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## ***Humulus japonicus* Invasion**

### **The invasion of Japanese hop (*Humulus japonicus*) in a restored floodplain forest**

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## Abstract

Japanese hop (*Humulus japonicus* Sieb. & Zucc.) is an emerging invasive plant that has been observed to invade and spread throughout wetlands. As an annual vine, *H. japonicus* can smother native vegetation, forming dense stands and reducing biodiversity. At a restored floodplain forest in Joslin, Illinois, formerly used as an experimental site to test the effectiveness of different reforestation methods, *H. japonicus* has invaded stands of the previously dominant invasive, reed canarygrass (*Phalaris arundinacea* L.). We conducted an observational field study to examine the spatio-temporal dynamics of *H. japonicus* invasion relative to gradients in canopy cover and species composition. Ten transects, with half the transect extending into and half extending beyond *H. japonicus* patches, were established in October 2022. Seven quadrats per transect were surveyed for vegetation cover and canopy cover in October 2022, June 2023, and October 2023. Transects were evenly split between forested and open areas based on the reforestation treatments. *H. japonicus* cover significantly increased from October 2022 to October 2023, which has resulted in a slight decrease and replacement of *P. arundinacea* across the site. Shade reduced *H. japonicus* cover, indicating its preference for sunlit conditions. Species richness was higher in forested transects compared to open ones, most likely due to the absence of both *P. arundinacea* and *H. japonicus* in shaded transects. Along transects, quadrats that had been invaded by *H. japonicus* differed in species composition from quadrats that had not been invaded in both October 2022 and October 2023. *H. japonicus* cover was much lower in June than October, suggesting that temporal niche partitioning may allow *P. arundinacea* to persist, and indicating that monitoring for *H. japonicus* should occur late in the growing season. Both invasive species are shade intolerant, suggesting that planting fast-growing trees should be an effective long-term solution for controlling invasion.

**Keywords:** competition; floodplain forest; *Humulus japonicus*; *Phalaris arundinacea*; plant invasion

## Management Implications

Japanese hop (*Humulus japonicus*), a vine originating from East Asia, poses a threat to wetland and riparian ecosystems by smothering vegetation and decreasing biodiversity. Current management of *H. japonicus* includes a combination of mechanical and chemical control.

However, there is limited research on *H. japonicus* ecology, interactions with other species, and its role as an invader in wetlands. Therefore, the objectives of this study were to quantify the invasion of *H. japonicus*; determine the effects of invasion on *P. arundinacea* cover, plant species richness, and community composition; and examine environmental preferences, specifically shade tolerance, of *H. japonicus*.

Key findings include an increase in *H. japonicus* cover between years and a corresponding decrease in *P. arundinacea* cover, indicating the replacement of *P. arundinacea*. The replacement of a dense, rhizomatous, perennial grass by an annual vine has potential implications for ecosystem functions including soil stability, and *H. japonicus* invasion has the potential to increase erosion in riparian areas. Additionally, the invasion of *H. japonicus* reduced species richness and altered species composition. Increased canopy cover reduced *H. japonicus* cover, meaning that restoring canopy cover and planting fast-growing trees could effectively manage *H. japonicus* populations.

## Introduction

The introduction of invasive species in wetland ecosystems has detrimental impacts, including altering ecosystem function (Vitousek et al. 1997) and reducing biodiversity (Zedler and Kercher 2004). Wetlands are especially vulnerable to biotic homogenization (Price et al. 2018), which occurs when local, native species are replaced by few generalist, nonnative ones across many sites (McKinney and Lockwood 1999). With an increase in anthropogenic activity, ecosystems are more likely to be invaded by nonnative species that replace native species (Lázaro-Lobo and Ervin 2021), resulting in biotic homogenization.

For floodplain forest ecosystems, biotic homogenization occurs at an even faster rate than nearby upland forests (Johnson et al. 2016). This has led to biological homogenization at a regional scale, with the introduction of the invasive reed canarygrass (*Phalaris arundinacea* L., Poaceae) (Price et al. 2018). A common wetland invader, *P. arundinacea* is a long-lived perennial grass that spreads aggressively due to its expansive network of underground rhizomes (Lavergne and Molofsky 2004). Invasion by *P. arundinacea* is widespread throughout much of North America including Alaska, Canada, and the temperate, conterminous U.S., and invasion negatively affects plant diversity and floristic quality, resulting in changes in community composition (Spyreas et al. 2010).

Despite the prolific spread of *P. arundinacea* in wetlands, one emerging invasive plant that has been observed to invade and cover wetland vegetation, including *P. arundinacea*, is the Japanese hop (*Humulus japonicus* Sieb. & Zucc., Cannabaceae) (personal observation). An annual vine, *H. japonicus* has been observed to form dense monotypic mats (Guyon and Cosgriff 2022) that outcompete plant communities with species of conservation concern (EMPPPO 2019). The vine overgrows all vegetation, even overtopping shrubs and trees and suppressing their regeneration (Guyon and Cosgriff 2022; Kim & Kim 2009). For species growing underneath *H. japonicus*, limited light and high moisture leads to death and rapid decomposition (Kim and Kim 2009), resulting in decreases in biodiversity, species richness, and functional richness (Fried et al. 2019). As it continues its invasion in wetlands, increases in the range distribution and local abundance of *H. japonicus* are likely to contribute to biotic homogenization across vulnerable ecosystems. Additionally, as *H. japonicus* replaces both native and invasive vegetation whose roots stabilize soil, ecosystems are more vulnerable to erosion, which can alter ecosystem functions (Cellone et al. 2016).

*H. japonicus* was introduced from eastern Asia to Europe and North America for medicinal and ornamental purposes (EMPPPO 2019). In North America, *H. japonicus* has established in eastern Canada and the United States, west to North Dakota and south to Kansas (USDA 2023), making it a major species of concern. *Humulus japonicus* easily colonizes disturbed, flood-prone ecosystems that have open canopy gaps (Guyon and Cosgriff 2022) and is found in wetlands as it is mainly dispersed by water along rivers (EMPPPO 2019) and grows best on riversides with alluvial soils (Balogh and Dancza 2008; EMPPPO 2019). Since *H. japonicus* grows best in full light, restoring canopy cover is the most effective long-term treatment for controlling invasion in forested floodplains, wetlands, and riparian zones that are subject to repeated or ongoing colonization pressure (Guyon and Cosgriff 2022). Measures taken to prevent the establishment and spread of *H. japonicus* in these types of areas include preserving native tree cover and avoiding disturbances that allow for its establishment (Fried et al. 2018).

