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Roe deer prefer mixed-sex willow stands over monosexual stands but do not discriminate between male and female plants[☆]

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ABSTRACT

Male and female plants of dioecious species often experience differential herbivory, possibly due to differences in defences such as secondary metabolite composition or nutritional quality. These plant sex effects on herbivory have been extensively studied for plant individuals, but not for stands/populations. For mobile herbivores, such as deer, stands may be a more relevant scale to study than individual plants. We predicted that male *Salix viminalis* plants should be subject to more extensive roe deer (*Capreolus capreolus*) browsing than female plants due to weaker defence in male plants. Furthermore, we expected that mixed-sex stands should experience more damage than monosexual stands due to positive effects of diet mixing on browsing by generalists. We tested for differences in roe deer browsing in plots that were either monosexual male or female, or a mix of male and female plants in a replicated field experiment. Roe deer browsing was estimated after one growth season with heavy herbivory. We also measured plant secondary metabolite concentrations and nitrogen content in leaves from all experimental clones to test the assumption that the sexes differed in defence or nutrients. Mixed-sex plots were more extensively browsed than monosexual plots. However, there was no difference in browsing between male and female plant individuals within mixed-sex plots or between monosexual plots. Plant secondary metabolite profiles differed between male and female plants, while nitrogen content did not. Our findings suggest that the diversified plant secondary metabolite contents of mixed-sex plots may have led to more extensive herbivory. Higher browsing of plant sex mixes may impact both natural and commercial *S. viminalis* stands with different sex ratios.

1. Introduction

Sex-biased herbivory is common for plants and has long received attention (reviewed by Cornelissen and Stiling, 2005). However, reported studies of sex-biased herbivory have mainly dealt with effects on individual plants while effects over larger spatial scales, such as stands or populations, are not well-understood. Investigating plant sex effects at larger spatial scales is important, because plant sex ratios are often skewed and vary among species, populations, and stands, ranging from strongly female- to strongly male-biased (Barrett et al., 2010). Strong biases can especially be found within vegetatively reproducing species (Alliende and Harper, 1989). Male-biased sex ratios are more common than female-biased sex ratios in dioecious plants but biases in both

directions, and of sex ratios in equilibrium, are common (Field et al., 2012). Sex-biased abundances are at least in part dependent on genetic factors in several dioecious species (Alström-Rapaport et al., 1997; Åhman, 1997; Barrett et al., 2010). However, sex-biased abundances can also be explained partly by a higher mortality among plants of either sex (Lloyd, 1974), possibly due to herbivory (Elmqvist and Gardfjell, 1988).

An important difference between male and female plants of dioecious species can be in how well-defended they are against herbivores. For example, female and male dioecious plants often differ in plant secondary metabolite concentrations (e.g. Nybakken and Julkunen-Tiitto, 2013), and females tend to be better-defended both chemically and structurally (reviewed by Cornelissen and Stiling, 2005). Because

female plants have higher reproductive costs (reviewed by Obeso, 2002), they are expected to grow more slowly (Lloyd and Webb, 1977). This can at least partly be explained by the Resource Availability Hypothesis, predicting that defence, growth and reproduction are traded off against each other (Coley et al., 1985). Meanwhile, a higher reproductive effort is often associated with a higher resource allocation to defence at further cost of growth, leading to the expectation that females are better-defended than males. Differences in defences has in turn been suggested as an explanation for why female plants often experience less herbivory than males (e.g. Cornelissen and Stiling, 2005).

Foraging choices by herbivores should balance intake of plants in a way that provides necessary nutrients while minimizing ingestion of harmful compounds (Marsh et al., 2006). Freeland and Janzen (1974) proposed that generalist herbivores diversify their diets in terms of toxin contents through consuming a diversity of plant species because they have a limited capacity to process high amounts of any particular plant secondary metabolite (the Detoxification Limitation Hypothesis). Studies of generalist herbivore diet choices and physiological effects of mixed diets across plant species have provided some support for the Detoxification Limitation Hypothesis (e.g. Dearing and Cork, 1999; Miura and Ohsaki, 2004; Unsicker et al., 2008; Pankoke et al., 2012; Sotka and Gantz, 2013). Thus, one may hypothesize that intersexual differences in defensive plant secondary metabolite content may lead to differences in browsing patterns across stands of different sex ratios. If total plant secondary metabolite concentration is important for herbivores, and female plants contain higher levels of secondary metabolites, monosexual female populations or stands should be subject to less herbivory than mixed or male ones. If, on the other hand, avoiding accumulation of single compounds is more important for herbivores, mixed-sex stands should experience more herbivory than monosexual stands.

