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Research paper

Maximizing pollinator diversity in willow biomass plantings: A comparison between willow sexes and among pedigrees

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ABSTRACT

Shrub willow has potential to be a substantial source of renewable biomass for production of bioenergy, biofuels, and bioproducts. At the same time, scientists and land managers are increasingly challenged to achieve multiple ecosystem functions in bioenergy plantings. Due to willow's early-season bloom period, a time when natural forage for bees can be limited, biomass plantings offer a potential benefit of floral resources for pollinators, some of which are known to be experiencing recent declines. To assess which willow cultivars and sex may provide the most benefit to pollinators, we conducted a common-garden experiment (Northeast U.S.) comparing bee (Hymenoptera: Anthophila) visitation among seven pedigrees and both sexes of short rotation coppice willow. We quantified abundance, richness, diversity, evenness, and species composition in plots planted with each pedigree and sex. Over seven sampling dates, we found an abundant and diverse bee assemblage comprised of 4675 individuals representing > 56 species, at least 10 of which are rare or in decline in the northeastern United States. Willow sex was an important factor in bee visitation differences, with male willows supporting 39% greater abundance, 20% greater richness, and 9% greater diversity of bee visitors. Greater bee richness and diversity on male willows was due to an abundance-driven accumulation of species, as determined via rarefaction. Ordination analyses indicated composition of bee species assemblages differed among both willow pedigree and sex. Thus, our findings suggest male willow of varying pedigrees may be optimal for strategic plantings intended to meet bioenergy goals while also benefiting pollinators.

1. Introduction

Biomass production is an increasingly utilized means for mitigating fossil fuel use and meeting global energy demands [1]. Estimates indicate as of 2010, woody biomass production covered ~9% of the world primary energy supply and 65% of the world renewable primary energy consumption [2]. Projections show it is possible to accommodate 33% of the world's primary energy consumption with bioenergy and by 2050, 18% of the world's primary energy resources may be solely derived from agriculturally grown woody biomass [2].

Dedicated woody energy crops, such as willow (*Salix spp.*) and poplar (*Populus spp.*), grown in a short rotation coppice (SRC), can be a sustainable way to produce biomass [3]. With development of an expanding bioenergy sector, it is critical to understand how woody crop production affects land-use concerns, such as food production, biodiversity, materials production, and carbon sequestration [1,4,5]. Shrub willow (*Salix spp.*) has several attributes that make it appealing for

biomass production, including its ability to produce high yields in a few years, ease of vegetative propagation, broad genetic base, short breeding cycle, and ability to coppice (or re-sprout) after multiple harvests [3].

Woody crops have myriad potential applications [6] including: decreasing soil erosion and mitigating water pollution [7], serving as a wind/snow buffer [8], and bioremediation [9]. Sustainable bioenergy systems can also promote biodiversity [10], featuring reduced biocidal inputs, low management intensity [11], a 3–4 year harvest cycle, and minimal tillage [12]. Various studies have analyzed impacts of SRC willow agroecosystems on various groups of taxa, e.g., birds [13–15], small mammals [16], and general invertebrate assemblages [17,18]; these indicate SRC plantings provide reliable nesting, feeding, and breeding habitat. Rowe et al. [18,19], while not focusing on bees, noted high abundance of visiting Hymenoptera, suggesting willow may provide a resource for pollinators. Manning et al. [20] devised planting strategies featuring second generation bioenergy crops, including SRC

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willow and poplar, interwoven in a multifunctional landscape to maximize ecosystem services and associated biodiversity.

Pollination is a vital ecological service provided by multiple groups of taxa, with recent estimates indicating 87.5% of all flowering plants [21] and 35% of the global plant-based food supply [22] require animal-mediated pollination. Bees (Hymenoptera: Anthophila) are dominant pollinators of wild plants and crops in terrestrial ecosystems and are in need of conservation [23]. Global insect pollinator declines have been documented throughout the 20th century [24,25] and are associated with land-use change, increased pesticide use, persistence of pesticides in the environment, invasive species introductions, and spread of emerging diseases [26,27]. By and large, these land-use changes are due to agricultural intensification [28–30]. Thus, a deeper understanding of land management practices for a given agricultural ecosystem and a broader landscape design perspective can be used to enhance sustainability with regards to pollinator biodiversity. The potential of willow to provide nutrients for pollinators early in the season suggests strategic integration of SRC willow into multifunctional landscapes may promote pollinator health.

