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## Creating Perennial Flower Strips: Think Functional!

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### Abstract

In last decades, farmland biodiversity came under large threat. To counteract farmland biodiversity loss and other environmental impacts of intensive agriculture, European farmers can apply Agri-environmental schemes. One of these is the creation of flower strips, a part of the cropping field where flowers are sown or naturally settled. Flower strips are known to increase biodiversity in the agricultural landscape, notably attracting specific insects groups, such as pollinators and natural enemies that can provide valuable pollination and biocontrol services to the crop. However, the plant species composition and management of the strips can have a large influence on the identity and amount of useful insects present in the strips, suggesting the need to develop tailored flower strips to maximize the services delivered. Functional diversity (FD) is sometimes proposed as a promising approach, focusing on plant functional traits rather than plant species itself. Yet, it is not certain that sowing a set of plant species results in the desired vegetation with the desired functional trait composition. Species from soil seed bank or dispersing from neighboring vegetation can settle in the strip, while sown species might not always be equally adapted to local conditions. To test this, we developed seed mixtures with four different levels of FD, based on flower traits, and sowed them as flower strips in a conventional arable field. We monitored the vegetation to calculate the FD of the realized vegetation. While the absolute FD values of the realized vegetation were lower than the expected FD values, the realized vegetation showed the same FD gradient as expected from the sown mixtures, indicating that it is possible to manipulate FD in flower strips.

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## 1. Introduction

Agriculture has known a lot of changes in recent decades. An important direction of change is intensification. Farms increased in size, their management became more mechanized, field size increased and crop rotations were simplified. This intensification has led to habitat destruction and fragmentation and a reduction of landscape diversity and biodiversity (Kruess & Tscharntke, 1994; Stoate et al., 2001; Tscharntke et al., 2005). To counteract this loss of biodiversity and the consequent loss of ecosystem services, European authorities created the system of Agri-Environmental Schemes. Agri-Environmental Schemes were designed to convince farmers to reduce the environmental risks of modern agricultural practices and to preserve nature and cultivated landscapes (European Commission, 1998; European Commission, 2005). One example of Agri-Environmental Schemes is the creation of flower strips, a part of the cropping field where flowers are sown or naturally settled. Flower strips are known to increase biodiversity in the agricultural landscape, notably attracting specific insects groups, such as pollinators and natural enemies that can provide valuable pollination and biocontrol services to the crop (Haaland et al., 2011).

However, the plant composition and management of the strips can have a large influence on the identity and amount of useful insects present in the strips, suggesting the need to develop tailored flower strips to maximize the services delivered (Korpela et al., 2013; Tschumi et al., 2014). Functional diversity (FD) is sometimes proposed as a promising approach, focusing on plant functional traits rather than plant species itself (e.g. Fontaine et al., 2006; Campbell et al., 2012). Yet, it is not certain that sowing a set of plant species can result in the desired vegetation (De Cauwer et al., 2005; Lepš et al., 2007). Species from soil seed bank or dispersing from neighboring vegetation can settle in the strip, while sown species might not always be equally adapted to local conditions (Münzbergová & Herben, 2005). As a consequence, when sowing a wildflower strip with a certain plant species mixture, it is not sure that the established vegetation will have the desired functional trait composition or the desired FD level (Fukami et al., 2005). We tested in a replicated field experiment whether it is possible to create different levels of plant FD in sown flower strips.

## 2. Materials and Methods

### 2.1. Experimental setup

To create a plant FD gradient, four plant species mixtures were prepared with equal species richness but contrasting diversity in functional traits. A list of 20 forb plant species was composed from perennial herbaceous species that were commonly found in grasslands, used in Agri-Environmental Schemes in Wallonia, Belgium and that were available from the market. Because the focus of the flower strip experiment was the provision of food sources to flower visiting pollinators and natural enemies, a set of seven floral functional traits was selected. These traits were (1) flower color, (2) flower class according to Müller (1881), (3) UV reflection in the peripheral part of the flower (5 classes), (4) presence of a UV pattern, (5) the month of the flowering start, (6) flowering duration in months and (7) the maximal height of the plant. For all 20 species, functional trait values for these traits were retrieved from TRY database (Kattge et al., 2011) for the former four traits and from Lambinon et al. (2008) for the latter three traits. Based on these traits, FD of every possible combination of seven plant species was calculated using Rao quadratic entropy index (Botta-Dukát, 2005) with equal importance of the traits and equal abundance of the plant species. The combinations with lowest and highest FD were selected, as well as the combinations with functional diversity closest to the 33<sup>rd</sup> and the 67<sup>th</sup> percentile of the range, resulting in four plant species mixtures with contrasting FD: very low (VL), low (L), high (H) and very high (VH) (Table 1). For these mixtures, in total 17 out of the 20 listed plant species were used.

