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LETTER

Intraspecific chemical diversity among neighbouring plants correlates positively with plant size and herbivore load but negatively with herbivore damage

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Abstract

Intraspecific plant diversity can modify the properties of associated arthropod communities and plant fitness. However, it is not well understood which plant traits determine these ecological effects. We explored the effect of intraspecific chemical diversity among neighbouring plants on the associated invertebrate community and plant traits. In a common garden experiment, intraspecific diversity among neighbouring plants was manipulated using three plant populations of wild cabbage that differ in foliar glucosinolates. Plants were larger, harboured more herbivores, but were less damaged when plant diversity was increased. Glucosinolate concentration differentially correlated with generalist and specialist herbivore abundance. Glucosinolate composition correlated with plant damage, while in polycultures, variation in glucosinolate concentrations among neighbouring plants correlated positively with herbivore diversity and negatively with plant damage levels. The results suggest that intraspecific variation in secondary chemistry among neighbouring plants is important in determining the structure of the associated insect community and positively affects plant performance.

Keywords

Associational resistance, biodiversity–ecosystem function, *Brassica*, chemical polymorphism, community genetics, glucosinolates, herbivory, plant diversity, plant–neighbour interactions, trophic cascades.

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INTRODUCTION

It has been widely recognised that biological diversity plays an essential role in structuring communities and ecosystem processes (Tilman *et al*. 1996, 2006; Naeem & Li 1997; Yachi & Loreau 1999; Snyder *et al*. 2006; Haddad *et al*. 2011). Both inter- and intraspecific genetic diversity serve as a reservoir for variation in traits involved in ecological interactions across environments. Heterogeneity in the biotic and abiotic environment, in turn, influences the evolution of populations maintaining trait diversity (Wimp *et al*. 2004; Crawford *et al*. 2007; Hughes *et al*. 2008; Lankau & Strauss 2008). In terrestrial ecosystems, plants provide most of the resources for higher trophic levels and shape the composition of communities. Empirical studies have shown that intraspecific plant diversity, to a large extent, drives the assembly of invertebrate communities associated with a plant species, resulting in increased plant fitness (Crutsinger *et al*. 2006; Johnson *et al*. 2006; Kotowska *et al*. 2010; Ferrier *et al*. 2012; Lamit *et al*. 2015). Remarkably, intraspecific diversity can be as important for the associated community as interspecific diversity (Cook-Patton *et al*. 2011),

Genetic diversity among neighbouring plants has been shown to affect the associated community at different trophic levels and functional groups (herbivores: e.g. Johnson *et al*. 2006; Ferrier *et al*. 2012; carnivores of herbivores: e.g. Crutsinger *et al*. 2006; Johnson *et al*. 2006; and pollinators: e.g. Genung *et al*. 2012). The community changes because of increasing genetic plant diversity can be understood as a result of the indirect genetic effects which shape the structure and function of communities and can explain the non-additive effects observed in diversity experiments (Bailey *et al*. 2014). However, the underlying mechanisms and the plant traits that explain community responses to increased diversity are poorly understood (Hughes *et al*. 2008; Abdala-Roberts & Mooney 2014; Moreira *et al*. 2016).

In experiments manipulating intraspecific plant diversity, the general trend is that plants in more diverse plots tend to have a more diverse associated community and a higher individual plant fitness (Hughes *et al*. 2008; Moreira *et al*. 2016). Genotypic variation may influence the distribution and damage levels of herbivores on focal plants through processes referred to as associational resistance or susceptibility (Barbosa *et al*. 2009). Here focal plants gain protection by growing next to less palatable plants or become more susceptible to herbivory by a spillover effect from attractive or palatable neighbour plants (Barbosa *et al*. 2009; Plath *et al*. 2012; Zakir *et al*. 2013; Ruttan & Lortie 2015). Variation in chemical and

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physical traits may act directly upon the arthropods or indirectly through plant–plant interaction (e.g. resource competition, camouflage) and could explain some of the observed diversity effects on the associated insect community.

Different plant traits may be responsible for the diversity effects. For example, plant secondary metabolites (PSM) are often studied for their role in defence against insect herbivores and other plant antagonists (Fraenkel 1959; Schoonhoven *et al.* 2005; Iason *et al.* 2012), but they have been less well studied as factors influencing community structure. Variation in PSMs can directly affect the abundance and composition of the associated herbivore community and the consequent amount of damage by influencing the ability of herbivores to find and/or colonise specific plant phenotypes (Finch *et al.* 2003). The effects of PSMs are not restricted to the ecological interactions with a focal plant, but they can also affect interactions indirectly through chemical changes as a result of plant–plant communication (Heil & Karban 2010; Jactel *et al.* 2011; Zakir *et al.* 2013; Schuman *et al.* 2015) or soil legacy effects (Kostenko *et al.* 2012), resulting in associational resistance or susceptibility in neighbouring or successive plants, respectively. In addition to the effects of individual PSMs, diversity in PSMs can also determine the interactions with herbivores (Lankau & Strauss 2007; Poelman *et al.* 2009; Moore *et al.* 2014; Richards *et al.* 2015) and recently the importance of chemical diversity for other biodiversity levels has been brought to attention (Schuman *et al.* 2016). Although the influence of PSMs has been explored in the context of genetic diversity effects (Parker *et al.* 2010), PSM diversity among neighbouring plants has received less attention.

