



Management of Grassland-like Wildflower Strips Sown on Nutrient-rich Arable Soils: The Role of Grass Density and Mowing Regime

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Abstract

Wildflower strips (WS) are proposed in many European countries as a strategy to enhance biodiversity and ecosystem services in arable fields. To create and maintain WS on nutrient-rich cultivated soils reveals challenging. Flowered species may be outcompeted by grasses due to high phosphorus content in soil. We studied during 5 years seed mixture (grass density in the seed mix) and mowing regime influenced the ability of WS to provide environmental benefits (flower provision for insects and landscape purposes, reduction of soil nutrient load) and respond to farmer concerns (noxious weed promotion, forage production). Lowered grass density increased flower abundance, but not diversity, only in the first 3 years. In the last 2 years mowing effects became determinant. Flower cover and richness were the highest under the twice-a-year mowing regime. This regime also increased forage quantity and quality. Flower colour diversity was conversely the highest where mowing occurred every two years. Potassium in the soil decreased under the twice-a-year mowing regime. Other nutrients were not affected. No management option kept noxious weed to an acceptable level after 5 years. This supports the need to test the efficacy of specific management practices such as selective clipping or spraying. Mowing WS twice a year was retained as the most favourable treatment to maintain species-rich strips with an abundant flower provision. It however implies to mow in late June, i.e. at the peak of insect abundance. It is therefore suggested to keep an unmown refuge zone when applying this management regime.

Keywords Agri-environment Schemes · Plant diversity · Soil nutrients · Weed management · Wildflower strips

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Introduction

In the face of environmental degradation due to agriculture intensification, many European countries adopted Agri-environment Schemes (AES) as a response (Batáry et al. 2015). Among those schemes, flower strips are often proposed as a strategy to enhance flower-feeding insect biodiversity (Haaland et al. 2011) as well as ecosystem services, such as pollination (Nicholls and Altieri 2013; Uyttenbroeck et al. 2017), natural pest control (Landis et al. 2000; Hatt et al. 2017, 2018) and improvement in landscape aesthetics (Junge et al. 2015). The type of strips and their management may vary between countries, depending on their policy (Haaland et al. 2011). Several countries adopted AES prescription for perennial field margins made of indigenous forbs and grasses managed by mowing, with the aim to keep meadow-like vegetation (Smith et al. 2010; Haaland et al. 2011; Tarmi et al. 2011). In this way, Walloon (South Belgium) AES management prescriptions for wildflower strips include the use of species typical from

local lowland hay meadows (Piqueray et al. 2016) and mowing as a management. They therefore aimed at creating habitats analogous to these ecosystems, known to be particularly attracting to insects, but in strong decline in Europe (Ridding et al. 2015; Staab et al. 2015). Doing this, they may contribute to the preservation of species related to this ecosystem, alongside historical site preservation and restoration (Kiehl and Pfadenhauer 2007; Staab et al. 2015). Such strips are likely to provide a further advantage for farmers in the form of forage production (De Cauwer et al. 2006a). However, farmers can be at a loss as to how using forage from their strips (Bruinberg et al. 2002; De Cauwer et al. 2006a). Some other concerns may arise from farmers adopting wildflower strip AES. One major concern is about the risk to promote noxious weeds (Smith et al. 2010). Despite several implications in terms of farmer acceptance of AES, these multiple aspects have rarely been studied (Uyttenbroeck et al. 2016), but see works by De Cauwer et al. (2006a, b, 2008). It is therefore imperative to know how to implement and manage flower strips in order to make them both effective regarding their aims and acceptable to farmers.

To create and maintain wildflower strips analogous to hay meadows on nutrient-rich cultivated soils reveals somewhat challenging. Indeed, especially the high phosphorus content is known to be unsuitable to the development of species-rich grasslands (Janssens et al. 1998). In such fertile conditions, grasses tend to become dominant at the expense of flowering species (Schellberg et al. 1999; Mountford et al. 2016). Especially, the growth of legumes may be restricted due to their low competitive abilities against grasses in nutrient-rich ecosystems (Zanetti et al. 1996). This in turn affects forage quality, as fewer legumes in the forage can lead to lower protein content. The inclusion of grasses in wildflower strips seed mixture is therefore a questionable management practice (Staab et al. 2015). On the one hand, it may affect the emergence of the sown flowers which would affect both insect attraction and landscape aesthetics improvement. But on the other hand, grass species may be useful at the implementation of field margins, as they can help controlling weed emergence through clonal field occupation (Hansson and Fogelfors 1998). The question arises therefore which proportion of grass to sow in order to promote flowers while maintaining weeds at an acceptable level.

