Multi-species crop mixtures increase insect biodiversity in an intercropping experiment

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Abstract

Recent biodiversity declines require action across sectors such as agriculture. The situation is particularly acute for arthropods, a species-rich taxon providing important ecosystem services. To counteract negative consequences of agricultural intensification, creating a less hostile agricultural “matrix” through growing crop mixtures can reduce harm for arthropods without yield losses. While grassland biodiversity experiments showed positive plant biodiversity effects on arthropods, experiments manipulating crop diversity and management intensity to study arthropods are lacking. Here, we experimentally manipulated crop diversity (1-3 species, fallows), crop species (wheat, faba bean, linseed, oilseed rape) and agrochemical input (high vs. low) and studied responses of arthropod biodiversity. Increasing crop diversity increased arthropod diversity and arthropod numbers. Mass-flowering crops attracted more arthropods than legumes or cereals. Integrating intercropping into agricultural systems could lead to a massive increase in flower visits (up to 15 million visits/ha), indicating benefits of intercropping for insect biodiversity and associated ecosystem services.
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**Keywords:** arthropods, flower visitors, intercropping, diversity, crop identity

**Main**

Arthropods and especially flower visiting insects provide a range of important ecosystem services. Since more than one third of the global food production comes from crops that depend on pollinators (Klein *et al.* 2007), increasing cropping system diversity in space or time may help to balance environmental sustainability and agricultural production. Modern agricultural landscapes are often dominated by large expanses of crop monocultures (Eurostat 2018), where food or habitat resources for flower visiting arthropods are generally scarce (Nicholls & Altieri 2013). Some mass flowering crops, such as oilseed rape (canola) or sunflower, can support some flower visitor species, but only for limited time periods (Westphal *et al.* 2003) and without providing safe sites for reproduction and hence population growth. Herbicide application removes weeds efficiently from agricultural fields and thus leads to clean landscapes where only a fraction of species can survive due to habitat and resource losses (Nicholls & Altieri 2013). Input of other agrochemicals, such as fertilizers, insecticides and fungicides increase yield, but often at the expense of overall agrobiodiversity, potentially contributing substantially to recent insect declines (Benton *et al.* 2002; Tscharntke *et al.* 2005; Dicks *et al.* 2021). There are, however, countermeasures focusing on the concept of sustainable intensification, a process (or system) where yields are increased without harmful environmental impacts. Integrated pest management and conservation agriculture (including diversified crop rotations) have been practiced for a long time already with mixed biodiversity benefits (Lichtenberg *et al.* 2017; Dainese *et al.* 2019; Beillouin *et al.* 2021). One approach to support biodiversity in agriculture is intercropping (Martin-Guay *et al.* 2018; Wuest *et al.* 2021), where two or more crop species are grown on the same piece of land. As large expanses of cropland worldwide are dominated by cereal monocultures, growing mixtures of cereals with another crop (e.g. legumes) may have positive impacts on the quality of the matrix (Perfecto *et al.* 2009) in which natural habitats are embedded.

Intercropping has been shown to increase flower visitor or natural enemy abundance and diversity (Norris *et al.* 2018; Brandmeier *et al.* 2021) while at the same time enhancing yield stability and productivity (Yu *et al.* 2015; Raseduzzaman & Jensen 2017; Liet *et al.* 2020) or reducing needs for chemical fertilizers when cereals are intercropped with legumes (Hauggaard-Nielsen *et al.* 2008). Despite these benefits, intercropping has remained surprisingly unpopular in industrialized countries, though it is widely used in low-input tropical agroecosystems (Hauggaard-Nielsen *et al.* 2009) and in traditional smallholder farming systems in the Global South (Brooker *et al.* 2015). When implemented at larger scales in the landscape, intercropping may be an important measure to support arthropod populations by increasing the availability of food and nesting resources.