The aims of this study were to investigate to what extent plant chemical diversity among neighbouring plants accounts for species abundance and diversity of the associated invertebrate community, both herbivores and their natural enemies, and whether this has consequences for traits considered important for plant fitness such as size and herbivore damage. Plant–insect interactions have been extensively studied in the wild cabbage, *Brassica oleracea* (Moyes *et al.* 2000; Gols *et al.* 2008b; Newton *et al.* 2009a) and its derived crops (Bukovinszky *et al.* 2008; Hambäck *et al.* 2009; Poelman *et al.* 2009; Broekgaarden *et al.* 2010). Like other brassicaceous species, *B. oleracea* contains glucosinolates that interact with plant myrosinase upon tissue damage producing a range of compounds (Wittstock & Halkier 2002; Halkier & Gershenzon 2006) that are toxic to many generalist herbivores and directly or indirectly affect their growth and development (Agrawal & Kurashige 2003; Gols & Harvey 2009; Hopkins *et al.* 2009; Winde & Wittstock 2011). Natural populations of *B. oleracea* exhibit substantial quantitative and qualitative genetic variation in foliar glucosinolates (Mithen *et al.* 1995). This variation has been shown to affect the presence and abundance of herbivores on individual plants and among populations in the field (Moyes *et al.* 2000; Moyes & Raybould 2001; Newton *et al.* 2009a, 2010).

In a common garden experiment, we established plots with plants from the same (monocultures) or different populations (dicultures and tricultures) using three natural *B. oleracea* populations that differ substantially in constitutive and herbivore-inducible glucosinolate concentrations (Gols *et al.* 2008b; Harvey *et al.* 2011). We monitored the associated invertebrate community throughout the growing season and characterised glucosinolate profiles of individual plants. Specifically, we explored the following questions:

(1) Does increasing chemical diversity among neighbouring *B. oleracea* plants influence the associated herbivore and carnivore community, and does it have consequences for plant size and plant damage levels?
(2) Do generalist and specialist herbivores respond differentially to increasing chemical diversity among neighbouring plants?
(3) To what extent do glucosinolates in focal plants and glucosinolate variation among neighbouring plants affect the herbivore community and plant damage levels?

MATERIAL AND METHODS

Biological system

The selected *B. oleracea* populations, Kimmeridge (50°60′ N, 2°13′ W), Winspit (50°59′ N, 2°03′ W) and Old Harry (50°64′ N, 1°92′ W), are located within a range of 15 km in Dorset, England. These populations are genetically sub-structured and gene flow is low but significant among populations (Raybould *et al.* 1999). Seeds from a bulk random sample collected from at least 20 plants per population along a transect were sown in trays on 14 April 2014 and grown under glasshouse conditions at Wageningen University. After 5 weeks, the seedlings were transplanted into the experimental field located next to the university campus (51°59′ 22″ N, 5°39′59″ W). The experimental design consisted of 63 plots (4 × 4 m each), separated by rows (3 m width) of grass (*Lolium* and *Poa* species). Each plot contained 25 plants in a square array of 5 × 5 plants placed 75 cm apart.

Common garden experiment

For exploring how chemical differences among neighbouring plants affected the associated invertebrate community, we manipulated the frequency of the three plant populations within the plots. Twenty-seven plots contained only one plant population (9 plots for each of the three plant populations), 27 plots consisted of two plant populations (9 plots for each of the three possible combinations) and 9 plots contained plants from each of the three populations. Hereafter, these diversity treatments are referred to as monocultures, dicultures and tricultures respectively. The plots were spatially arranged according to a fully randomised design.

In the dicultures, plants from the same population were planted in alternate diagonals of the 5 × 5 plant matrix. Thus, each plant individual inside the plot core of nine plants had four neighbouring plants of another population at each side. The same procedure was followed up for tricultures, where each plant individual in the plot core had two plants of each of the other populations as neighbouring plants.

Invertebrate community monitoring

The invertebrate community was monitored on the nine central plants of each, however some plants died over time (at the end of the season, 38 plants from the plot cores had died;

no difference in mortality was found between plant population, diversity treatment or their interaction; generalised linear model (GLM): all $P > 0.05$). The upper and lower surfaces of each leaf of these plants were visually inspected and the number of individuals of each invertebrate species per plant was recorded. Herbivores were identified at the species level (Table S1), whereas the carnivores were classified at different taxonomic levels (Table S2). The common parasitoid species were identified based on cocoon morphology. The monitoring was performed six times during the whole season from June to August, in weeks 23, 24, 25, 26, 28 and 32 of 2014, with a blind protocol. For each plant, we obtained the abundance per herbivore and carnivore groups and we calculated the Shannon's diversity index (H) for the herbivore community only, because many carnivores were not identified at the species level. The cabbage aphid *Brevicoryne brassicae* is a specialist sap feeding insect and the most common in cabbage crop fields (Bukovinszky *et al*. 2008). Its main parasitoid *Diaeretiella rapae* (a braconid wasp) was very abundant accordingly. Even after log-transformation these species still disproportionally influenced the overall abundance of herbivores and carnivores, therefore, we excluded them from herbivore and carnivore abundances and analysed them separately.

Plant measurements

Number of leaves was counted and maximum height and width were measured for the nine central plants in each plot. Plant size was calculated as the volume of a cylinder that would fit each plant given its maximum height and diameter in centimetres. Total damage was estimated visually as a proportion of the total leaf area and through comparisons with photographs of plants with a wide range of damage levels. The proportion of damaged leaf area ranged from 0 to 1 with levels taken to the nearest first decimal, making up 11 levels in total. Both plant size and damage levels were measured during the six time points across the season. In week 33 (17 weeks after germination), when monitoring had finished, leaf samples were taken for glucosinolate analysis (for details see the Supporting Information) from the nine central plants (or less plants due to mortality). Sixty plots were sampled since three plots had been flooded previously. We sampled leaves at the end, since repeated sampling would have disturbed the insects and altered the plants' condition. Moreover, the glucosinolate composition is known to be stable throughout the season (Gols *et al*. unpublished results); although the concentrations change ontogenetically and in response to herbivory, this is considered systematic among plant populations. From each plant, 12–15 discs (diameter 2.5 cm) were punched with a cork borer from five fully developed leaves, wrapped in tin foil, immediately flash frozen in liquid nitrogen and then stored at −20 °C.