Furthermore, the mowing regime has been shown to be a main driver of plant community composition in grassland ecosystems (Kahmen et al. 2002). Mowing can indeed decrease the inter- and intraspecific competition, therefore permitting the co-existence of numerous species in a small scale (Tälle et al. 2016). In many countries, wildflowers strips are left unmanaged (Scheper et al. 2015). In others, annual late-summer or winter mowing may be prescribed

(Haaland et al. 2011; Tarmi et al. 2011). However, a recent study pointed out that early-summer mowing was more efficient to maintain forbs in nutrient-rich contexts (Kirmer et al. 2018). It also offers the advantage that flowers may regrow in the late-summer and that a second forage harvest may be available to farmers in autumn. This results in a twice-a-year mowing frequency, identified by Uchida and Ushimaru (2014) as an intermediate disturbance regime (between land abandonment and intensification) particularly favourable to maintain plant and insect species richness in agricultural lands. Also, in the absence of soil fertilisation, increased biomass uptake through multiple cuttings is likely to diminish soil nutrient stocks (Oelmann et al. 2007), therefore conducting to soil condition more suitable for the maintenance of meadow species (Critchley et al. 2002). Moreover, adapting mowing date may help to prevent weed infestation (Smith et al. 2010). Forage yield and quality are also likely to be decreased in cases of delayed mowing (Bruinberg et al. 2002). There is therefore a need to test how mowing regime is able to modulate benefits generated by wildflower strips.

In this study, we aimed to explore, through an experimental design, how management options in grassland-like wildflower strips may influence their ability to provide environmental benefits (flower provision for insects and landscape purposes, reduction of soil nutrient load) and respond to main farmer concerns (noxious weed promotion, forage quantity and quality). We first hypothesize that decreasing grass density should result in increasing flower provision, but should also promote weed development. Second, increasing mowing frequency should increase flower provision, forage quantity and quality and help controlling weeds. It should result in soil nutrient depletion due to increasing forage exportation. Through the verification of these hypotheses, we aimed at determining which option may optimise ecological benefits and farmer acceptance for AES.

Materials and Methods

Study Site and Experimental Design

The experiment was implemented in April 2010. It was located in Gembloux (Belgium, Wallonia; 50°33'45"N; 4°42'22"E; alt. 170 m; annual mean temperature ca. 9 °C, annual rainfall ca. 800 mm). Soil at the site is very fertile (WRB soil group: retisol). It was previously occupied by an intensive arable field devoted to growing cereals and row crops (previous 3-year rotation: potatoes, spelt and winter barley). Two seeds mixtures, differing in amount of grass seed present, were tested (Table 1): (1) the seed mixture with high grass density (G_{high}) was composed of 85%

Table 1 List of species and sowing densities in the G_{high} and G_{low} modalities

Species	Type	G_{high} (kg/ha)	G_{low} (kg/ha)	Colour
<i>Agrostis capillaris</i> L.	Grass	3	1.5	/
<i>Festuca rubra</i> L.	Grass	15	7.5	/
<i>Poa pratensis</i> L.	Grass	7.5	3.75	/
<i>Achillea millefolium</i> L.	Forb	0.15	0.15	White
<i>Centaurea cyanus</i> L.	Forb	0.3	0.3	Blue
<i>Centaurea jacea</i> L.	Forb	0.45	0.45	Purple
<i>Daucus carota</i> L.	Forb	0.45	0.45	White
<i>Glebionis segetum</i> (L.) Fourr.	Forb	0.3	0.3	Yellow
<i>Leucanthemum vulgare</i> Lam.	Forb	0.9	0.9	White
<i>Malva moschata</i> L.	Forb	0.45	0.45	Pink
<i>Papaver rhoeas</i> L.	Forb	0.3	0.3	Red
<i>Silene latifolia</i> subsp. <i>alba</i> (Mill.) Greuter & Burdet	Forb	0.75	0.75	White
<i>Lotus corniculatus</i> L.	Legume	0.1725	0.1725	Yellow
<i>Medicago lupulina</i> L.	Legume	0.15	0.15	Yellow
<i>Trifolium pratense</i> L.	Legume	0.1275	0.1275	Purple
Total		30	17.25	

Colour is the flower colour considered to compute colour diversity

grasses and 15% flowers. This proportion, as well as the applied sowing density (30 kg/ha) correspond to the recommendation of the seed provider; (2) the seed mixture with lower grass density (G_{low}) had the same flower seed density and composition as G_{high} but the grasses seed density was divided by two. The G_{low} was therefore sown at 17.25 kg/ha (Table 1). The three mowing regimes applied were: (1) once a year in August–September (MOW1); (2) once every two years in August–September (MOW0.5); (3) twice a year in late June and in August–September (MOW2). MOW1 corresponds to the prescription that was applied in the Walloon wildflower strips AES at the beginning of the experiment. MOW0.5 and MOW2 were introduced as alternative management options. Mowing regime application began in 2011. In 2010 (first year), all plots were mown only in September. Mowing was accomplished by a plot harvester (Haldrup© with Busatis © cutting blade) and forage was removed. The experiment was therefore made of six treatments (2 sown grass densities*3 mowing regimes). It contained four replicates, corresponding to four blocks, and was therefore composed of 24 experimental plots. Plot size was 60 m² (6 × 10 m). Within each block, plots were placed according to a strip-plot design, with seed mix placed longitudinally and mowing regimes transversely within each block (Fig. 1).