Table 1. Species used in the four mixtures.

Species	VL	L	H	VH
<i>Achillea millefolium</i>	x	x	x	x
<i>Anthriscus sylvestris</i>	x		x	x
<i>Crepis biennis</i>		x		
<i>Galium verum</i>	x	x		
<i>Geranium pyrenaicum</i>			x	
<i>Heracleum sphondylium</i>	x			
<i>Hypochaeris radicata</i>		x		
<i>Knautia arvensis</i>	x	x		
<i>Leontodon hispidus</i>		x	x	
<i>Leucanthemum vulgare</i>	x		x	
<i>Lotus corniculatus</i>				x
<i>Lythrum salicaria</i>		x		x
<i>Malva moshata</i>				x
<i>Medicago lupulina</i>				x
<i>Origanum vulgare</i>			x	
<i>Prunella vulgaris</i>			x	x
<i>Trifolium pratense</i>	x			

VL: very low functional diversity (FD); L: low FD; H: high FD; VH: very high FD.

To create the plant species mixtures in the field, four seed mixtures were prepared, each containing equal seed mass (0.5 kg/ha) of the seven forb plant species. By sowing equal seed mass per species, we aim to create perfect evenness between the forb plant species, assuming that plant species with lower seed mass have seeds with higher mortality and thus need more seeds to establish the same abundance as plant species with higher seed mass. Furthermore, three grass species, *Festuca rubra* L., *Agrostis* spp. and *Poa pratensis* L. were added to the seed mixtures (11.5 kg/ha, 5 kg/ha and 5 kg/ha respectively). As the focus of the wildflower strips was to provide food sources for flower visitors, the floral traits of these grasses were not taken into account. Seeds were obtained from ECOSEM, Belgium. The seed mixtures were sown in an experimental field located in the experimental farm of Gembloux Agro-Bio Tech, 50°34'03''N; 4°42'27''E at 150 m elevation. The four mixtures and one control containing only the grass species were sown in a 5x5 Latin square design, consisting of five flower strips of 125x8 m with conventional cropping zones of 27 m between the strips and each of the five flower strips consisting of five 25x8 m plots. This results in five replicates of four FD treatments (VL, L, H and VH) and a control treatment. The control plots were not considered in this particular study because only sowing the grass species results in a lower total seed mass that was brought in the control plots while sowing, which could have influenced the vegetation dynamics. On 6 June 2013, the grass species were sown with a Wintersteiger plot seeder and the forb plant species with a Nodet precision seeder. Strips were mown yearly once in June and once in September with removal of hay.

## 2.2. Sampling

The vegetation development was monitored in 2014 in the 25 flower strip plots to evaluate the realized vegetation. For this, three permanent quadrats of 1x1 m were created in each plot. In June and September before mowing, the permanent quadrats were surveyed by estimating the proportion of horizontal cover of each forb plant species.

## 2.3. Statistical analysis

For each plot, the average cover of each forb plant species was calculated by summing up the cover in each of the three permanent quadrats in both survey periods, and dividing by six. For the not-sown plant species appearing in the permanent quadrats, the trait values of the seven functional traits were retrieved from the TRY database (Kattge et al., 2011) and from Lambinon et al. (2008). This results in a new species x trait matrix with all observed species and their trait values. With the average plant species cover as abundance and the floral traits for all plant species, the

realized FD was calculated for each plot with the Rao quadratic entropy index to compare it with the expected FD of the treatment. As Rao's index is sensitive to the amount of species in the species x trait matrix, the expected FD of the four sown species mixtures was recalculated with the new species x trait matrix. The difference between the treatments for their mean realized FD was tested with Kruskal-Wallis rank sum tests and Nemenyi tests.

To investigate the effect of the not-sown species occurring in the vegetation, the realized FD based on only the sown species was calculated by giving the not-sown species zero abundance in the calculation. To investigate the effect of non-equal abundance of the sown species on the FD gradient, the FD based on only sown species and with presence/absence as abundance values of the sown plant species was calculated. For both of these realized FD measures, the difference between the treatments for their mean values was also tested with Kruskal-Wallis rank sum tests and Nemenyi tests.

The number of forb plant species present in each plot was calculated as the total amount of species in the three quadrats and the two sampling periods together. The difference between the means of species number per FD treatment was tested with a Kruskal-Wallis rank sum test and Nemenyi tests to verify whether a realized FD gradient was caused by a difference in species richness.