Statistics

All statistical analyses were performed in R (R Core Team, v 3.2.3, Vienna, Austria). We first explored to what extent population origin of focal plants and the number of populations within plots (hereafter diversity treatment), included as fixed factors, explained the variation in invertebrate community properties (herbivore and carnivore abundance, and herbivore diversity) and plant traits (size and damage levels) at the individual plant level. As each core plant was monitored repeatedly (six times in total), data were analysed with repeated measures analyses using linear mixed models (*lme4* package for fitting linear mixed models and GLMs) adding week as a fixed factor and plant ID as a random factor. As plant size may affect colonisation by the herbivores, an additional model for herbivore and *B. brassicae* abundance was run where plant size was included as a covariate. Models analysing abundance of carnivores and *D. rapae* included the abundance of herbivores as a covariate. For the model analysing plant damage, the abundance of carnivores and chewing herbivores, and the herbivore diversity were included as covariates. Statistical models also included the interaction terms between week, diversity treatment and plant population. The accompanying graphs of these analyses are given in the Supporting Information.

The glucosinolates were measured only once at the end of the season so glucosinolate data were analysed using a fixed effects linear model. A multivariate approach was used to analyse differences in the glucosinolate composition among plant populations. Here, we used partial least squares regression with discriminant analysis (PLS-DA, *mixOmics* package, González *et al*. 2011) which reduces the dimensions of the multivariate data taking into account the separation by groups (in this case plant populations) and allows to explore which glucosinolate variables contribute most to the differences among groups.

Second, we investigated whether there were correlations between glucosinolate and herbivore community properties, as well as plant damage levels. For these analyses, we used the values per plant averaged across the season, as an estimate of the community and plant responses throughout the season. As dietary breadth and feeding guild may also influence how insects respond to glucosinolates, we performed multiple regressions with abundance of generalist and specialist herbivores, and the dominant aphid *B. brassicae* as response variables. Similarly, effects of glucosinolates on herbivore diversity and plant damage were analysed. The explanatory variables were the total concentration, composition and variation of glucosinolates within plots and their interactions with diversity treatment. We used the first component of the PLS analysis as an estimate of glucosinolate composition. The variation in glucosinolates within plots was estimated as the coefficient of variation of the total glucosinolate concentrations among the sampled plants within plots (CV_{conc}). The CV_{conc} increased when more plant populations were combined within plots ($F_{(1,58)} = 40.34$, $P < 0.0001$) and there were also differences in CV_{conc} among plant–population combinations ($F_{(3,30)} = 4.93$, $P = 0.0067$; Fig. S1). Since dicultures and tricultures had overlapping mean CV_{conc} values, which were higher than those in monocultures (Fig. S1), we grouped dicultures and tricultures into a single level (polyculture) and included the diversity treatment as a two-level factor. For the models analysing the herbivore community attributes, we included plant size as a covariate. The analyses are described in detail in the Supplementary Information.

RESULTS

Invertebrate community

Abundance

Herbivore abundance (excluding the aphid *B. brassicae*) increased until week 28 (mid-July) and was lower in the final monitoring week (week 32, mid-August) than in week 28 (time effects: $\chi^2_{(1)} = 855$, $P < 0.0001$; Fig. 1a). Increasing plant diversity of the plots increased the abundance of the herbivores ($\chi^2_{(1)} = 8.42$, $P = 0.004$; Fig. 1b). In general, Winspit plants harboured fewer herbivores than Kimmeridge and Old Harry plants ($\chi^2_{(2)} = 19.20$, $P < 0.0001$; *post hoc* Tukey: $P = 0.0002$ and $P = 0.0014$ respectively). However, there was a triple interaction between diversity treatment, plant population and time ($\chi^2_{(2)} = 67.6$, $P < 0.0001$; Fig. S2). Kimmeridge and Winspit plants had more herbivores in more diverse plots, but this effect was evident in Old Harry plants only later in the season. When plant size was added as a covariate in the model, there were no differences in abundance of herbivores among plant populations ($\chi^2_{(2)} = 4.75$, $P = 0.09$), whereas plant size was highly significant in explaining herbivore abundance ($\chi^2_{(1)} = 727$, $P < 0.0001$). Here, the effects of plant diversity treatment and the triple interaction were also significant ($\chi^2_{(1)} = 3.92$, $P = 0.048$ and $\chi^2_{(2)} = 60.9$, $P < 0.0001$; Fig. S3), as in the previous model.

A separate analysis, including only the aphid *B. brassicae*, showed that its abundance increased during the season ($\chi^2_{(1)} = 13810$, $P < 0.0001$, Fig. S4) and was highly influenced by plant population ($\chi^2_{(2)} = 43.2$, $P < 0.0001$). Old Harry plants harboured more cabbage aphids than Kimmeridge and Winspit plants (*post hoc* Tukey: $P < 0.0001$, both comparisons). The abundance of this aphid was affected by a triple interaction among plant population, diversity treatment and time ($\chi^2_{(2)} = 496$, $P < 0.0001$). Kimmeridge plants showed a lower number of cabbage aphids in more diverse plots, but this effect was reverted with time. The opposite trend was shown for the Winspit plants, while no effect was observed in the abundance of cabbage aphids in Old Harry plants. Similar results were obtained when plant size as covariate was included (Fig. S5).