While research on *H. japonicus* has been conducted in Europe (e.g. Balogh and Dancza 2008; Georgescu et al. 2021; Urziceanu et al. 2022) and Asia (e.g. Ju et al. 2006; Kim & Kim 2009), research on *H. japonicus* in the North America is limited, especially compared to other invasive wetland plant species such as *P. arundinacea* or the common reed (*Phragmites australis* [Cav.] Trin. ex Steud.). With its status as an emerging species of concern, and such an aggressive

invader, *H. japonicus* should be further researched to fill a current gap in literature. There is a need for observational field studies of its ecology as an invasive species, its interaction with other plants, and its role as an invader in wetlands. Studying its invasion dynamics and patterns within a field setting rather than an experimental setting allows for species interactions to be observed under natural environmental conditions. More effective management strategies for *H. japonicus* invasion can be developed through invasion monitoring.

We conducted an observational study in a restored floodplain forest from October 2022 to October 2023 to track the spatio-temporal dynamics of *H. japonicus* invasion relative to gradients in canopy cover and species composition. Our specific objectives were 1) to quantify the invasion or decline of *H. japonicus* by measuring its cover along the same transects between years, 2) to investigate the change in *P. arundinacea* cover in response to *H. japonicus* invasion, 3) to determine the effects of *H. japonicus* invasion on plant species richness and community composition, and 4) to examine the relationship between *H. japonicus* cover and canopy cover in a field setting.

## Materials and Methods

### *Study Site*

The Joslin wetland mitigation site is a 6.1-ha floodplain wetland located along the Rock River in Henry County, northwest Illinois, USA (41.5542 N, -90.1835 W). Previously agricultural land, the site is now a compensatory mitigation wetland, meaning that the site has been restored into a wetland to provide compensation for losses of aquatic resources (Corps and EPA 2008; Matthews et al. 2020). Across the site, topography and hydrology are similar, and the soil type is Sawmill silty clay loam (Matthews et al. 2020).

The restoration site was designed by the Illinois Department of Transportation and restored in 1998 as an experiment to test the effectiveness of five reforestation methods: balled-and-burlapped tree plantings, bare root tree plantings, seedling plantings, acorn plantings, and passive restoration (Matthews et al. 2020). Fifteen years after restoration, in treatments with less expensive methods, i.e. passive restoration and acorn plantings, *P. arundinacea* invaded and dominated the vegetation cover. In treatments with more costly methods, i.e. bareroot and balled-and-burlapped tree plantings, there was less *P. arundinacea* invasion due to greater canopy cover, resulting in greater plant species richness (Matthews et al. 2020). While *P. arundinacea*

previously dominated large areas of the Joslin field site, a new, emerging invader, *H. japonicus*, was observed to have invaded many of the *P. arundinacea* patches by 2021 (personal observation). Invasion by *H. japonicus* has resulted in decreased *P. arundinacea* abundance.

In 2022, ten transects, extending roughly east to west, were established and sampled. Transects were evenly split between forested and open areas based on the initial tree-planting treatments described by Matthews et al. (2020) (Figure 1). Seven 1-m × 1-m quadrats were surveyed per transect, with the mid-point of each transect positioned at the boundary of *H. japonicus* expansion, marking the edge of *H. japonicus* patches as it replaces *P. arundinacea* stands (*H. japonicus* cover >20%). From the mid-point, two halves of a 24-m transect were established, with 12 m extending into *H. japonicus* growth and 12 m beyond the *H. japonicus* invasion front, into an area where it has the potential to expand. Quadrats were placed every 4 m, with the mid-point (0 m), 4 m, 8 m, and 12 m at the center of each quadrat (Figure 2). A marking flag was placed in the center of the mid-point quadrat (0 m) and labeled with the transect number. Additionally, a Garmin GPS unit was used to mark the ends and middle of each transect.

#### *Vegetation and Canopy Cover Sampling*

Field sampling was conducted on October 5, 2022, June 6, 2023, and October 2, 2023. Within each quadrat, vegetation percent cover was estimated for each individual plant species as the percentage of ground within a quadrat covered by the species, rounded to the nearest 5%. Additionally, tree canopy cover, measured as the percentage of occupied overhead area using a spherical densiometer, was collected in each quadrat in October 2022 and October 2023. Additionally, in October 2023, light intensity readings were obtained by collecting a 15-second average reading for each quadrat using a LI-COR Quantum Sensor (model LI-190). LI-COR readings were collected during times without cloud cover to ensure consistency, although some variability in sky conditions may still have been present. Densiometer data were used to explore the relationship between *H. japonicus* cover and shade, while LI-COR data were used to support measurements made via densiometer.

#### *Statistical Analysis*

All statistical analysis was conducted in R version 4.3.1 (R Core Team 2023). Figures were visualized with the collected raw data using the *ggplot2* package (Wickham 2016). Light intensity recorded using the LI-COR sensor was related to canopy cover from the densiometer

using a Kendall rank correlation due to non-normality of the light intensity data. Additionally, a linear mixed effects model was conducted to determine if canopy cover varied from October 2022 to October 2023. The response variable was canopy cover in October 2023 while the fixed effect was canopy cover in October 2022 and the random effect was transect.

To determine the effects of year (October 2022 and October 2023), canopy cover, distance along the transect (indicated by quadrat), and transect on *H. japonicus* cover, *P. arundinacea* cover, and species richness, separate linear mixed-effect models were conducted using the Kenward-Roger approximation method with the *lme4* package in R (Bates et al. 2015).

Initially, the effect of position along the transect was tested using three alternative variables: quadrat as a continuous numerical variable, a categorical variable split into negative (-12 m, -8 m, -4 m), neutral (0 m), and positive quadrats (4 m, 8 m, 12 m), and using *H. japonicus* cover as a proxy for distance along the transect. The best variable for position along the transect (quadrat) was determined for each response variable (*H. japonicus* cover, *P. arundinacea* cover, and species richness) based on AIC values. Quadrat as a categorical variable was determined to be the best predictor of *H. japonicus* cover. *H. japonicus* cover was found to be the best predictor of both *P. arundinacea* cover and species richness. Then, for each response variable, linear mixed-effects models were constructed with year, canopy cover, and distance along the transect (or its proxy) as fixed effects and transect as a random effect. Model selection was done through backwards elimination ( $p$ -value for removal  $\geq 0.05$ ) using the *lmerTest* package (Kuznetsova et al. 2017). All models were validated using QQ and residual plots, as well as formal tests of normality (Shapiro-Wilk). For models explaining *P. arundinacea* cover and species richness, shade and *H. japonicus* cover values were standardized while *P. arundinacea* cover and species richness values were log + 1 transformed to reduce heteroskedasticity. Effect plots were created to understand interactions between variables and points were extrapolated from the linear model predictions using raw data.