Floristic Survey

In each of the 24 plots, six permanent 1 m²-quadrats were placed for vegetation surveys. In these quadrats, the

horizontal cover of all the sown species was recorded each year from 2010 to 2014 in early June, at the peak of vegetation. Unsown weed species were not recorded individually, but instead as a global problematic weed species cover (annual arable weeds, *Cirsium arvense* and *Rumex crispus/obtusifolius*). As indicators for flower-feeding insects and landscape interests, we computed the following metrics:

- Flowering plant species richness (insects)
- Total flowering plant cover (insects and landscape)
- Flowering plant Shannon's diversity (insects)
- Flower colour Shannon's diversity (landscape)

Flower colour diversity indeed proved to be an important feature in landscape aesthetics (Stilma et al. 2009; Junge et al. 2015). Flower colour was assessed based on field observation and is provided in Table 1. To compute the colour diversity, horizontal cover of the species with identical colour was summed to obtain a total cover for each colour. Species richness and Shannon's diversity were calculated, as they have been shown to be relevant for flower-feeding insects in previous studies (Ebeling et al. 2008; Fründ et al. 2010).

Forage Yield and Forage Analyses

The plot harvester directly provided fresh matter yields (FMY) for each mown plot, each year between 2010 and 2014, in three distinct 1.5 × 6 m strips within the plot. In each plot, one ca. 1 kg fresh forage sample was taken at the moment of mowing. It was weighed and then dried in an oven (65 °C for 36 h) in order to measure dry matter content (DMC) and subsequently calculate dry matter yield (DMY).

In 2014, dried samples were ground in a hammer mill (1 mm screen; Waterleau, BOA, Belgium) and then ground again in a Cyclotec mill (1 mm screen; FOSS Electric, Hillerød, Denmark). They were then submitted to NIRS analysis (XDS spectrometer, FOSS Electric, Hillerød, Denmark), and the absorption data was recorded as log 1/R from 1100 to 2498 nm, every 2 nm (WINISI 1.5, FOSS Tecator Infrasoft International LCC, Hillerød, Denmark). Chemical characteristics of forage, i.e. proteins (% DM), fibres (% DM) according to the Van Soest method, i.e. neutral detergent fiber (NDF) and acid detergent lignin (ADL) and digestible organic matter (DOM, % DM, cellulase method) were then estimated using the NIRS calibrations previously developed at CRA-W for hay and grass (Table 2). The correspondence between the predicted sample and the NIR spectral database was evaluated through the standardised H value (distance between the predicted sample and the centroid of the spectral database) according to Shenk and Westerhaus (1991). NIRS predictions were correct when averaged H value of predicted samples was

Fig. 1 Schematic representation of the experimental design in Gembloux (Belgium; 50° 33'45"N; 4°42'22"E). Grass buffer zones were maintained to facilitate circulation and working in the experimental plots

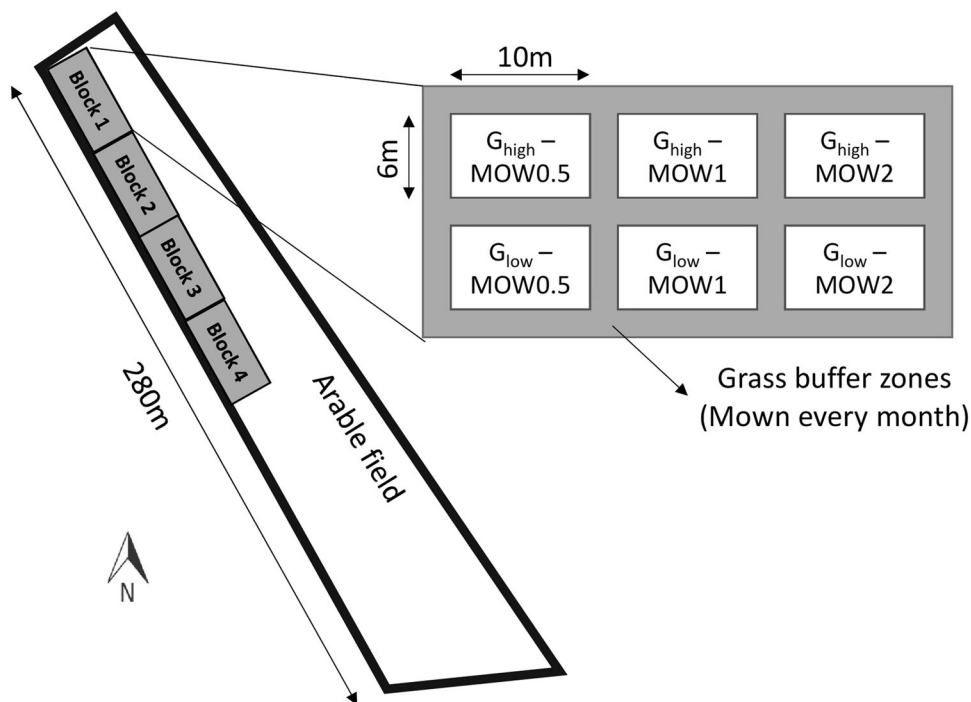


Table 2 NIRS calibration characteristics for estimating the chemical composition of wildflowers strips

Constituent	<i>N</i>	Mean	SD	Range	SEC	<i>R</i> ²	SECV
Protein (% DM)	3273	14.24	5.75	3.32–34.16	0.84	0.98	0.86
NDF (% DM)	1698	48.72	7.35	18.86–70.24	2.02	0.92	2.05
ADL (% DM)	1222	3.21	1.53	1.00–9.07	0.58	0.86	0.6
DOM (% DM)	2487	76.91	10.13	39.37–95.43	2.34	0.95	2.38

N number of sample in the NIRS database, *SD* standard deviation of the population in the NIRS database, *SEC* standard error of calibration, *R*² coefficient of determination, *SECV* standard error of cross validation

lower than 3. For the MOW2 regime, retained values were the means weighted by DMY at each date (June and September).