It has been proposed that willow provides important early-season nutrient resources for wild pollinator populations in North America [31]. Ostaff et al. [32] provide data indicating frequency of pollinator visitation in willow was notable, with many *Andrena* bees and flower flies (Diptera: Syrphidae) present. Pollinator preferences for male willows were documented, suggesting a protein-rich source of pollen (which is only produced by male flowers) was especially attractive [32,33]. Yet whether this preference for male flowers is generalizable across a full assemblage of pollinator species, and whether particular pedigrees of willow are more attractive than others, is currently unknown. Such information is critical if specific willow pedigrees and sex are being considered for the simultaneous goals of bioenergy production while also maximizing resources for pollinators.

This study assessed presence, abundance, and distribution of a full assemblage of bees among willow sex and several popular cultivars currently being used in bioenergy plantings. Due to known differences in resources provided between willow sexes (i.e. pollen and nectar in male flowers compared to only nectar in female flowers), we expected associated bee abundance and diversity would differ by willow sex. We also hypothesized that physical traits (i.e. relative catkin abundance and estimated biomass) among pedigrees and between willow sexes could potentially influence resources provided and therefore attractiveness to pollinators. Thus, we assessed how variation among these traits were related to pollinator assemblages. Overall, the objective of our study was to provide information to inform how to maximize both bioenergy goals and bee biodiversity in willow bioenergy plantings.

2. Materials and methods

2.1. Study system and design

The Solvay settling basins in Camillus NY are a historically significant site where deposition of waste from soda ash (Na_2CO_3) production occurred from 1881 to 1986. The soda ash facility had profound impacts on local ecosystems as result of high Cl^- , Na^+ , and Ca^{2+} ionic waste loading [34]. The site has a range of annual precipitation between 1055 and 1164 mm. In May 2013, a common-garden field experiment was established at the settling basins (lat. 43°04'02.0", long. 76°15'28.0"). A total of 9360 willows were planted representing 30 willow cultivars, selected for their growth potential to function as an evapotranspiration cover, to prevent percolation and leaching of chloride [35], and to produce biomass for renewable energy.

The shrub willows were coppiced after their first growing season to promote generation of multiple stems on each plant. Shrub willow cultivars were consolidated into seven species-related groupings (hereafter called pedigrees) and sex (Appendix A). Thirty 7.8 m × 7.9 m cultivar plots were randomly assigned in 4 blocks (n = 120). Three

double-rows of willow were planted within each plot. Double-rows were 1.8 m apart, contained 13 plants spaced 0.76 m between the double-row and 0.6 m along the row, for a total of 78 willows per plot. The plots' combined total area encompassed 0.74 ha. One cultivar plot (KP × female) was omitted from sampling and analyses due to lack of coppice growth caused by a hardpan forming in places at this site, thus limiting soil depth for plant growth.

2.2. Cultivar characteristics

On the day each cultivar plot was considered to be at "peak flowering," the plot was categorically indexed on a scale of 1–3 with 1 representing plots with < 33% of the willow stems within each sample plot having catkins, 2 with 33–66% of willow with catkins, and 3 having > 66% with catkins as an approximation of proportional catkin abundance in each cultivar plot (adapted from a previously developed scale [33]). Willows are dioecious and so sex of flowering individuals was also noted. Stem diameters were measured at 30 cm height with digital calipers to the nearest 0.01 cm for 10 plants in the middle double-row. Stem diameters and number of stems per plot were used to estimate biomass of each plot. Dry stem biomass of each cultivar plot was estimated by an allometric equation ($R^2 = 0.9760$, $p < 0.0001$), developed for a diverse set of willow cultivars at different locations following procedures from an earlier study [41], using stem diameter and number of stems per plot:

$$\text{Mass (Mg)} = e^{-2.25313 + 2.62534 * \ln(\text{diameter(mm)}) * (1003 * 10^3)}$$

2.3. Bee collection

In 2015, when willows were entering their second year of above-ground coppice growth on a three year old root system, elevated white and blue pan traps were used to collect pollinators on seven collection dates in 2015 over the 3.5 wk willow flowering period (April to May). Pan traps are efficient at providing the greatest abundance and species richness of various passive sampling methods for bees [36]. White was chosen as a color of high reflectance and blue as an alternate color, as it catches bees not caught in white/yellow traps, to optimize collection [37,38]. In each plot, one white and one blue trap were set at catkin level (usually between 1 and 2 m, n = 238), where active pollinator foraging occurs, to capture pollinator visitors throughout willow plots [37].