To determine whether species composition differed in quadrats invaded by *H. japonicus* (negative quadrats) vs. quadrats not invaded by *H. japonicus* (positive quadrats), a permutational multivariate analysis of variance (PERMANOVA) was conducted. Analyses were done separately for October 2022 and October 2023. A Bray-Curtis dissimilarity index between quadrats was calculated for each analysis, and additionally, a nonmetric multidimensional scaling (NMDS) ordination with two dimensions was conducted using the MetaMDS function of

the R package *vegan* (1000 random starts) (Oksanen et al. 2022). NMDS plots were created using *ggplot2* and base R (Wickham 2016). Because we were interested in determining the effect of *H. japonicus* on species composition, *H. japonicus* was excluded from the species list and analysis. However, some quadrats contained only *H. japonicus*. Therefore, to ensure at least one species was present in each transect/quadrat combination, a dummy species was used in the analysis and given a small percentage of cover (0.01%) to all quadrats.

Transects acted as a random effect while *H. japonicus* cover and shade were fixed effects. To account for this design, transect was the first term in the model so variation among transects would be accounted for before testing other terms and permutations were restricted so that quadrats were freely permuted within transects, but not across transects (permutations=999). Terms were tested in order starting with transect, *H. japonicus* cover, and shade. To ensure that each step used the same permutations, *set.seed* was used. The model was analyzed using the *adonis2* function within the *vegan* package (Oksanen et al. 2022).

## Results and Discussion

### *Invasion of H. japonicus and replacement of P. arundinacea*

There was a positive correlation between the readings from the LI-COR sensor and the readings made using the spherical densiometer (Kendall's Tau = 0.30, p-value = <0.001), indicating that increased canopy led to decreased light availability to the herbaceous layer. Additionally, canopy cover in October 2022 was a significant predictor of canopy cover in October 2023 (ANOVA with transect included as random effect;  $F = 31.92$ ,  $df = 1$ , p-value < 0.001). There was not a significant difference in canopy cover in October 2022 compared to October 2023, meaning that any changes in *H. japonicus* cover, *P. arundinacea* cover, and species richness were not due to changes in canopy cover.

To determine changes in *H. japonicus* and *P. arundinacea* cover from October 2022 to October 2023, we compared the cover of each species, separately, under all explanatory variables. The model explaining *H. japonicus* cover included fixed effects of year, canopy cover, quadrat position (categorical), date:canopy cover interaction, and a canopy cover:quadrat interaction. The model explaining *P. arundinacea* cover included fixed effects of canopy cover, quadrat position (*H. japonicus* cover), and a canopy cover:quadrat interaction. The random effect of transect had significant impacts on both *H. japonicus* and *P. arundinacea* (Table 1, Table 2).



*H. japonicus* cover was significantly impacted by transect, year, canopy cover, and quadrat (Table 1), while *P. arundinacea* cover was significantly impacted by transect and quadrat, which was represented by *H. japonicus* cover (Table 2).

*H. japonicus* percent cover was high October 2022, much lower in June 2023, and greatest in October 2023 (Figure 3a). Mean *H. japonicus* cover greatly increased from 7.76% in June 2023, the beginning of the growing season, to 43.9% in October 2023, the end of the same growing season. Generally, *H. japonicus* cover increased at each quadrat along the transect (Figure 3a), meaning that it grew in areas where it had previously colonized as well as into areas where it had yet to colonize in 2022. From October 2022 to October 2023, mean *H. japonicus* cover increased from 32.4% to 43.9%, indicating an expansion of invasion of *H. japonicus* across the site. Quadrat as a categorical variable significantly impacted *H. japonicus* cover (Figure 3a). In positive quadrats, *H. japonicus* mean cover increased from 9.33% to 21.8% in 2023, meaning that *H. japonicus* has continued to spread throughout the field site. Even in negative quadrats, *H. japonicus* grew more densely in 2023, increasing its mean percent cover from 57.2% to 65.9%.

For both October 2022 and October 2023, areas at the site with higher canopy cover had lower *H. japonicus* cover (Figure 4). However, there was a significant interaction between year and canopy cover, indicating that *H. japonicus* cover was greater under low canopy cover in October 2023 compared to October 2022 (Table 1, Figure 4a). This may be due to the colonization of areas under low canopy cover that had not yet been invaded by *H. japonicus* in October 2022 but had been colonized by October 2023. There was also a significant interaction between canopy cover and quadrat (Table 1, Figure 4a). In the negative quadrats, *H. japonicus* cover decreased more rapidly as canopy cover increased compared to the positive quadrats. Because the positive quadrats were beyond the *H. japonicus* invasion front in October 2022, it is possible that *H. japonicus* had not yet reached equilibrium with the environmental conditions, such as shade, in the positive quadrats. Given more time, the relationship between *H. japonicus* cover and canopy cover is expected to be similar in both positive and negative quadrats. Another reason for this interaction could be due to the presence of *P. arundinacea* present in the positive quadrats in October 2022. Because *P. arundinacea* was on the other side of the invasion front, it may hinder the spread of *H. japonicus* due to competition, regardless of canopy cover. Because *P. arundinacea* develops dense stands and a thick layer of thatch, it inhibits seedling

establishment by other species (Thomsen et al. 2012). However, based on its ability to invade established stands of *P. arundinacea*, we speculate that, given sufficient light, *H. japonicus* invasion into other, native vegetation would be even more rapid.

Because *H. japonicus* was observed invading *P. arundinacea* stands at the Joslin field site, changes in *P. arundinacea* cover were examined from October 2022 to October 2023. As *H. japonicus* cover increased from October 2022 to October 2023, *P. arundinacea* cover decreased, suggesting the invasion of *H. japonicus* and the replacement of *P. arundinacea*. *P. arundinacea* mean cover was stable at 19.3% in October 2022 and 19.3% in June 2023, then decreased to 16.5% in October 2023, though the change between years was not significant (Table 2, Figure 3b). *H. japonicus* cover, which differed along the transect due to the study design, negatively affected *P. arundinacea* cover. *P. arundinacea* was found in quadrats within the *H. japonicus* invasion front in June 2023. However, by October 2023, *P. arundinacea* mean cover had decreased in these negative quadrats, declining from 10.6% to 5.03% (Figure 3b). Thus, *P. arundinacea*, a perennial grass, established earlier in the growing season while *H. japonicus*, an annual vine, established later but was able to outgrow *P. arundinacea* by the end of the growing season. In quadrats beyond the *H. japonicus* front, *P. arundinacea* cover decreased from 26.0% in October 2022 to 25.4% in October 2023, suggesting that *H. japonicus* was able to expand its invasion, albeit only slightly (Figure 3b).