Soil Analyses

In March 2014, at the resumption of the growing season, soil samples were collected in each experimental plot ($n = 24$) using a 2 cm-diameter auger. Five soil samples were randomly collected at 20 cm of soil depth. The five soil samples were merged in a composite sample.

Mobile nitrogen ($N\text{-NO}_3$) was measured on fresh samples sieved to 8 mm. Concentrations were determined in a soil-solution mixture at 1:5 w:v ratio after extraction with KCl (0.1N) and agitation for 30 min. After a 30 min decantation and subsequent filtration (filter: Whatman® 602H1/2), nitrate was reduced into nitrite using a cadmium column. Nitrite was analysed by the modified Griess-Ilosvay method (Bremner 1965; Guiot 1975). All soil samples were dried at 40 °C and sieved to 2 mm. A subsample of each sample was

finely ground (<200 μm) for C and N analyses. Total organic carbon (C_{tot}) and total nitrogen content (N_{tot}) were measured by dry combustion (ISO10694 and ISO13878, respectively for C_{tot} and N_{tot}). The available potassium (K_{av}) and phosphorus (P_{av}) concentrations were determined in a soil-solution mixture at a 1:5 w:v ratio after extraction with $\text{CH}_3\text{COONH}_4$ (0.5 M) and EDTA (0.02 M) at pH 4.65 and agitation for 30 min (Lakanen and Erviö 1971). The concentration of K was measured by flame atomic absorption spectrometry while the concentration of P was measured by colourimetry at 430 nm.

Data Analyses

We tested effects of sown grass density, mowing regime, and their interaction using mixed-effects models with block as a random effect. When yearly data was available (i.e. floristic data and forage yield), year effect was included in models. Analyses were also computed for each year

Table 3 ANOVAs for variables measured at each of the 5 years of the experiment (2010–2014)

Effects		Flowering plant richness	Plant diversity	Flowering plant cover	Weed cover	Colour diversity	DMY
Year	<i>F</i> [4;12]	225.65	109.65	103.2	54.11	81.84	36.89
	<i>P</i>	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Seed mix	<i>F</i> [1;3]	0.07	0.55	9.63	0.07	2.66	0.04
	<i>P</i>	0.804	0.513	0.053	0.808	0.201	0.853
Mowing	<i>F</i> [2;6]	12.21	4.95	7.7	1.2	1.88	380.72
	<i>P</i>	0.008	0.054	0.022	0.365	0.233	<0.001
Year*Seed mix	<i>F</i> [4;12]	0.22	0.15	0.63	1.4	0.29	1.81
	<i>P</i>	0.924	0.958	0.653	0.294	0.882	0.191
Year*Mowing	<i>F</i> [8;24]	7.51	3.31	10.26	1.4	3.55	46.54
	<i>P</i>	<0.001	0.011	<0.001	0.248	0.008	<0.001
Seed mix*Mowing	<i>F</i> [2;6]	0.74	1.73	1.21	1.45	2.34	0.86
	<i>P</i>	0.517	0.255	0.362	0.305	0.177	0.468
Year*Seed Mix*Mowing	<i>F</i> [8;24]	0.46	2.29	0.6	1.02	2.37	1.55
	<i>P</i>	0.871	0.056	0.766	0.45	0.049	0.191

Effects of year, seed mix, mowing and their interactions are provided. *F*-values, with degrees of freedom under square brackets and associated *P*-values are provided

Significant effects are in bold

separately and for total (sum) forage yield values. ANOVAs were computed on mixed-effect models in order to determine effects significance. Analyses were computed using Minitab 16 (Minitab Inc.).

Results

Flowering Species Response

Our models revealed year effects ($P < 0.001$) for all tested variables where yearly data was available (Table 3). Concerning flowering species related variables (abundance, richness, diversity and colour diversity) interaction between year and mowing regime was significant.

Species richness was significantly influenced by mowing regime only in 2013 ($P < 0.05$, Table 4) and 2014 ($P < 0.01$, Table 4). It tended to decrease with time, whatever the mowing regime. However, the decrease tended to be lower with increasing mowing frequency. Consequently, in the last year (2014), the species richness was the highest in MOW2 and the lowest in MOW0.5. MOW1 had an intermediate value. The response was similar for plant diversity, but to a lower extent. In 2014, it was also slightly higher for MOW2, but only with marginal significance ($0.05 < P < 0.1$, Table 4). Also, colour diversity responded this way. However, in this case, the highest value was observed for MOW0.5 in 2014. In 2012, the interaction between mowing regime and seed mix was significant for both colour and plant diversity ($P < 0.05$, Table 4). Flowering plant cover was influenced by sown grass density in the first three years

2010–2012 (Table 4), with higher values for G_{low} seed mix (Table 4). In the last year (2014), difference in flowering plant cover was only due to mowing regime, with the highest value for MOW2 ($P < 0.01$, Table 4).

