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RESEARCH ARTICLE

Quantifying direct and indirect effects of early-season herbivory on reproduction across four brassicaceous plant species

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Abstract

1. Insect herbivores can directly affect plant reproduction by feeding on reproductive tissues, or indirectly by feeding on vegetative tissues for which plants are unable to compensate. Additionally, early arriving herbivores may have cascading effects on plant reproduction by altering the later arriving community. However, the dynamic interplay between plant development and the assembly of herbivore communities remains underexplored. Hence, it is unclear whether non-outbreak levels of ambient herbivory early in the development of plants can impact plant fitness and to what extent these effects are mediated through changes in plant development and subsequent herbivory.
2. By excluding the herbivore community in an exclosure experiment and by manipulating early-season herbivory in a common garden field experiment replicated across four Brassicaceae species and 2 years, we tested whether early-season herbivory by caterpillars (*Pieris rapae*) or aphids (*Myzus persicae*) affected development, reproduction, and the herbivore communities associated with individual plants. In addition, we tested a causal hypothesis to assess the relative importance and temporal interplay between variation in herbivore communities and variation in plant development in determining plant reproduction.
3. Early-season herbivory affected plant reproduction in the exclosure experiment, with effects being highly dependent on the plant species, the herbivore species and the year. However, we found no such effects in the field experiment. The exploratory path analysis indicated that variation in plant reproduction is best predicted by variation in plant development, explaining 80% of the total effect on seed production. This suggests early-season herbivory had limited effects on later plant development, and plants were able to attenuate the impact of early-season herbivory. However, no clear compensatory mechanism could be identified.
4. While early-season herbivory has the potential to affect plant reproduction through changes in plant development or the subsequent development of the

associated community, these effects were small and varied across closely related species. This suggests that plant species may be exposed to different levels of natural selection by early-season herbivores through plant- or community-mediated effects on reproduction.

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KEYWORDS

community assembly, insect herbivores, legacy effects, mustard family, path analysis, plant fitness, plant-mediated interactions, plant strategies

1 | INTRODUCTION

Over their lifetimes, plants interact with a diverse and highly dynamic community of insect herbivores. As the costs of herbivory are dependent on the plant's ontogeny and environment, plants may not always be able to fully compensate for the consumptive effects of herbivores, resulting in reduced reproductive output (Boege & Marquis, 2005; Lehndal & Agren, 2015; Ochoa-Lopez et al., 2015). Herbivores can therefore directly affect plant reproduction by feeding on reproductive tissues or seeds, or indirectly by feeding on vegetative tissues important for the growth and development of plants.

However, the indirect effects of herbivory on plant reproduction need not be exclusively mediated by the plant itself. Herbivore-induced changes in plant phenotype have been shown to affect the plant's susceptibility to later attacks, connecting plant herbivores through a network of indirect interactions (Huang et al., 2017; Kessler & Halitschke, 2007; Ohgushi, 2005). These changes in the phenotype of plants emerge through induced plant responses involving myriad chemical and morphological traits to enhance resistance to current or future attackers (Karban, 2011; Schuman & Baldwin, 2016), or result from antagonists manipulating plant responses or modifying the plant tissues on which they feed (Behmer, 2009; Dussourd, 2017; Lill & Marquis, 2003). In addition to shifts in the identity of attackers, induced plant responses that occur early in the season can affect the trajectory of insect population growth that persists throughout the development of the plant (Karban, 1993; Wold & Marquis, 1997). Although direct interactions with specific herbivore individuals are often highly transient, priority effects caused by individual attackers may thus influence the subsequent interactions plants are exposed to, even after the causal biotic interaction ceases to persist (Han et al., 2020; Wurst et al., 2015; Yoneya et al., 2023).

These priority effects in which early colonisers affect the trajectory of community assembly are increasingly recognised as potential drivers of ecological and evolutionary dynamics (Kafle et al., 2018; Mertens, Boege, et al., 2021; Poelman & Kessler, 2016). A growing number of studies identified priority effects of transient herbivory on either above- or belowground herbivore communities (Barber et al., 2012; Stam et al., 2018). These effects can cascade through the carnivore community (Hernandez-Cumplido et al., 2016), the fungal community (Abdala-Roberts et al., 2019; Kostenko et al., 2012), the pollinator and florivore community (Chauta et al., 2017; Hoffmeister et al., 2016; Rusman et al., 2020) and can ultimately affect plant reproduction (Machado et al., 2018; McArt et al., 2013; Rusman et al., 2020; Stam et al., 2019). The ecological costs plants suffer through altered interactions with antagonists and mutualists can accumulate throughout the plant's lifetime, with mediated effects possibly outweighing the direct (i.e. consumptive) costs of the initial attack on plant fitness (Poelman & Kessler, 2016).

The notion that transient attack by a particular herbivore or herbivore species can impact plant fitness through mediated effects reveals a potential key role for early-season herbivores in driving ecological and evolutionary dynamics. Their effects on reproduction can be modulated by their priority effects on the assembly of the subsequent community, as well as through changes in the plant's development ensuing from the damage to valuable tissues early in the development of plants. Furthermore, as plant responses to initial herbivory are determined by herbivore functional traits such as the feeding guild of the early-season herbivore, characteristics of the initial attacker are likely to be important in determining the emergent effects on subsequent plant development, interactions with the community and plant reproduction (Ali & Agrawal, 2012; de Bobadilla et al., 2022; Mertens, Fernández de Bobadilla, et al., 2021). For example, by directly consuming valuable plant tissues, leaf-chewing herbivores such as caterpillars are likely to have a different and conceivably more substantial direct impact on early plant development than less destructive sap-feeding herbivores such as aphids (Ochoa-Lopez et al., 2015). In addition to direct damage to tissues, the mode of herbivore feeding also determines plant physiological responses, with potential effects on the subsequent development of the plant (Iqbal et al., 2017; Rivas-San Vicente & Plasencia, 2011). While it is well-established that plant responses to different types of herbivores emphasise different parts of a complex network of phytohormonal biosynthesis pathways (Erb & Reymond, 2019), the potential fitness consequences of attack by specific herbivores or, more broadly, herbivores belonging to different feeding guilds cannot be predicted from physiological responses to an initial attacker alone (Mertens, Fernández de Bobadilla, et al., 2021).

Because the effects of early-season herbivory on plant reproduction are mediated by the interplay between changes in subsequent plant development and the assembly of the plant-associated community, effects are expected to be dependent on the biology and ecology of the plant and the initial herbivore, and the environment in which these interactions take place. Indeed, while an increasing number of studies confirm the importance of the timing and identity of herbivore attack in determining the effects on subsequent plant development and reproduction, these studies are not congruent in their conclusion on the sign and strength of these effects (Adhikari & Russell, 2014; Garcia & Ehrlen, 2002; Pearse et al., 2018; Rasmussen & Yang, 2022; Rusman et al., 2020). The effects of early-season herbivory on plant reproduction may be more consistent among closely related plant species. This is because these species are more likely to follow comparable life-history strategies and co-evolutionary processes between plants and their associated herbivore communities often cause closely related plants to interact with similarly structured herbivore communities (Cirtwill et al., 2020; Ehrlich & Raven, 1964; Mertens, Bouwmeester, et al., 2021). A comparative approach across plant species would provide insight into the generality and consistency of indirect effects mediated by the plant and associated community on plant reproduction and improve our understanding of the trade-offs in life history strategies plants face.

In addition to evaluating the consistency of effects of early-season herbivory on plant reproduction, gaining insight into the processes mediating these effects will be essential to interpret the disparity in results reported by different studies and achieve a predictive understanding in terms of the ecological outcome of these interactions. However, disentangling the effects of herbivores on plant reproduction mediated by changes in the development of plants from those mediated by changes in the community with which plants interact is challenging, as effects of herbivores on plant performance often include an induced response that results in both changes in plant development and altered interactions with the plant-associated community (Gruntman & Novoplansky, 2011; West & Louda, 2018). To assess the relative importance and dynamic interplay between processes mediating the effects of early-season herbivory on plant reproduction, studies will need to take the temporal structure of plant development and community assembly into account.

In this study, we tested the effects of early-season herbivory on plant reproduction in four closely related annual Brassicaceae species replicated in two growing seasons (2017 and 2018), for plants grown in an open-field common-garden and plants grown in tents (i.e. excluding effects mediated by the plant-associated community). We hypothesise that early-season herbivory will negatively impact plant reproduction and that herbivory by chewing herbivores will have a more substantial impact than herbivory by aphids. We further hypothesise that the effects of early-season herbivory will be mediated by both changes in plant development and the associated herbivore community. To test these hypotheses, we manipulated the presence of either *Myzus persicae* aphids or *Pieris rapae* caterpillars on plant seedlings, or left plants untreated. We investigated (i) whether early-season herbivory affected plant female reproduction and whether effects were dependent on the presence of the plant-associated community, (ii) whether we could formulate and validate a causal hypothesis in the form of a path analysis describing community-mediated and plant-mediated effects on plant reproduction, (iii) whether early-season herbivory affected the estimated mean values of variables used in this path model and (iv) whether early-season herbivory affected the composition or structure of the subsequent herbivore community associated with individual plants. Our results provide novel insights into the relative importance of community-mediated and plant-mediated effects in determining plant reproductive success, and we discuss its implications for the evolution of plant defence strategies. By constructing a mechanistic model on the temporal interplay between plant development and the assembly of the associated herbivore community, we provide a testable causal hypothesis for future studies.

