquatica*. At Baie du Doré the native plant cover decreased by 15% in the control plots, while increasing by 20% in the herbicide-treated plots. While at Rondeau Provincial Park, native plant cover decreased by 16% in the control plots and by 65% in the herbicide-treated plots. In contrast, the non-native plant cover at Baie du Doré increased by 50% in the control plots and decreased by 100% in the herbicide-treated plots. At Rondeau Provincial Park, non-native plant cover similarly increased by 55% in control plots and decreased by 25% in the herbicide treated plots.

Although the mean coefficient of conservatism (*CCs*) remained relatively low, with the highest observed mean *CCs* value being 1.5 of a possible 10, the increase in value from the control plots reflects that *P. australis* ($CC_s = 0$) is no longer the dominant species in the plots. After the herbicide treatment, *Hydrocharis morsus-ranae* (European Frog-bit; $CC_s = 0$) became the dominant species in the treated plots at Rondeau Provincial Park. Secondary invasion by species like *H. morsus-ranae* have been documented previously (e.g., Bonello and Judd 2020; Robichaud and Rooney 2021b), as the suppression of *P. australis* without active planting or reseeding creates an open niche for secondary invaders to establish within (Kettenring and Tarsa 2020). For example, in monitoring the effects of glyphosate treatment in Rondeau Provincial Park a secondary invasion by *H. morsus-ranae* was also observed in the first years after *P. australis* suppression (Robichaud and Rooney 2021b). However, at Baie du Doré a secondary invasive species did not establish in the plots, instead the annual *Impatiens capensis* (Spotted Jewelweed; $CC_s = 4$) became most abundant. The observed difference between the two sites may be due to the pre-treatment plant community composition (Pearson et al. 2016). For example, *H. morsus-ranae* was observed in nearly all pre-treatment plots at Rondeau Provincial Park but not in the plots from Baie du Doré. This suggests that pre-treatment surveys may help predict where secondary invasions are likely to occur following *P. australis* suppression and could be useful in generating guidance on where seeding or planting could be most valuable to facilitate native plant community recovery. Additionally, the increase in water depths at Rondeau Provincial Park between 2022 (19 cm; std. 13 cm) and 2023 (32 cm; std. 11 cm) was likely a contributing factor. Because *H. morsus-ranae* is a floating macrophyte, these deeper water conditions likely favored its establishment in the absence of *P. australis*. Unlike Rondeau Provincial Park, water depths at Baie du Doré decreased between 2022 (23 cm; std. 10 cm) and 2023 (13 cm; std. 9 cm), resulting in moist conditions which could favor germination of annuals like *I. capensis*. Interestingly, *I. capensis* was not present in the 2022 pre-treatment plots at Baie du Doré, although we would not expect it to coexist with the dense *P. australis* targeted for treatment. Instead, *I. capensis* likely either dispersed into the treated area following herbicide application (e.g., its seeds can travel up to 2 m through ballistic dispersal; Hayashi et al. 2009). Alternatively, it may have been waiting in the seedbank for suitable conditions to arise, as *I. capensis* seeds can remain viable in the seedbank for up to three years (Perglová et al. 2009).