Flower visitor richness and species composition depend on the local plant community (Rotchés-Ribalta *et al.* 2018), and it was shown that not only crop diversity, but also crop identity affects arthropods (Meyer *et al.*, 2019). Additional community attributes such as interaction network complexity can help to understand relationships between crop diversity and ecosystem functioning, such as pollination success or crop yield (Saunders & Rader 2019). Besides crop diversity and identity, a large body of literature has examined effects of management intensity (e.g. organic vs. conventional farming) on flower visitors. However, only few studies so far compared monocultures and mixtures under high vs. low input management (Brandmeier *et al.*2021). In our experiment, we focus on biodiversity benefits from intercropping. We experimentally manipulated
management intensity (high vs. low), crop diversity within the cropping system (crop monocultures, two- and three-species mixtures and, as control plots, plots where no crops were sown. These plots in particular were overgrown by weeds, depending on the application of herbicides as part of the management treatment) and crop identity (wheat, faba bean, linseed, oilseed rape) in a series of intercropping trials to test effects of increasing crop diversity and different crop identities in monocultures and mixtures on arthropod and especially flower visitor diversity and community structure. We expect higher arthropod diversity and higher arthropod abundance with increasing crop diversity (hypothesis 1). We assume that some crops are more appealing than others, due to floral resource provisioning and differences in growth habit, leading to higher diversity and abundances in these plots and therefore more complex plant-flower visitor networks (hypothesis 2).

Materials and Methods

Experimental design

We set up a field experiment as part of a series of multi-year intercropping trials in 2019 close to Münster, Germany (51°58’32.5”N 7°33’57.4”E) and controlled crop diversity, crop species and management intensity in a randomized split blocks design. Plots were sown in monocultures and mixtures of summer wheat (Triticum aestivum L.), faba bean (Vicia faba L.), linseed (Linum usitatissimum L.) and oilseed rape (Brassica napus L.) in mixed intercropping in a substitutive design (Table S11). We used different sowing densities for crop mixtures. For wheat, faba bean and linseed a 50/50 and 33/33/33 ratio was used in two- and three-species mixtures, while for oilseed rape, due to its high competitiveness, a sowing density of 20% was used in mixtures. A total of 104 plots each measuring 3x4 m were sown in four replicate blocks with a sowing machine (Wintersteiger Plotseed S) at a row spacing of 12.5 cm with 8 rows per m at a sowing depth of 3.5 cm on May 14th in 2019. We assigned management intensity at random to half-blocks: One half of each block received high intensity management, made of one pre-emergence spray of herbicides (4.4 L/ha Stomp Aqua with 455 g/L Pendimethalin as active agent) and nitrogen fertilizer applied as a solution of urea and ammonium nitrate (70 kg N/ha); the other half received no treatment (low intensity). We adjusted application levels to the common amounts for our region, but reduced fertilizer quantity to account for legumes in our mixtures. Crop mixture and inherent levels of crop diversity were the lowest level of replication and were assigned to random positions within each half-block (Figure S2).

We used two different methods to sample arthropods: flower visitor observations and pan traps for four different cropping systems: fallow, no crop was sown (but weeds established especially under low intensity management); crop monocultures; 2 species, two-species mixtures; and 3 species, three-species mixtures and for up to 13 different crop mixtures, respectively. Note that flower visitor observations were conducted on monocultures of wheat, bean and linseed and their two- and three-species mixtures (eight levels), while pan traps were used to sample arthropods on a wider range of crop mixtures, including mixtures with oilseed rape (13 levels, Table S11).

Flower visitor observations

Observations were carried out between July 2nd-10th at appropriate weather conditions (warm, sunny, dry). One m² of each plot was observed for 15 min. Due to time limitations and withering of linseed, only one replicate out of four could be observed twice. We assessed numbers of flower visits and flower visitor species (if possible, orders otherwise), on monocultures of wheat, faba bean and linseed, their two- and three-species mixtures and control plots where no crop was sown, but where weeds established (Table S9). We recorded which plant species was visited for both crops and weeds for each plot and counted every contact with floral organs as one visit. When the ears of wheat plants were visited, this was also counted as a flower visit, as it has been shown that hoverflies and bees also visit wind-pollinated plants (Saunders 2018). For data analyses, we summed all observations per crop diversity (four levels, see Staab et al., 2015) and per crop mixture (eight levels).

Pan traps
Pan traps were installed for four time periods (June 28th–July 1st, July 5th–8th, July 12th–15th, July 23rd–26th) as an indirect measure of abundance (Scherber & Beduschi 2021) to assess insects on plots containing wheat, faba bean, linseed and oilseed rape, their mixtures and plots with no crop sown. We had to exclude the third round, because heavy rain flooded the traps. We used plastic bowls sprayed with UV yellow paint (MONTANA BLACK INFRA COLORS infra yellow), filled with water and a drop of detergent (Frosch). Traps were placed at ground level in the vegetation of each plot and were left active for 72 h; then we transferred insects into 70% ethanol in the field and sorted them up to order/suborder level in the laboratory using binoculars (Leica EZ4-HD). We summed individuals from all three sampling periods per crop diversity (four levels) and per crop mixture (13 levels, Table S12).