Carnivore abundance (excluding the parasitoid *D. rapae,* the most important parasitoid of the aphid *B. brassicae*) increased with time ($\chi^2_{(1)} = 3720$, $P < 0.0001$, Fig. S6) and was affected by the origin of the focal plant ($\chi^2_{(2)} = 8.88$, $P = 0.012$) and their interaction term ($\chi^2_{(2)} = 17.9$, $P = 0.0001$). Old Harry plants had more carnivores than Winspit plants (*post hoc* Tukey: $P = 0.019$), but this effect waned with time. The interaction between diversity treatment and time was also significant ($\chi^2_{(1)} = 25$, $P < 0.0001$). Carnivores were more abundant in high diversity plots but these effects faded with time. The abundance of herbivores was positively associated with carnivore abundance ($\chi^2_{(1)} = 165$, $P < 0.0001$). The waning effect of diversity treatment with time on abundance was more prominent for *D. rapae* (parasitoid of *B. brassicae*) than for the other carnivores, especially on Old Harry and Winspit (Fig. S7).

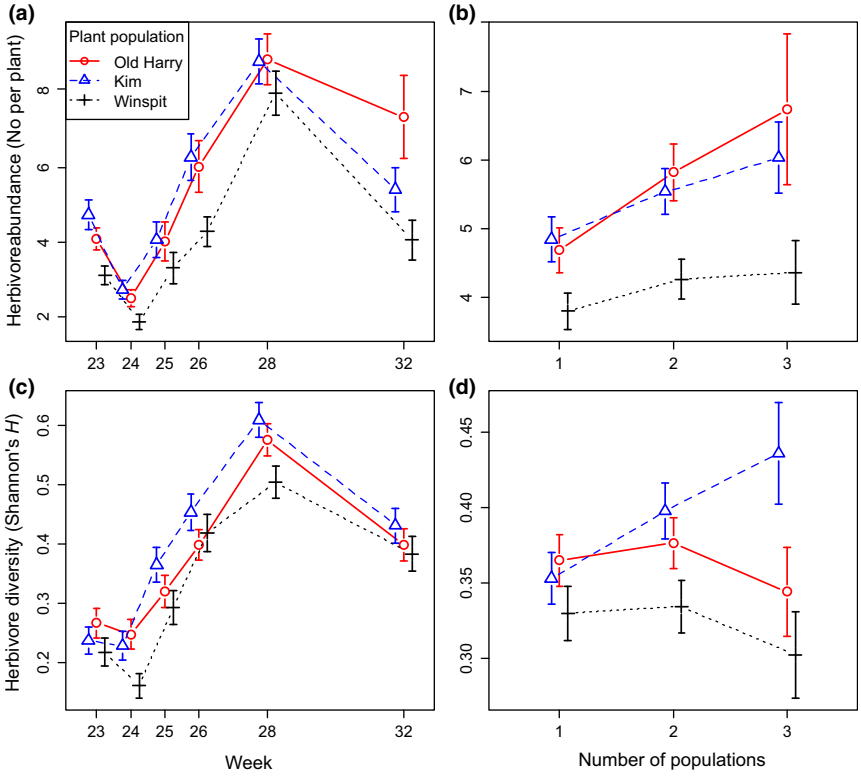

Figure 1 Abundance (a and b) and Shannon's diversity index (c and d) of the herbivore community (excluding the most dominant species, the aphid *Brevicoryne brassicae*) associated with individual plants of three wild cabbage populations averaged across the season (a and c) and among diversity treatments (b and d, monocultures of one, dicultures of two or tricultures of three populations). $N = 529$, error bars (± 1 SEM). The same legend applies for all panels.

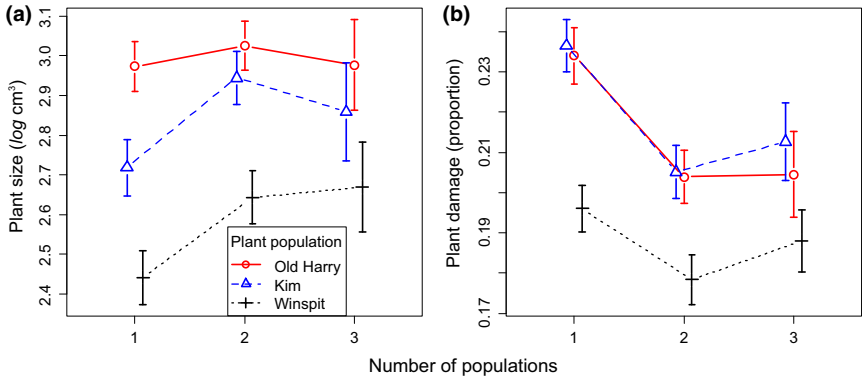

Figure 2 Mean plant size (a) and damage levels inflicted (b) to cabbage plants originating from three wild populations and planted in different diversity combinations averaged across the season (monocultures of one population, dicultures of two populations or tricultures of three populations). $N = 529$, error bars (± 1 SEM). The same legend applies for both panels.

Herbivore diversity

The Shannon's diversity index increased with time until week 28, after which it decreased at the final time point of measuring ($\chi^2_{(1)} = 150$, $P < 0.0001$; Fig. 1c). Herbivore diversity differed among the plant populations ($\chi^2_{(2)} = 9.62$, $P = 0.008$, Fig. 1d). Species diversity was higher on Kimmeridge plants than on Winspit plants (*post hoc* Tukey: $P = 0.0066$). The three-way (population–diversity–time) interaction term was also significant ($\chi^2_{(2)} = 7.64$, $P = 0.022$; Fig. S8). The strongest opposing effects of diversity treatment occurred later in the season on Kimmeridge and Winspit plants (Fig. S8).

Plant traits

Plant size

Plants grew with time ($\chi^2_{(1)} = 22921$, $P < 0.0001$) and were larger in more diverse plots ($\chi^2_{(1)} = 8.02$, $P = 0.005$; Fig. 2a). Plant populations differed in size ($\chi^2_{(2)} = 57.7$, $P < 0.0001$). Winspit plants were smaller than Old Harry and Kimmeridge plants (*post hoc* Tukey: $P < 0.0001$, both comparisons). Time interacted with plant population ($\chi^2_{(2)} = 24.3$, $P < 0.0001$; Fig. S9) as the size difference between populations was reduced with time.