While our main goal was to evaluate the initial suppression efficacy of RPAS-based herbicide application to *P. australis* in wetlands, long-term monitoring is necessary to assess the outcome of *P. australis* suppression for the vegetation community (Jordan 2022); although notably a review found that 40% of studies on *P. australis* management lasted only one year (Kettenring and Adams 2011; Hazelton et al. 2014). Based on existing *P. australis* management literature using systemic herbicides (Derr 2008; Mozdzer et al. 2008; Knezevic et al. 2013), and the reported trajectory of vegetation recovery (Bonello and Judd 2020; Robichaud and Rooney 2021b), we anticipate that the RPAS-treated sites will follow a similar succession, provided adequate follow-up treatments and monitoring take place (Lombard et al. 2012). However, while the dominance of *H. morsus-ranae* at Rondeau Provincial Park and *I. capensis* at Baie du Doré may be due to hydrologic disturbance, the health of the seedbank, or sources of native plant propagules (Rohal et al. 2019), the differences in these biodiverse coastal wetlands in the first year after herbicide treatment highlight the need for further examination of the complex factors involved in plant recovery following *P. australis* suppression in dynamic wetland environments. Further research is needed to evaluate the broader implications of RPAS-based herbicide applications at a larger extent than was feasible under our Research Authorization (limited to 2 ha of intended treatment area per site) and continued monitoring should be implemented to assess native plant recovery. Additionally, research in lower density *P. australis* patches (< 20 live stems m^{-2}) or systematically assessing the effects of fragmentation and interspersion (e.g., Robinson et al. 2009) on the herbicide efficacy could increase the generalizability of our results which were collected from relatively contiguous patches with higher live *P. australis* stem density (minimum of 20 live stems m^{-2}).

Our results have important implications for wetland management and conservation efforts, particularly in the context of invasive species control and habitat restoration. The substantial reduction in *P. australis* live stem density and canopy height following RPAS-based herbicide application represents successful initial *P. australis* suppression, but the widely reported need for follow-up treatments to maintain long-term suppression of *P. australis* (e.g., those summarized by Kettenring and Adams 2011; Lombard et al. 2012) could also be reduced with RPAS-based herbicide applications. The few surviving ramets observed in our study will mean that the area requiring follow-up treatment is reduced compared to helicopter application methods. Further, RPAS technology has valuable potential for follow-up spot spraying of

remnant *P. australis* ramets after initial herbicide treatments. Minimizing repeated herbicide applications is crucial to reduce the total amount of herbicide entering wetlands for *P. australis* management and lowering risks of *P. australis* evolving herbicide resistance and the threat of herbicides accumulating in the environment.

RPAS technology is acknowledged for its ability to be used in association with dull, dirty, or dangerous activities on-demand without jeopardizing the safety of pilots, which in the case of *P. australis* management can include the health of those taking part in herbicide applications or bystanders. Given the success of this first application of RPAS-based herbicide application to *P. australis* in Canada (> 99% reduction in live stem density one year after treatment), RPASs have the potential to play a critical role in invasive species mapping and management (Takekawa et al. 2023). While our focus was on the efficacy of RPAS-based imazapyr applications, there is a need to quantify the off-treatment effects on adjacent vegetation based on herbicide (e.g., droplet size) and flight (e.g., height) parameters to better inform land managers of the impacts of these combined technologies on the broader ecosystem.

Competing Interests

Competing interests: The authors declare none.

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Tables

Table 1. Dunn's test results of planned comparisons among control and remotely-piloted aircraft system (RPAS)-based herbicide treated plots, pre-treatment (2022), and post-treatment (2023) for live Phragmites australis stem density and canopy height. Each comparison group had a sample size of 18 plots. Adjusted p value is based on the Bonferroni correction method for multiple planned comparisons.

Anticipated effect of treatment	Planned comparison		Response variable	Adjusted *p* value
No effect	2022 control	2022 treatment	Live *P. australis* stem density	1.000
			Canopy height	1.000
No effect	2022 control	2023 control	Live *P. australis* stem density	1.000
			Canopy height	1.000
Effect from herbicide treatment	2023 control	2023 treatment	Live *P. australis* stem density	<0.001
			Canopy height	<0.001
Effect from herbicide treatment	2022 treatment	2023 treatment	Live *P. australis* stem density	<0.001
			Canopy height	<0.001

Table 2. Dunn's test results of planned comparisons among control and remotely-piloted aircraft system (RPAS)-based herbicide treated plots, pre-treatment (2022), and post-treatment (2023) for species richness (S), Shannon-Weiner diversity (H'), Simpson's reciprocal diversity index (1/D), Pielou's evenness (J), mean coefficient of conservatism value (CC_s). Each comparison group had a sample size of 18 plots. Adjusted p value is based on the Bonferroni correction method for multiple planned comparisons.