Statistical analyses

Data analysis was done in R (version 3.6.1). We checked distributional assumptions using the fitdistrplus package (Delignette-Muller & Dutang 2015) and inspected model residuals for constant variance.

We used generalized linear mixed-effects models (package glmmTMB, Brooks et al., 2017) with tweedie errors and management nested in blocks as random effects to analyze data on flower visitor diversity and total arthropod diversity, which is expressed as Shannon’s entropy (Jost 2007) and its numbers equivalent (exponential of Shannon’s diversity) in the whole manuscript. This index was calculated using the package vegan (Oksanen et al. 2019). As fixed effects we used 1) crop diversity*management and 2) crop mixture*management in separate models.

We generated plant-flower visitor networks using the bipartite package (Dormann et al. 2009) for all crop mixtures and management intensities separately (N=4). For calculations of network metrics, we pooled our data per block, crop mixture and management intensity, and then analyzed effects of crop mixture and management on network metrics using the same model structure as above with generalized poisson and compois errors. We used the number of interactions, number of flower visitor species, and Shannon’s diversity of interactions as metrics to describe the networks, as more complex indices need a minimal network size to function reliably (Dormann et al. 2009), which was not the case in this study.

The number of flower visits and the total number of arthropod individuals were analyzed as described above with generalized poisson errors. Crop biomass was analyzed using tweedie errors. Means were compared using Type II Wald chisquare tests from the car library (Fox & Weisberg 2019).

Results

Arthropod diversity in monocultures, two- and three-species mixtures

Increasing crop diversity under high intensity management in cropping systems positively affected flower visitor and arthropod diversity (Fig. 1, Tables S1, S2). Except for three-species mixtures, arthropod diversity was always higher under low than under high management intensity.

Effects of crop and mixture identity on arthropod diversity

Observation data showed that presence of faba bean and linseed, in monoculture or mixture, lead to an increase in flower visitor diversity (Fig. 2a). Pan trap data showed that arthropod diversity was influenced by an interaction between crop mixture and management: Arthropod diversity was highest in linseed-oilseed rape mixtures, faba bean-oilseed rape mixtures and linseed monocultures under low intensity management; and in oilseed rape monocultures and wheat-faba bean-linseed mixtures under high intensity management (Fig. 2b). Both methods show that crop mixtures significantly influenced arthropod diversity (Tables S1, S2).

Plant-flower visitor networks and network metrics

The number of flower visits was higher under low than under high intensity management (Table S3). On untreated (low intensity management) plots, fewest visits were observed on wheat monocultures (35 visits).
and fallows (“no crop”) plots (53 visits). Number of visits increased when linseed was present. Wheat-faba bean-linseed mixtures were visited most frequently (612 visits, Fig. 3a, Table S3).

Under high intensity management, fewest visits were observed when no flowering crop was sown (i.e. two visits on wheat monocultures and 16 visits on “no crop” plots). With increasing proportion of linseed (from 33% in three-species mixtures and 50% in two-species mixtures to 100% in monocultures), more visits were observed (i.e. 353 visits in linseed monocultures, Fig. 3b, Table S3). Overall, bees mainly visited linseed, while hoverflies mainly visited weeds and faba beans. The mean number of interactions as well as the number of flower visitor species and Shannon’s diversity of interactions was lowest in high intensity wheat monocultures and no crop plots. Values for all indices increased considerably in mixtures containing linseed as well as in linseed monocultures. Shannon’s diversity of interactions was also high in faba bean monocultures. In most cases, values were higher for low compared to high intensity management (Fig. 4, Tables S5, S6).

**Arthropod abundances**

Flower visitor observations showed that abundances were usually higher under low intensity management (Fig. 5a, b), while pan trap data (all arthropods) showed a reversed trend (Fig. 5c, d). Pan traps which were placed on linseed plots contained fewer arthropods than traps on plots sown with other crops. Conversely, observations on plots containing linseed showed highest numbers of flower visits (Tables S7, S8).

**Crop biomass**

Crop biomass was influenced by an interaction between crop mixture and management, but not by crop diversity (Tables S9, S10). Under high management intensity, crop biomass increased with crop diversity, while under low management intensity, biomass was highest in two-species mixtures. Notably, biomass was low in oilseed rape monocultures and in mixtures containing oilseed rape (except linseed-oilseed rape mixtures, Fig. S1).