There was also a significant interaction between canopy cover and quadrat (Table 2, Figure 4b). As *H. japonicus* cover in quadrats decreased, the relationship between *P. arundinacea* cover and canopy cover changed from a positive to negative relationship. This means that for quadrats beyond the *H. japonicus* invasion, *P. arundinacea* cover decreased with increased canopy cover, consistent with previous studies demonstrating the shade intolerance of *P. arundinacea*. However, for quadrats within *H. japonicus* growth, *P. arundinacea* cover increased with increased canopy cover, which may be due to the decreased competitive ability of *H. japonicus* in shaded but occupied quadrats.

The invasion of *H. japonicus* across the study site has resulted in the replacement of *P. arundinacea*. Prior to the invasion of *H. japonicus* around 2015, *P. arundinacea* previously dominated open-canopy areas that had been planted with acorns or left unplanted in 1998 (Matthews et al. 2020; Spyreas et al. 2010). *H. japonicus* was present at the site by 2015 and still present in 2020 (Charles 2021), though the domination of *H. japonicus* and intrusion into *P.*

*arundinacea* stands was not observed until 2022. These previous observations demonstrate a decrease in *P. arundinacea* cover as *H. japonicus* has invaded throughout the site.

Our study suggests that the invasion of *H. japonicus* in this restored wetland is ongoing. Our results show an increase in *H. japonicus* cover throughout the site between years, which corresponded with a slight decrease in *P. arundinacea* cover. However, *P. arundinacea* was able to persist as an invader at the site, which may be due to temporal niche partitioning, when species' niches are separated by time (Carothers et al. 1984). Its rapid growth and early establishment in June, compared to the later establishment of *H. japonicus* in the growing season, allows it to survive, even in October when *H. japonicus* has completely covered it. It is important to note that its invasion dynamics, specifically its interactions with *P. arundinacea* and response to canopy cover, may be different for sites that are at equilibrium, where *H. japonicus* spread has stabilized.

One concern about the replacement of *P. arundinacea* by *H. japonicus* is the alteration of ecosystem functions. *P. arundinacea* was introduced into the United States for soil stabilization and erosion control to provide aid for susceptible ecosystems (Lavergne and Molofsky 2004). *P. arundinacea* has a dense root system and spreads laterally via rhizomes (Apfelbaum and Sams 1987; Lavergne and Molofsky 2004), while *H. japonicus* is an annual plant with a shallow root system (Pannill et al. 2009). Previous work has found that under low water flow conditions, *P. arundinacea* produced adventitious roots that protected soils from erosion by higher, swifter flows (Ree 1976). The replacement of dense roots that protect waterways from erosion by shallow roots that are easily washed away by flooding events leaves soil bare and increases erosion in wetlands. Erosion has detrimental consequences for wetlands, including wetland loss (Cellone et al. 2016) and sediment mobilization (Castillo et al. 2002).

#### *Effects of H. japonicus invasion on species richness and plant community composition*

The model explaining species richness included fixed effects of year and quadrat position (*H. japonicus* cover). Species richness was significantly impacted by year, quadrat, and the random effect of transect (Table 3). Transects were established in both forested and open areas, and species richness differed among transects, with higher species richness along forested transects compared to open transects, defined by the original tree-planting treatments. In October 2022, species richness averaged 3.09 species per quadrat in forested transects and 1.54 species per quadrat in open transects, while in October 2023, species richness averaged 4.23 species per

quadrat in forest transects and 1.63 species per quadrat in open transects. Species richness also differed significantly between years (Table 3). From October 2022 to October 2023, species richness increased from an average of 2.31 to 2.93 species per quadrat (Figure 5). *H. japonicus* cover, which differed along the transect, also significantly affected species richness (Table 3). Species richness was greater where there was less *H. japonicus* cover, which includes quadrats beyond the *H. japonicus* invasion front in October 2022 (Figure 5).

Interestingly, there was an increase in species richness from year to year, even with the increase in *H. japonicus*. The increase in richness might be explained by interannual variation in weather and the general spatial and temporal variability of annual ground cover vegetation inherent to floodplain ecosystems, which can result in large fluctuations in species richness (Jonas et al. 2015). Species richness increased with higher canopy cover, most likely due to the shade intolerance of both *H. japonicus* and *P. arundinacea*. Additionally, species richness was reduced in areas where *H. japonicus* cover was greater due to its strong competitive abilities that allow it to be a dominant species (Balogh and Dancza 2008).

Not only did *H. japonicus* invasion affect species richness, it also affected species composition. There were differences in species composition along transects, as half the transect was within the *H. japonicus* invasion front and the other half was beyond the invasion front. The PERMANOVA indicated that there was a significant difference in species composition between transects and along transects in both October 2022 and October 2023 (Table 4). Transect significantly impacted species composition due to the location of the transect in forested vs. open areas. Species composition was also significantly impacted by *H. japonicus* cover (Table 4). Similarly, the NMDS plots show slight differences between quadrats invaded by *H. japonicus* and quadrats not invaded by *H. japonicus* (Figure 6). There is a tendency for non-invaded quadrats to appear on the right of NMDS axis 1, and invaded quadrats to appear on the left, which suggests some differentiation of species composition. *P. arundinacea* tended to appear on the left of the NMDS plots with invaded quadrats, while other species, such as *Sicyos angulatus* L., *Pilea pumila* (L.) A. Gray and *Galium aparine* L. (Figure 6a), and *P. pumila*, *Morus alba* L., *Bidens frondosa* L., and *Toxicodendron radicans* (L.) Kuntze (Figure 6b), appeared on the right of the plots, in quadrats not invaded by *H. japonicus* or *P. arundinacea* that form monospecific stands.

*H. japonicus* control methods

Understanding the negative relationship between *H. japonicus* abundance and canopy cover helps inform *H. japonicus* management. Our results were consistent with previous knowledge about the ecology of *H. japonicus* suggesting that it has a preference for sunlit conditions, often being found in areas with gaps in canopy cover (Pannill et al. 2009; Pasiecznik 2022). Therefore, the findings from this study support previous recommendations for *H. japonicus* management by increasing canopy cover by planting native trees (Guyon and Cosgriff 2022; Pannill et al. 2009). However, due to the climbing nature of *H. japonicus* as a vine, trees need to be fast growing to establish canopy cover quickly. Even more importantly, *H. japonicus*, if established early, could overtop, and possibly kill planted trees. A previous study by Guyon & Cosgriff (2022) looked at controlling *H. japonicus* by planting two fast-growing tree species, eastern cottonwood (*Populus deltoides* W.Bartram ex Marshall) and American sycamore (*Platanus occidentalis* L.), in combination with herbicidal treatments. They determined that planting containerized trees may be viable for long-term *H. japonicus* control, when combined with herbicide application for three to five years to allow native trees to grow tall enough and escape getting overtopped. Additionally, monitoring invaded and restored sites is essential for long-term *H. japonicus* control. Monitoring should occur later in the growing season to be effective because *H. japonicus* reaches peak cover near the end of the growing season. If monitored too early, *H. japonicus* dominance will be underestimated.