Weed Cover

Weed cover showed a yearly variation, but was not influenced by management options. It was the highest in the first year (2010). The lowest values were observed in 2011 and 2012 (0.9% and 1.4%, respectively). It then increased again in 2013 and 2014 until 9.2% in average (Table 4), mainly due to *Cirsium arvense* expansion.

Forage Quantity and Quality

The mowing regime significantly influenced the forage DMY ($P < 0.05$, Table 4), except in the first year (2010) when a single mowing regime (once in September) was applied to all plots. Over the 5 year period, wildflower strips cut twice a year (MOW2) had the highest DM forage production compared to the low mowing rate. Grass proportion in the seed mix did not influence the forage DMY (Table 4).

As confirmed by the averaged H value of predicted samples lower than 3 ($H = 2.44$), NIRS calibrations developed from hay and grass samples could be used for predicting wildflower strip characteristics. Cutting regime of wildflower strips significantly impacted the chemical characteristics and the digestibility of forage. Forage from MOW2 had a higher protein content ($P < 0.001$, Table 4), lower fibre content (NDF) ($P < 0.001$, Table 4) and lower

Table 4 Mean values (\pm SD) by mowing regime and by seed mix of all tested variables

	Mean values \pm SD					ANOVA		
	By mowing regime			By seed mix		Mowing	Seed mix	Mowing*seed mix
	MOW0.5	MOW1	MOW2	G _{high}	G _{low}			
Flowering plant richness								
2010	12.0 \pm 0.0	12.0 \pm 0.0	12.0 \pm 0.0	12.0 \pm 0.0	12.0 \pm 0.0			
2011	9.1 \pm 0.4	9.1 \pm 0.4	9.3 \pm 0.5	9.1 \pm 0.3	9.3 \pm 0.5			
2012	7.3 \pm 0.5	7.8 \pm 1.3	7.8 \pm 0.9	7.7 \pm 1.1	7.5 \pm 0.8			
2013	6.8 \pm 0.9	7.0 \pm 0.5	7.8 \pm 0.5	7.2 \pm 0.7	7.2 \pm 0.8	*		
2014	4.9 \pm 1.0	6.4 \pm 1.3	7.0 \pm 0.8	6.2 \pm 1.2	6.0 \pm 1.5	**		
Plant diversity								
2010	2.31 \pm 0.07	2.30 \pm 0.07	2.33 \pm 0.03	2.32 \pm 0.05	2.31 \pm 0.07			
2011	1.93 \pm 0.10	1.88 \pm 0.12	1.93 \pm 0.15	1.94 \pm 0.11	1.88 \pm 0.12			
2012	1.56 \pm 0.14	1.49 \pm 0.13	1.46 \pm 0.12	1.52 \pm 0.14	1.49 \pm 0.12			*
2013	1.26 \pm 0.24	1.28 \pm 0.14	1.53 \pm 0.11	1.38 \pm 0.17	1.34 \pm 0.25			
2014	1.04 \pm 0.22	1.07 \pm 0.23	1.27 \pm 0.19	1.14 \pm 0.17	1.11 \pm 0.28	°		
Flowering plant cover [%]								
2010	116.6 \pm 12.7	121.0 \pm 21.0	124.5 \pm 13.9	112.5 \pm 14.5	128.9 \pm 13.2		*	
2011	79.0 \pm 11.4	86.4 \pm 16.8	78.1 \pm 22.3	72.3 \pm 13.0	90.0 \pm 16.4		°	
2012	81.0 \pm 10.9	83.1 \pm 13.9	76.3 \pm 11.3	74.5 \pm 12.2	85.8 \pm 9.0		*	
2013	43.0 \pm 9.4	46.8 \pm 10.1	53.8 \pm 13.7	45.8 \pm 12.7	49.9 \pm 10.7			
2014	42.9 \pm 12.1	53.2 \pm 21.6	86.6 \pm 15.4	54.2 \pm 20.2	67.5 \pm 28.2	**		
Weed cover								
2010	34.9 \pm 18.1	28.5 \pm 15.5	28.3 \pm 10.4	33.1 \pm 16.4	28 \pm 12.9			
2011	0.7 \pm 0.6	0.8 \pm 0.6	1.3 \pm 2.9	1.3 \pm 2.3	0.5 \pm 0.6			
2012	2.0 \pm 1.8	1.8 \pm 2.4	0.4 \pm 0.4	1.3 \pm 1.8	1.6 \pm 1.9			
2013	5.4 \pm 5.5	2.8 \pm 3.1	1.7 \pm 1.0	2.3 \pm 2.1	4.3 \pm 5.0			
2014	14.0 \pm 11.4	5.6 \pm 5.5	8.1 \pm 3.2	7.8 \pm 4.8	10.7 \pm 10.4			
Colour diversity								
2010	1.58 \pm 0.07	1.57 \pm 0.06	1.60 \pm 0.02	1.60 \pm 0.02	1.56 \pm 0.07			
2011	1.18 \pm 0.02	1.10 \pm 0.07	1.15 \pm 0.06	1.14 \pm 0.07	1.14 \pm 0.06			
2012	1.00 \pm 0.21	1.10 \pm 0.11	1.05 \pm 0.10	1.09 \pm 0.15	1.01 \pm 0.15			*
2013	0.91 \pm 0.12	0.88 \pm 0.08	0.95 \pm 0.10	0.94 \pm 0.09	0.88 \pm 0.1			
2014	0.91 \pm 0.16	0.67 \pm 0.14	0.72 \pm 0.20	0.78 \pm 0.14	0.75 \pm 0.24	*		
DMY [10³kg/ha]								
2010	3.8 \pm 0.3	3.9 \pm 0.4	4.0 \pm 0.3	4.0 \pm 0.3	3.9 \pm 0.4			
2011	–	5.4 \pm 1.0	8.3 \pm 2.0	7.0 \pm 1.9	6.7 \pm 2.5	**		
2012	6.0 \pm 1.1	6.9 \pm 0.6	9.5 \pm 0.6	7.2 \pm 1.7	7.8 \pm 1.7	***		
2013	–	4.7 \pm 0.7	9.7 \pm 0.9	7.1 \pm 2.4	7.3 \pm 3.1	**		
2014	5.0 \pm 1.1	4.2 \pm 0.6	6.0 \pm 0.7	5.2 \pm 0.9	4.9 \pm 1.3	*		
Sum	14.8 \pm 1.8	25.1 \pm 1.5	37.5 \pm 2.6	25.7 \pm 9.3	25.9 \pm 10.4	***		
Proteins [%]	8.0 \pm 0.9	7.1 \pm 0.6	10.3 \pm 0.8	8.1 \pm 1.5	8.9 \pm 1.6	***		
NDF [%]	55.7 \pm 1.4	56.1 \pm 1.1	45.8 \pm 1.3	53.0 \pm 5.1	52.0 \pm 5.1	***		
ADL [%]	7.7 \pm 0.6	7.7 \pm 0.3	6.1 \pm 0.1	7.2 \pm 0.9	7.1 \pm 0.9	**		
DOM [%]	49.8 \pm 3.5	50.0 \pm 1.9	61.4 \pm 0.8	53.1 \pm 6.4	54.4 \pm 5.7	***		
N _{tot} [%]	0.119 \pm 0.004	0.119 \pm 0.005	0.121 \pm 0.003	0.119 \pm 0.006	0.12 \pm 0.003			
N-NO ₃ [mg/kg]	0.264 \pm 0.083	0.251 \pm 0.391	0.331 \pm 0.209	0.262 \pm 0.211	0.302 \pm 0.295			
P _{av} [mg/100 g]	30 \pm 3.2	30.2 \pm 1.7	28.7 \pm 1.4	29.5 \pm 2.2	29.7 \pm 2.4			