Anticipated effect of treatment	Planned comparison		Response variable	Adjusted p value
No effect	2022 control	2022 treatment	Species richness	1.000
			Shannon-Weiner diversity	1.000
			Simpson's reciprocal diversity index	1.000
			Pielou's evenness	0.544
			Mean conservatism value	1.000
No effect	2022 control	2023 control	Species richness	0.760
			Shannon-Weiner diversity	1.000
			Simpson's reciprocal diversity index	1.000
			Pielou's evenness	1.000
			Mean conservatism value	0.314
Effect from herbicide treatment	2023 control	2023 treatment	Species richness	0.025
			Shannon-Weiner diversity	0.031
			Simpson's reciprocal diversity	0.050

			index	
			Pielou's evenness	0.220
			Mean conservatism value	0.101
Effect from herbicide treatment	2022 treatment	2023 treatment	Species richness	0.003
			Shannon-Weiner diversity	<0.001
			Simpson's reciprocal diversity index	<0.001
			Pielou's evenness	<0.001
			Mean conservatism value	1.000

Figures
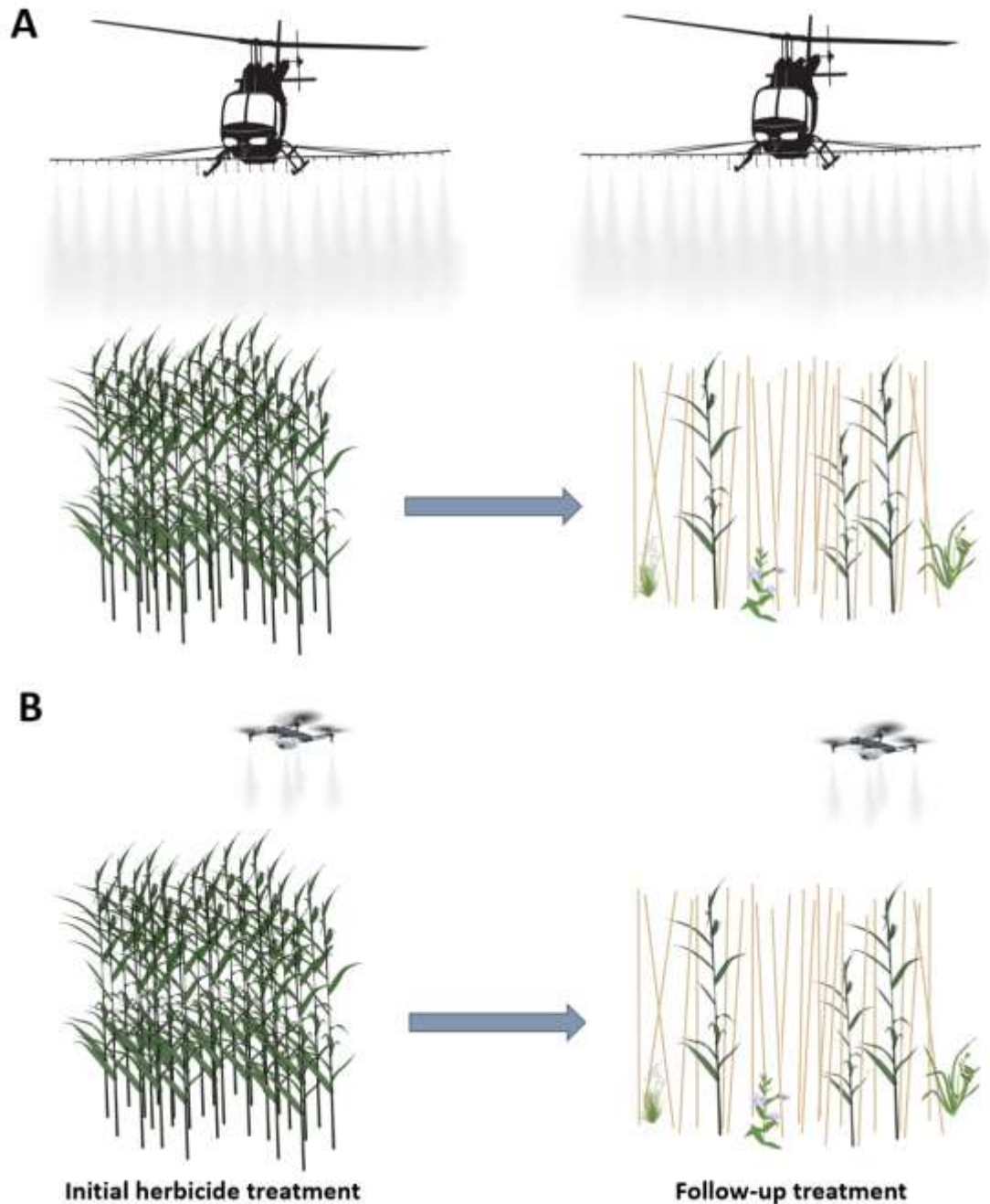
Figure 1. Graphic of complexities associated with *Phragmites australis* follow-up treatments after the initial herbicide treatment. The initial herbicide treatment shows a dense monoculture *P. australis* stand that can be effectively treated by either helicopter (A) or RPAS-based (B) herbicide applications. The follow-up treatment shows standing dead as a result of the herbicide treatment, with remnant *P. australis* ramets and native vegetation in close proximity.