2 | MATERIALS AND METHODS

2.1 | Study system

We tested the effects of early-season herbivory on plant reproduction, plant development and the herbivore communities associated with four annual Brassicaceae species: *Brassica nigra* W.D.J. Koch, *Raphanus raphanistrum* L., *Sinapis arvensis* L., and *Rapistrum rugosum* (L.) All. (see Table S1). These species are annual herbaceous plants native to the Netherlands, have similar ecological niches, are overlapping in their development and phenotype and share a substantial part of the community of herbivore species (Mertens, Bouwmeester, et al., 2021). For each plant species, we collected seeds from at least 25 mother plants propagated by open pollination at the experimental fields of Wageningen University, The Netherlands (51°59′26.5″ N; 5°39′50.5″ E). Seeds were sown in trays with potting soil (Lentse Potgrond) and germinated in a glasshouse. After germination, plants were transplanted to peat soil cubes. Three-week-old plants were placed under a roofed shelter to acclimatise them to field conditions. Plants along with the peat soil cubes were transplanted to the experiments when they were 4 weeks old, at which time they had four to eight fully formed leaves depending on the plant species (mid-May; Week 21 of 2017 and 2018). To control the timing and identity of early-season herbivores, we inoculated the plants with either three adult wingless *M. persicae* (green peach aphids), two neonate *P. rapae* caterpillars (small cabbage white) or left plants untreated at the moment of transplantation. Both insect species commonly infest the early vegetative stages of the four plant species under natural conditions in abundances comparable to our treatment (Mertens, Bouwmeester, et al., 2021). The insects were obtained from stock cultures kept under greenhouse conditions (22 ± 1°C, 50%–70% r.h., L16:D8) at the Laboratory of Entomology, Wageningen University. The aphids were reared on *Raphanus sativus* (radish), and caterpillars were reared on *B. oleracea* var. *gemmifera* cv. Cyrus (Brussels Sprouts).

2.2 | Herbivore exclosure set-up

To assess the effects of early-season herbivory on plant reproduction while excluding effects mediated by the plant-associated community, we installed an exclosure experiment. Plants were planted in mesh tents (measuring 4 m length × 4 m width × 2.5 m height), with nine plants in each tent planted in monoculture at 1-m equidistance. Mesh size of the tents was 0.6 mm, which excluded all arthropods while allowing light and air into the tent. Mesh tents were located at the same experimental site as the open field experiment. All plants in a tent received the same treatment at the moment of transplantation, where they were inoculated with either three *M. persicae* aphids, two *P. rapae* caterpillars or were left untreated. The inoculation treatments were applied by placing the insects on a fully developed leaf and enclosing this leaf in a mesh bag. To control for a possible effect of enclosing leaves, we also enclosed one of the leaves of plants that were left untreated. After 14 days, we removed the treatment by excising the bagged leaf with a razor. Bagging the herbivore infested leaf enhanced the chance of keeping the tent free of herbivores after the treatment is applied. To ensure pollination, we placed commercially available *Bombus terrestris* hives (*Natupol Smart*; Koppert Biological Systems; Rodenrijs, The Netherlands) and *Lucilia sericata* fly pupae (*Natupol Fly*; Koppert Biological Systems; Rodenrijs, The Netherlands) in the tents at the start of flowering. The number of plants in the exclosure experiment was year-dependent due to unintentional infestations of plants and the limited number of mesh tents available (Table 1, Table S1). To assess the effects of early-season herbivory on plant reproduction, we measured the total number of seeds produced by each plant as a proxy for plant fitness. We harvested plants after the start of plant senescence but before the siliques started losing seeds. For each plant, the total number of seeds was estimated by extrapolating the weight of 100 seeds to the total seed biomass and rounding to the nearest natural number.

2.3 | Open field set-up

In parallel to the exclosure experiment, we installed a common garden experiment open to the full plant-associated arthropod community. The soil type at the experimental site is loamy sand and is embedded in an agricultural area maintained by local farmers (Unifarm). When no experiments are conducted at the site, it is used for organic agriculture and included in a crop rotation scheme. Hence, the field is regularly tilled, and the soil enriched by biological fertilisation through, for example, legume cover crops. Prior to installing the experiments, the field site was tilled using a cultivator, further homogenising the topsoil. No additional fertilisation was applied. The 12 possible combinations of four plant species and three treatments (inoculated with aphids, caterpillars or left untreated) were randomly assigned to plots consisting of nine plants in monoculture planted 1 m apart. Each of the 12 combinations was replicated eight times, resulting in 96 plots in each field season. Plots measured 3 m × 3 m and were separated from each other and the field edge by 4-meter-wide grass lanes. To obtain edge uniformity, we planted a strip of *B. nigra* in high density (six plants per square meter, 1 m wide) around the experimental field. In addition, we installed a mesh fence and kites to prevent herbivory by vertebrates. Plants were inoculated with three *M. persicae* aphids, or two *P. rapae* caterpillars by placing the insects on a fully developed leaf. As a pilot experiment showed that bagged leaves on plants in the open field were prone to breaking because of weather conditions (e.g. stronger wind in the open field compared with the exclosure set-up), the inoculated leaves were not bagged, and the herbivores were left free to move on the plant.

We recorded the development of herbivore communities on the five central plants in each plot (excluding the four corner plants) by monitoring individual plants from seedling to seed set. In cases where a plant died before the second monitoring round, we monitored one of the corner plants instead. Recording of the herbivore communities started 2 days after the experiment was installed. We monitored the development of herbivore communities on individual plants by weekly counts early in the season and by biweekly counts later in the season. Insects were identified in situ to species or family level. If accurate identification was not possible, we included the observations as morphospecies in our data (Table S2). In addition to observations of the herbivore community, we recorded a set of plant parameters as proxies for plant biomass and development: plant height (measured from the ground to the top of the plant), diameter (measured as the distance between the two most distal leaves), the number of true leaves and the number of flowering and seed-carrying branches (aggregated as reproductive branches). The height and diameter of plants were used to derive the volume of a cone, representing plant biomass as a single-volume parameter in the subsequent analyses. To assess

TABLE 1 Replication statement for the two experiments presented. See Table S1 for additional information.

Experiment	Scale of inference	Scale at which the factor of interest is applied	Number of replicates at the appropriate scale
Community exclosure	Individual plant	Tent	3 or 4 tents for each plant species by treatment combination in each of 2 years
Open-field experiment	Individual plant	Plot	8 plots for each plant species by treatment combination in each of 2 years

the effects of early-season herbivory on plant reproduction, we estimated the total number of seeds produced by each plant using the same protocol described for the herbivore exclosure experiment. Finally, we counted the total number of damaged leaves as a proxy for herbivore pressure.

2.4 | Statistical analysis

As we directly manipulated the presence of *P. rapae* and *M. persicae* as part of the open-field experiment, we excluded these herbivore species from the herbivore community observations. Both herbivore species commonly colonise the plants early in their development and, and as a result, it was impossible to distinguish the individual herbivores or their offspring we introduced in the open field from natural colonisation by other individuals. Including these species in our analyses would substantially bias the interpretation of the communities associated with differently treated plants. In addition, we removed plants for which the infestation treatment was considered unsuccessful (the insects applied as treatment were not observed in any of the two first observation rounds) from our data set and removed plants that were monitored less than four times during the field season (Table S1). Analyses were carried out in R (R Core Team, 2014) using the *lme4* (Bates et al., 2015), *nlme* (Pinheiro et al., 2012), *PiecewiseSEM* (Lefcheck & Freckleton, 2015), *ggplot2* (Wickham, 2009), *emmeans* (Lenth et al., 2019), *mgcv* (Wood, 2011), *gamm4* (Wood et al., 2017), *Multcomp* (Hothorn et al., 2008), *car* (Fox & Weisberg, 2018), *vegan* (Oksanen et al., 2012), *BiodiversityR* (Kindt & Coe, 2005) and *bipartite* (Dormann et al., 2008) packages.

2.4.1 | Effect of early-season herbivory on plant reproduction

To test whether early-season herbivory affected plant reproduction, we analysed the square-root-transformed number of seeds for each of the plant species separately using mixed effect models. Models included treatment, year of the field season and their interaction as explanatory variables and included plot identity or identity of the mesh tent as random intercept. As the number of seeds produced by plants in the open field was not directly comparable to that of seeds produced by plants in the tents, we analysed the open field and herbivore exclosure experiments separately. These experiments were not directly comparable because the methodology differed between the experiments (bagging leaves and removing the inoculation after 14 days in the herbivore exclosure experiment, while herbivores were left free in the open field set-up), and the effects of early-season herbivory may be modulated by the presence of the meshed tent. In addition, pollinators were supplied in the tents to ensure plants could reproduce, while we relied on natural pollination in the open-field set-up. In each analysis, we formulated a set of models to include either a variance structure that was year-dependent, treatment-dependent, or dependent on both factors combined, or assumed homogeneity of variance among all factor levels (Bates et al., 2015). We selected the model with the lowest Akaike information criteria (AIC) score and used diagnostic plots to verify that model assumptions were met (Harrison et al., 2018; Johnson & Omland, 2004; Zuur et al., 2009). We estimated the effect size and significance of fixed factors using type II Wald χ^2-tests. Pairwise post hoc comparisons among treatments were performed for each year separately using Tukey's honest significant difference test.