Pan traps were filled halfway with water and two drops of Dawn® original blue unscented dishwashing detergent were added to break the surface tension. Traps were evenly dispersed in the center double-row of each cultivar plot to avoid decreasing capture rate [39]. Pan traps were deployed between 9:00–10:00 a.m. and left for 24 h. Collection of pollinators occurred every three days from onset of flowering to inflorescence drop-off, as weather permitted. Sampling was conducted on seven dates from 4/19/2015 to 5/15/2015. Specimens were transferred to Whirl-Pak® bags and stored in 95% ethanol. As pan traps were collected, pollinators from white and blue bowls were combined to the plot level (n = 833, 119 plots × 7 days). Plots within the waste beds predominantly contained willow or other plants not yet flowering, and as previous research has documented bees having 150–600 m flight ranges [40], we do not expect bees were flying through to get to other plants during the time period sampled. Thus, the assumption is that bees collected were most likely actively foraging, but by using pan traps, our collection may include a background assemblage of bees that may not have been actively visiting willow catkins.

Collected bees (Hymenoptera: Apoidea: Anthophila) were identified using Discover Life (<http://www.discoverlife.org>), as well as Mitchell's "Bees of the Eastern United States I" (1960) and "Bees of the Eastern United States II" (1962). *Lasioglossum* (Dialictus) were identified to the subgenus level, with only some individuals identified to species, thus

Table 1

Bee species collected from 44 male and 75 female SRC willow plots established near Camillus, NY and number of different willow pedigrees in which each species was detected.

Family	Species	No. Willow Pedigree	Willow Sex		
			F	M	
Halictidae	<i>Agapostemon (Agapostemon) texanus</i> (Cresson, 1872)	2	0	2	
	<i>Agapostemon (Agapostemon) virescens</i> (Fabricius, 1775)	2	1	1	
	<i>Augochlorella aurata</i> (Smith, 1853)	7	56	46	
	<i>Halictus (Seladonia) confusus</i> (Smith, 1853)	7	352	329	
	<i>Halictus (Odontalictus) ligatus</i> (Say, 1837)	6	11	5	
	<i>Halictus (Protohalictus) rubicundus</i> (Christ, 1791)^a	7	111	88	
	<i>Lasioglossum (Dialictus) spp.^b</i>	7	1313	1007	
	<i>Lasioglossum (Evyllaes) cinctipes</i> (Provancher, 1888)	6	11	10	
	<i>Lasioglossum (Lasioglossum) coriaceum</i> (Smith, 1853)	7	22	20	
	<i>Lasioglossum (Leuchalictus) leucozonium</i> (Schränk, 1781)	7	21	19	
	<i>Lasioglossum (Sphecodogastra) quebecense</i> (Crawford, 1907)	2	2	0	
	<i>Sphecodes minor</i> (Robertson, 1898)	1	1	0	
	Andrenidae	<i>Andrena (Andrena) algida</i> (Smith, 1853)	1	0	1
		<i>Andrena (Thysandrena) bisalicensis</i> (Viereck, 1922)	6	17	22
		<i>Andrena (Conandrena) bradleyi</i> (Viereck, 1907)	3	5	8
		<i>Andrena (Melandrena) carlini</i> (Cockerell, 1901)	7	40	23
		<i>Andrena (Plastandrena) crataegi</i> (Robertson, 1893)	1	1	0
		<i>Andrena (Holandrena) cressonii</i> (Robertson, 1891)	7	10	15
		<i>Andrena (Melandrena) dunningi</i> (Cockrell, 1898)	7	61	55
<i>Andrena (Tylandrena) erythrogaster</i> (Ashmead, 1890)		5	9	5	
<i>Andrena (Andrena) frigida</i> (Smith, 1853)		7	132	89	
<i>Andrena (Trachandrena) hippotes</i> (Robertson, 1895)		7	112	112	
<i>Andrena (Cnemidandrena) hirticincta</i> (Provancher, 1888)		1	0	1	
<i>Andrena (Trachandrena) imitatrix</i> (Cresson, 1872)		1	1	0	
<i>Andrena (Andrena) mandibularis</i> (Robertson, 1892)		6	49	41	
<i>Andrena (Trachandrena) mariae</i> (Robertson, 1891)		1	0	1	
<i>Andrena (Larandrena) miserabilis</i> (Cresson, 1872)		7	26	37	
<i>Andrena (Tylandrena) perplexa</i> (Smith, 1853)		1	1	1	
<i>Andrena (Micrandrena) personata</i> (Robertson, 1897)		6	10	12	
<i>Andrena (Trachandrena) rugosa</i> (Robertson, 1891)		6	10	13	
<i>Andrena (Andrena) tridens</i> (Robertson, 1902)		1	0	1	
<i>Andrena (Thysandrena) w-scripta</i> (Viereck, 1904)		5	5	8	
<i>Andrena (Micandrena) ziziae</i> (Robertson, 1891)		1	0	2	
<i>Andrena spp.</i>		3	2	1	
Colletidae		<i>Colletes inaequalis</i> (Say, 1837)	6	29	19
Apidae	<i>Apis (Apis) mellifera</i> (Linnaeus, 1758)	3	2	3	
	<i>Bombus (Pyrobombus) impatiens</i> (Cresson, 1863)	5	11	4	
	<i>Ceratina (Zadontomerus) calcarata</i> (Robertson, 1900)	6	40	33	
	<i>Ceratina (Zadontomerus) dupla</i> (Say, 1837)	7	49	33	
	<i>Ceratina spp.</i>	1	1	0	
	<i>Nomada armatella</i> (Cockerell, 1903)	2	0	2	
	<i>Nomada cressonii</i> (Robertson, 1893)	6	12	5	
	<i>Nomada denticulata</i> (Robertson, 1902)	1	1	0	
	<i>Nomada depressa</i> (Cresson, 1863)	5	5	3	
	<i>Nomada fervida</i> (Smith, 1854)	2	1	1	
	<i>Nomada gracilis</i> (Cresson, 1863)	5	12	6	
	<i>Nomada luteoloides</i> (Robertson, 1895)	3	3	2	
	<i>Nomada parva</i> (Robertson, 1900)	1	1	1	
	<i>Xylocopa (Xylocopoides) virginica</i> (Linnaeus, 1771)	1	2	0	
	Megachilidae	<i>Osmia (Osmia) cornifrons</i> (Radoszkowski, 1887)	6	12	6
		<i>Osmia (Melanosmia) pumila</i> (Cresson, 1864)	4	5	4