Figure 2. Map of Provincially Significant Wetlands used for on-the-ground vegetation surveys for evaluation of RPAS-based applications of Habitat® Aqua to invasive *Phragmites australis*.
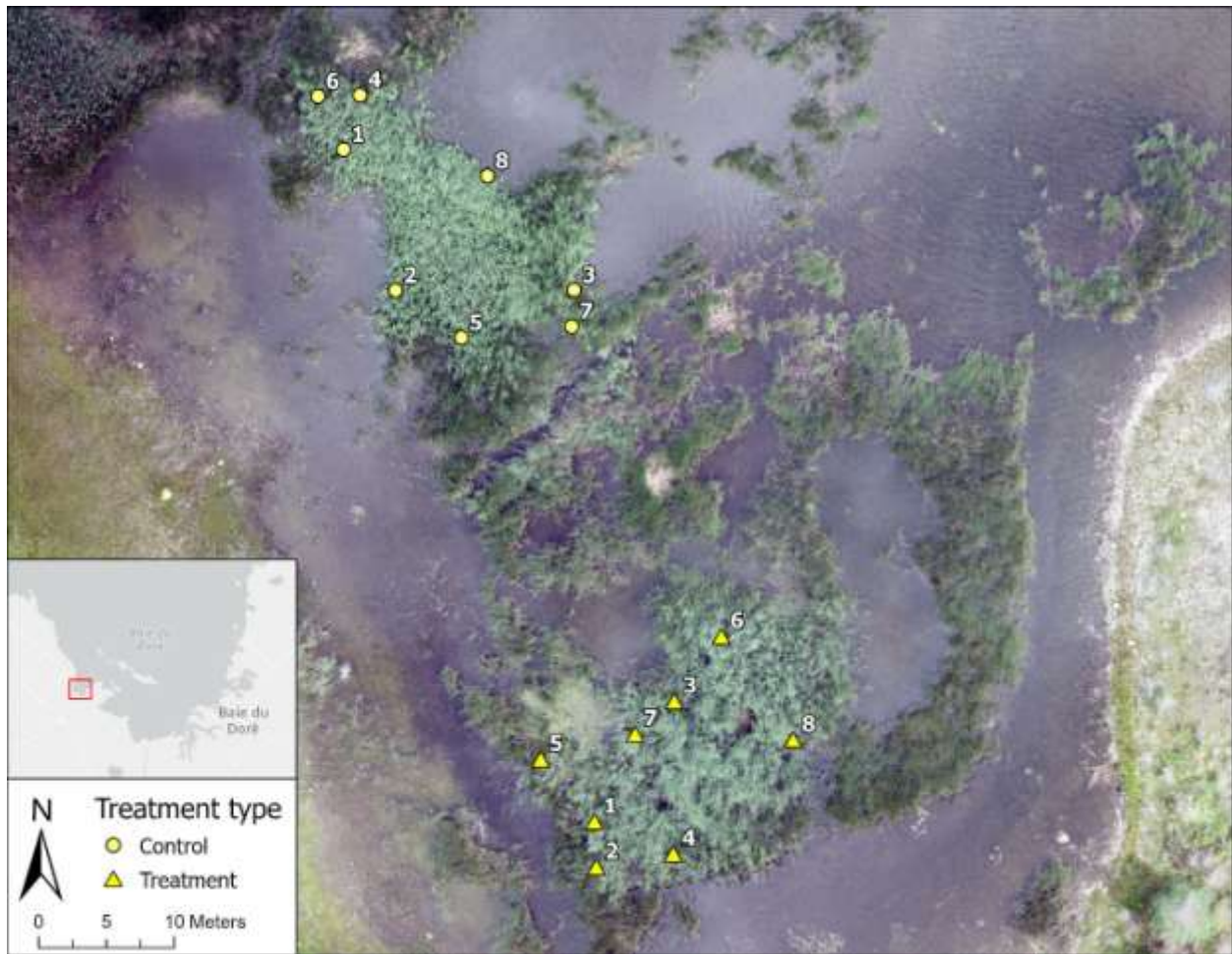
Figure 3. Locations of experimental control (n=8) and treatment (n=8) plots for RPAS-based herbicide treatment in Baie du Doré wetland. The northern patch was left as the control (untreated) and the southern patch was designated as the treatment side to be sprayed with Habitat® Aqua by an RPAS. Yellow circles represent control plots and yellow triangles represent treatment plots. Numbers represent pairing by water depth between the treatment and control plots. Basemap imagery was collected by Ontario Power Generation's Advanced Inspection and Maintenance team in 2022.