2.4.2 | Plant- and community-mediated effects on seed production

To disentangle the effects of herbivores on plant reproduction mediated by changes in plant development from those mediated by changes in the plant-associated herbivore community, we formulated a set of causal models that relate plant performance and herbivory early in the season to plant performance and herbivory later in the season, and ultimately plant reproduction at the end of the growing season. Path analysis is a multivariate technique that allows modelling the multivariate dependency between variables based on a priori causal hypothesis (Shipley, 2016). Once such a hypothesis is consistent with the data, it can be used to quantify the direct and indirect effects of herbivory and plant development on reproduction. We formulated a set of path models describing our initial causal hypothesis (Figure 1). These path models differed in how they included year and plant-species effects on the intercept of variables in the path model while constraining the path coefficients to be the same across year and plant species, that is, the strength of the relationships between the variables (Table S3). To avoid an overparameterization of the path model, we aggregated observations for the different variables that were repeatedly measured over the growing season, that is, the volume of plants, the number of leaves, the number of damaged leaves and the abundance and richness of the folivore community, into early-season (observation rounds 1 and 2; Weeks 1 and 2 after the start of the experiment in both 2017 and 2018), mid-season (rounds 3 and 4; between Week 3 and 5 in 2017 and between Week 3 and 4 in 2018) and late-season (rounds 5 and 6; between Week 6 and 8 in 2017, and between Week 5 and 7 in 2018) proxies by taking the maximum parameter value as observed for each individual plant within each subdivision. The volume of plants, the number of reproductive branches, and the number of seeds were square-root-transformed, and the abundance of herbivores associated with leaves, the number of damaged leaves, and the abundance of florivores and seed predators were transformed using a $\log(x+1)$ transformation. Variables were then centred and standardised to a mean value of 0 and a standard deviation of 1. The path model was constructed by specifying linear mixed models including the identity of plots as random effect, and accounting for the heterogeneity of residuals by using variance functions when resulting in a better model fit based on AIC. The path model was then evaluated by AIC and Fisher's *C* global goodness-of-fit statistic with the associated

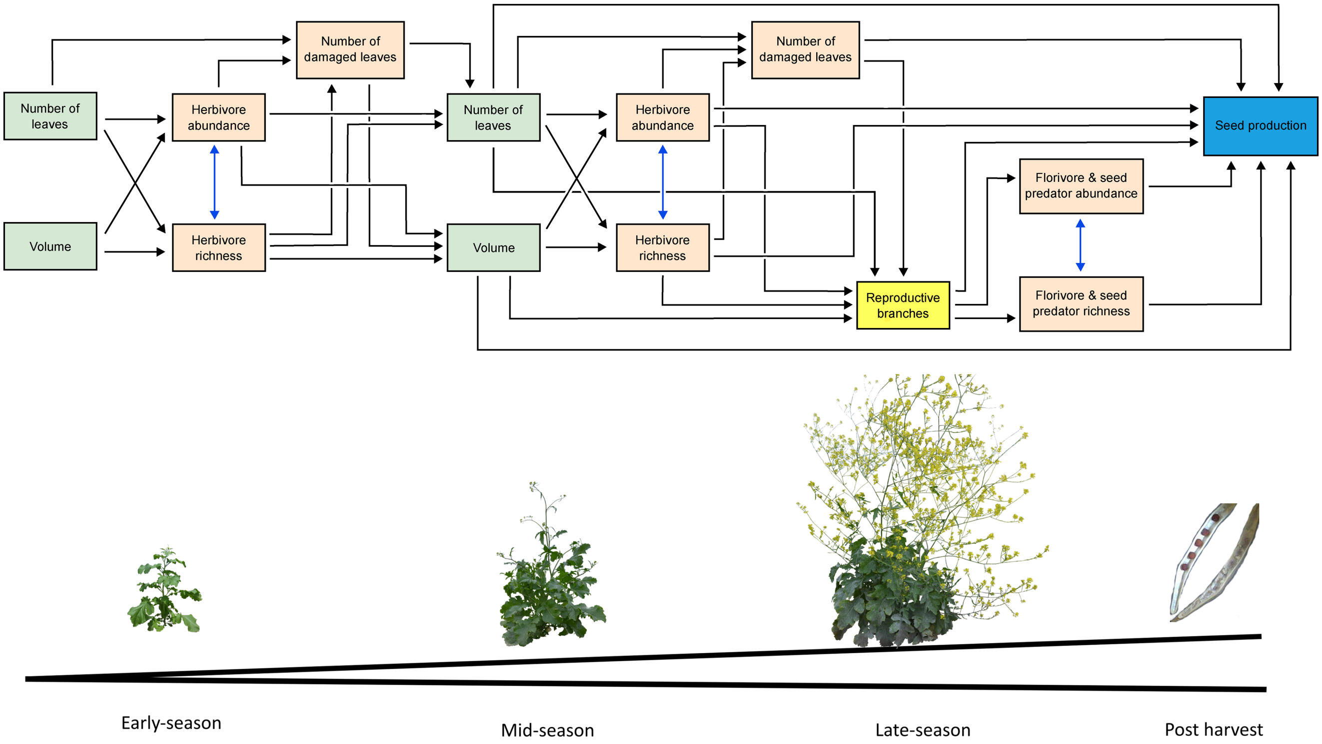


FIGURE 1 Path diagram of the causal hypothesis, expressing the assumed relation between the measured proxies for plant biomass, the observed herbivore community and the total number of damaged leaves early in the plant development, plant biomass, the observed herbivore community, and the number of damaged leaves in the middle of the plant growing season, the number of reproductive branches and associated florivores and seed predators late in the season, and plant reproduction for plants in the open field experiment. Black arrows represent unidirectional relationships among variables while double-headed blue arrows show correlated errors. Variables related to plant development, the observed herbivore community and leaf damage, the number of reproductive branches, and seed production are represented in green, red, yellow and blue rectangles, respectively.

p-value, where $p > 0.05$ indicates that the data are sufficiently well-represented by the path model (Lefcheck & Freckleton, 2015; Shipley, 2009). To test the validity of the causal assumption when relaxing the constrain on path coefficients, we fitted the causal model to each plant species, the 2 years, and all combinations of plant species and year separately (Table S3). Model parsimony when either retaining or relaxing the constraint on path coefficients was evaluated using AIC (Douma & Shipley, 2021; Shipley & Douma, 2020).

As our initial causal hypothesis presented in Figure 1 was not supported by the data (Table S3), we proceeded to an exploratory approach by reformulating path models to be consistent with the data. We removed all paths in our initial hypothesis, which were not important and included all paths for which we found a significant independence claim and, importantly, whose causality could be supported by ecological knowledge. Variables that showed dependence but between which we could not presume a biologically sound causal relation were incorporated as correlated error. The final structure and fit of the optimised model depended on how the effects of plant species and year on the estimations of variables in the model were included in the path model (Table S4). The most parsimonious path model was selected based on AIC (Shipley & Douma, 2020).

To determine the relative importance of variables related to plant development and those related to the herbivore community in their effect on plant reproduction, we evaluated the direct and indirect effect of each variable in the model on seed production. Direct effects are represented by the direct path from the variable of interest to the number of seeds. An indirect effect represents the effect of the variable of interest to the number of seeds produced mediated by another variable and can be calculated as the product of the path coefficients along the path. The total indirect effect is the sum of all possible indirect effects. We used standardised path coefficients to allow comparison of effects among the different variables (Shipley, 2016). Finally, we fitted the optimised exploratory path model on the different year-by-species combinations to assess the generality of the model structure and consistency of direct and indirect effects on seed production across different plant species and the 2 years.

2.4.3 | Effect of early-season herbivory on path variables

To estimate the degree to which early-season herbivory could affect plant reproduction, we fitted univariate regressions for all variables in the path model (mixed effect models). All regressions included treatment, year of the field season and their interaction as explanatory variables and included plot identity as random intercept. For each of the path variables tested, we formulated a set of models differing in how they accounted for heterogeneity of variance among factor levels and selected the model with lowest AIC. We estimated the effect size and significance of fixed factors using type II Wald χ^2-tests. Pairwise post hoc comparisons were performed for each year separately using Tukey's honest significant difference test.

To make a tractable path model, the diversity of the herbivore community and plant measurements were aggregated to early-, mid- and late-season proxies. To test the robustness of this choice, we performed additional analyses on the unaggregated form of variables, which were repeatedly measured while controlling for the time since the start of the experiment. A preliminary analysis of the relationship between each of the repeatedly measured variables (herbivore richness and abundance and richness and abundance of subsets of the community based on the herbivore feeding guild, the number of damaged leaves, the plant volume and number of leaves) and the day after the start of the experiment indicated a nonlinear relationship between time and the response variable. We therefore applied Generalised additive mixed models with Gaussian, gamma or negative binomial probability distribution and thin plate regression splines to assess dynamics over time (Wood, 2017; Zuur et al., 2009). These models included the treatment, year of the field season, and their interaction as fixed factors and included plant identity nested within plot identity as random intercepts to account for the dependency of observations and repeated measurements on the same plant. We then compared models fitted with a single smoothing function estimating the relation between the response variable and day after start of the experiment, with models that fitted a different smoothing function for each early-herbivory treatment level (three smoothers), year of the field season (two smoothers) or the interaction between treatment and year (six smoothers) using likelihood-ratio tests. The effect size and significance of variables in the model were estimated using likelihood-ratio tests. As we found strong variation across years relative to the effects of our treatments, we repeated the analysis for each field season separately.