We investigated how plant sex composition affects roe deer (*Capreolus capreolus* L.) browsing patterns in stands of a dioecious shrub used for bioenergy production, the common osier (*Salix viminalis* L.). Commercial plantations of *Salix* are often monoclonal and thus monosexual. Roe deer is a generalist that can cause serious damage to both wild *Salix* spp. plants as well as *Salix* short rotation coppice in Europe (Bergström and Guillet, 2002). We hypothesized that roe deer prefer male plants to female plants, and that a sexually mixed diet would be preferred to a monosexual diet. We expected male and female *S. viminalis* to differ in defence because it belongs to a genus where intersexual quantitative differences in plant secondary metabolites are well-documented (Nybakken et al., 2012; Ruuhola and Nybakken, 2013; Nybakken and Julkunen-Tiitto, 2013). We recorded natural browsing intensity in experimental *S. viminalis* field plots either with only males, only females or a balanced sex ratio and related herbivory patterns to intersexual variation in plant secondary metabolites. In addition, we measured leaf nitrogen (N) content as a proxy for nutritional quality. We tested the predictions that (i) female *S. viminalis* contain higher total plant secondary metabolite concentrations and (ii) individual male plants in mixed-sex stands are browsed to a higher extent. We also predicted (iii) that plant secondary metabolite profiles differ between sexes and (iv) that secondary metabolite diversity is higher in sex mixes. Finally, we tested the Detoxification Limitation Hypothesis-based prediction (v) that plant sex mixes are more extensively browsed than monosexual stands.

2. Materials and methods

The common osier (*S. viminalis* (L.)) is a dioecious shrub-like tree species that is naturalized in large parts of Europe and used in short rotation coppice forestry for bioenergy production. Natural populations and commercial plantations are often subject to herbivory from insects and mammals, and the first year is a critical phase because biomass removal, especially during summer, can reduce biomass production substantially (Guillet and Bergström, 2006). Previous studies have

established variation between sexes in terms of plant secondary metabolites in other *Salix* spp.; female leaves of *S. lasiolepis* (Boecklen et al., 1990) and *S. pentandra* (Hjältén, 1992) have been reported to have higher concentrations of total phenolic substances than males. However, there are also examples of the opposite pattern within the genus: Nybakken and Julkunen-Tiitto (2013) found that total phenolic acid concentrations were higher in male than in female *S. myrsinifolia*. The latter study also found that leaves from females contain higher total concentrations of salicin and SaOH-diglucoside than leaves from males. In addition to leaf chemistry, shoot tissue plant secondary metabolite concentrations often vary between sexes of *Salix* spp.; total phenol concentration is higher in female *S. lasiolepis* (Price et al., 1989) and salicortin concentrations have been found to be highest in stems of females of both *S. rigida* (Elmqvist et al., 1991) and *S. myrsinifolia* (Nybakken and Julkunen-Tiitto, 2013). For the present study we used cuttings of 20 female genotypes and 19 male genotypes (20 males collected, but one was later excluded − see explanation below) collected in March 2013 from clone archives holding clones originally collected in other locations in Sweden, outside Uppsala, Sweden (Latitude: 59°80, Longitude: 17°66). The cuttings were stored in −5 °C dark rooms until planted in fields and a greenhouse.

Roe deer (*Capreolus capreolus* (L.)) is a common herbivore in large parts of the natural and naturalized range of *S. viminalis* and causes extensive biomass losses in *Salix* short rotation coppice plantations (Dimitriou et al., 2011). Fencing commercial *Salix* short rotation coppice plantations is an expensive management option and it is therefore important to find a method to reduce the negative impact of roe deer on the economic sustainability of bioenergy production. Roe deer avoids high total tannin concentrations and it has been suggested that they actively avoid high concentrations of single toxins by diversifying their diet (Verheyden-Tixier and Duncan, 2000), which would imply detoxification limitations.