Protecting wetland ecosystems is extremely important, especially for ecosystem resilience against future invasions. To manage *H. japonicus* populations, mechanical control is often used, though this is only effective for small, concentrated populations (Guyon and Cosgriff 2022). Chemical control, including both pre- and post-emergent herbicides, can be effective (Pannill et al. 2009; Steffen and Edgin 2017), though this is only short term (Guyon and Cosgriff 2022). Additionally, for ecosystems with frequent flooding, such as floodplain forests, the effects of herbicides may be short-lived and affect native species (Guyon and Cosgriff 2022). Biological control, which is only effective when there is high host specificity, is difficult and not feasible for *H. japonicus* control due to its similarity to the native common hop (*Humulus lupulus* L.) (Guyon and Cosgriff 2022; Pannill et al. 2009; Steffen and Edgin 2017). Prescribed burns are ineffective and may even stimulate *H. japonicus* growth (Steffen and Edgin 2017). However, *H. japonicus* is an annual invasive vine, not a perennial, so controlling its seedbank, which remains

viable in soil for up to 3 years (Pannill et al. 2009; Urziceanu et al. 2022), could potentially control *H. japonicus* populations.

While managing *H. japonicus*, consequences of its removal, such as increased soil erosion in riparian wetlands, need to be mitigated. *H. japonicus* populations must be eradicated and native vegetation, including grasses and sedges that spread laterally through rhizomes to effectively hold soil must be re-established (Zuazo and Pleguezuelo 2009). Fast-growing species, including trees, are also effective at erosion control and *H. japonicus* management due to fast growth of both aboveground and belowground biomass (Burylo et al. 2012). However, care must be taken when removing invasive species and re-establishing native vegetation in invaded ecosystems. The removal of invasive species often creates a “weed-shaped hole” that allows reinvasion by the same invader or different invaders (Buckley et al. 2007). Several studies (e.g. Hulme and Bremner 2005; Magnoli et al. 2013; Pavlovic et al. 2009) have found an increase in invasive, rather than native plant species, after the removal of an invasive plant species. Therefore, there is the need to quickly re-establish native species in wetlands to not only restore biodiversity, but also prevent the reinvasion of invasive species.

#### *Future directions*

Increased anthropogenic disturbances will only facilitate the spread of invasive species into new ecosystems (Zimmermann et al. 2014). Especially for emerging invaders, limiting the introduction and dispersal of the species is more cost-effective and efficient relative to post-invasion removal (Davies and Sheley 2007). Identifying suitable habitat, and for species that are dispersed via water, determining dispersal corridors along waterways, is important for preventing their spread to new ecosystems (Urziceanu et al. 2022). Tools such as species distribution models (Srivastava et al. 2019) and invasion risk maps (Rodríguez-Merino et al. 2018; Urziceanu et al. 2022) can be used to mitigate the effects of current and future *H. japonicus* invasion.

An important part of invasive species management is restoring native vegetation while controlling invasive plant populations (Catford 2016; Funk et al. 2008). Therefore, there is a need for further studies focusing on the interactions between *H. japonicus* and species that it co-exists with, both native and invasive species, such as *P. arundinacea*. With rapid global change affecting ecosystems and species interactions (Kuebbing & Nuñez 2015), it is even more important to study how these interactions shift under variable environmental conditions to further

inform management and restoration. Continued research on *H. japonicus* is essential for a complete understanding of its ecology, role as an invader, and interactions with native species.

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### **Competing Interests**

The authors have no relevant financial or non-financial interests to disclose.

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*Consent to participate:* Not applicable.

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## Tables

**Table 1.** Analysis of variance (ANOVA) results for *Humulus japonicus* cover. Marginal  $R^2=0.43$ .

Source	df	MS	<i>F</i>	<i>p</i>
Transect	1			<b>&lt;0.001</b>
Year	1	6763.1	10.43	<b>0.0016</b>
Canopy Cover	1	3677.9	5.67	<b>0.019</b>
Quadrat (categorical)	2	8636.4	13.31	<b>&lt;0.001</b>
Year × Canopy Cover	1	3507.6	5.41	<b>0.022</b>
Canopy Cover × Quadrat	2	2380.7	3.67	<b>0.028</b>

**Table 2.** Analysis of variance (ANOVA) results for log+1 transformed *Phalaris arundinacea* cover. Marginal  $R^2=0.27$ .