2.4.4 | Effect of early-season herbivory on herbivore community composition and structure

In a final set of analyses, we explored whether the composition and structure of herbivore communities differed among plant species and treatments in each of the 2 years. To visualise the observed herbivore community, we constructed a bipartite interaction network linking herbivore species and plant species and used multivariate ordinations (non-metric multidimensional scaling with three dimensions) of the herbivore communities observed on individual plants. We assessed community composition (i.e. incidence of herbivore species) based on the Sørensen dissimilarity matrix, and community structure (composition weighted for species abundances) by calculating the Euclidean distance of Hellinger-transformed cumulative abundance data for each plant individual (Legendre & Gallagher, 2001). Using these distances, we tested for differences among differently treated plants of the same plant species through a nonparametric permutational multivariate analysis of variance with 1000 permutations (PERMANOVA) (Anderson, 2001). To ensure valid permutation of communities, we specified the dependency of observations on plants in the same plot in a stratified permutational design. As this permutation design requires an equal number of samples in each permutable group, we randomly sampled three plants per plot to obtain an equal sample size and then performed the stratified PERMANOVA analysis. This procedure of randomisation followed by PERMANOVA was repeated 1000 times and we report the median, first and third quantiles, and 5th and 95th percentiles of the results obtained by repeated permutation analysis.

3 | RESULTS

3.1 | Effect of early-season herbivory on plant reproduction

Effects of early-season herbivory on plant reproduction strongly depended on whether plants were grown in the exclosure set-up or in the open field. In the community exclosure experiment, we found that early-season herbivory affected plant reproduction (Table 2), although the sign (i.e. positive or negative) and strength of the effect was strongly dependent on the plant species and year. Interestingly, inducing herbivores could have a positive effect on seed production compared with non-treated plants in one plant species and a negative effect on seed production in another plant species or year (Figure 2). For example, in the 2018 growing season, we found that early-season herbivory by *M. persicae* aphids on *R. raphanistrum* resulted in a 54% decrease in the square-root-transformed number of seeds produced relative to untreated plants, while in *S. arvensis*, it resulted in a 64% increase in seed production. Similarly, whether the effect induced by a specific early-season herbivore was positive or negative was year-dependent. For example, in 2017, we observed that early season herbivory by *P. rapae* caterpillars feeding on *B. nigra* resulted in a 43% reduction in seed set, while in 2018 herbivory by *P. rapae* resulted in a 91% increase in seed production compared with plants which were left untreated. While the effects of the inducing herbivore in the exclosure experiment could be substantial, we did not find any evidence for an overall effect of early-season herbivory on seed production for plants grown in the open field set-up (Table 2). This was confirmed by the post-hoc analysis, showing that only the pairwise difference between untreated *R. rugosum* plants and plants challenged by *P. rapae* caterpillars in the 2018 season was significant (1 out of 48 pairwise comparisons). Overall, these results indicate that the effect of early-season herbivory on plant reproduction was strongly dependent on the inducing herbivore species, the mediating plant species and whether plants grew in tents or the open field.

3.2 | Plant- and community-mediated effects on seed production

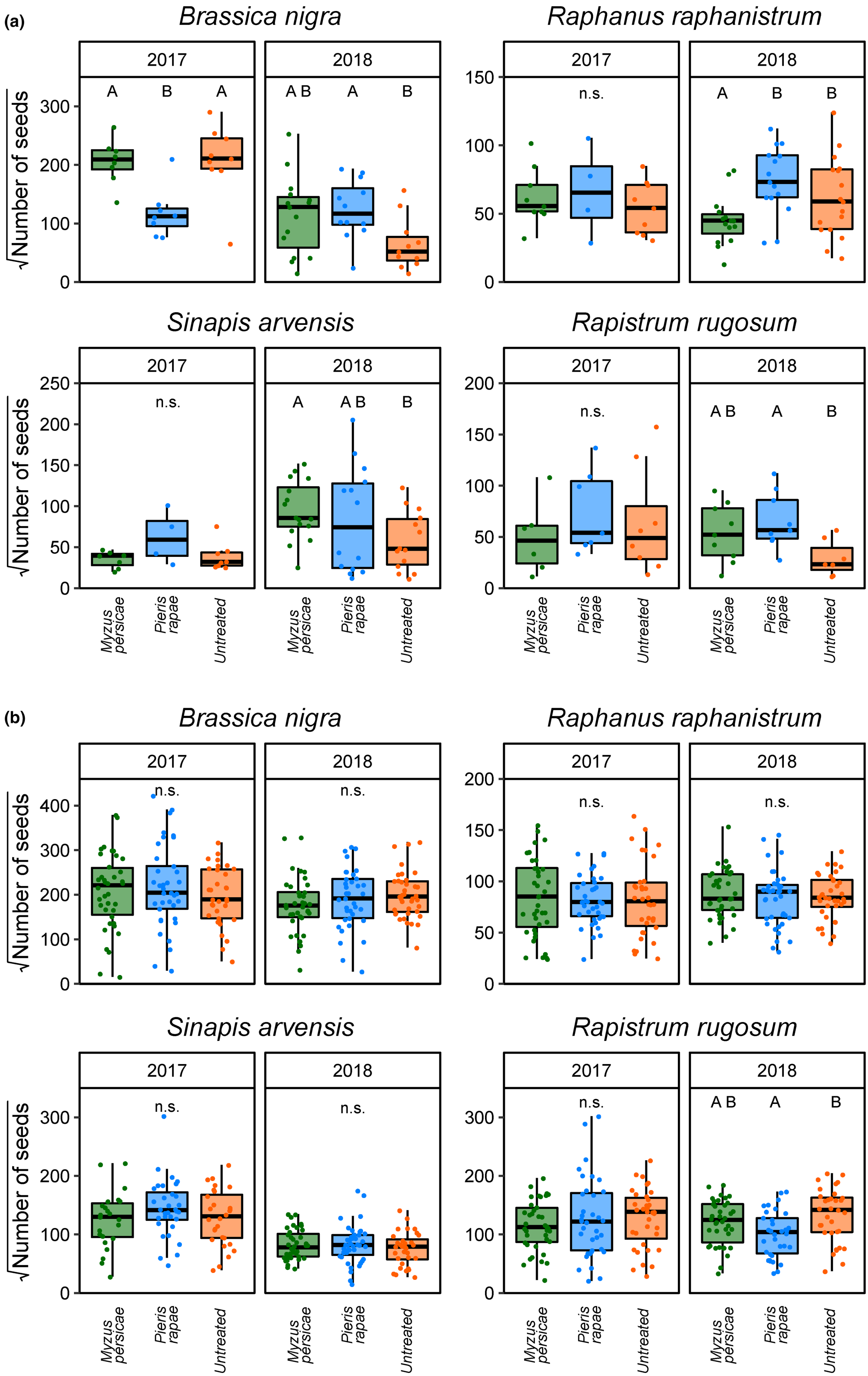
Exploration of path models fitted on the full data or subsets of the data based on combinations of the plant species and field seasons, revealed that our a priori causal hypothesis (Figure 1) expressing the relation between the measured proxies for plant biomass, the observed folivore herbivore community, the number of damaged leaves, the number of reproductive branches and associated florivores and seed predators, and ultimately seed production, was not supported by the data (Table S3). When using an exploratory approach to formulate a causal hypothesis, several changes were made compared to our initial model (cf. Figure 1 and Figure S1). First, the optimised descriptive path model included a substantially higher number of causal relations compared with our initial causal hypothesis (50 paths compared to 35 paths in the initial causal hypothesis). Second, causal relationships between early-season biomass and mid- or late-season variables to seed production had to be added, suggesting alternative indirect paths through which early-season variables can affect plant reproduction that were not captured by the measured mid- and late-season variables (Figure S1). For example, early season biomass in terms of plant volume, as well as the number of leaves had a direct causal relation with the number of reproductive branches plants produced. Plants with more leaves early in the season produced more reproductive branches, while plants with a higher volume early in

TABLE 2 Overview of effect size of early-season herbivory (Treatment), year of the field season (Year), and their interaction (Treatment*Year) on square-root transformed number of seeds produced by the different plant species in the community exclosure and open-field experiments.

Experiment	Plant species	Variance function	Explanatory variable	χ^2	df	*p*-value
Community exclosure	*Brassica nigra*	None	Treatment	3.64	2	0.1625
			Year	31.55	1	**<0.0001**
			Treatment*Year	19.61	2	**<0.0001**
	Raphanus raphanistrum	Treatment	Treatment	25.61	2	**<0.0001**
			Year	0.06	1	0.8107
			Treatment*Year	6.88	2	**0.0321**
	Sinapis arvensis	Treatment*Year	Treatment	6.68	2	**0.0354**
			Year	3.12	1	0.0773
			Treatment*Year	3.26	2	0.1962
	Rapistrum rugosum	None	Treatment	5.83	2	0.0543
			Year	0.48	1	0.4877
			Treatment*Year	1.27	2	0.5303
Open field	*Brassica nigra*	Treatment*Year	Treatment	1.25	2	0.5364
			Year	2.19	1	0.1387
			Treatment*Year	2.58	2	0.2755
	Raphanus raphanistrum	Treatment*Year	Treatment	0.50	2	0.7802
			Year	0.20	1	0.6554
			Treatment*Year	0.02	2	0.9889
	Sinapis arvensis	Year	Treatment	1.73	2	0.4215
			Year	65.75	1	**<0.0001**
			Treatment*Year	1.70	2	0.4284
	Rapistrum rugosum	Treatment*Year	Treatment	3.71	2	0.1566
			Year	0.24	1	0.6279
			Treatment*Year	3.55	2	0.1697

Note: Models were adjusted to account for heterogeneity of model residuals using a variance function across different treatments, years, all factor levels, or were not adjusted when no heterogeneity found (indicated as none). Values in bold indicate significant factor effects ($p < 0.05$).