Table 4 (continued)

	Mean values \pm SD					ANOVA		
	By mowing regime			By seed mix		Mowing	Seed mix	Mowing*seed mix
	MOW0.5	MOW1	MOW2	G_{high}	G_{low}			
K_{av} [mg/100 g]	29.2 \pm 2.2	27.7 \pm 4.1	23.8 \pm 1.2	26.9 \pm 3.4	26.8 \pm 3.8	*		
C_{tot} [g/kg]	15.8 \pm 1.7	16.5 \pm 2.3	15.6 \pm 1.2	15.8 \pm 2	16.1 \pm 1.6			

Yearly values are displayed when available. ANOVA is the result of ANOVAs made on mixed-effect models (with Block as random grouping effect). Different letters indicate significant differences. For weed cover, different letters indicate significant difference in mean values for each year. DMY data were not available for MOW0.5 in 2011 and 2013 due to absence of mowing

DMY dry matter yield, NDF neutral detergent fiber, ADL acid detergent lignin, DOM digestible organic matter

*** $P < 0.001$; ** $0.01 > P > 0.001$; * $0.05 > P > 0.01$; ° $0.1 > P > 0.05$; not displayed: $P > 0.1$

indigestible fibre content (ADL) ($P < 0.01$, Table 4). Finally, the digestible organic matter (DOM) was significantly higher in MOW2 than in MOW0.5 and MOW1 ($P < 0.001$, Table 4). As in the case of DMY, decreasing the proportion of grass seeds in the seed mixture did not influence the chemical composition of the forage.

Soil Nutrients

Plots with the highest mowing rate tended to experience a K_{av} depletion. For this nutrient, we observed contents varying from 23.8 mg/100 g in MOW2 to 27.7 mg/100 g in MOW1 29.2 mg/100 g in MOW0.5 ($P < 0.05$, Table 4). None of the other soil variables were affected by management options.

Discussion

Grass Seed Density Effect

Our study revealed effects of grass seed density on flowering plant abundance in the first three years, with a higher flower cover in the seed mix with reduced grass proportion. This was primarily due to improved development of the legumes *Lotus corniculatus* and *Trifolium pratense* during the first years after strip implementation (Table 5). This is congruent with the general low competitive ability of legumes under eutrophic conditions (Zanetti et al. 1996). However, this was not observed beyond the fourth year after sowing, as mowing regime effects became more and more pronounced and overwhelmed initial sowing conditions. No effect was found on colour and species diversities. It was previously shown by Staab et al. (2015) that detrimental effect of grass on flower species diversity appeared when grass biomass proportion is higher than 90%. In our case, it is likely that this threshold was not reached even with our higher grass density.