Abundance of 56 + bee species collected spring 2015 from 240 pan traps over 7 sampling dates.

^a Rare species, and species known to be in decline, are bolded per Bartomeus et al. (2013).

^b (*Dialictus*): *abanci*, *anomalum*, *coeruleum*, *imitatum*, *lineatulum*, *pilosum*, among others.

Lasioglossum (*Dialictus*) was consolidated into a single species grouping for subsequent analyses (so 51 species instead of 56). This clade is widely recognized as particularly difficult to identify with little consensus as to consistent morphological identifying characteristics. To verify identifications, representative specimens were compared to expertly identified material deposited in the Cornell University Insect Collection (<http://cuic.entomology.cornell.edu/>). Voucher specimens are stored in the Entomology Museum at SUNY ESF.

2.4. Statistical analyses

One-way ANOVA was utilized to determine if the randomized block design or cultivars were significant factors in bee visitation response

variables (abundance, richness, evenness, Shannon-Wiener diversity). Bee visitation response variables assessed bees per plot across all seven collection dates. E_{var} was used as the index for evenness as it performs the best of all the evenness indices over the widest range of circumstances [42]. Each cultivar is a combination of pedigree \times sex (Appendix A). The randomized block design did not have a significant effect on bee abundance ($F_{3,118} = 0.57$, $P = 0.64$), richness ($F_{3,118} = 1.84$, $P = 0.15$), Shannon-Wiener diversity ($F_{3,118} = 2.3$, $P = 0.081$), or evenness ($F_{3,118} = 0.29$, $P = 0.83$), and so was not included in subsequent analyses.

Abundance and richness model residuals were non-normal. As a result, generalized linear models by poisson distribution were generated with willow sex and pedigree as factors associated with abundance

and richness. Due to overdispersion, abundance was transformed by natural log and instead modeled linearly. Linear models were generated with willow sex and pedigree as factors associated with abundance, diversity, and evenness. Model selection via AIC was used to determine the most parsimonious model [43]. Further, we used ANOVA to evaluate significance of our strongest model factor, willow sex. Next, because of the nonlinear relationship between abundance and richness, we used individual-based rarefaction to test effects of cumulative abundance on cumulative richness using the ‘vegan’ package in R [44].

Kruskal-Wallis and Mann-Whitney tests were used to test for differences in categorical catkin scores associated with pedigree and sexes of willow, respectively. Post-hoc Dunn's comparisons for the Kruskal-Wallis test were completed in R using the ‘PMCMR’ package [45]. Mann-Whitney was used to determine if estimated biomass was significantly associated with sex of willow.