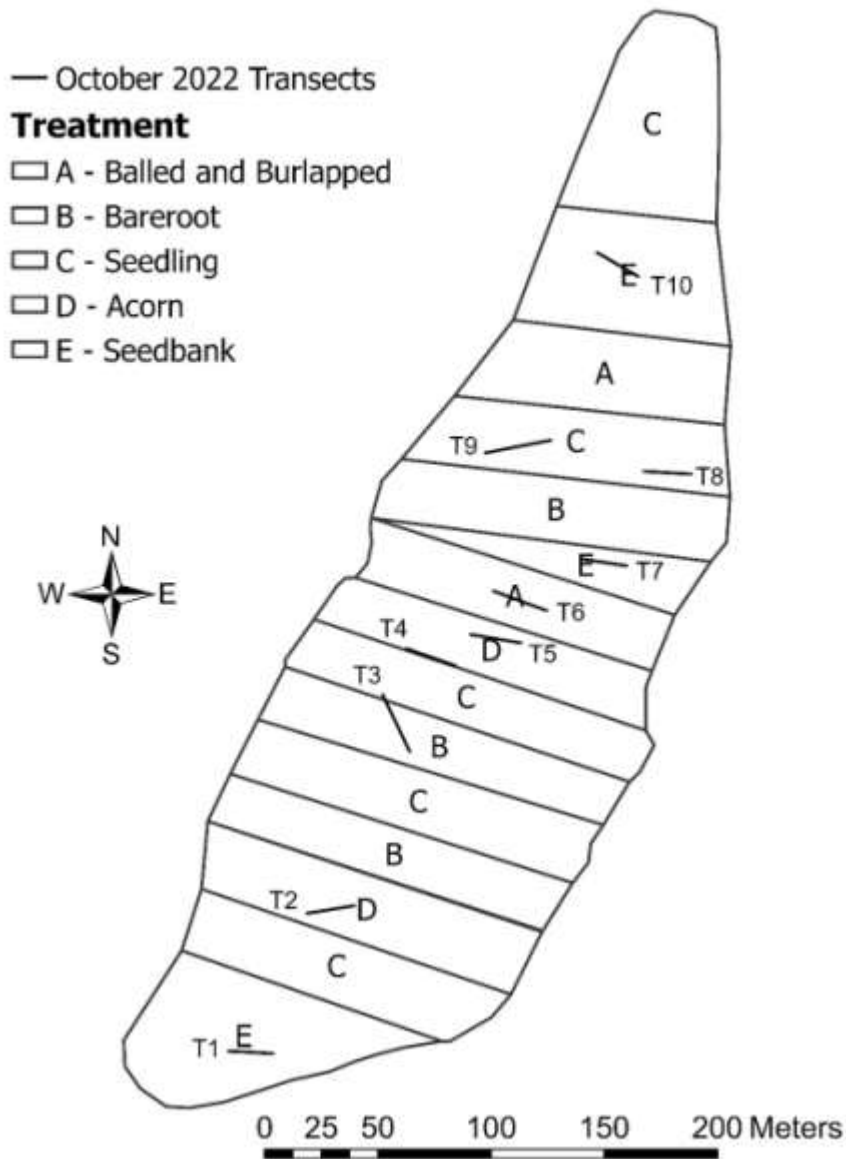
Source	df	MS	<i>F</i>	<i>p</i>
Transect	1			<b>&lt;0.001</b>
Canopy Cover	1	0.182	0.12	0.73
Quadrat ( <i>H. japonicus</i> Cover)	1	54.86	37.48	<b>&lt;0.001</b>
Canopy Cover × Quadrat ( <i>H. japonicus</i> Cover)	1	40.509	27.68	<b>&lt;0.001</b>

**Table 3.** Analysis of variance (ANOVA) results for log+1 transformed species richness. Marginal  $R^2=0.31$ .

Source	df	MS	<i>F</i>	<i>p</i>
Transect	1			<b>&lt;0.001</b>
Year	1	1.28	5.97	<b>0.016</b>
Quadrat ( <i>H. japonicus</i> Cover)	1	17.05	79.39	<b>&lt;0.001</b>

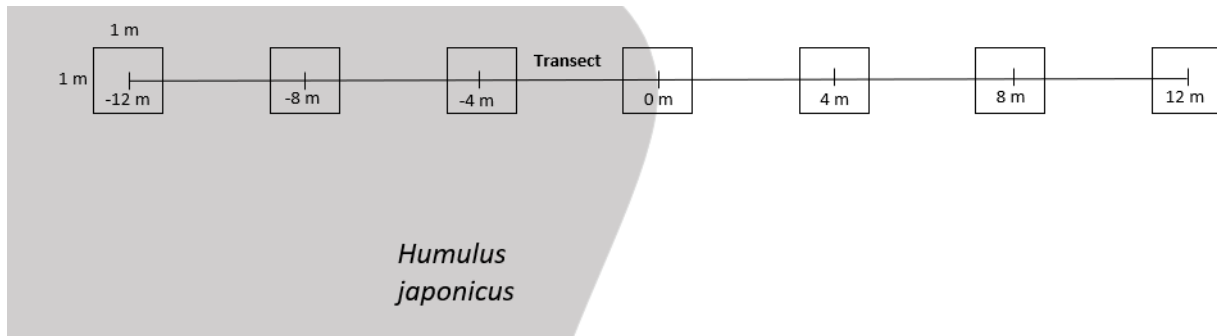
**Table 4.** Permutational multivariate analysis of variance (PERMANOVA) results for vegetation community composition differences between plots invaded and not invaded by *Humulus japonicus*.

Source	2022				2023		
	df	SS	<i>F</i>	<i>p</i>	SS	<i>F</i>	<i>p</i>
Transect	9	6.33	2.28	<b>0.001</b>	7.50	2.73	<b>0.001</b>
Quadrat ( <i>H. japonicus</i> cover)	1	2.69	8.71	<b>0.001</b>	2.22	7.25	<b>0.001</b>
Shade	1	0.25	0.80	0.50	0.38	1.23	0.27
Residual	58	17.91			17.73		