The study area (Latitude: 58°83′N, Longitude: 17°78′E), is located east of Uppsala, Sweden. It is an open agricultural landscape surrounded by mixed coniferous and deciduous forests. Adjacent to the experimental area were agricultural fields with crops that included *Hordeum vulgare*, *Brassica napus*, and *Trifolium* spp. We frequently observed roe deer in, and in the proximity of, the experimental plots throughout the growing season. The other herbivores, which caused much less of the visible damage, included the European hare (*Lepus europaeus*) and various insect herbivores, most of which were coleopterans and lepidopterans. Hare browsing is easily distinguished from roe deer browsing by the former leaving cleaner, angled, cuts on lower parts of stems (Åhman and Bertholdsson, 2001). We observed this on plants that had died during the course of the field experiment and on few surviving plants.

To quantify plant secondary metabolite concentrations, and investigate potential differences between plant sexes and clones, we analysed leaf chemistry of greenhouse grown *S. viminalis* clones. We planted cuttings collected and stored as for the field experiment in January 2014. Five cuttings per genotype were planted in individual plastic pots (11 × 11 × 12 cm, Göttinger) with planting soil (Hasselfors™). Prior to planting, the soil-filled pots were heated in groups of six in a microwave oven at 900 W for 6 min to prevent soil contamination by microorganisms or plant seedlings from affecting secondary metabolite production or growth of *S. viminalis* plants, thus equalizing initial growth conditions. We grew plants randomly placed on trays in a greenhouse at 20° C with 18 h of light per day and collected fully expanded single leaves from each plant after 6 weeks. The leaves were dried for two days in 18° C, and milled using a ball mill (CryoMill, Retsch) with three 12 mm diameter steel balls for 2 min. We divided milling time over two rounds, with a one minute pause in between rounds, to avoid high temperatures that could potentially alter chemical composition. We stored the leaf material in transparent plastic vials in a cardboard box until extraction. 29 plants were not large enough after six weeks; these were either grown for an additional four

weeks before leaves could be collected or replaced with new plants from which leaves were collected after four weeks. One female and four male genotypes (78054, 78195, 79036, 80019 and Öland 7) were excluded from the chemical analysis because they had been identified as genotypically identical to others or incorrectly sexed in the clonal archive. The excluded genotypes were replaced by genotypes (77683, 81092, 811208, Anki and Orm) collected from the same experimental gardens. Genotype 81092 was later excluded because the species was incorrectly determined. The new genotypes were grown separately one month after the first leaf collections using the same methods as above. Secondary phenolic metabolites were extracted using a Precellys-homogenizer with 100% methanol and analysed with HPLC as described in Nybakken et al. (2012).

To quantify N content in leaves as a measure of nutritional quality, we planted three plants of each clone in conditions similar to those for plants used in plant secondary metabolite measurements. We measured SPAD-values, representing absorbance by chlorophyll, which are closely correlated to N concentration in *Salix* leaves (Weih and Rönnberg-Wästjung, 2007; Liman et al., 2017) using a chlorophyll meter (Minolta SPAD 502 Plus) after six weeks. The same five clones as excluded from plant secondary metabolite analysis were excluded from the statistical analysis but not replaced, since the decision of replacing the five clones was made after termination of the experiment.

In May 2013 we planted 30 plots, each with 40 cuttings, using two cuttings of each of the same 20 genotypes as used in the greenhouse, in an open agricultural landscape outside of Uppsala, Sweden. Each plot measured 10 × 10 m. We planted the *S. viminalis* cuttings in rows with distances of 70 cm, a distance used in many commercial plantations (Moritz et al., 2016), between each cutting and row. To exclude competition and ensure establishment of the plants, we covered the ground between and around the cuttings using a weed control mat in each plot. We divided the experiment's 30 plots into ten spatially coherent blocks, each containing three plots. Within each block we randomized three sex ratio treatments among plots: (1) female only, (2) male only, and (3) an even sex mix. Thus, each block contained one all-female plot, one all-male plot, and one mixed-sex plot. The sex mixes were random compositions of 10 genotypes of same genotypes as for the monosexual plots per sex. Each plot consisted of seven rows, each with six plants, except for the last, which had four plants (Fig. 1). Because all plants had been browsed (Fig. 1), we used remaining plant height of the plants as a proxy for resource-depletion by the browsers. We measured the plant heights in October 2013. Because the extent of hare *L. europaeus* browsing was negligible we ascribed all effects on height to roe deer browsing.