Permutational multivariate analysis of variance (pMANOVA) using a Bray-Curtis distance matrix (100 permutations) was used to test for multivariate differences in bee species composition between sexes and among pedigrees of willow. Heterogeneity of dispersion was first checked among groups for species composition data with the betadisper function for sex ($F_{1,118} = 3.53, P = 0.063$) and pedigree ($F_{6,118} = 0.40, P = 0.88$). An NMDS goodness of fit plot was used to compare non-metric and linear fits for sex (Non-Metric $R^2 = 0.97$, Metric $R^2 = 0.92$) and pedigree (Non-Metric $R^2 = 0.98$, Metric $R^2 = 0.92$). Due to a stronger R^2 with the nonmetric fit, a nonmetric multidimensional scaling (NMDS) ordination was carried out using a data set matrix of 119 willow plots and 51 bee species to visualize differences in bee species between sexes and among pedigrees of willow. NMDS makes no assumptions as to normality in the distribution of variables [46], instead it ordines objects based on rank distances so similar objects are closer to each other, based on Bray-Curtis weighting. Overlays were based on correlations of matrix data and individual species with axes of the ordination. Length and direction of lines indicate relative significance of relationships of each species to each axis. A SIMPER analysis was conducted to identify factors contributing most to differences between sexes. Analyses and NMDS were completed in R using the ‘vegan’ package.

3. Results

A total of 4675 bees were collected and identified to the lowest taxonomic level possible (mostly species but in some cases subgenus). We found > 56 species of bees, representing 13 genera and 5 families with 17.9% ($n = 10$) being rare or known to be in decline in the northeastern United States (Table 1). Bee visitation response variables (abundance, richness, diversity, evenness) reported throughout the results assessed bees/plot across all seven collection dates.

Willow cultivars had no significant difference in mean bee species richness (10.5 ± 1.58 (SE) bees/plot, $F_{29,118} = 1.15, P = 0.31$) or diversity ($H' = 1.7 \pm 0.16, F_{29,118} = 1.41, P = 0.11$). There was an overall significant difference in abundance (39.3 ± 8.67 bees/plot, $F_{29,118} = 1.76, P = 0.023$) and evenness ($E_{var} = 0.6 \pm 0.05, F_{29,118} = 1.67, P = 0.036$), though Tukey's HSD did not differentiate significant differences in pairwise comparisons among the 30 cultivars for abundance or evenness. This is likely a result of 30 comparisons with relatively low replication ($n = 4$). Due these results, analysis was conducted on data aggregated by pedigree and sex.

In model selection analysis, sex of willow alone was the best predictor of abundance, richness, and diversity, but not evenness (Appendix B). Willow pedigree alone was less effective at predicting abundance, richness, and evenness, and was only a marginal improvement over the null model for predicting diversity.

Willow sex did have a significant effect on parameters assessed for bees. Male willows had a greater bee abundance ($F_{1,118} = 11.4, P = 0.001$), richness ($F_{1,118} = 9.31, P = 0.003$), and diversity ($F_{1,118} = 5.2, P = 0.024$), than female willows (Fig. 1). Evenness was not

significantly different among sexes ($F_{1,118} = 0.12, P = 0.73$), which implies the greater Shannon diversity index in male willows was driven by differences in richness. Willow pedigree had no significant effect on bee abundance ($38.5 \pm 5.81, F_{6,118} = 1.22, P = 0.30$), richness ($10.3 \pm 0.93, F_{6,118} = 1.58, P = 0.16$), diversity ($1.6 \pm 0.08, F_{6,118} = 2.04, P = 0.066$), or evenness ($0.57 \pm 0.03, F_{6,118} = 0.09, P = 0.50$).

To further understand differences between male and female willow, rarefaction was conducted at the plot level to determine whether abundance or specialization on hosts would more clearly explain differences in bee species richness. We found greater bee abundance in male plots drove the pattern of greater species richness because rarefied richness was not significantly different between sexes (male = 3.3 ± 0.07 , female = $3.2 \pm 0.06, F_{1,118} = 2.37, P = 0.13$).

Estimated biomass did not differ significantly between willow sexes (male = 5.0 ± 0.5 Mg/ha, female = $5.2 \pm 0.4, F_{1,115} = 0.12, P = 0.73$). Median catkin score for both male and female willows was 3, however, 17% of female plots had a score of 1, relative to 2% of male plots. This led to significantly greater catkin scores (Mann-Whitney, $W = 4048, P = 0.0035$) for males compared to female willows (Appendix D). Additionally, there were significant differences in catkin scores among willow pedigrees ($H_{6,118} = 31.6, P = 0.001$; Appendix E) with KP pedigree and KPM having the highest catkin scores (highest proportion of 3 scores) and VCC-HYB having the lowest catkin scores (highest proportion of 1s).