Plant damage

Plants were less damaged in more diverse plots (diversity effect: $\chi^2_{(1)} = 4.48$, $P = 0.034$; Fig. 2b). However, the origin of the focal plant *per se* also affected damage levels ($\chi^2_{(2)} = 11.8$, $P = 0.003$). Winspit plants were less damaged than Kimmeridge and Old Harry plants (*post hoc* Tukey: $P = 0.002$ and $P = 0.03$ respectively). Damage levels correlated positively with increasing abundance of leaf chewing herbivores ($\chi^2_{(1)} = 4.86$, $P = 0.027$), and negatively with herbivore diversity ($\chi^2_{(1)} = 13.2$, $P = 0.0003$), but damage levels did not correlated with carnivore abundance ($\chi^2_{(1)} = 0.41$, $P = 0.52$). There was also a significant interaction effect between time and plant diversity ($\chi^2_{(1)} = 7.45$, $P = 0.006$), and time and plant population ($\chi^2_{(2)} = 16.1$, $P = 0.0003$). The effect of plant diversity on

Table 1 Glucosinolate concentrations and loadings of the partial least square discriminant analysis (PLS-DA) for the eight foliar glucosinolates detected by HPLC

			Concentration			PLS	
Glucosinolate		Class	Old Harry	Kimmeridge	Winspit	Component 1	Component 2
Gluconapin	3-butenylglucosinolate	Aliphatic (butyl side chain)	18.20 (15.11)	0.61 (0.80)	43.70 (16.39)	0.87	0.27
Glucoraphanin	4-methylsulfinylbutyl glucosinolate		0.92 (2.45)	0.42 (0.48)	0.29 (1.02)	−0.09	0.32
Progoitrin	(R)-2-Hydroxy-3-butenyl glucosinolate		5.28 (7.14)	2.71 (2.78)	1.08 (2.01)	−0.24	0.52
Glucoiberin	3-methylsulfinylpropyl glucosinolate	Aliphatic (propyl side chain)	0.53 (0.85)	0.72 (1.05)	0.27 (0.59)	−0.23	0.01
Sinigrin	Allyl glucosinolate		3.99 (3.80)	3.13 (2.47)	3.66 (5.49)	0.04	0.15
4-Methoxyglucobrassicin	4-Methoxy-3-indolylmethyl glucosinolate	Indole	0.13 (0.08)	0.19 (0.12)	0.16 (0.08)	−0.09	−0.39
Glucobrassicin	3-Indolylmethylglucosinolate		11.06 (5.06)	7.76 (3.98)	8.97 (4.35)	0.03	0.48
Neoglucobrassicin	1-Methoxy-3-indolylmethyl glucosinolate		0.71 (1.86)	1.74 (2.89)	3.56 (3.61)	0.34	−0.39
Total glucosinolates			40.82 (15.14)	17.29 (8.45)	61.69 (18.39)		

The class of the glucosinolate refers the amino acid precursor (methionine for the aliphatic glucosinolates and tryptophan for the indole glucosinolates) and the side chain group for the aliphatic glucosinolates. The mean concentration (μmol/g DM) of individual glucosinolates is given for each plant population.

plant damage decreased with time, while the difference in damage between plant populations increased with time (Fig. S10).

Glucosinolates

Eight glucosinolates were identified in the leaves of the three plant populations (Table 1). The total glucosinolate concentrations differed among the plant populations ($F_{(2,494)} = 389$, $P < 0.0001$) with no significant effect of the number of populations present in a plot ($F_{(1,494)} = 0.08$, $P = 0.78$; Fig. 3a) or the interaction term between these two factors ($F_{(2,494)} = 1.2$, $P = 0.3$). Total glucosinolate concentrations were lowest in leaves of Kimmeridge plants, intermediate in Old Harry plants and highest in Winspit plants (*post hoc* Tukey tests: $P < 0.0001$, all comparisons). The PLS-DA on the glucosinolates revealed a grouping pattern in a two-dimensional space (Fig. 3b). Kimmeridge plants were well separated from Winspit plants, whereas the glucosinolate composition of Old Harry plants exhibited some overlap with the other two populations. Samples of the Kimmeridge plants were characterised by relatively high concentrations of glucobrassicin and sinigrin (*c.* 45 and 16% of total content, Table 1). Winspit samples differentiated mainly on the basis of the first PLS-DA component characterised by relatively high concentrations of gluconapin (*c.* 70% of total content). The glucosinolate profiles of Old Harry plants were more variable, some samples separated along the second PLS-DA component, whereas a second group of samples tended to have glucosinolate profiles closer to those characteristic for Winspit. The dominant glucosinolate in Old Harry leaf tissues was also gluconapin (*c.* 45% of total content) followed by glucobrassicin (27%, Table 1).