Contrarily to our initial assumption, grasses did not hamper noxious weed, as weed cover was not significantly influenced by grass proportion in the seed mix. Earlier studies on weed suppression by flower strips showed that weeds only overdevelop in the case of spontaneous unsown flower strips, while sown flower strips efficiently cover the soil against weeds (Denys and Tschardt 2002; De Cauwer et al. 2008). After the third year, such a conclusion could have been driven from our experience, as annual weed emergence on the first year was rapidly pulled up to a ca. 1% weed cover. However, from the fourth year, weed cover increased again to ~10% on average on the fifth year, i.e. the end of AES commitment. This was moreover mainly due to *C. arvensis*, a particularly pernicious species that is likely to spread into arable fields where it is difficult to control (Tiley 2010). This rather high cover level is therefore likely to discourage farmers from continuing with their implemented flower strips.

Mowing Effect

Colour diversity, reflecting potential for landscape aesthetics (Junge et al. 2015), was the highest under the MOW0.5 mowing regime after 5 years of application. A tradeoff is therefore observed with flower abundance and richness that were the lowest under this mowing regime. The highest values for these variables were observed under the twice-a-year mowing regime. *T. pratense* was one of the main drivers for this pattern, as it responded the best to the MOW2 regime (Table 5). This result is particularly relevant as this species is considered to be a keystone species for bumblebee conservation in Europe and could facilitate the pollination of wild and cultivated plants (Kleijn and Raemakers 2008; Rundlöf et al. 2014).

The increased abundance of this species in the MOW2 regime may also partly explain the better forage quality in those plots. Of course, it is well known that digestibility correlates positively with the utilisation frequency, either

Table 5 Mean species cover each year, by mowing regime and by seed mix

	Year	Species cover				
		By mowing regime			By seed mix	
		MOW0.5	MOW1	MOW2	G _{high}	G _{low}
<i>Achillea millefolium</i> L.	2010	4.50	4.68	4.92	5.12	4.28
	2011	5.15	5.58	4.17	6.24	3.69
	2012	8.93	9.79	6.21	7.07	9.55
	2013	8.41	11.15	13.80	10.79	11.45
	2014	2.03	4.25	11.86	5.75	6.35
<i>Centaurea cyanus</i> L.	2010	13.44	13.57	12.57	12.50	13.88
	2011	0.13	0.06	0.10	0.01	0.18
	2012	0.00	0.00	0.00	0.00	0.00
	2013	0.00	0.00	0.00	0.00	0.00
	2014	0.00	0.00	0.00	0.00	0.00
<i>Centaurea jacea</i> L.	2010	6.73	7.25	6.94	7.11	6.83
	2011	4.56	5.73	5.17	5.42	4.89
	2012	24.58	30.40	20.60	23.90	26.49
	2013	22.94	24.77	13.88	19.28	21.78
	2014	25.40	30.96	19.15	24.68	25.65
<i>Daucus carota</i> L.	2010	8.15	6.96	7.53	6.75	8.34
	2011	4.73	4.85	5.33	4.67	5.28
	2012	1.06	0.21	0.46	0.53	0.63
	2013	1.61	0.59	0.26	0.62	1.03
	2014	0.20	0.07	0.48	0.18	0.33
<i>Glebionis segetum</i>	2010	11.56	11.96	11.73	11.85	11.65
	2011	0.00	0.00	0.00	0.00	0.00
	2012	0.00	0.00	0.00	0.00	0.00
	2013	0.00	0.00	0.00	0.00	0.00
	2014	0.00	0.00	0.00	0.00	0.00
<i>Leucanthemum vulgare</i> Lam.	2010	4.49	4.67	4.73	4.77	4.49
	2011	10.29	13.92	8.04	9.79	11.71
	2012	17.62	15.73	16.40	15.57	17.60
	2013	0.00	0.00	0.00	0.00	0.00
	2014	0.00	0.06	0.00	0.00	0.04
<i>Lotus corniculatus</i> L.	2010	11.46	10.55	11.25	7.48	14.69
	2011	15.75	13.31	14.42	9.68	19.31
	2012	6.79	16.61	25.47	15.16	17.42
	2013	0.81	0.74	1.50	1.13	0.90
	2014	0.13	0.54	1.15	0.65	0.56
<i>Malva moschata</i> L.	2010	2.65	2.11	2.78	2.50	2.53
	2011	1.98	1.48	1.65	1.85	1.56
	2012	5.81	2.19	0.58	3.04	2.69
	2013	2.96	1.95	0.28	1.64	1.82
	2014	6.71	1.59	0.70	2.88	3.12
<i>Medicago lupulina</i> L.	2010	18.65	18.57	18.19	17.06	19.88
	2011	9.90	11.25	11.31	10.24	11.40
	2012	0.00	0.07	0.13	0.09	0.04
	2013	0.06	0.00	5.96	2.71	1.31
	2014	0.00	0.58	6.65	1.73	3.10
<i>Papaver rhoeas</i> L.	2010	15.52	17.44	18.13	17.72	16.33
	2011	0.00	0.00	0.00	0.00	0.00
	2012	0.00	0.20	0.06	0.09	0.09
	2013	0.00	0.07	0.00	0.04	0.00
	2014	0.00	0.07	0.00	0.00	0.04
	2010	6.33	7.79	8.73	6.26	8.97
	2011	5.77	6.69	5.52	5.44	6.54