Ordination of the 119 willow plots in 51 bee species space indicated bee species composition was significantly different between willow sexes (pMANOVA $F_{1,118} = 2.59, R^2 = 0.022, P = 0.03, \text{Stress} = 0.16$, Fig. 2). Bee species composition was also significantly different among pedigrees (pMANOVA, $F_{6,118} = 1.43, R^2 = 0.072, P = 0.0495, \text{Stress} = 0.16$, Fig. 3). A joint plot overlay revealed species highly correlated ($r > 0.2$) with both Axis 1 and Axis 2. SIMPER analysis showed the most abundant bee species, *Lasioglossum* (*Dialictus*), *Halictus confuses*, *Andrena hippotes*, *Andrena frigida*, *Andrena rubicundus*, and *Andrena dunningi*, contributed to 2/3 of the differences in bee species found between male and female willows (Appendix C, lines in Figs. 2 and 3). Overall, SIMPER analyses among pedigrees followed the same general trend as SIMPER between sexes, with *Lasioglossum* (*Dialictus*), *Halictus confuses*, and *Andrena hippotes* as main contributors, accounting for ~50% of variability explained (Appendix C). Bee species composition differed among the seven willow pedigrees, despite no differences in bee abundance, richness, and Shannon diversity among pedigrees. Distribution of specific bee species was influenced and varied among pedigrees. However, for both sex and pedigrees, pMANOVA R^2 values were low, indicating other variables not measured influenced bee species composition.

4. Discussion

4.1. Willow trait effects on bee diversity

A primary finding in our system was significant differences in bee species associated with willow sexes. These differences were largely driven by abundance, not host specialization of visitors. Ostaff et al. [32] also found a greater abundance of bees, notably *Andrena*, associated with male willow and suggested it was likely a result of male willows providing both pollen and nectar, while females only produce nectar. This effect may be dependent on the necessity of pollen foraging to bee natural history with strong preferences for male willow early in the season to provision for offspring. While willow sexes may partition different resources and pollinators may exhibit loyalty (flower constancy) to specific species [24], cultivars and pedigrees here may have not provided distinguishing traits. Cultivars in this trial were bred and selected for yield, insect resistance, and stem form. Flower characteristics were not selection criteria in this process and relatively little is known about them, but is an area of interest for future studies.

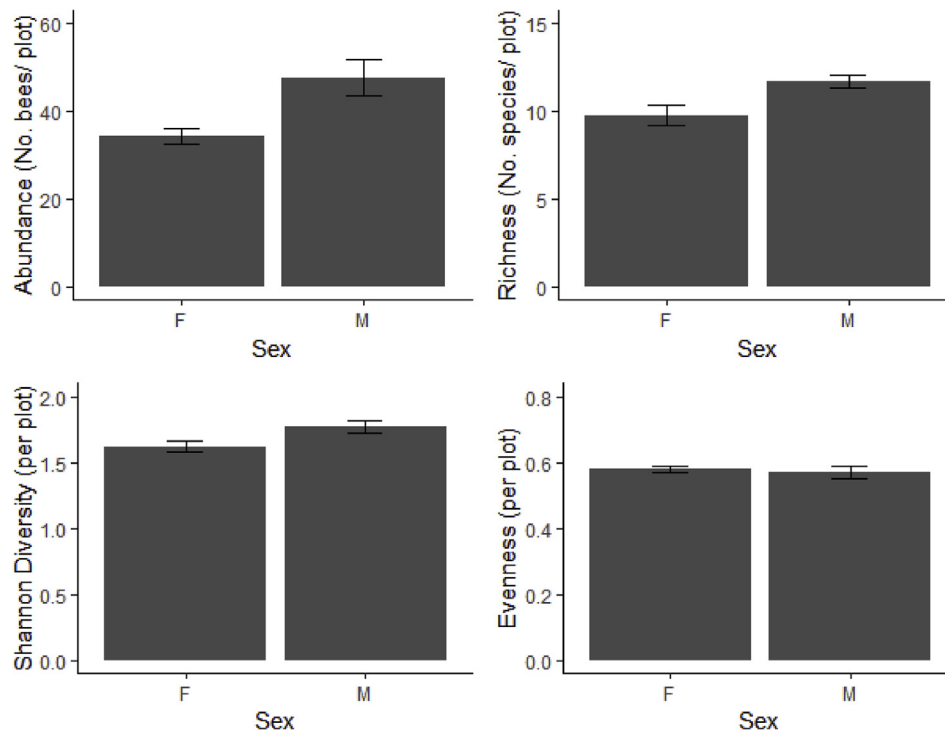


Fig. 1. Willow sex as a factor associated with bee abundance, richness, Shannon diversity, and evenness with reported means \pm SE. Bees were collected 168 h across 7 sampling dates.