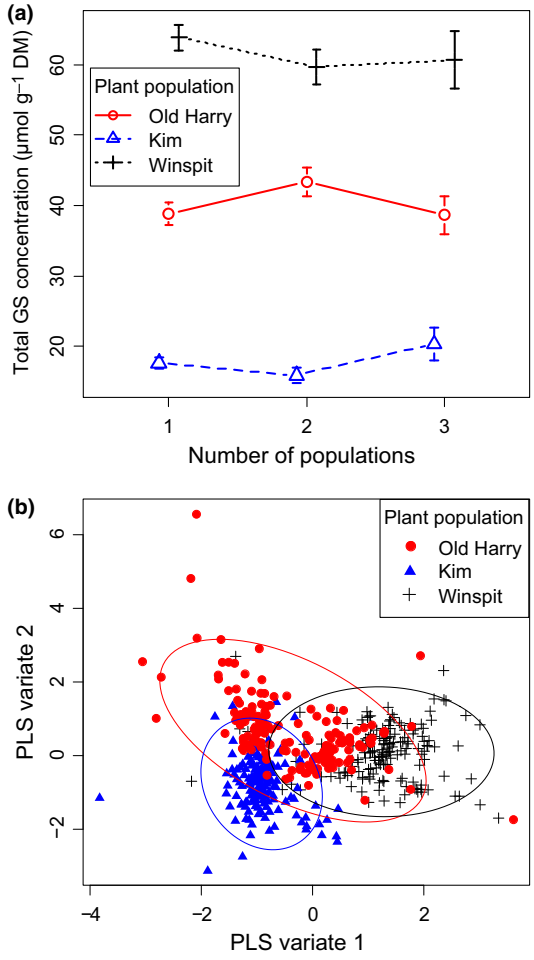

Figure 3 Foliar glucosinolate concentration and composition. (a) Mean total glucosinolate concentration (µmol per gram of dry leaf mass) per plant population among diversity treatments measured at the end of monitoring season. $N = 500$, error bars (± 1 SEM). (b) Sample contribution plots based on PLS-DA of all eight foliar glucosinolates. Samples were classified according to their population origin. The ellipses (obtained with the plotting function of the R package *mixOmics*) represent the distribution of the dots with a confidence of 95%.

Effects of plant chemistry

When considering the effects of plant chemistry using the means across all weeks for the other variables, abundance of generalist herbivores correlated negatively with the first PLS-DA component but only in polycultures ($\chi^2_{(1)} = 4.68$, $P = 0.03$; Fig. S11). The interaction between the total glucosinolate concentration and diversity treatment was significant, with fewer generalist herbivores in plants with high glucosinolate concentrations only in monocultures ($\chi^2_{(1)} = 10.2$, $P = 0.0014$; Fig. S11). The abundance of specialist herbivores (excluding *B. brassicae*) correlated positively with total glucosinolate concentration ($\chi^2_{(1)} = 5.58$, $P = 0.0018$) regardless of diversity treatment (Fig. S12). *Brevicoryne brassicae* abundance correlated positively with the first PLS-DA component ($\chi^2_{(1)} = 57.2$, $P < 0.0001$), and negatively with both total glucosinolate concentration ($\chi^2_{(1)} = 97.4$, $P < 0.0001$) and CV_{conc} (the latter effect was more pronounced in polycultures; $\chi^2_{(1)} = 94.6$, $P < 0.0001$; Fig. S13).

Herbivore diversity was influenced by an interaction between CV_{conc} and diversity treatment ($F_{(1,491)} = 5.63$, $P = 0.018$); in polycultures, higher CV_{conc} values were associated with higher herbivore diversity (Fig. 4), whereas in monocultures this pattern was reversed. The abundance of all herbivore groups and the herbivore diversity were positively correlated with plant size (all $P < 0.0001$).

Plant damage correlated negatively with the first PLS-DA component ($F_{(1,488)} = 11.78$, $P = 0.0007$; Fig. 5a) and with herbivore diversity ($F_{(1,488)}$=13.61, $P = 0.0003$; Fig. 5b). CV_{conc} had an effect on plant damage that depended on the diversity treatment ($F_{(1,488)}$=13.2, $P = 0.0003$); plants in plots with higher CV_{conc} were less damaged in polycultures, but this effect was not observed in monocultures (Fig. 5c). The interactions of diversity treatment with other variables were not significant (Table S3).

DISCUSSION

Increasing levels of chemical diversity among neighbouring plants positively correlated with the abundance of invertebrates and plant size, and at the same time correlated negatively with the amount of damage. While glucosinolate composition in leaf tissues influenced herbivore abundance and the amount of herbivore damage, increasing variation in glucosinolate concentrations among neighbouring plants

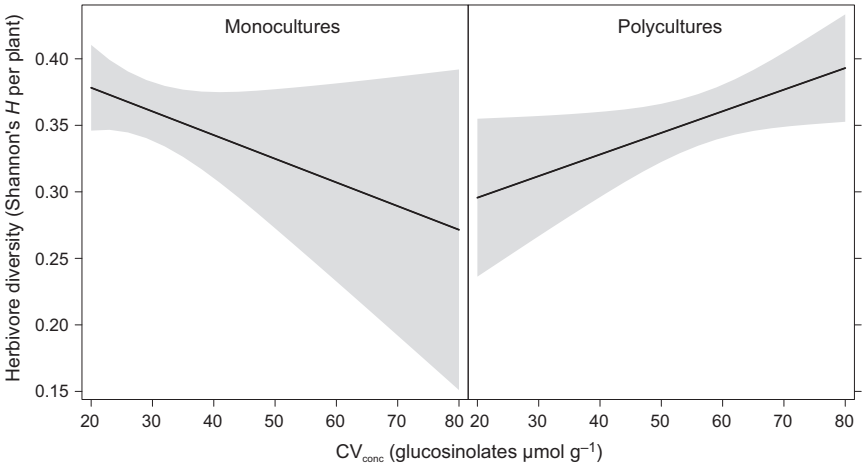

Figure 4 Effects of the linear model estimating the relationship between the CV of glucosinolate concentrations within plots and herbivore diversity. Estimated predictions are shown separately for the monocultures and polycultures (dicultures plus tricultures) in adjacent panels. The shaded bands indicate the 95% confidence interval of the response.

further affected herbivore diversity and plant damage. In addition, the plant populations not only differed in their glucosinolate profiles, but also in growth potential which further contributed to the observed effects. Thus, plants and the associated community were influenced by the interaction between the phenotype of the focal plant and that of its neighbours.