Data treatment was conducted in R (R Core Team, 2013).

### 3. Results and Discussions

In total, 35 plant species were found, of which 14 were sown species. The three sown species which did not appear in permanent quadrats, were *Anthriscus sylvestris* (L.) Hoffmann, *Lythrum salicaria* L. and *Trifolium pratense* L. It is possible that they needed more time to germinate or that the site conditions were not favorable enough for them to settle. The sown species *Heracleum sphondylium* L., appeared with only one individual and in a plot where it was not sown. Among the ten most abundant species were four not-sown species, namely *Cirsium arvense* (L.) Scop., *Sinapis alba* L., *Malva sylvestris* L. and *Rumex obtusifolius* L. *C. arvense*, *M. sylvestris* and *R. obtusifolius* have been reported as common weed species (Donald, 1994; Zaller, 2004; Zahedi & Ansari, 2011). This suggests that enough bare soil was available during the initial vegetation development for weeds to colonize the flower strips. *Sinapis alba* is commonly used as cover crop (Haramoto & Gallandt, 2004) and has been cultivated in the experimental field during the years preceding the experiment. This may have enabled this species to emerge from the soil seed bank.

Considering all the plant species found in the quadrats, the realized FD was significantly different over the treatments ( $H=12.04$ ;  $P=0.007$ ). Figure 1.a shows the increasing realized FD with the treatments. This shows that it is possible to manipulate the FD level in flower strips. The realized FD was always lower than the expected FD.

Considering only the sown plant species found in the quadrats, the realized FD was significantly different over the treatments ( $H=14.63$ ;  $P=0.002$ ). Figure 1.b shows the increasing realized FD with the treatments. Only for the VL treatment, the realized FD is lower when considering only sown plant species than when considering all plant species ('VL' in Figure 1.b and Figure 1.a), but the pairwise comparisons of the Nemenyi test did not show a different pattern. This suggests that additional plant species that colonized the plots mainly brought more diversity in traits to the plots with lowest FD treatment. Indeed, in the lower FD treatments, the chance may be higher that additional plants bring new traits to the vegetation.

The realized FD calculated with only presence/absence data of sown plant species was significantly different over the treatments ( $H=18.23$ ;  $P<0.001$ ). Figure 1.c shows the increasing realized FD with the treatments. The trend was more pronounced than when the not-sown plants are included and with the relative abundance of the plant species (Figure 1.a and 1.b) and this was confirmed by the pairwise comparisons of the Nemenyi test. It suggests that the desired evenness of the sown species was not well established in the field. Some sown species had lower abundance than others or did not even emerge. The latter is clearly visible in Figure 2, where the mean realized number of sown species was always lower than the expected seven species. This might affect more the treatments with a higher FD value, as they have lower functional redundancy and losing a species consequently leads more likely to a loss of trait diversity.