FIGURE 2 Seed production by plants belonging to four different plant species measured in two field seasons (2017 and 2018), (a) when the herbivore community was excluded, and (b) when plants could interact with their full natural communities. Plants were challenged early in the season by *Myzus persicae* aphids (green), *Pieris rapae* caterpillars (blue) or were left untreated (orange). Pairwise comparisons were performed separately for each plant species by field season combination. Boxplots show median, first and third quantiles, and 95% interval. Dots represent individual observations. Boxes within each panel with no letters in common are significantly different based on Tukey's HSD ($p < 0.05$), whereas n.s. indicates no significant differences were found.


(a)
Brassica nigra
Raphanus raphanistrum
2017
2018
A B A
A B A B
n.s.
A B B
√Number of seeds
0
100
200
300
50
150
Sinapis arvensis
Rapistrum rugosum
n.s.
A A B B
n.s.
A B A B
250
Myzus persicae
Pieris rapae
Untreated
(b)
Brassica nigra
Raphanus raphanistrum
n.s.
400
Sinapis arvensis
Rapistrum rugosum
A B A B

the season produced fewer branches when controlling for indirect effects (Table S5). Third, the exploratory analysis suggests that variation in herbivory is predicted to not substantially affect plant reproduction (Figure 3; Table S6). Moreover, the effects of the different variables related to herbivory on the number of seeds produced were predominantly mediated by variables related to plant development, with herbivory of reproductive tissues having no effect on seed production (Figure 3; Table S6). Interestingly, also the number of damaged leaves was estimated to have only small effects on the number of seeds produced. Finally, the volume of plants early in the season directly (negatively) affected seed set. Taken together, the exploratory model fitted on all data independent of plant species and year of the field season indicated that plant development had the strongest causal relations with seed production, while effects induced by herbivory were less strong (Figure 3; Table S6). Plant characteristics are predicted to account for 80.39% of the total effects on seed production (63.73% direct, 16.67% indirect) while herbivore pressure accounted for 19.61% of the effects on seed production (3.92% direct, 15.69% indirect).

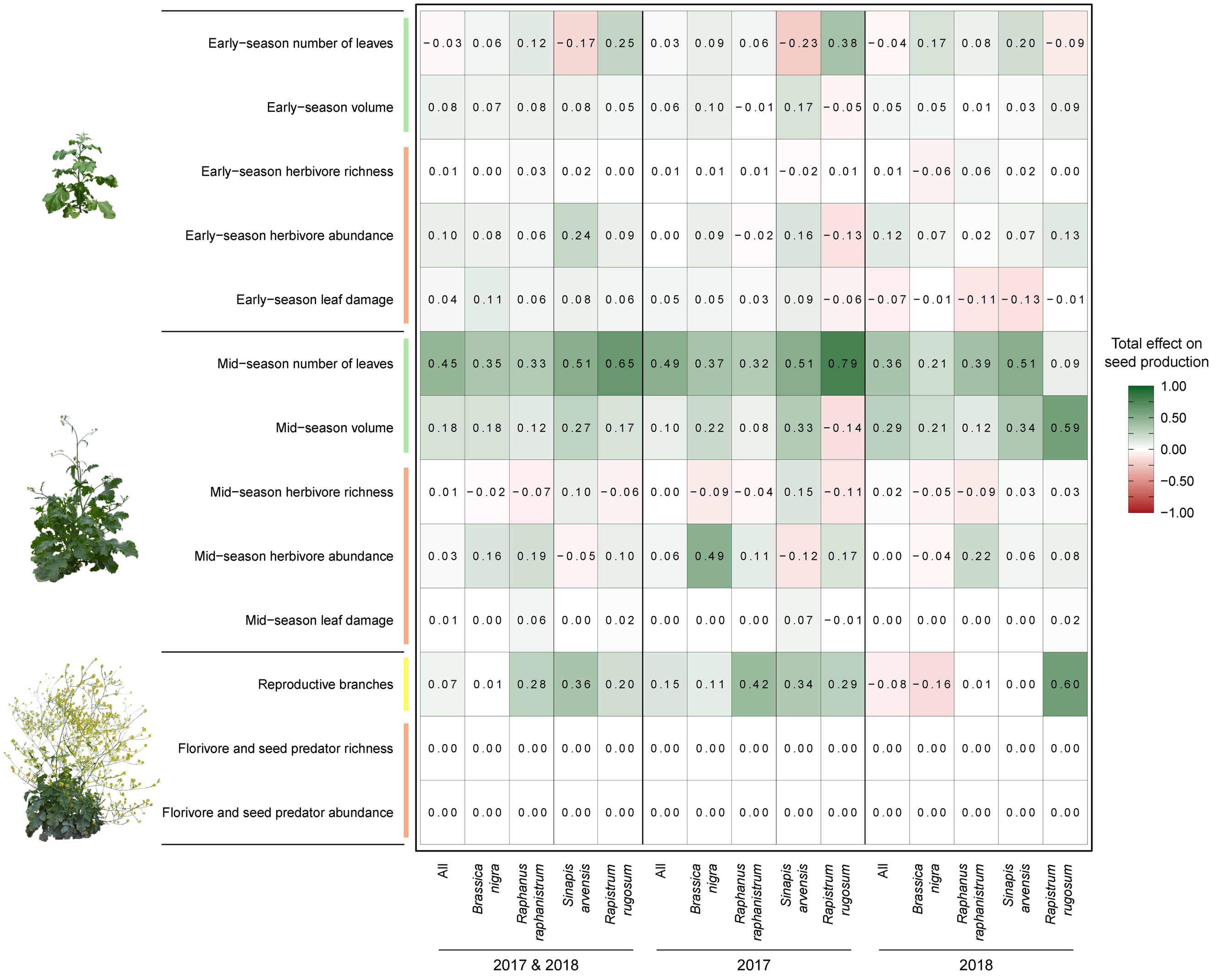
Fitting the optimised exploratory piecewise path model to different subsets of the data to remove equivalence constrains on path coefficients revealed that the estimation of path coefficients depended on the plant species, the year or the plant species by year combination (Table S5). The dependence of path coefficients on the year and plant species had substantial influence on the estimation of direct and indirect effects on seed set (Figure 3; Table S6). However, plant development remained more important in predicting plant reproduction than variation in the richness or abundance of the herbivore community. Even though the estimated values of path coefficients depended on the subset of the data to which the model was fitted, constraining path coefficients to be equal among plant species and the 2 years was found to result in the most parsimonious model based on AIC (path coefficients fully constrained: AIC = 157; equivalence constraint removed between years but not plant species: AIC = 325; equivalence constraint removed between plant species but not between years: AIC = 685; equivalence constraints on path coefficients removed for both year and plant species: AIC = 1329). However, this generalisation may be the result of the increased uncertainty in the path coefficients with the smaller sample sizes of each subset. In addition, it is likely that not all path coefficients are dependent on the plant species or year.

3.3 | Effect of early-season herbivory on path variables

The herbivory treatments had a significant effect on the total number of damaged leaves early in the season, with plants inoculated with caterpillars having a significantly higher number of damaged leaves in the 2018 season (Tables S7 and S8). However, for most plant species, we found no evidence of significant effects of early-season herbivory on the other variables used in the path analysis, including the total number of damaged leaves later in the season (Table S7). The exceptions were found for *S. arvensis* and *R. rugosum*. For *S. arvensis*, we found that our treatments had a significant effect on the mid-season volume of plants, a significant effect on the abundance of florivores, and had a year-dependent effect on the number of reproductive branches. For *R. rugosum*, we found that early-season herbivory affected the abundance and richness of the florivore community in a year-dependent way. These overall effects were substantiated by the post hoc analysis (Table S8). In 2018, *S. arvensis* plants challenged by *M. persicae* aphids had a significantly higher mid-season volume than plants challenged by *P. rapae* caterpillars, and in 2017 *S. arvensis* plants treated with aphids produced significantly fewer reproductive branches than caterpillar-treated plants. In addition to these effects on variables related to plant development we found that in 2018, *S. arvensis* plants challenged by *P. rapae* encountered significantly fewer florivores and seed predators than plants which were challenged by *M. persicae* or were left untreated early in the season. Even though we did not observe any significant overall effects of our treatments on the mid-season volume of *R. rugosum* plants, we did find post-hoc differences between treatments in the 2018 field season. Here, *R. rugosum* plants which were challenged by aphids had a significantly lower mid-season volume compared to plants challenged by caterpillars. In 2017, *R. rugosum* plants which were left untreated encountered a more species rich florivore and seed predator community compared with plants challenged early in their development by either *M. persicae* aphids or *P. rapae* caterpillars.

We then proceeded with the analysis of the repeatedly measured variables as observed over time, that is, not aggregated in early-, mid- and late-season proxies. The models describing a year-specific relation between the different response variables and the day since the start of the experiment generally resulted in the best fit to our data (Tables S9 and S10). With the exception of *B. nigra*, the change in the number of damaged leaves over time was best described by including a different smoothing function for each treatment (for *R. rugosum*), or each of the year-by-treatment combinations (for *R. raphanistrum* and *S. arvensis*) (Table S9). In addition, the change over time in the number of leaves produced by *B. nigra* was best described by including a different smoother for each treatment. When analysing both years simultaneously, our treatments had significant year-dependent overall effects on the number of damaged leaves observed for *B. nigra*, *S. arvensis* and *R. rugosum* plants, the abundance of herbivores on *S. arvensis* and *B. nigra* and on the volume of *R. rugosum* plants (Table S9). In addition, our treatments significantly affected the number of damaged leaves in *R. rugosum*, independent of the year. The abundance of leaf-chewing herbivores on *S. arvensis* plants and the abundance of sap-feeding herbivores on *R. rugosum* plants were significantly affected by our treatments in a year-dependent way (Table S10).