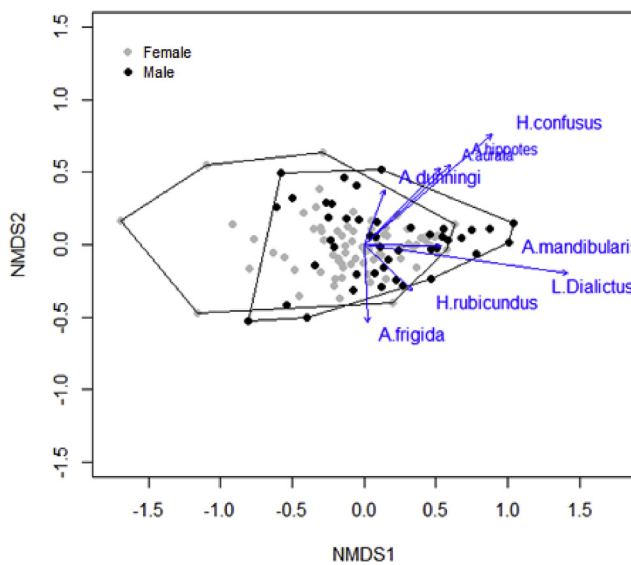


Fig. 2. Ordination of 51 bee species sampled from 44 male and 75 female willow plots in an SRC willow planting near Camillus, NY. Lines indicate species association with axes ($r > 0.20$) using a joint plot overlay.

Proportion of stems with catkins was significantly different between sexes and among pedigrees. This may indicate forage availability [33], which is supported further as males had a significantly greater catkin score than females. Proportion of stems with catkins may have played a role as bees might be more likely to encounter male catkins simply because male plots had more catkins. While proportion of catkins per plot may have a role, as indicated by our analyses, other factors such as mean number of stems per stool, mean catkins per stem [33], and/or catkin color may drive responses in visitation by particular bee species. Willow cultivars used in SRC systems generate a wide range of number of stems per stool [47] or per hectare (27, 885–72, 115 stems ha^{-1})

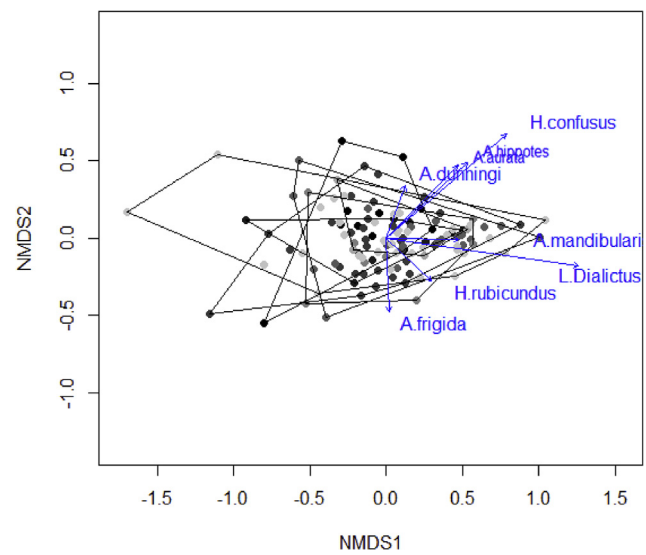


Fig. 3. Ordination of 51 bee species among 7 pedigrees of willow. Total area occupied by each pedigree is visualized by polygons. Species composition differed significantly among pedigrees. Lines indicate species association with axes ($r > 0.20$) using a joint plot overlay.

[36]. Despite that, estimated stem biomass (Mg/ha) did not appear to differ significantly between sexes after one year of aboveground growth. In some systems plant biomass has been positively correlated with diversity [48].

4.2. Bees

SRC willow cultivars supported an abundant and diverse bee fauna, several of which are rare or known to be in decline. Due to our consolidation of *Lasioglossum* (*Dialictus*), our finding of > 56 species associations is very likely an underestimate. In published studies of bee

diversity in agroecosystems [49], few mass flowering crops have a comparable or greater bee richness to what we found in SRC in this study (e.g. apple, blueberry). High bee biodiversity levels can ensure plant-pollinator phenological synchrony and pollination function [50]. In some cases, these wild bee species may be dominant flower visitors of crops [51]. *Andrena* bees, one of the dominant genera we found with several declining species, have been shown to be more effective at pollination of some crops than honey bees [52]. *Halictus rubicundus*, another declining species we captured, is also an efficient crop pollinator [53]. Planting forage habitat for bees adjacent to pollinator-dependent agriculture can conserve wild pollinators in resource poor agricultural landscapes [54].

SRC has been shown to increase vegetation and arthropod abundance and diversity when replacing annual biomass crops [55]. Integration of biomass crops into agricultural landscapes and other managed landscapes could provide a wide range of ecosystem services [20,56], including nesting and forage habitat, both of which are necessary for subsistence of native wild pollinators. Furthermore, timing of willow bloom, which is immediately prior to bloom of several economically important tree fruit crops, e.g., apple and cherry, could result in spillover of pollination services [57]. It is of note, however, that pollen grains of *S. brachycarpa-glauca* are small and can form a film clogging the stigma surface and thus interfering with germination of conspecific pollen of *Polemonium viscosum* [58], which may be potentially detrimental if there is overlap in bloom period. While little is known about this kind of interaction among willow species in this study and fruit trees, further studies of benefits and potential drawbacks associated with integrating willow in agricultural landscapes with fruit crops and other bee pollinated species is needed.