Increasing plant diversity within a plot increased the abundance of herbivores on focal plants as has been reported for other systems (Utsumi *et al*. 2011). The abundance of herbivores also depended on the plant population origin of the focal plant. Furthermore, herbivore responses to each population in relation to diversity treatment differed across the experimental period. Host plant preference of herbivores has been shown to be dependent on the genotype of neighbouring *B. oleracea* plants (Hambäck *et al*. 2009). The effect of diversity treatment on the diversity of herbivores was weak and also depended on the population origin of the focal plant. The community was characterised by species often associated with cabbage (wild and cultivated, Moyes *et al*. 2000; Newton *et al*. 2009a; Poelman *et al*. 2009) and colonisation (as opposed to abundance) of cabbage plants by these species may be less affected by variation in glucosinolate chemistry. Volatile communication between plants could also contribute to the herbivore response, as seen in mixed fields of different barley genotypes (Glinwood *et al*. 2011).

The abundance of carnivores was expected to be closely linked to that of the herbivores. Both herbivore abundance *per se* and time explained the largest part of the variation in abundance of the carnivores. Genotypic plant diversity can increase the species richness and abundance of carnivores, so they may exert higher pressure on herbivores in more diverse plant assemblages (Crutsinger *et al*. 2006; Johnson *et al*. 2006). The abundances of both the herbivore and carnivore community were positively affected by the diversity treatments but their temporal responses were contrasting. These effects could be explained by the fact that the abundance of prey/hosts increases over the season and the more subtle differences among the host plants may be overruled when prey or hosts are abundant.

The composition of the herbivore community was dominated by the sap-sucking aphid *B. brassicae*. As was found for the other herbivore community members, the abundance of this aphid was interacting with diversity treatment, plant population and time. The presence of more suitable plants for this species (i.e. Old Harry plants) may have reduced the acceptance of neighbouring less suitable plants (i.e. Kimmeridge), evidence supporting the attractant-decoy hypothesis (Ruttan & Lortie 2015). The cabbage aphid also showed a strong positive response to specific glucosinolate composition, but a negative response to total glucosinolate concentrations which supports previous evidence that plant chemistry plays an important role regulating populations of this species (Newton *et al*. 2009b). The contrasting abundance dynamics of *D. rapae*, the parasitoid of *B. brassicae*, and the host itself in response to diversity treatment and population origin over time further suggest that, though abundance of hosts plays a major role in explaining abundance of the parasitoid, the more subtle difference in host plants is perceived differently by the herbivore and the parasitoid. Previous work has shown that *D. rapae* is strongly affected by host abundance and little by variation in chemical diversity of the host plant (Newton *et al*. 2009b).

Surprisingly, more diverse plots supported more herbivores but were less damaged. At the end of the season, the abundance of herbivores was higher in more diverse plots while the difference in damage due to diversity treatment was more evident early in the season, thus both responses were temporally uncoupled. The positive relationship between the abundance of chewing herbivores and damage levels, suggests that this feeding guild is largely causing the damage, predominantly early in the season when damage appears to be most sensitive to the diversity treatments. Plant growth may have exceeded additional damage caused by chewing herbivores later in the season. Alternatively, herbivores in more diverse environments face higher heterogeneity in availability and quality of resources which can reduce consumption (McArt & Thaler 2013), but also they potentially interact more frequently in a more diverse and abundant herbivore community. Thus, it is