**Discussion**

Data from our agricultural field experiment show the importance of increased crop diversity in cropping systems for arthropod diversity and abundances. Independent of the sampling methods employed, we found that higher crop diversity increased flower visitor diversity under high intensity management (hypothesis 1). On the other hand, crop identity played an important role as well, as some mixtures were visited more frequently than others (hypothesis 2). In our study, especially linseed was visited often, in both mixtures and in monocultures. This suggests that when a mass flowering crop is available, this crop is more important than other crops (in our case faba bean) or weeds, masking their effects. Consequently, plant-flower visitor networks that we created were dominated by linseed; when this crop was available, most interactions were measured, most flower visitor species were found and therefore Shannon’s diversity of interactions was high. Weeds were significantly reduced under high-input management, and in these plots linseed played an even more important role as on plots without treatment. Increasing weed abundances on fields where no flowering crop is grown (i.e. wheat monocultures) can maintain flower visitor populations and ensure pollination services (Bretagnolle & Gaba 2015). As an alternative to accepting tolerable levels of weed densities in the field (Nicholls & Altieri 2013), intercropping could be integrated into conventional farming systems. Our predictions indicate that the number of flower visits could increase from about 4,700 visits/ha in wheat monocultures up to 566,000 visits/ha in wheat-bean-linseed mixtures. In low input systems, the number of visits could be as high as 1.5 million visits/ha in wheat-faba bean-linseed mixtures.

Previous studies showed that ecosystem service delivery is positively influenced by the richness of service-providing organisms like flower visitors (Dainese et al. 2019). Although honeybees (mainly the European honeybee *Apis mellifera* L.) are kept worldwide to provide crop pollination, other insects (such as wild bees, flies, beetles, wasps) contribute a lot more to total pollination than previously thought (Rader et al. 2016; Page et al. 2021). Floral abundance and richness are important for crop pollination services from unmanaged flower visitors (Kremen et al. 2007; Garibaldi et al. 2014), thus wild pollinator communities should be
supported by increasing the floral abundance and richness in their environment. Obviously, monocultures of mass flowering crops such as linseed or oilseed rape can serve as a food resource for particular species (Bombus) and for a limited period of time, but floral abundance can be increased using crop mixtures, too (comparing mixtures to cereal monocultures). The advantage of crop mixtures compared to a mass-flowering monoculture is that the monoculture (e.g. oilseed rape) has to be treated with higher amounts of fertilizers and pesticides. In mixtures, these amounts have to be reduced, especially if legumes are present. This could potentially lead to a system change in agriculture, leading away from intensively treated monocultures to less intensively treated mixtures. Pollinators, as mobile organisms, react to diversification at different spatial scales. Diversification methods have already been shown to benefit flower visitors and pollination services by enhancing floral diversity at the local scale (Garibaldi et al. 2014; Isbell et al. 2017). At the landscape scale, an increasing distance from a natural habitat leads to lower wild bee richness, visitation numbers and fruit set (Garibaldi et al. 2011). Therefore, integrating flower-rich agricultural fields into conventional farming can provide important and well-connected resources for flower visitors as well as other arthropods.

Using yellow pan traps we showed that crop identity affected arthropod diversity and abundances. On oilseed rape monocultures and mixtures (especially wheat-oilseed rape and wheat-faba bean-oilseed rape mixtures) arthropod abundances were high, while plots including linseed attracted less individuals. These results can be explained by the poor crop performance of oilseed rape, which suffered from the late sowing date and only grew sparsely on some plots. Thus, plots with oilseed rape contained more bare ground and pan traps were easily visible, leading to a higher attraction than in e.g. linseed plots. While pan traps are suggested to be an efficient method for large-scale agricultural systems and to reduce collector biases (Westphal et al. 2008), they are group-specific and the catching success depends on color (Moreira et al. 2016). Thus, for our plot-based trials observations seem more appropriate. The observed subplot represents a sufficient part of the whole plot and we were able to generate plant-flower visitor networks from qualitative observation data (Nielsen et al. 2011). On the other hand, observations are very time-consuming and can lead to biases when conducted by multiple people (Westphal et al. 2008). Using high resolution camera traps could be an efficient and standardized method to assess flower visits on multiple plots on the same time. When using high technology cameras or camera traps with adequate resolution, flower visitors can be determined up to species or family level (Droissart et al. 2021) with low sampling effort.