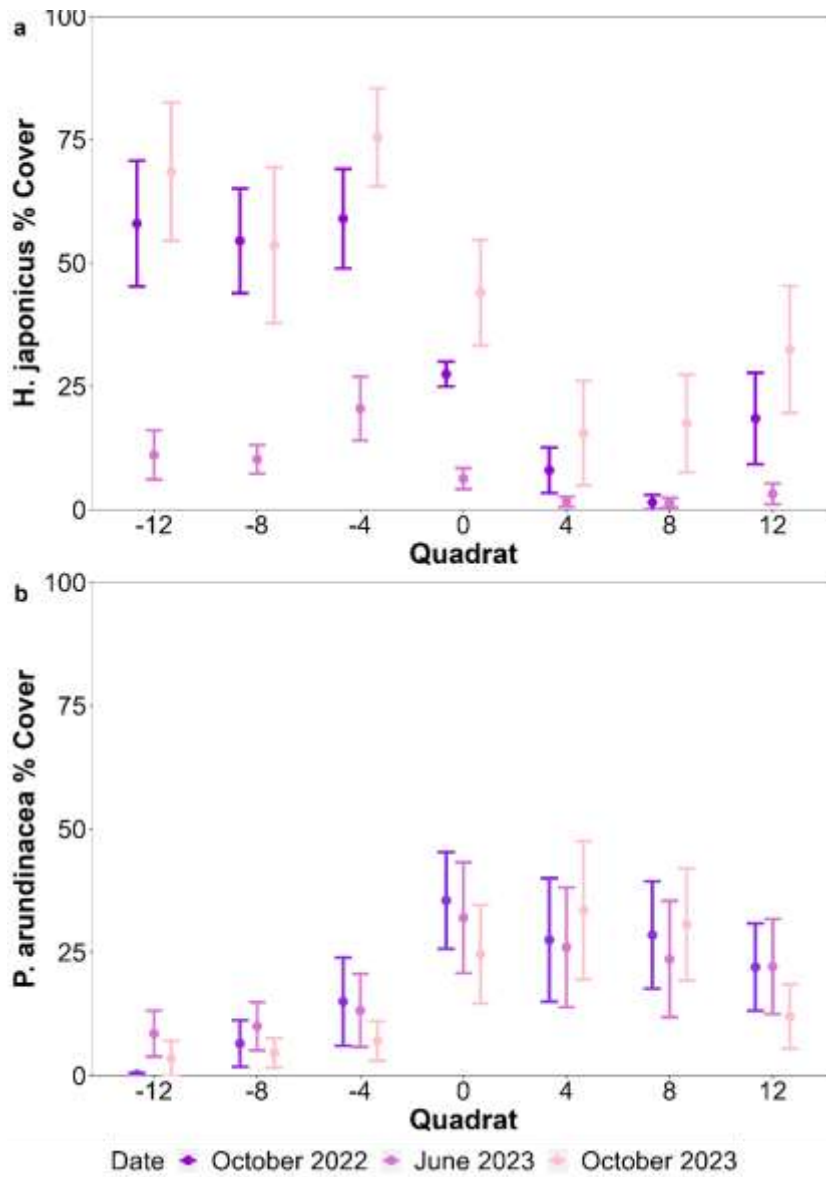


**Figure 1.** Map of field site in Joslin, Illinois, USA divided by restoration treatment: a) balled-and-burlapped tree plantings, b) bare root tree plantings, c) seedling plantings, d) acorn plantings, and e) passive restoration, or seed bank. Ten transects were located at the Joslin field site in October 2022, June 2023, and October 2023 across the different treatments. Transects were split between treatments resulting in open and forested canopies.

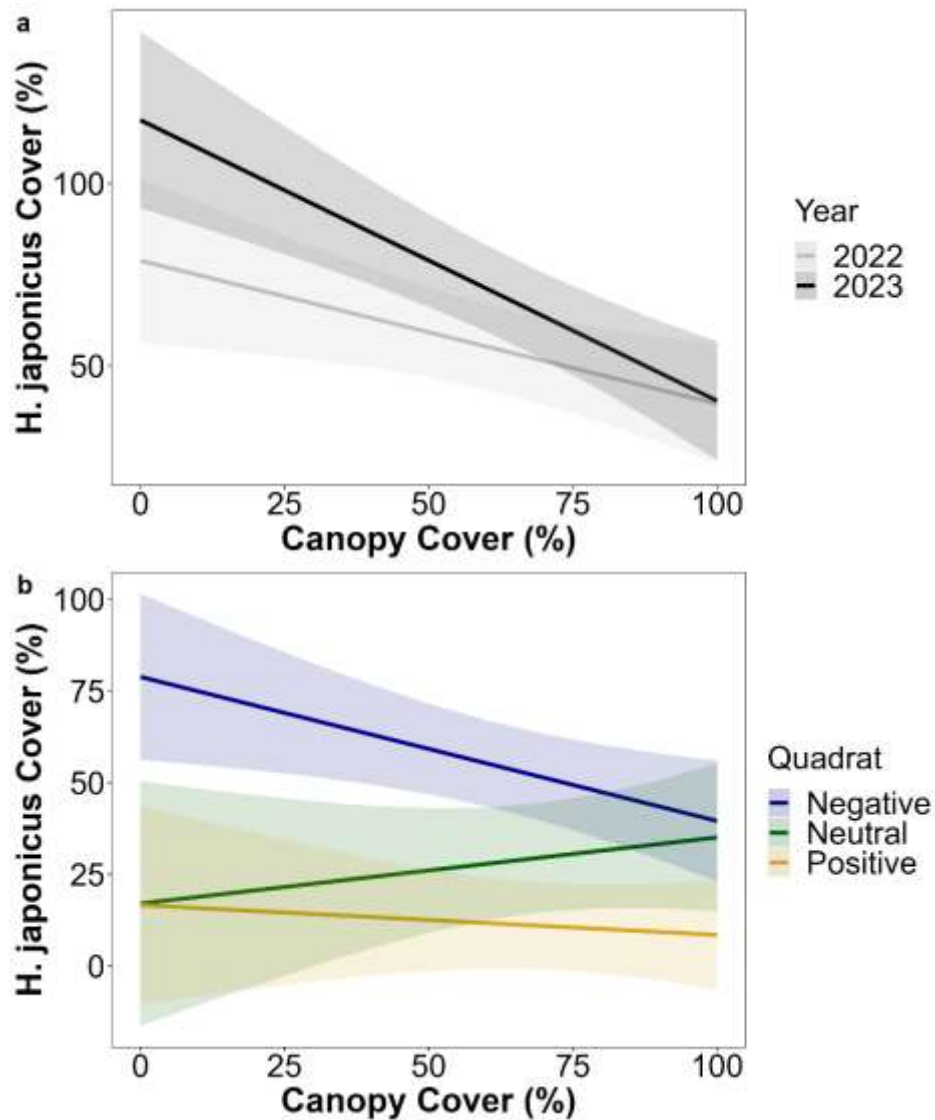




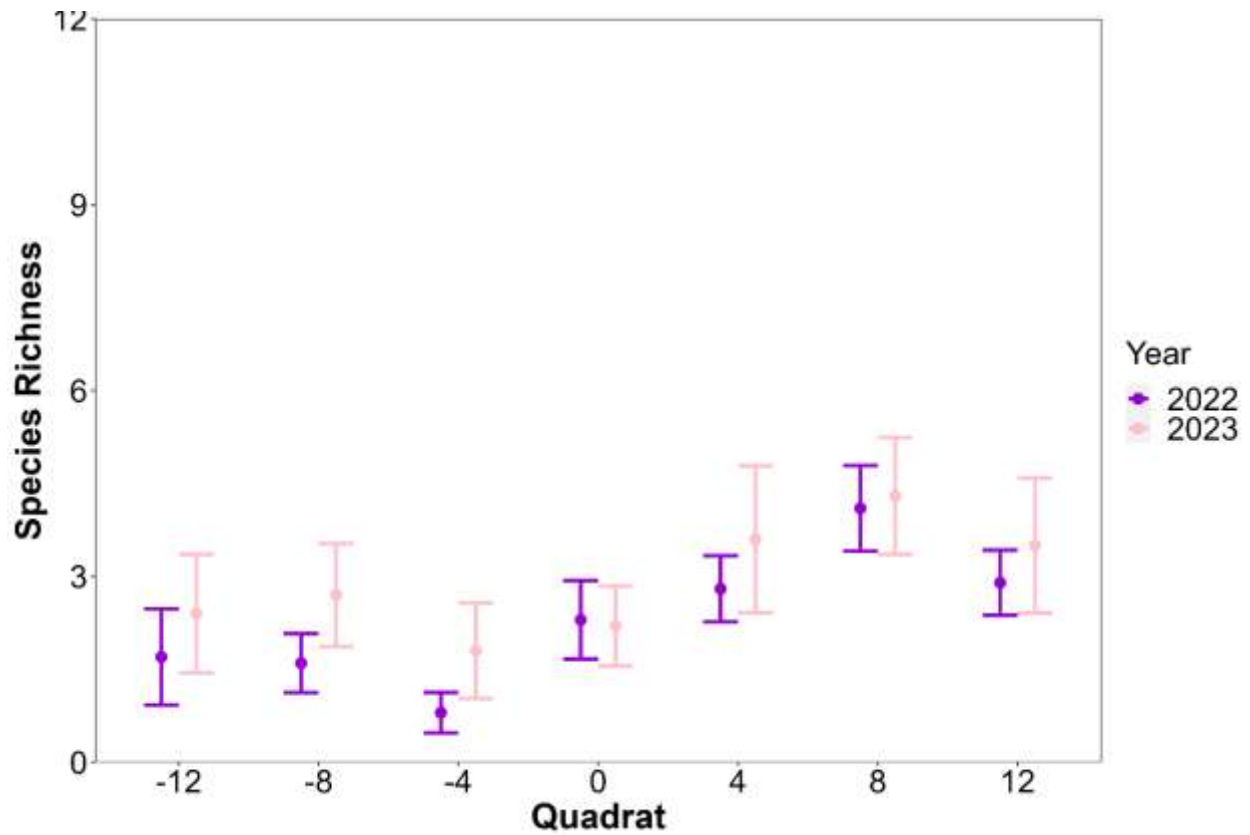
**Figure 2.** Quadrat and transect set-up for vegetation and canopy cover sampling. Quadrats were sampled every 4 meters, extending roughly east and west, into and out of *Humulus japonicus* patches. Quadrat 0 is at the invasion front of *Humulus japonicus*, whereas negative quadrats were sampled within *Humulus japonicus* patches and positive quadrats were sampled in patches where *Humulus japonicus* had not yet invaded.