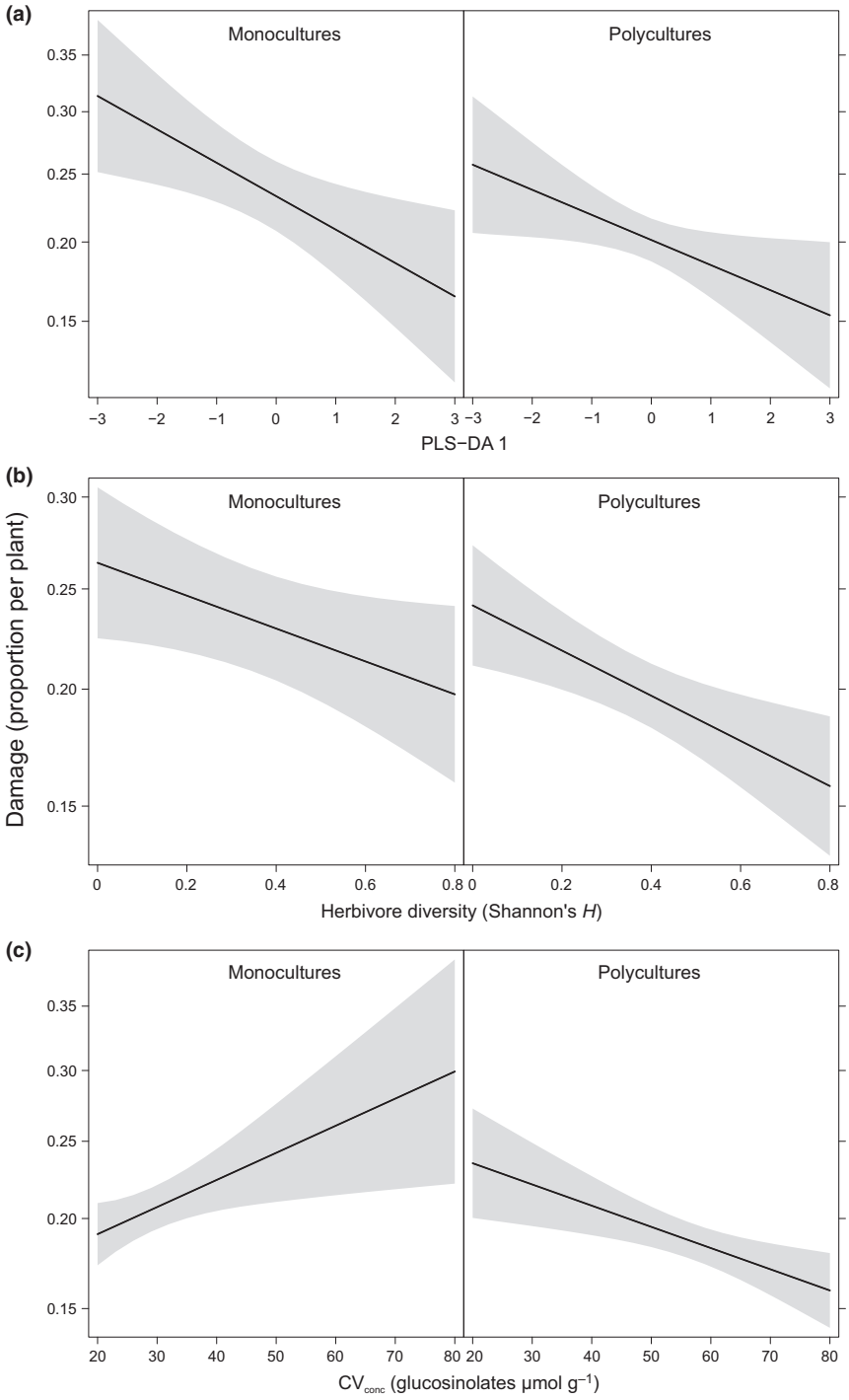

Figure 5 Effects of the linear model estimating the relationships between plant damage and (a) the first PLS-DA component, (b) herbivore diversity and (c) the CV of the glucosinolate concentrations within a plot. Estimated predictions are shown separately for the monocultures and polycultures (dicultures plus tricultures) in adjacent panels. The shaded bands indicate the 95% confidence interval of the response.

possible that both resource heterogeneity and interference competition contribute to a decrease in damage levels.

Another factor that could explain the waning effect of diversity treatment on plant damage is the increase in chemical defence in response to herbivory, here the induction of glucosinolates, which is well documented for *Brassica*, including the studied populations (Gols *et al*. 2008a,b; Textor & Gershenzon 2009). However, total glucosinolate concentration did not change with diversity treatments, suggesting that differential induction in relation to diversity treatment was negligible.

The populations also exhibited variation in plant size. Statistical models including both population and plant size revealed that plant size largely explained population related

differences on herbivore abundance. However, manipulation of plant diversity also influenced the size of the plants, which increased with higher plant diversity. Competition for resources above-ground (i.e. light and space), as well as belowground (nutrients) and soil microbiota associated with the plants may have differed in plots exposed to the various diversity treatments, which in turn could enhance the access to resources below-ground and benefit plant growth (Eisenhauer *et al*. 2010). In addition, plant–plant interactions could have affected growth of neighbouring plants, as shown in different barley genotypes (Ninkovic 2003). Glucosinolates in roots also differ in these cabbage populations (van Geem *et al*. 2016), therefore, glucosinolate variation could result in belowground allelopathic effects between neighbouring plants.

Dietary breadth is considered an important factor influencing host plant selection and insect performance (Ali & Agrawal 2012). Where specialists are predicted to be positively affected by plant-specific secondary metabolites, the opposite relationship is predicted for generalists. The abundance of specialist and generalist herbivores in relation with glucosinolate concentration showed indeed contrasting patterns. Concentrations of gluconapin and neoglucobrassicin, as well as total glucosinolate concentrations correlated negatively with the abundance of generalists, whereas total glucosinolate concentrations correlated positively with the abundance of specialists. In previous laboratory assays (Gols *et al*. 2008b), concentrations of glucosinolates correlated negatively with the performance of the generalist *Mamestra brassicae*, thus results obtained in laboratory support the findings in the field.

The risk of being damaged appears to be influenced by the chemical diversity of the plants surrounding a focal plant as indicated by the CV_{conc}. It has been proposed that chemical diversity can be actively selected for, since plants with a higher diversity of PSMs will be more likely to have compounds that are active against a range of herbivore species (Jones *et al*. 1991). This phenomenon may not only depend on the chemical profile of the focal plant, but also on that of its neighbours, which in turn may have consequences for other plant fitness-related traits (Schuman *et al*. 2015). Schuman *et al*. (2016) highlighted the importance of diversity in plant chemistry on higher trophic levels and the lack of manipulative experiments exploring this idea. Our data not only support the hypothesis that chemical diversity in plant communities affects structuring of the associated community (Richards *et al*. 2015) but also showed that not all herbivores respond similarly to the diversity treatments.

To summarise, not only the properties of a focal plant are important for determining the type and intensity of its ecological interactions, but also the properties of the neighbouring plants and even more the variability among neighbouring plants (Genung *et al*. 2012; Bailey *et al*. 2014; Schuman *et al*. 2015). Despite the fact that the plants used in this experiment do not represent strict genotypes, there is enough genetic differentiation in chemical and size traits among the plant populations for detecting the effects of population mixing in dicultures and tricultures compared to monocultures. The results of this study suggest a role of associational resistance and interference competition when considering the influence of neighbouring plants, but further studies are needed to elucidate the underlying mechanisms. With the present data we stress that linking the factors considered important for plant development and survival under natural conditions, such as the influence of plant chemical defence on herbivores and carnivores, and the interactions between neighbouring plants, is necessary for understanding the assembly of plant associated communities at different trophic levels.

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AUTHORSHIP

CBS and EHP conceived the study and designed experiments; CBS, EHP and RG performed field experiments; MR and JG provided analytical materials and performed glucosinolate analysis; CBS performed the statistical analyses; CBS, EHP and RG wrote the manuscript with input and revisions from all the authors.

DATA ACCESSIBILITY STATEMENT

The results of this study will be archived in a public repository such as Dryad.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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