When analysing the 2 years separately, the effect of the treatments on the number of damaged leaves was confirmed in the 2018 season for all plant species except *R. raphanistrum* (Table S10), showing that plants challenged by *P. rapae* caterpillars had a higher number of damaged leaves than plants challenged by *M. persicae* aphids



	2017 & 2018					2017					2018				
	All	*Brassica nigra*	*Raphanus raphanistrum*	*Sinapis arvensis*	*Rapistrum rugosum*	All	*Brassica nigra*	*Raphanus raphanistrum*	*Sinapis arvensis*	*Rapistrum rugosum*	All	*Brassica nigra*	*Raphanus raphanistrum*	*Sinapis arvensis*	*Rapistrum rugosum*
Early−season number of leaves	−0.03	0.06	0.12	−0.17	0.25	0.03	0.09	0.06	−0.23	0.38	−0.04	0.17	0.08	0.20	−0.09
Early−season volume	0.08	0.07	0.08	0.08	0.05	0.06	0.10	−0.01	0.17	−0.05	0.05	0.05	0.01	0.03	0.09
Early−season herbivore richness	0.01	0.00	0.03	0.02	0.00	0.01	0.01	0.01	−0.02	0.01	0.01	−0.06	0.06	0.02	0.00
Early−season herbivore abundance	0.10	0.08	0.06	0.24	0.09	0.00	0.09	−0.02	0.16	−0.13	0.12	0.07	0.02	0.07	0.13
Early−season leaf damage	0.04	0.11	0.06	0.08	0.06	0.05	0.05	0.03	0.09	−0.06	−0.07	−0.01	−0.11	−0.13	−0.01
Mid−season number of leaves	0.45	0.35	0.33	0.51	0.65	0.49	0.37	0.32	0.51	0.79	0.36	0.21	0.39	0.51	0.09
Mid−season volume	0.18	0.18	0.12	0.27	0.17	0.10	0.22	0.08	0.33	−0.14	0.29	0.21	0.12	0.34	0.59
Mid−season herbivore richness	0.01	−0.02	−0.07	0.10	−0.06	0.00	−0.09	−0.04	0.15	−0.11	0.02	−0.05	−0.09	0.03	0.03
Mid−season herbivore abundance	0.03	0.16	0.19	−0.05	0.10	0.06	0.49	0.11	−0.12	0.17	0.00	−0.04	0.22	0.06	0.08
Mid−season leaf damage	0.01	0.00	0.06	0.00	0.02	0.00	0.00	0.00	0.07	−0.01	0.00	0.00	0.00	0.00	0.02
Reproductive branches	0.07	0.01	0.28	0.36	0.20	0.15	0.11	0.42	0.34	0.29	−0.08	−0.16	0.01	0.00	0.60
Florivore and seed predator richness	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Florivore and seed predator abundance	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

FIGURE 3 Overview of the total effect of variables included in the descriptive path model expressing the relation between the measured proxies for plant biomass, the observed herbivore community, and the total number of damaged leaves early in the plant development, plant biomass, the observed herbivore community and the number of damaged leaves in the middle of the plant growing season, and the number of reproductive branches and associated florivores and seed predators late in the season (path model is presented in Figure S1). The total effect of each variable is calculated by summing its direct effect on seed production and the indirect effects mediated by other variables in the model (presented in Table S5). These effects were calculated from standardised path estimates obtained by fitting the descriptive path model to each of the year-by-plant species combinations, and can be interpreted as the effect an increase of one standard deviation of the variable has on the square-root-transformed number of seeds produced by a plant. Variables related to plant development, the observed herbivore community and leaf damage, and the number of reproductive branches are annotated with a green, red, or yellow line next to the variable names, respectively.

or plants which were left untreated (Table S11). In the 2017, field season *B. nigra* challenged by *P. rapae* caterpillars produced a higher number of leaves than plants that were left untreated. Our treatments could also affect the richness or abundance of (subsets of) the herbivore community associated with plants. *Sinapis arvensis* plants challenged by *M. persicae* aphids early in the season encountered a higher average abundance of herbivores than plants challenged by *P. rapae* caterpillars in the 2018 growing season (Tables S10 and S11). In 2017, both the species richness and abundance of sap-feeding herbivores was significantly higher on *R. rugosum* plants which were left untreated compared with plants challenged by early-season herbivores. Finally, in 2018, *S. arvensis* plants challenged by *P. rapae* encountered a more species rich chewing herbivore community compared to plants challenged by *M. persicae*, and the abundance of chewing herbivores on caterpillar-treated plants was treatment-dependent for all plant species except *R. raphanistrum* (Table S11).

Taken together, these results show that early-season herbivory can affect the richness and abundance of the herbivore community, as well as the plant traits we measured. However, the detectability and strength of effects depends on the plant species and the biotic and abiotic environment, as represented by the year-dependency of results. Importantly, simplifying the repeatedly measured variables by aggregation into early-, mid- and late-season values obscured the detectability of treatment effects.

3.4 | Effect of early-season herbivory on herbivore community composition and structure

Initial exploration of the herbivore communities associated with the four plant species revealed substantial variation in the prevalence of herbivore species over time (Figure 4). This turnover in the herbivore community in the field corresponded with changes in the ontogeny of plants. The network between the total herbivore community and the different plant species is well connected (i.e., no modularity of interactions between herbivores and specific plant species) and similar across the 2 years (Figure 4). Herbivore communities associated with individual plants significantly differed in their composition and abundance-weighed community structure depending on the plant species (results from PERMANOVA composition: Pseudo-$F=37.34$, $R^2=0.01$, df $=3$, $p=0.001$; structure: Pseudo-$F=61.74$, $R^2=0.15$, df $=3$, $p=0.001$) and year (composition: Pseudo-$F=198.23$, $R^2=0.18$, df $=1$, $p=0.001$; structure: Pseudo-$F=222.97$, $R^2=0.18$, df $=1$, $p=0.001$; Figures S2–S5). Hence, we proceeded to test for treatment effects by analysing each plant species by year separately. The overall effect of our treatments on the composition or structure of herbivore communities was not statistically significant for any of the plant species, and only minimal variation in the composition or structure of the cumulative herbivore community associated with individual plants could be attributed to early-season herbivory (Table 3, Table S12, Figures S2–S5).

4 | DISCUSSION

We show that early-season herbivory can affect plant reproduction and that these effects are largely dependent on the plant species, the inducing herbivore species, and the biotic and abiotic environment in which plants grow (Figure 2). When the insect community was excluded, early-season herbivory affected plant reproduction in three out of the four plant species with effects of up to a sixfold change in seed set, suggesting that plants are not inherently able to compensate for this damage. However, effects of early-season herbivory on plant reproduction were diminished when plants could interact with their full associated community. An exploratory path analysis of the direct and indirect causal effects between plant development, the herbivore community pressure and the number of seeds plants produced provides a mechanistic insight into how effects of early-season herbivory could be attenuated in the open field experiment. Variation in the levels of observed herbivore pressure in terms of species richness and abundance and the number of damaged leaves represented 19.61% of the predicted impact on seed production, and these effects were mediated by plant development. Variation in plant development and biomass was predicted to be much more likely to impact plant reproduction (80.39% of the total effect on seed production, Figure 3, Table S6). Importantly, priority effects induced by early-season herbivory comparable to what plants are exposed to under natural conditions could affect plant development or the herbivore community, but these effects were infrequent and year- and plant species-dependent (Tables S7–S11). In addition, the effects on variables included in the exploratory model were relatively small, and causal effects induced by variables that were affected by our treatments were likely cancelled out by other causal paths involving variables that were unaffected by our treatment, resulting in minimal net effects on plant reproduction. Finally, even though a path model constraining path coefficients to be equal for all plant species and both years was most parsimonious, the strength of causal effects in the exploratory path model differed substantially when fitted to subsets of the data, further emphasising the context dependency of mediated effects (Figure 3, Tables S5 and S6). Taken together, our findings show that priority effects induced by early-season herbivory are predicted to affect plant reproduction through changes in the development of plants and—to a lesser extent—changes in plant-associated herbivore communities. However, the detection, sign, and strength of effects on plant development and herbivore communities was highly context dependent, and plants were able to compensate for potential effects on plant reproduction.

Herbivory in life stages when plants are less tolerant to biotic stress can readily be hypothesised to have important and long-lasting consequences for plant development and ultimately plant reproduction. While studies suggest that early-season herbivory has the most substantial effects on plant development, herbivore community assembly, and seed production compared to herbivory in later stages of plant development, effects on plant reproduction are not consistent and range from negative to neutral or even positive (Adhikari & Russell, 2014; Garcia & Ehrlen, 2002; Pearse et al., 2018; Rasmussen & Yang, 2022; Rusman et al., 2020). For example, a study on *B. nigra* showed that herbivory by a diverse set of insects in the early ontogenetic stages of plants resulted in a reduced seed production compared to herbivory in later ontogenetic stages (Rusman et al., 2020), while a study involving milkweed and monarch caterpillars found that the reproduction of plants challenged early in their development was comparable to that of plants, which were left unchallenged (Rasmussen & Yang, 2022). Our exclusion experiment confirms that early-season herbivory can affect plant reproduction, but also highlights the context dependency of effects (Figure 2). Variation in selection pressure

FIGURE 4 Overview of the number of plants on which each herbivore species occurred (i.e. prevalence) at a given monitoring round (panels a and d) with their respective scales, and the associated interaction network between the herbivore species (blue bars) and plant species (green bars; panels b and c). Lines connecting herbivore species and plant species in the network represent realised interactions, with the width of these lines representing the frequency of the interaction. The percentage of plants belonging to the different plant species in each stage of ontogeny at a given monitoring round was classified as vegetative (dark green), budding (light green), flowering (yellow), or seeding (orange) (panels e and f). We constructed these figures for 2017 (panels a, b, and e) and 2018 (panels c, d, and f) separately.