4.3. Management recommendations

While it is prudent to include pedigree variation in plantings, contrary to other findings [33], our results indicated plantings intended to support flower-visiting insects can be solely male. Though seeds from females may provide a source of nutrients for other animals [16], willow seed lifespan is very short, and we found fewer pollinators in samples from female plots. Planting only one sex of willow may also curtail feral population establishment risk. This risk is low though as a disproportionate number of the highest yielding SRC willow genotypes in U.S. breeding programs are triploid. Since willows are dioecious they must outcross to form seed, and although triploids have been shown to produce viable progeny through crosses with a diploid or tetraploid pedigrees, this occurs rarely [59], thus, it appears there is minimal risk of willow hybridizing with native willow species.

Effects on bee abundance, richness, diversity, and species composition in our study were driven in part by heterogeneity provided by willow sexes and pedigrees. Some of the unexplained variation presented here may be due to landscape scale effects, e.g., plant composition, habitat configuration. Furthermore, local and landscape factors interact and likely also influence abundance, richness, diversity, and bee species composition [49]. Agricultural intensification can change bee species composition towards fauna dominated by common taxa [60]. Regardless, a network of floral resources is required throughout the season to support wild pollinators, not just when commercial crops are in flower [61]. Restoration of habitat heterogeneity is also important to restore biodiversity in agricultural landscapes [48]. Wild bees can be responsible for a majority of crop flower visitation and some of the species we found associated with SRC willow are known to be in decline. Additionally, SRC willow was visited by many of the same wild bee species known to pollinate local New York commercial crops. As such, we agree with Ostaff et al. [32] that addition of willow to managed landscapes, perhaps as buffer strips, would be beneficial as early season nutrient resources at a time of limited alternatives. These buffer strips have the potential to also provide other services, e.g., mitigation of nonpoint source pollution from agricultural fields [62].

Our results indicate sex and pedigree of SRC willow plantings influence abundance, richness, and diversity of bees. Elucidating bee species associations with SRC willow has management implications for supporting biodiversity. Our recommended approach to managing willow and optimizing ecosystem services for pollinators is to predominantly plant male willows, thus minimizing unintended dispersal of non-native willow cultivars while maximizing pollinator visitation. If possible, we also recommend establishing a matrix of plots with non-simultaneous harvest cycles to ensure flowering across years [33], and to delay harvest until after willow flowering occurs in the early spring. Further information on pollinator interactions with specific cultivars as well as knowledge about differences in quality and quantity of nectar and pollen are needed to develop more specific recommendations.

With numerous problems facing bees and concerns with sustainably meeting global energy demands, SRC willows offer an opportunity to develop multifunctional landscapes supporting bees, providing other ecosystem services, as well as producing biomass as a form of renewable energy. While this research provides useful insights into pollinator interactions with SRC willow, an improved understanding of pollinator interactions with willow and other agricultural crops across the landscape is needed to inform best management practices for incorporating willow into multifunctional agricultural ecosystems. For example, edge plantings of willow may support nesting activity and enhanced food resources [63] and so use of willow as buffer strips or wind breaks needs context-based comparisons with alternate land enhancement strategies [12]. Pollinator fauna of agroecosystems can be diverse and challenging to thoroughly sample [64] and so additional pollinator sampling in SRC shrub willow systems may identify additional declining bee species positively associated with SRC crops. Additionally, we recommend research on unintended effects of other ecosystem services (e.g., phytoremediation) to ensure one service is not provided at the expense of another. The design of multifunctional landscapes requires balancing different services provided to maximize overall benefit. Additional benefits to pollinators when willow is introduced into the landscape are just being elucidated and will need to be balanced with other services provided by willow across the landscape.

5. Conclusions

This study is one of the first to identify bee species (Hymenoptera: Apoidea: Anthophila) and investigate preferences of bees in SRC willow grown for biomass production in North America. We verified willow sex was an important factor in bee visitation differences, with male willows supporting 39% greater abundance, 20% more richness, and 9% greater diversity of bee visitors. Bee species composition also differed among willow pedigree and sex and differences between sex were driven by abundance, not host specialization. Our findings suggest male willow of varying pedigrees is optimal for strategic plantings to combine bioenergy production goals, maximize pollinator diversity, and provide resources for rare and threatened pollinator species.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.biombioe.2018.07.013>.

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