The statistical software R 3.2.2 (R Development Core Team, 2015) was used for all analyses. We performed a canonical correspondence analysis to compare male and female plant secondary metabolite profiles. The canonical correspondence analysis model was created using the cca() function in the "vegan" package and included concentrations of the 19 compounds that were found in all samples. Plant sex and clone were used as factors. We used a permutation test (n = 9999) to test for significance of each factor. The CCA figure was created using the "ggplot2" package for R. We used mean values of CCA1 and CCA2 coordinates for each clone to demonstrate what effects mixing clones of the two sexes has on variation in secondary metabolites. To investigate differences in dispersion between monosexual or mixed-sex plots, we calculated the standard error values for vectors containing double sets of all clone mean CCA coordinates for males, and similar vectors for female clone coordinates. In addition, we calculated standard errors for CCA1 and CCA2 coordinates for an average mixed-sex plot by using vectors of double sets of 10 random clones of each sex, averaged over 10.000 iterations.

To compare concentrations of individual compounds, and to compare total phenolic and lignan concentrations, in male and female plants we used Linear Mixed Models. All linear mixed models were produced and tested using the lmer() function in the "lme4" package

Fig. 1. Photography of browsed Salix viminalis plant in one of the experimental plots. In front of the plant, roe deer (Capreolus capreolus) hoof prints are visible. Photo credit: Kim K. Moritz.

and *type II Wald chi-square* tests using the Anova() function in the "car" package. Log-transformed concentrations were used as response variables, sex was used as a fixed factor and clone as a random factor. Because an analysis showed that all replaced plants except for 81092, for which the morphological and chemical profile indicated a different species than *S. viminalis*, were not different from other genotypes within the same sex, four of the five replacement genotypes were kept in the analysis. To test for differences in N-content, we compared male and female plant SPAD values in a linear mixed model, using SPAD values as a response variable, with sex as a fixed factor and clone as a random factor.

We used a Linear Mixed Model for testing the main effect of plot sex ratio treatment on mean plant heights after one growing season as a proxy for roe deer herbivory, using the lmer() function in the "lme4" package for R. We used log-transformed mean heights for each plot as a response variable, plot sex ratio treatment as a fixed factor, and block as a random factor. We tested the model for plot sex ratio treatment effects using the Anova() function in the "car" package for R. To visualize differences between plot sex ratio treatments we produced a figure showing means and standard errors of the log-transformed data using the "ggplot2" package.

We tested for individual plant sex effects on herbivory using a mixed linear model using the lmer() function in the "lme4" package and testing it using the Anova() function in the "car" package. Surviving individual plants' heights were log-transformed and used as a response variable, plant sex as a fixed factor and plot nested within block as a random factor.

To test for effects of sex ratio effects and individual sex on survival we used individual survival as a response variable, sex ratio treatment and individual sex as fixed factors, and plot nested within block, and genotype, as random factors in a generalized linear model with a binomial error distribution and a log-link. Because we expected plant survival to be related to browsing, we also performed a Pearson correlation test between average survival and height for clones used in the

Table 1

Secondary metabolites in Salix viminalis leaves. Female and male estimates are concentration estimates (mg/g) from linear mixed models of sex differences. χ2 and P-values (bold if significant) are results of effects of sex in linear mixed models (degrees of freedom = 1 for each model). Sex is the plant sex with higher concentrations of a compound.