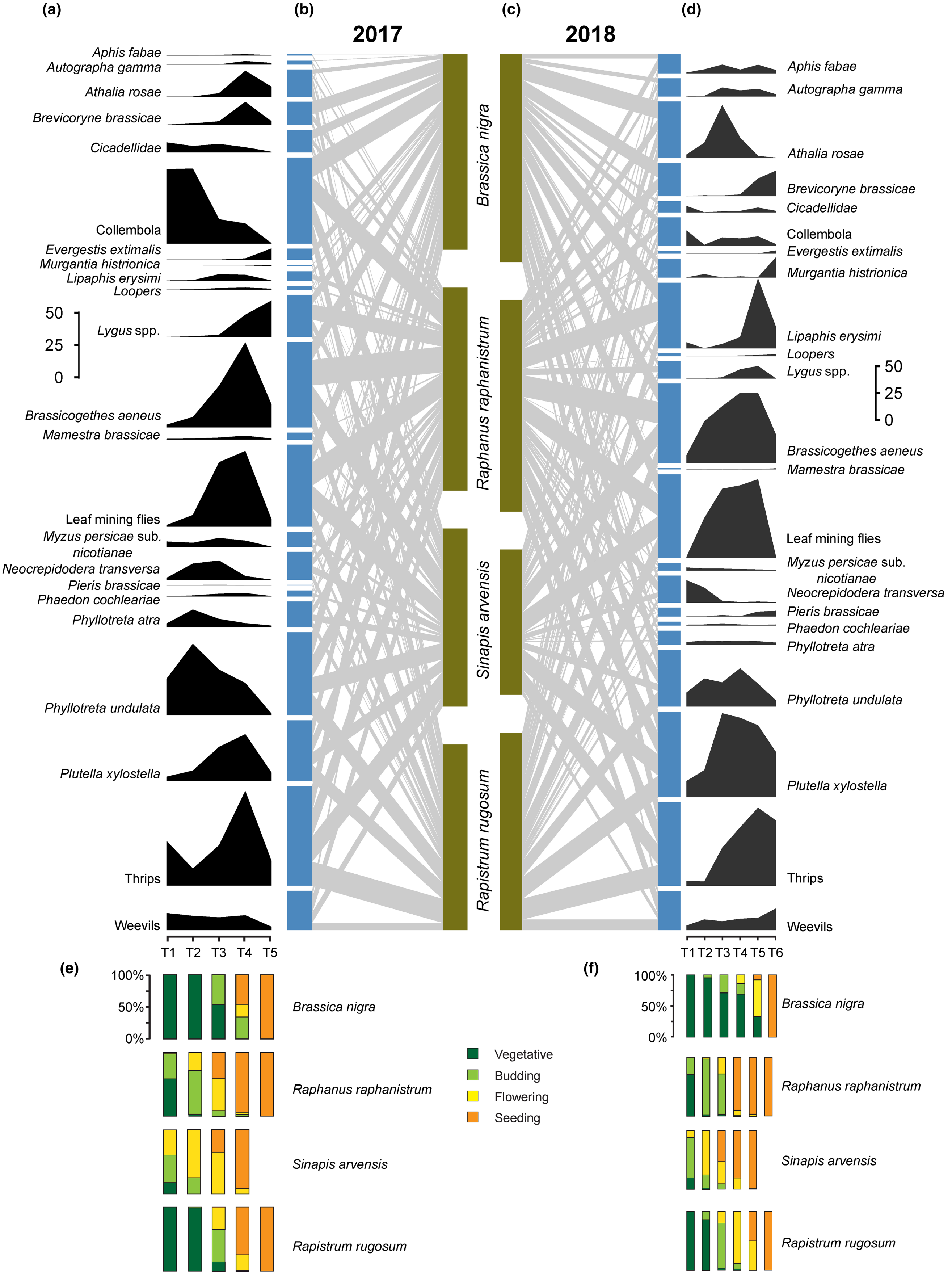

(a)
(b)
2017
(c)
2018
(d)
Aphis fabae
Autographa gamma
Athalia rosae
Brevicoryne brassicae
Cicadellidae
Collembola
Evergestis extimalis
Murgantia histrionica
Lipaphis erysimi
Loopers
Lygus spp.
Brassicogethes aeneus
Mamestra brassicae
Leaf mining flies
Myzus persicae sub. nicotianae
Neocrepidodera transversa
Pieris brassicae
Phaedon cochleariae
Phyllotreta atra
Phyllotreta undulata
Plutella xylostella
Thrips
Weevils
50
25
0
Brassica nigra
Raphanus raphanistrum
Sinapis arvensis
Rapistrum rugosum
T1 T2 T3 T4 T5
T1 T2 T3 T4 T5 T6
(e)
(f)
100%
50%
0%
Vegetative
Budding
Flowering
Seeding

TABLE 3 Results of the PERMANOVA testing the effects of early-season herbivory treatments on the composition (incidence) and structure (weighted abundance) of the full herbivore community associated with individual plants in each plant species by year combination.

		Composition				Structure			
Year	Plant species	Pseudo-*F*	R^2	df	*p*-value	Pseudo-*F*	R^2	df	*p*-value
2017	*Brassica nigra*	1.07	0.03	2	0.776	0.95	0.03	2	0.877
	Raphanus raphanistrum	1.09	0.03	2	0.698	1.01	0.03	2	0.868
	Sinapis arvensis	1.51	0.06	2	0.297	1.32	0.05	2	0.586
	Rapistrum rugosum	1.33	0.04	2	0.554	0.96	0.03	2	0.917
2018	*Brassica nigra*	1.15	0.03	2	0.592	0.98	0.03	2	0.600
	Raphanus raphanistrum	1.24	0.04	2	0.578	1.57	0.04	2	0.406
	Sinapis arvensis	1.00	0.03	2	0.635	2.06	0.06	2	0.058
	Rapistrum rugosum	0.91	0.03	2	0.881	0.97	0.03	2	0.775

Note: To account for dependency of observations, we applied a stratified permutation design (1000 permutations) with random sampling to ensure equal replication across treatment levels. The table presents the median pseudo-*F* value with associated R^2 and statistical significance in terms of *p*-value. A more complete overview of pseudo-*F* values per quantile is given in Table S9.

induced by early-season herbivory may then arise because plant species or individuals are not equally prepared for, or adapted to, the same stress, or because plants are selected to face different trade-offs over their development which were manifested by excluding the plant-associated community (Cipollini et al., 2014; Lu et al., 2016; Wright & McConnaughay, 2002). Even though the plant species in our study are closely related and consequently show similarities in their phenotype and development, inter- and intraspecific variation in development and chemical traits is still substantial (Mertens, Bouwmeester, & Poelman, 2021). While our study does not allow us to confidently correlate variation in specific plant functional traits to the effects of early-season herbivory on plant reproduction, differences in adaptation to early-season herbivores through plant chemical defences and life-history strategies is likely to be an important explanation for the conditionality of effects (Ahuja et al., 2011; Blažević et al., 2020). Alternatively, tolerance to early-season herbivory may be resource-driven when inter- and intraspecific variation in plant responses to local environmental conditions is substantial. While the agricultural history and preparation of the field site makes that variation in soil properties or nutrient availability is small and an unlikely explanation for the conditionality of effects, we did not measure soil conditions to support this claim. In addition, it is important to note that abiotic conditions in the exclusion experiment may have been uncommon for plants growing under natural conditions. The tents used to prevent the natural insect community from interacting with the plants may affect local levels of precipitation, airflow and water retention in the soil, which potentially affects how plants perceive their local environment (Sharma et al., 2020).

When plants were allowed to interact with their full associated community, the effect of early-season herbivory was strongly attenuated, revealing only marginal effects on plant reproduction. The most profound effect of early-season herbivory on plant fitness in a community context was found for *R. rugosum*, where in the 2018 season, plants challenged by *P. rapae* had reduced seed production compared to plants that were left untreated. The diminished effects of early-season herbivory on plant reproduction in our open field study may be explained by several non-exclusive hypotheses.

First, the pressure induced by our treatment may have been within the tolerance levels of plants, allowing plants to compensate for the damage to tissues and achieve equal reproductive success to that of untreated plants (Garcia & Eubanks, 2019; Strauss & Agrawal, 1999). Even when plants are unable to fully compensate for the effects induced by early-season herbivory through changes in their development, causal effects on seed production mediated by other factors related to plant development at different times in the ontogeny of plants may outweigh or cancel out effects mediated by variation in plant development, resulting in minimal net effects of early-season herbivory on plant reproduction (Hambäck et al., 2015). However, the effects of early-season herbivory on plant reproduction observed in the herbivore exclosure experiment provides evidence against this hypothesis (Figure 2).