In conclusion, we suggest to integrate intercropping into agricultural systems, as we showed that arthropod diversity benefits from increased crop diversity, especially under high intensity management. Moreover, it is important to find suitable crop mixtures (ideally including mass-flowering crops) which provide important resources to arthropods.

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Data availability statement

Datasets and R Codes will be published on Zenodo upon acceptance of this manuscript.

References


cropping in high input agriculture supports arthropod diversity without risking significant yield losses. *Basic Appl. Ecol.*, 53, 26–38.


**Figure Captions**

**Fig. 1** Biodiversity responses to increased crop diversity within cropping systems. a, Flower visitor and b, arthropod diversity for four different cropping systems (Fallow: no crop was sown, but weeds were present; Mono: crop monoculture, 2 crops; two-species mixture; 3 crops: three-species mixture) for high (red) and low (blue) management intensity. Graphs show raw data (open circles) and model fits (filled circles) with 95% confidence intervals, predicted from generalized linear mixed-effects models from a, flower visitor observations (N=64; Cropping system: $\chi^2=13.06$, p=0.005) and b, pan traps (N=104; Cropping system: $\chi^2=16.08$, p=0.001).

**Fig. 2** Biodiversity responses to crop mixtures. a, Flower visitor and b, arthropod diversity for a range of crop mixtures (0, fallow where no crop was sown; W, wheat; B, faba bean; L, linseed; O, oilseed rape; WB, wheat-faba bean; WL, wheat-linseed; WO, wheat-oilseed rape; BL, faba bean-linseed; BO, faba bean-oilseed rape; LO, linseed-oilseed rape; WBL, wheat-faba bean-linseed; WBO, wheat-faba bean-oilseed rape) for high (red) and low (blue) management intensity. Graphs show raw data (open circles) and model fits (filled circles) with 95% confidence intervals, predicted from generalized linear mixed-effects models for a, flower visitor observations (N=64; Crop mixture: $\chi^2=60.66$, p<0.001) and b, pan traps (N=104; Crop mixture: $\chi^2=40.98$, p<0.001).

**Fig. 3** Bipartite plant-flower visitor networks for plots sown with different crop mixtures for a, low and b, high intensity management. Networks were generated by summing all visits for each group. N=4 for each crop mixture and management intensity. Left section in networks represents plant species and right section represents flower visitors (see Table S4). Small bars indicate fewer visits than wider bars. Networks are sorted by total number of visits, starting with the fewest (upper left network) and ending with the most (lower right network) within the two types of management intensity.

**Fig. 4** Bipartite plant-flower visitor network indices in response to crop mixtures. Graphs show datapoints (open circles), model predictions (filled circles) and 95% confidence intervals from generalized linear mixed-effects models for each crop mixture (0, fallow where no crop was sown; W, wheat; B, faba bean; L, linseed; WB, wheat-faba bean; WL, wheat-linseed; BL, faba bean-linseed; WBL, wheat-faba bean-linseed) under high (red) and low (blue) management intensity. N=4. a, Number of interactions (Crop mixture: $\chi^2=176.65$, p<0.001; Crop mixture:Management: $\chi^2=17.04$, p=0.017), b, number of flower visitor species (Crop mixture: $\chi^2=95.79$, p<0.001) and c, Shannon’s diversity of interactions (Crop mixture: $\chi^2=47.61$, p<0.001).

**Fig. 5** Number of visits and arthropod numbers in response to a, c, four different cropping systems (Fallow: no crop was sown; Mono: crop monoculture, 2 crops; two-species mixture; 3 crops: three-species mixture) and b, d, different crop mixtures (0, fallow; W, wheat; B, faba bean; L, linseed; O, oilseed rape; WB, wheat-faba bean; WL, wheat-linseed; WO, wheat-oilseed rape; BL, faba bean-linseed; BO, faba bean-oilseed rape; LO, linseed-oilseed rape; WBL, wheat-faba bean-linseed; WBO, wheat-faba bean-oilseed rape) for high (red) and low (blue) management intensity. Graphs show raw data (open circles) and model fits (filled circles) with 95% confidence intervals, predicted from generalized linear mixed-effects models from a, b, flower visits
(N=64; Cropping system: $\chi^2=35.36$, p<0.001; Management: $\chi^2=8.68$, p=0.003 and Crop mixture: $\chi^2=176.65$, p<0.001; Crop mixture:Management: $\chi^2=17.04$, p=0.017) and c , d , all arthropods caught in pan traps (N=104; Crop mixture: $\chi^2=145.06$, p<0.001).