Compound	Female estimates	Male estimates	χ^2	P	Sex
(+)-Catechin	1.20117	0.90069	0.57	0.45	NA
Hyperin	0.13451	0.11151	0.25	0.61	NA
Isorhamnetin 3-acetylglucoside	0.14703	0.15115	0.01	0.94	NA
Isorhamnetin aglycone	0.03552	0.01439	5.56	**0.018**	Females
Kaempferol 3-glucoside	0.00154	0.00114	0.66	0.42	NA
Kaempferol 3-rhamnoside	0.06518	0.03078	2.31	0.13	NA
Kaempferol-aglycone + unidentified myricetin derivative	0.05292	0.02240	8.38	**0.004**	Females
Monocoumaroyl-astragalin	1.23376	0.91867	0.86	0.36	NA
Myricetin 3-galactoside	0.01531	0.01249	0.79	0.37	NA
Myricetin 3-rhamnoside	0.02915	0.01492	3.95	**0.047**	Females
Neolignan	0.04425	0.04607	0.04	0.85	NA
p-OH-cinnamic acid derivative	0.26895	0.24142	0.01	0.91	NA
Protocatechuic acid	0.00031	0.00028	0.30	0.59	NA
Quercetin 3-arabinoside	0.00691	0.00320	0.72	0.40	NA
Quercetin 3-glucuronide	0.00467	0.00315	2.25	0.13	NA
Quercitrin	0.03627	0.01869	2.25	0.11	NA
Unidentified chlorogenic acid derivate	0.02503	0.02227	0.03	0.86	NA
Unidentified lignan 1	0.04000	0.03933	0.01	0.94	NA
Unidentified lignan 2	0.00610	0.00557	0.05	0.82	NA
Unidentified quercetin-derivative	0.03833	0.01533	4.57	**0.032**	Females

field experiment.

Data available from the Dryad Digital Repository: [link] (Moritz et al., 2017).

3. Results

Chemical Analysis. We identified 22 plant secondary metabolites in our leaf samples (Electronic Supplementary material). Out of the plant secondary metabolites, four occurred in significantly higher concentrations in female plants (Table 1). Our canonical correspondence analysis showed that male and female plant secondary metabolite profiles differed (F = 6.23, P < 0.01, degrees of freedom (d.f.) = 1, Fig. 2). However, male and female plants did not differ in total
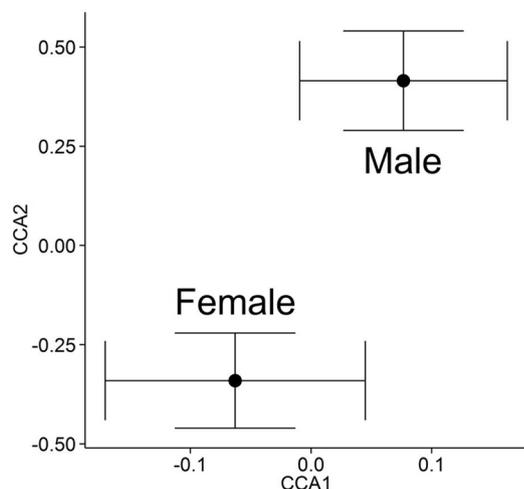
Fig. 2. Centroids and standard error bars of male and female plants for the first (CCA1) and second (CCA2) axis coordinates in a canonical correspondence analysis of phenolic and lignin compounds, clone and sex, explaining 37% of total variation.

concentrations of either phenolic acids (χ^2 = 1.92, P = 0.17, d.f. = 1, Fig. 3a) or lignans (χ^2 = 0.29, P = 0.59, d.f. = 1, Fig. 3b). Standard errors for calculated vectors of CCA1- and CCA2-coordinates were higher for mixed-sex plots (0.194, 0.240) than for male (0.117 0.161) and female (0.159, 0.160) plots. Clone affected phenolic profile (F = 5.91, P < 0.0001, d.f. = 37). SPAD-values, our proxy for N content in leaves, also did not differ between male and female plants (t = 1.25, P = 0.22).

Field Experiment. Plants in plots within the mixed-sex treatment were browsed down to lower heights than those in the plots within the monosexual treatments after one growing season (χ^2 = 87.9, P < 0.001, d.f. = 2; Fig. 4). There were no differences in roe deer browsing between male and female plants within the mixed-sex treatment (χ^2 = 0.01, P = 0.95, d.f. = 1).

Plant survival varied with plot sex ratio treatment (χ^2 = 7.88, P = 0.02, d.f. = 2). Plants in the monosexual female plot treatment had highest, and those in sex mix plots lowest, survival. There was, however, no effect of individual plant sex on survival (χ^2 = 1.02, P = 0.31, d.f. = 1). 57 plants in the sex-mixed treatment, 35 plants in the monosexual male treatment and 48 plants in the monosexual female treatment died. Of the 140 plants that died during the experiment, 58 were males and 82 were females. Survival was positively correlated with plant height (r = 0.36 p = 0.0325, n = 35).