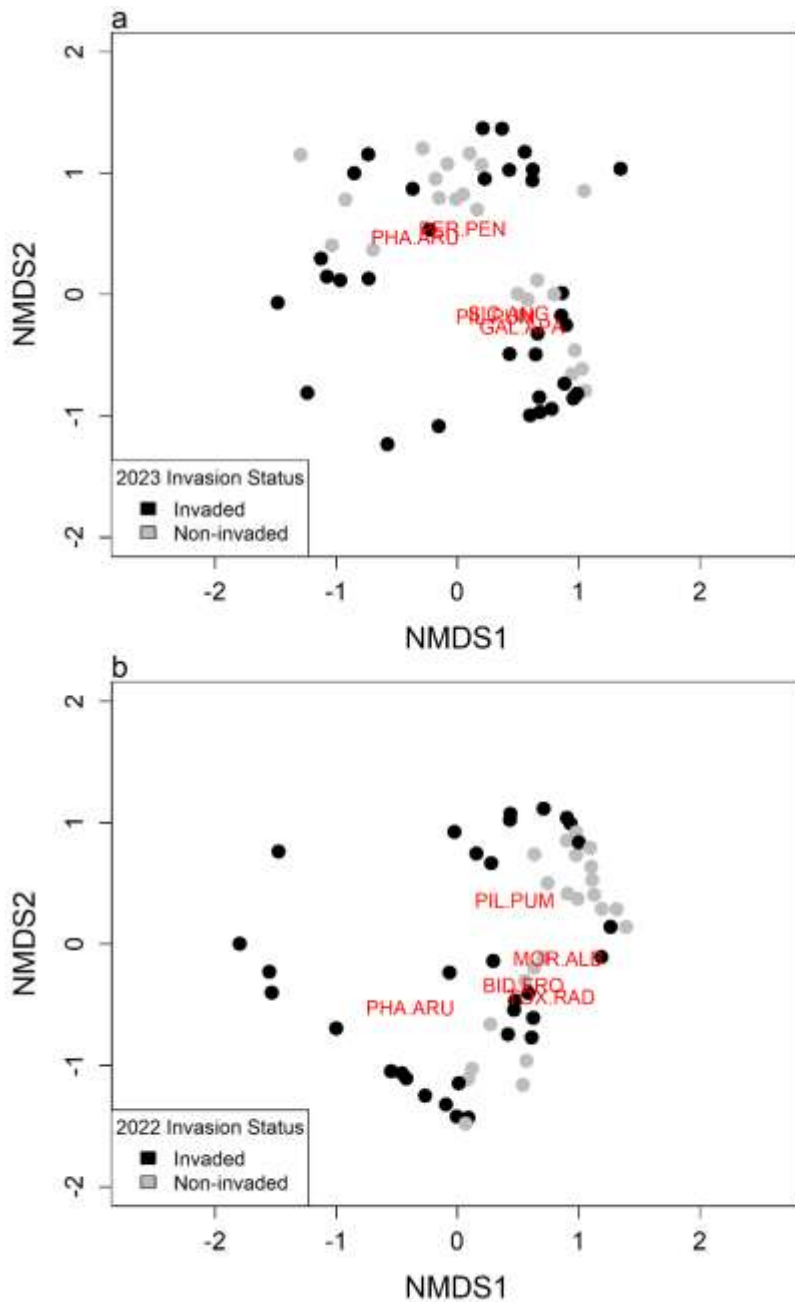
**Figure 3.** Standard error of the mean (SEM) of a) *Humulus japonicus* percent cover and b) *Phalaris arundinacea* percent cover in quadrats across all transects in October 2022, June 2023, and October 2023.



**Figure 4.** Effect plot showing *Humulus japonicus* cover in relation to canopy cover percentage in a) both October 2022 and October 2023 and b) negative (invaded in 2022), neutral, and positive quadrats (not invaded in 2022).



**Figure 5.** Standard error of the mean (SEM) of species richness in quadrats across all transects in October 2022 and October 2023. Species richness data do not include either *Humulus japonicus* or *Phalaris arundinacea*.



**Figure 6.** Non-metric multidimensional scaling (NMDS) for permutational multivariate analysis of variance (PERMANOVA) in a) October 2022 (stress=0.22) and b) October 2023 (stress =0.18). In 6a, species with relative cover >5% are shown in the plot (GAL.APA = *Galium aparine*, PER.PEN = *Persicaria pensylvanica*, PHA.ARU = *Phalaris arundinacea*, PIL.PUM = *Pilea pumila*, SIC.ANG = *Sicyos angulatus*). In 6b, species with relative cover >4% are shown in the plot (BID.FRO = *Bidens frondosa*, MOR.ALB = *Morus alba*, PHA.ARU = *Phalaris arundinacea*, PIL.PUM = *Pilea pumila*, TOX.RAD = *Toxicodendron radicans*).