Second, the effects on plant development and reproduction induced by the full plant-associated community may outweigh any effects induced by the initial herbivore treatment. Moreover, in cases where the community, or a subset of key herbivores in the community, is unresponsive to variation in plant traits induced by initial herbivory, the community of herbivores will be homogenised across treatments resulting in comparable effects on plant reproduction (Agrawal, 2005; Poelman & Kessler, 2016). Alternatively, subsequently arriving herbivores may prefer undamaged or better-developed plants, effectively homogenising herbivore pressure on seed production across different treatments in our experiment (Edwards & Wratten, 1983; Rubin et al., 2015). Indeed, the notion that herbivores may prefer taller, more apparent plants has been suggested for brassicaceous plants, with potential effects on plant fitness (Schlinkert et al., 2016). In addition to host choice in response to variation in plant apparency, the temporal structuring of herbivore communities is intimately connected with the availability of specific niches preferred by herbivores, which in turn is closely related to plant ontogeny (Figure 4; Ekholm et al., 2020; Tonkin et al., 2017). For example, the strong correlation between the start of flowering and interactions with the pollen beetle *Brassicogethes aeneus* may cause undamaged plants which develop fast, grow tall, and produce abundant inflorescences to attract high numbers of beetles. Feeding damage by these florivores can lead to inflorescences with damaged or undeveloped seeds, and therefore reduced seed production, effectively cancelling out the headstart these plants had (Rusman et al., 2020; Schlinkert et al., 2016; Williams, 2010). In support of this hypothesis, our multivariate analysis indicated that herbivore communities associated with the different treatments were not distinguishable in terms of their composition or structure for any of the plant species in either of the 2 years. We did find priority effects of early-season herbivory on the richness and abundance of herbivores or subsets of the herbivore community (Tables S7–S11). However, the exploratory path model suggested that the observed variation in herbivore pressure in terms of species richness, herbivore abundance and leaf damage was less likely to substantially affect seed production, providing an explanation for the minimal effects on plant reproduction observed in the open field.

A third hypothesis is that plant responses to later stressors were generally not compromised by trade-offs in the underlying physiology or development of responses to differential early-season herbivory. In this perspective, induced responses to initial herbivory are likely selected to incorporate the most likely trade-offs plants face, allowing them to dampen the effects cascading through the associated community (Karban, 2019; Mertens, Boege, et al., 2021; Orrock et al., 2015; Viswanathan et al., 2005). Plants may fine-tune responses to initial herbivory and the development of functional traits to best fit the most likely or most fitness-impacting future stressors, achieving reproductive success independent of our applied treatment. While a path model assuming equivalence in path coefficients among all plant species and both years was most parsimonious, the substantial variation in path coefficients when fitting the model to the different plant species suggests that the strength of direct and indirect effects linking plant development and herbivore pressure to plant reproduction may not have been generalizable across plant species. Variation among plant species in terms of the strength of plant- or community-mediated effects on seed production may indicate that herbivore pressure varies in how significant it acts as an agent of natural selection driving the evolution of plant life history strategies. In addition, while early-season herbivory may be conjectured to impose frequency-dependent selection on (induced) plant traits and life-history strategies, it is also likely that, for systems with high variability in insect communities over multiple generations, interannual variation in the biotic and abiotic environment leads to stabilised selection in which effects are neutral in their overall pressure on plant trait evolution (Mertens, Boege, et al., 2021).

A fourth hypothesis relates to the fact that this study limits its focus to the associated herbivore community. However, plants maintain ecological interactions with many different community members such as micro-organisms, carnivores and pollinators which are not assessed in our experiments. These ecological interactions have been shown to be involved in indirect plant defences or tolerance to herbivory and are important in determining plant reproduction (Howard et al., 2020; Kos et al., 2011). For example, induced responses to early-season herbivory can differentially affect the carnivore community, causing changes in top-down (predator-mediated) control on herbivore communities (Li et al., 2016; Lucas-Barbosa et al., 2016). Likewise, even though early-season herbivory has been shown to affect flower characteristics (Barragán-Fonseca et al., 2019; Rusman et al., 2019), a diverse pollinator community may ensure pollination by functional complementarity and thus limit herbivore-induced variation in plant reproduction (Santamaria & Rodriguez-Girones, 2007). These components of the plant-associated community may mediate the observed but unexplained causal relation between early-season variables such as plant volume and plant reproduction in our path models.

Finally, the attenuated effect sizes of early-season herbivory in the open field experiments can be explained by the natural occurrence of the two herbivore species we manipulated. These species are common and readily colonise the plant species used in our experiment under natural conditions (Mertens, Bouwmeester, et al., 2021). Hence, most plants in our experiment interacted with these species at some point in the early- to mid-season stages of their ontogeny. If the timing of attack by the early-season herbivore is not important, we could assume that all plants in our experiment were similarly induced. However, a recent study on *B. nigra* highlights the importance of the timing of herbivore attack, diminishing the plausibility of this hypothesis (Rusman et al., 2020).

5 | CONCLUSIONS

Our results show that in our study system, early-season herbivory could affect plant reproduction in the absence of the plant-associated insect community. However, these effects were attenuated when plants could interact with their associated community. By using path analysis, we predict that the absence of effects on reproduction should either be explained by the absence of effects of early-season herbivores on subsequent plant development, the dynamic interaction between plant development and herbivore pressure, or that such effects are compensated for. Early-season herbivory infrequently affected plant development, and when it did, plant reproduction remained unaffected. However, we could not identify a clear compensatory mechanism. Importantly, effects of early-season herbivory on plant development or the subsequent development of the associated herbivore community were small and not general across closely related species, suggesting that plant species may be exposed to different levels of natural selection by early-season herbivores through plant- or community-mediated effects on reproduction. However, these effects should be evaluated in context of interactions with a broader suite of community members and variation in abiotic conditions.

AUTHOR CONTRIBUTIONS

DM and EHP conceived the study and designed the experiment. BBJK, DM, YZ and SAZ collected the data. DM and JCD analysed the data. DM and EHP led the writing of the manuscript. All authors provided critical feedback on earlier drafts, contributed substantially to the final version of the manuscript and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.c866t1gf7 (Mertens et al., 2024).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Plant species used in our experiment, detailing the number of plants included per treatment (three *Myzus persicae* aphids, two *Pieris rapae* caterpillars, or plants left untreated) in the two field seasons as used in the statistical analysis.

Table S2. Herbivore insect species observed in our experiment.

Table S3. Overview of differently formulated path models, the degrees of freedom, and their fit expressed by AIC, and Fisher's *C* global goodness of fit statistic with the associated *p*-value.

Table S4. Overview of the fit and structure of path models after optimization when starting from path models with different constraints on the effects of plant species and year on the mean estimated for variables in the path models.

Table S5. Overview of path coefficients and their associated standard error (Estimate ± SE) and *p*-value for each of the causal relations retained in the optimised descriptive piecewise path model.

Table S6. Overview of the direct, indirect, and total effect of variables included in the optimized descriptive piecewise path model on seed production of plants in the open field experiment, calculated for the different subsets of the data based on plant species, year, or plant species by year combination.

Table S7. Overview of effect size of early-season herbivory (Treatment), year of the field season (Year), and their interaction (Treatment*Year) on the different variables related to plant development and the associated herbivore community as used in the path model.

Table S8. Overview of the estimated mean and associated standard error of the variables included in the path model for the three different treatments, i.e. plants which were challenged early in the season by *Myzus persicae* aphids, *Pieris rapae* caterpillars, or which were left untreated.

Table S9. Overview of the effect size as estimated by generalized additive mixed models of early-season herbivory (Treatment), year of the field season (Year), and their interaction (Treatment*Year) on herbivore species richness, herbivore abundance, the number of damaged leaves, the volume of plants, and the number of leaves observed for the different plant species.

Table S10. Overview of the effect size as estimated by generalized additive mixed models of early-season herbivory (Treatment), year of the field season (Year), and their interaction (Treatment*Year) on the richness and abundance of chewing herbivores and sap-feeding herbivores observed for the different plant species.

Table S11. Overview of the estimated mean and associated standard error of variables related to herbivore pressure as estimated by the GAMM analyses presented in Tables S9 and S10, for the three different treatments, that is, plants which were challenged early in the season by *Myzus persicae* aphids, *Pieris rapae* caterpillars, or which were left untreated.

Table S12. Results of the PERMANOVA analysis testing the effects of early-season herbivory treatments on the composition (incidence) and structure (weighted abundance, calculated as Hellinger-transformed herbivore abundance data) of the full herbivore community associated with individual plants in each plant species by year combination.

Figure S1. Exploratory piecewise path model obtained by optimizing our causal hypothesis where path coefficients and estimation of variables were constrained to be equal across all plant species and the 2 years.

Figure S2. Ordination of observed herbivore community composition (expressed by incidence of herbivores, panels A and B), and structure (expressed by Hellinger-transformed herbivore abundance data, panels C and D) in *Brassica nigra* according to three NMDS ordination axes (stress = 0.18 and 0.15 respectively).

Figure S3. Ordination of observed herbivore community composition (expressed by incidence of herbivores, panels A and B), and structure (expressed by Hellinger-transformed herbivore abundance data, panels C and D) in *Raphanus raphanistrum* according to three NMDS ordination axes (stress = 0.18 and 0.16 respectively).

Figure S4. Ordination of observed herbivore community composition (expressed by incidence of herbivores, panels A and B), and structure (expressed by Hellinger-transformed herbivore abundance data, panels C and D) in *Sinapis arvensis* according to three NMDS ordination axes (stress = 0.17 and 0.17 respectively).

Figure S5. Ordination of observed herbivore community composition (expressed by incidence of herbivores, panels A and B), and structure (expressed by Hellinger-transformed herbivore abundance data, panels C and D) in *Rapistrum rugosum* according to three NMDS ordination axes (stress = 0.18 and 0.16 respectively).

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