4. Discussion

Plantations that contained a mix of male and female plants received more roe deer browsing than the monosexual plots. As expected based on herbivory patterns, plants in mixed-sex plots had the lowest survival rate over the growing season. Because average survival rates were correlated (albeit weakly) to plant height, we conclude that browsing was likely a factor leading to decreased survival. Although survival rates may not be a consequence of roe deer browsing alone, there clearly are overall drawbacks for survival of *S. viminalis* when they grow in mixed-sex plots and roe deer browsing was by far the most dominant source of damage to plants. Mixing plant sexes may thus increase mortality, likely at least partly due to herbivory.

The more extensive browsing by roe deer in mixed-sex plots of willow compared to monosexual plots may be due to differences in secondary metabolite profiles rather than due to differences in total secondary metabolites or nutrient content between the sexes. We measured plant secondary metabolite contents and nutritional quality, both of which are expected to be important for dietary decisions of generalist herbivores (Freeland and Janzen, 1974). Our analysis of phenolic compounds and lignans of the male and female experimental genotypes revealed intersexual differences in chemical profiles in *S. viminalis*. Furthermore, standard errors for the Canonical Correspondence Analysis axes describing secondary metabolite profiles were larger in mixed-sex plots, indicating higher secondary metabolite diversity. Although we cannot make strong conclusions for a causal relationship between secondary chemistry and browsing based on our experimental design, our results thus support the Detoxification Limitation Hypothesis within a single dioecious plant species by showing that *S. viminalis* stands with more diverse secondary metabolite profiles are subject to more extensive roe deer browsing. This could have practical value for willow production systems because it implies that herbivory can be reduced by preferentially planting in single sex plots.

Effects of plant sex mixing on herbivory in our field experiment adds a new perspective to previous findings of studies generalist herbivore diet mixing. Previous studies found that sheep choose a species-diverse diet with mixed palatability over a monospecific diet with high palatability (Wang et al., 2010) and consume more food if offered two types of food mixes containing different toxins than one containing either toxin at the same concentrations (Burrit and Provenza, 2000; Villalba et al., 2004). Studies of diet mixing in, for example, mice (Freeland and Saladin, 1989) and caterpillars (Singer et al., 2002) further indicate that
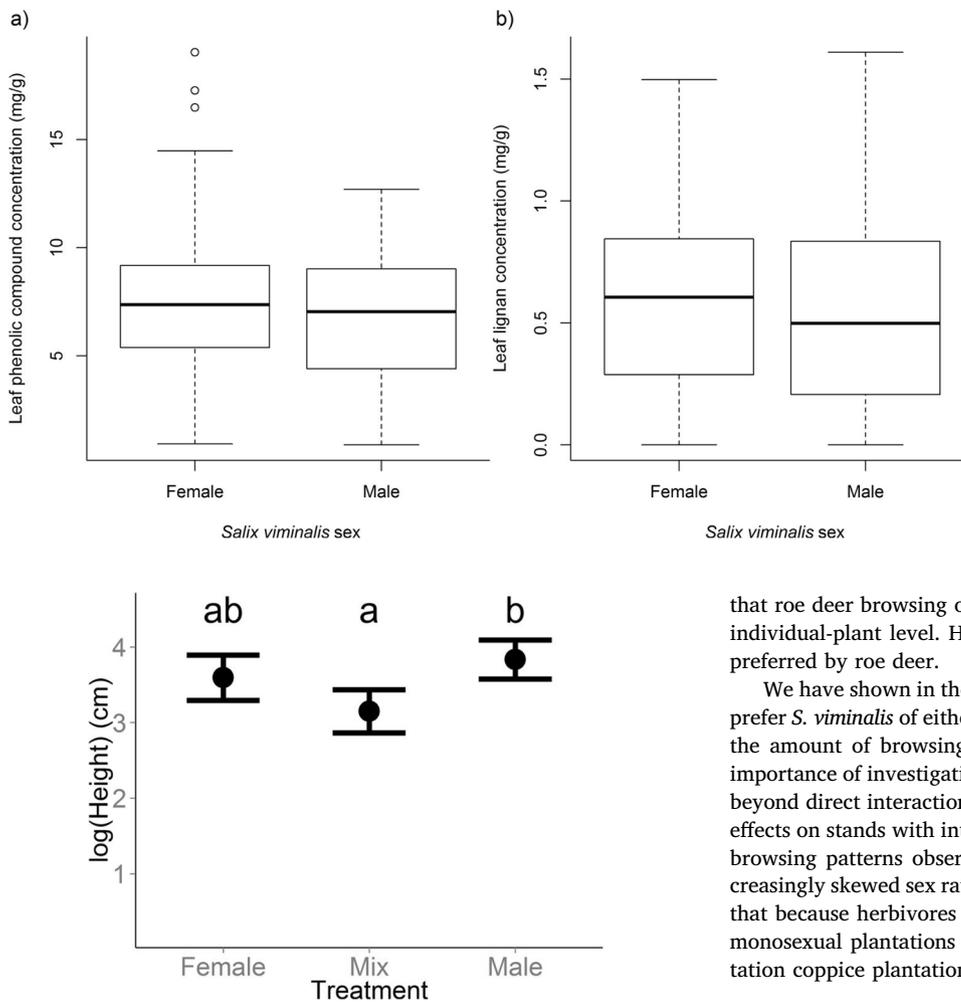
Fig. 3. Concentrations of total a) phenolic acid and b) lignan compounds in female and male *Salix viminalis*. Thick lines represent median values, box outlines represent first and third quartiles, and whiskers mark the ranges of non-outlier data. Rings represent outliers ($< 1.5\times$ interquartile range above the upper quartile).