Figure 4. Locations of experimental control (n=10) and treatment (n=10) plots for RPAS-based herbicide treatment in Rondeau Provincial Park wetland. The east side of the slough was left as the control (untreated) side and the west side of the slough was designated as the treatment side to be sprayed with Habitat® Aqua by an RPAS. Yellow circles represent control plots and yellow triangles represent treatment plots. Numbers represent pairing by water depth between the treatment and control plots. Basemap imagery was collected by Ontario Power Generation's Advanced Inspection and Maintenance team in 2022.
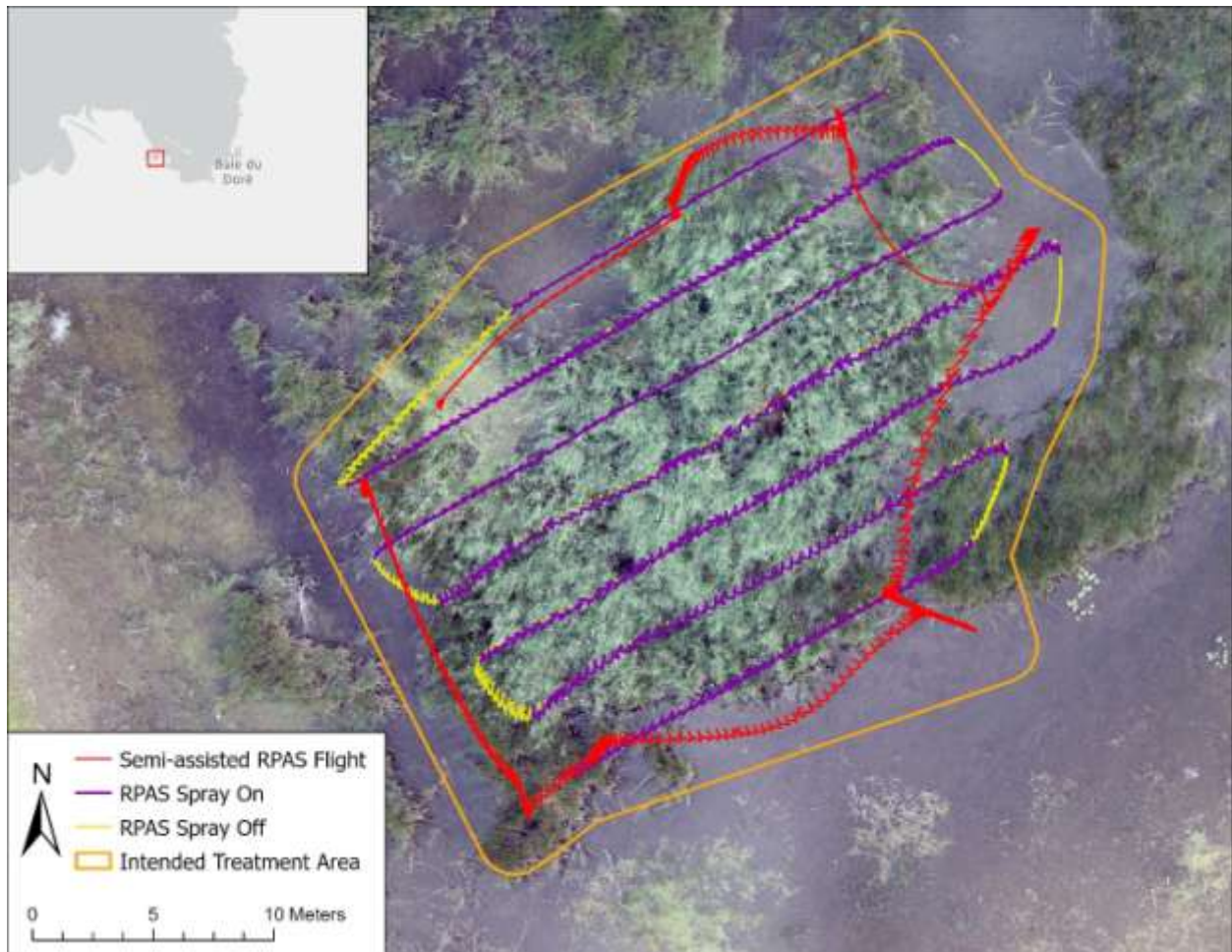
Figure 5. Semi-assisted flight lines (red; spraying herbicide along the ferry lines), automated flight lines (purple; spraying herbicide), and ferry lines (yellow; not spraying herbicide), used in the RPAS-based Habitat® Aqua application to the intended treatment area (orange polygon). Basemap imagery was collected by Ontario Power Generation's Advanced Inspection and Maintenance team in 2022.
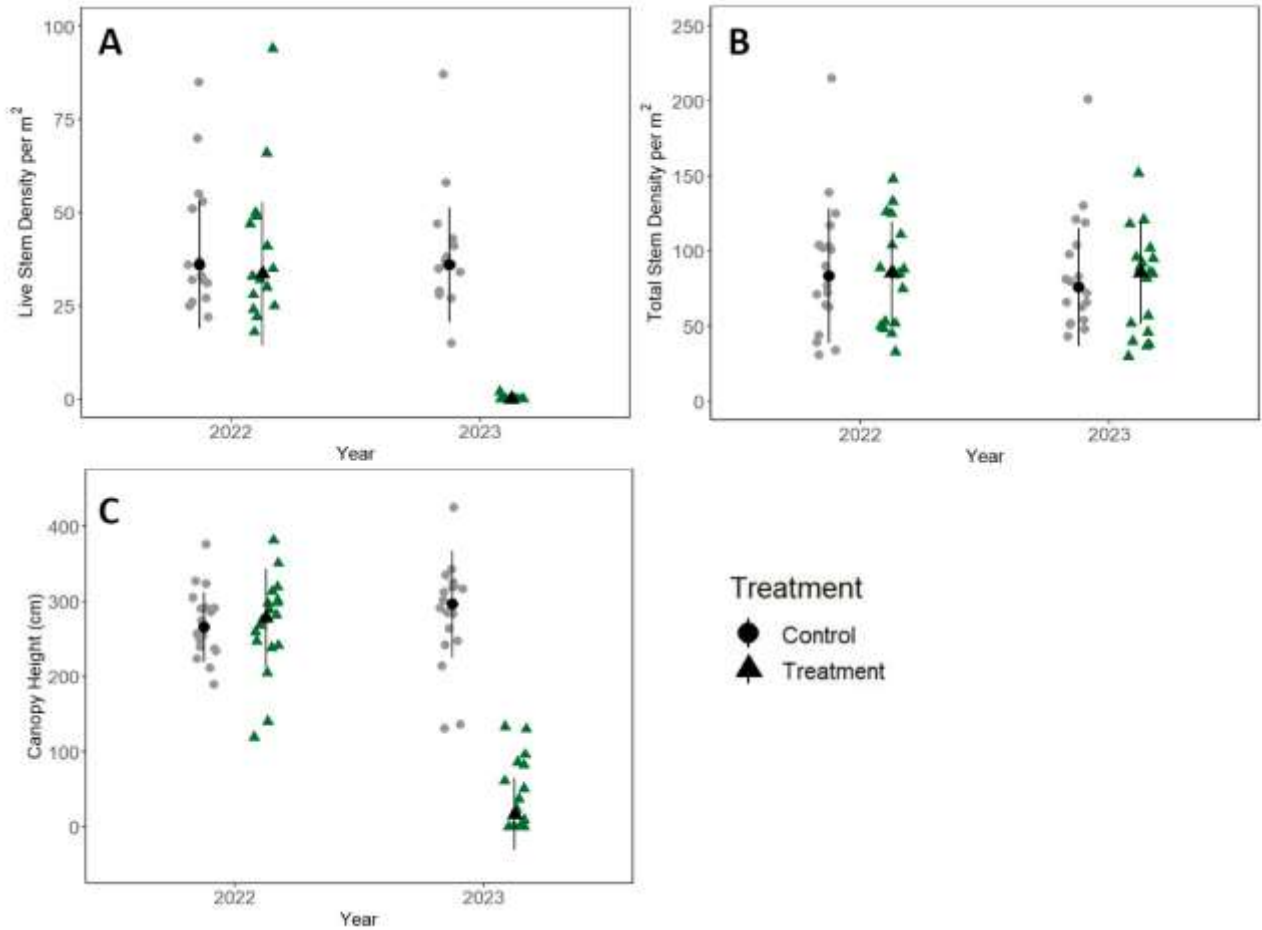