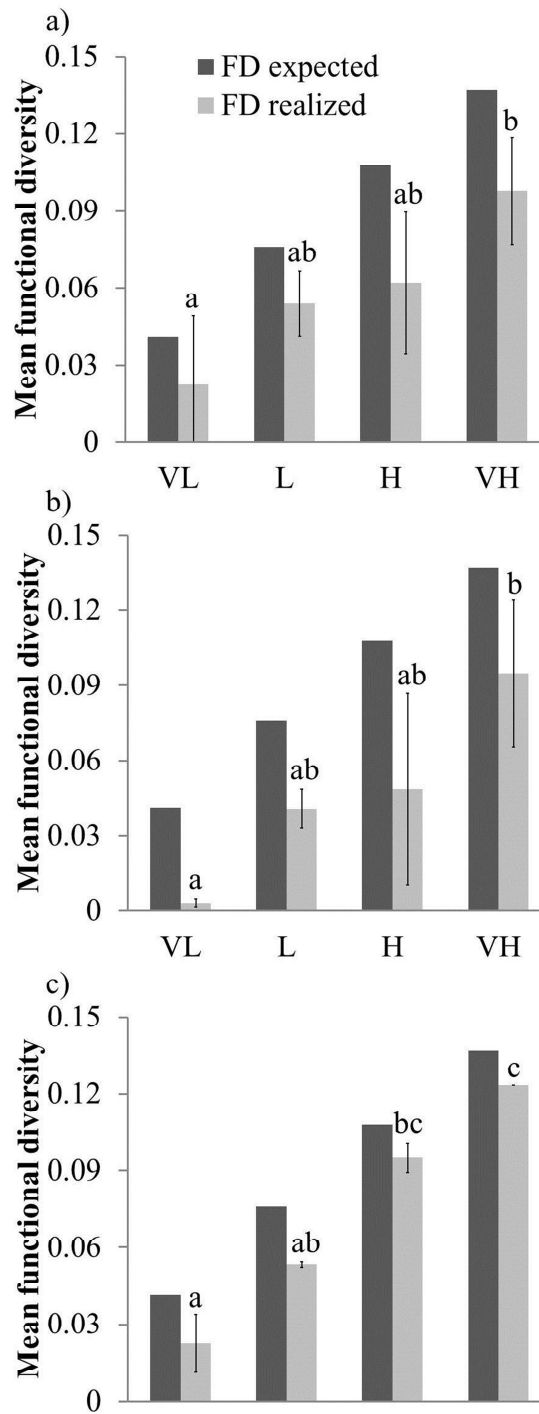


Fig. 1. Mean expected and realized functional diversity (FD) per treatment. The realized FD is the mean value for each treatment based on a) the abundance of all plant species, b) the abundance of only sown species and c) the presence/absence of only sown species; VL: very low FD; L: low FD; H: high FD; VH: very high FD; error bars show standard error of the mean, letters above error bars show results of Nemenyi pairwise comparison of the means (P<0.05).

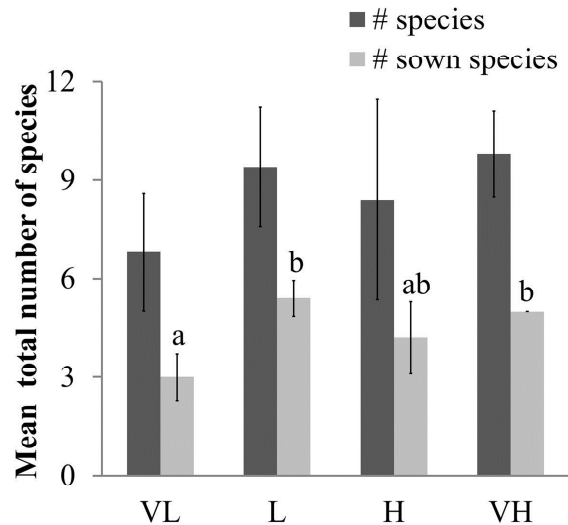


Fig. 2. Mean total realized number of forb plant species and sown forb plant species for the different treatments; VL: very low FD; L: low FD; H: high FD; VH: very high FD; error bars show standard error of the mean; letters above error bars show results of Nemenyi pairwise comparison of the means ( $P < 0.05$ ) for the sown species number (for the total species number no significant difference was found between the treatments).

No significant difference was found in the realized total number of forb plants species ( $H=4.91$ ;  $P=0.178$ ) over the treatments. However, there was a significant difference in the realized total number of sown ones ( $H=13.21$ ;  $P=0.004$ ), even if no clear trend was visible (Figure 2). It is possible that a little lower realized amount of sown plant species for the VL treatment has caused a lower realized FD value, but as mentioned before, the high functional redundancy can have reduced this effect. Because a vegetation with higher FD is expected to have more ecological niches filled (Mason et al., 2005), it may be possible that less additional species were able to colonize the plot with higher FD treatment, as observed by Van der Putten et al. (2000) and Lepš et al. (2007) for a higher species diversity. However, this was not observed in our experiment.

While it was shown that it is possible to manipulate FD in flower strips by sowing a mixture, a continued monitoring of the vegetation will show if the FD gradient will remain during further vegetation development. Indeed, abundance of species can change during the years after sowing (De Cauwer et al., 2005; Lepš et al., 2007) and vegetation succession can lead to convergence of the functional trait composition (Fukami et al., 2005).

As manipulating FD seems to be a possible tool to develop tailored flower strips, further research could focus on how plant FD steers the diversity of flower visiting pollinators and natural enemies and the related and other ecosystem services delivered by flower strips.

#### 4. Conclusions

A higher FD is not only leading to more biodiversity, it's also expected to deliver more ecosystem services. Manipulating FD can thus be an efficient way to maximize ecosystem service delivery, especially in Agri-Environmental Schemes. However, few studies investigated whether sowing a plant mixture results in a plant species composition with the desired FD. Here we have shown with an experimental study that it is possible to manipulate plant FD in flower strips by sowing a species mixture. The expected FD gradient was observed in the realized vegetation. However, the absolute FD values were lower than the expected FD values because sown species did not appear in even abundance, a part of them did not emerge and not-sown species appeared in the vegetation.

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