Fig. 4. Mean Salix viminalis heights ($\pm$ SE) in plots with male only, female only and mixed-sex treatments after one growth season with heavy Capreolus capreolus browsing.

diet mixing is important for herbivores. The present study describes the first experiment where diet mixing on a larger scale is studied. Future studies should investigate whether there is a causal relationship between sex-specific chemical profiles and generalist browsing patterns.

Although there were no differences in total concentrations of S. *viminalis* plant secondary metabolites, we did find significant differences between male and female plants in secondary metabolite profiles. Intersexual differences in plant secondary metabolite profiles of *S. viminalis* were mostly driven by quercetin glucoside derivates, a myricetin-kaempferol derivate and an isohamnetin derivate. A compound related to quercetin glucoside, quercetin diglucoside, has previously been found in higher concentrations in female *S. myrsinifolia* (Nybakken and Julkunen-Tiitto, 2013) and females being better-defended is common for dioecious plants (e.g. Cornelissen and Stiling, 2005). We do not expect differences between sexes or clones to be greenhouse-specific, but phenolic compound concentrations can change with environmental variables and a study including field sampling of plant materials would address this uncertainty. We did not find any intersexual differences in leaf N concentration but we acknowledge that there may be other nutritional variation between male and female plants. For example, concentrations of phosphorous (but not protein, potassium, calcium or magnesium) differ between male and female *S. lasiosepsis* (Boecklen et al., 1990). Contrary to our expectations, there was no intersexual difference in total phenolic acid or lignan content, and variation in herbivory rates within mixed-sex plots was not explained by the sex of individual plants. There were no individual plant sex effects on plant secondary metabolites or herbivory, which suggest

that roe deer browsing of *S. viminalis* is not affected by plant sex at an individual-plant level. However, at a stand level a mix of the sexes is preferred by roe deer.

We have shown in the present study that although roe deer does not prefer *S. viminalis* of either sex, mixing male and female plants increases the amount of browsing that the plants receive. This underlines the importance of investigating effects of plant sex and other traits at scales beyond direct interactions at an individual plant. Further evaluation of effects on stands with intermediate sex ratios would assess whether the browsing patterns observed in the present study also can lead to increasingly skewed sex ratios for stands of dioecious plants. We conclude that because herbivores preferred mixed-sex stands the deployment of monosexual plantations may reduce herbivory of *S. viminalis* short rotation coppice plantations.

Conflicts of interest

The authors do not have any conflicts of interest to declare.

Declaration of contributions

Kim K. Moritz wrote the manuscript, carried out field work, and performed the statistical analyses. Johan A. Stenberg, Christer Björkman, Amy L. Parachnowitsch and Matthew P. Ayres contributed to analysis and interpretation of results, and to writing the manuscript. Riitta Julkunen-Tiitto contributed to the chemical analysis, analysis and interpretation of results, and to writing the manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.envexpbot.2017.10.015.

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