Figure 6. Jitter plot comparison of live *Phragmites australis* stem density (A), total *P. australis* stem density (B), and canopy height (C) between control and RPAS-based herbicide treated plots, pre-treatment (2022), and post-treatment (2023). Note that total stem density includes both live and dead stems of *P. australis*. Grey circles represent control sites, and green triangles represent treated sites. Black symbols represent the median value, and error bars represent standard deviation. Created with ggplot2 (Wickham 2016).
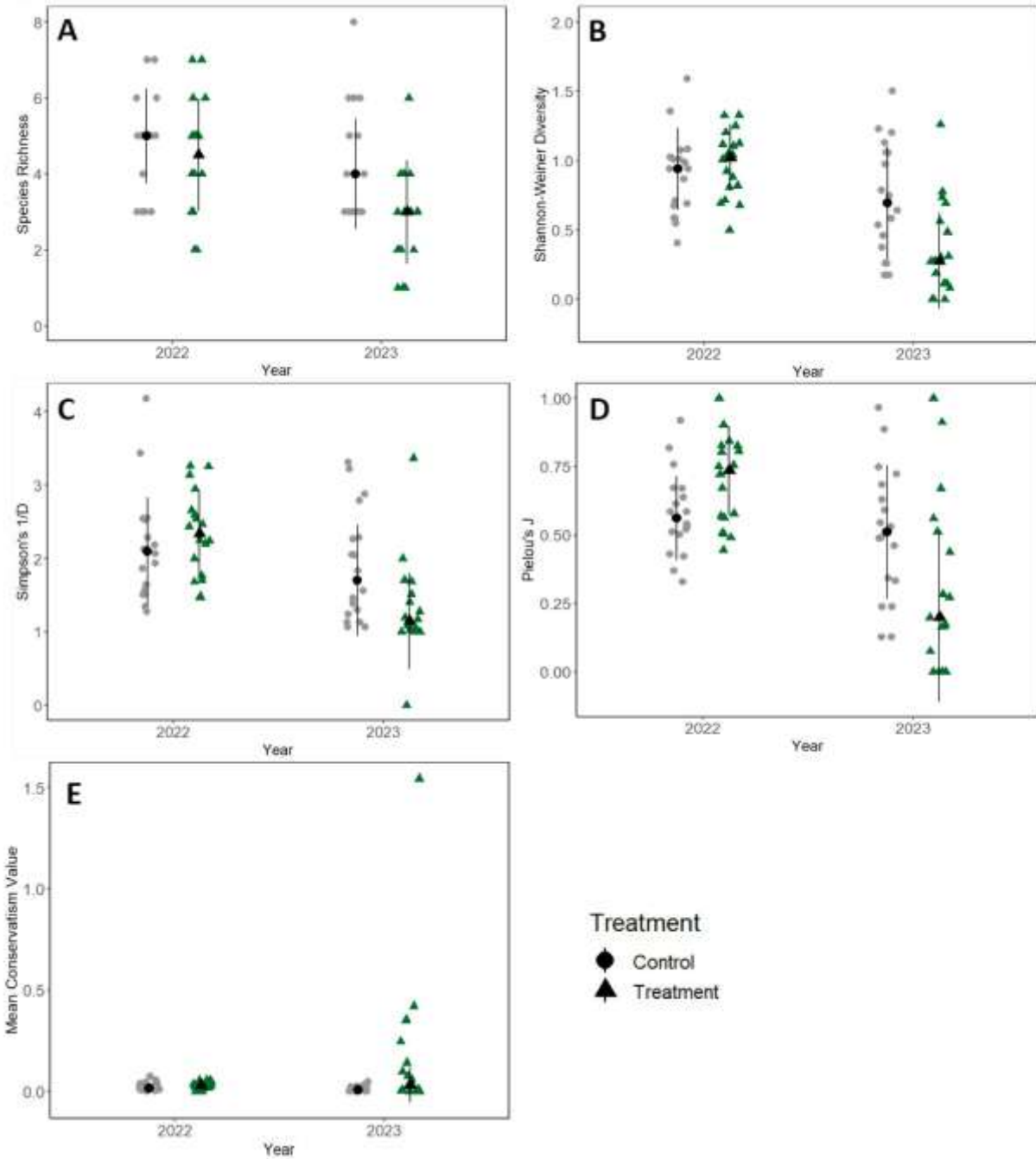
Figure 7. Jitter plot comparison of species richness (S; A), Shannon-Weiner diversity (H'; B), Simpson's reciprocal diversity index ($1/D$; C), Pielou's evenness (J; D), and mean coefficient of conservatism value (CC_s; E) between control and RPAS-based herbicide treated plots, pre-treatment (2022), and post-treatment (2023). Grey circles represent control sites, and green triangles represent treated sites. Black symbols represent the median value, and error bars represent standard deviation. Created with ggplot2 (Wickham 2016).