Table 5 (continued)

	Year	Species cover				
		By mowing regime			By seed mix	
		MOW0.5	MOW1	MOW2	G _{high}	G _{low}
<i>Silene latifolia</i> subsp. <i>alba</i> (Mill.) Greuter & Burdet	2012	16.24	7.93	4.90	8.79	10.59
	2013	5.83	5.48	3.28	4.64	5.08
	2014	8.44	4.36	2.53	4.47	5.76
<i>Trifolium pratense</i> L.	2010	13.08	15.44	17.06	13.42	16.98
	2011	20.73	23.52	22.38	18.94	25.47
	2012	0.00	0.00	1.48	0.32	0.67
	2013	0.39	2.03	14.86	4.99	6.53
	2014	0.00	10.65	44.04	13.89	22.57
<i>Festuca rubra</i> L.	2010	n.a.	n.a.	n.a.	n.a.	n.a.
	2011	29.44	22.48	32.21	43.03	13.06
	2012	37.30	36.56	47.70	49.33	31.72
	2013	48.15	49.94	70.56	66.19	46.24
	2014	52.77	56.63	64.56	67.04	48.93
<i>Poa pratensis</i> L.	2010	n.a.	n.a.	n.a.	n.a.	n.a.
	2011	1.69	1.46	1.42	1.88	1.17
	2012	0.42	0.62	0.51	0.14	0.88
	2013	3.96	5.70	0.94	3.56	3.50
	2014	2.59	9.71	5.36	3.23	8.55
<i>Agrostis capillaris</i> L.	2010	n.a.	n.a.	n.a.	n.a.	n.a.
	2011	2.21	2.44	2.19	2.94	1.61
	2012	0.26	0.24	0.14	0.14	0.29
	2013	3.57	3.01	0.02	0.50	3.90
	2014	13.95	11.98	1.17	4.52	13.54

mowing or grazing (Gardarin et al. 2014). Also, the stage of maturity of individual species when forages are mown, can explain the difference in digestibility (Bruinenberg et al. 2002). However, most species in our study, including the dominant *Festuca rubra*, belong to the plant functional type (PFT) C according to the classification proposed by Cruz et al. (2002). That type is characterised by low digestibility values in the beginning of the growing season but a slower decline during the growth of the plant. Therefore, over-maturity may be only part of the explanation for the difference in forage quality, species composition being another one. *Trifolium pratense* is known for its participation in DMY (De Cauwer et al. 2006a) and is frequently used in hay meadows with multi-cut management (Halling et al. 2004). Its digestibility is commonly high and less dependent on the ageing of the plant. Moreover, dicotyledonous species, that had higher cover in the MOW2 plots, have lower NDF content and higher pectin content, resulting in better digestibility (Bruinenberg et al. 2002). Forage production is not the main aim of wildflower strips. However, it may lead to a better integration of AES within usual farming procedures, and therefore make AES more acceptable (Sattler and Nagel 2010).

Concerning the soil variables, we only found that mowing regime influenced K availability, in the sense of an increased K depletion with increasing biomass exportation,

i.e. the MOW2 regime. Plant offtake plays a major role in K cycle. Repeated mowing without K supply is likely to provoke a K depletion within 3–10 years (Kayser and Isselstein 2005). For other soil variables, installation of flower strips and subsequent biomass exportation through mowing did not result in a clear soil nutrient depletion. It is well known from ecological restoration literature that repeated biomass exportation is unlikely to rapidly reduce availability of these nutrients in the soil (Marrs 1993; Walker et al. 2004; Piqueray and Mahy 2010). In case of conversion from arable land to grassland, P uptake from mowing can fail at diminishing plant-available P, as it can be replaced through mineralisation from larger non-available pools (Walker et al. 2004). P_{av} content in our study site (ca. 30 mg/100 g) was far higher than the 5 mg/100 g considered as the upper limit for maintaining species-rich grasslands in semi-natural systems (Janssens et al. 1998). We indeed observed a decrease in species richness and flower cover over the 5 years of the experiment, that was limited through multiple mowing. This confirms the need for a rather early mowing date to maintain flowering plant species under fertile conditions (Kirmer et al. 2018).

Conclusions and implications for management

Accordingly to our assumptions, mowing twice a year (in late June and in September) resulted in the most interesting option. It permitted enhancement of flowering plant cover and resulted in better forage production, both in quantity and quality. We showed that this option would likely contribute to decreased K availability in soils, but did not have an impact on other soil nutrients over 5 years. This option should therefore be promoted in wildflower management and was therefore accepted as an alternative management option in Walloon AES following this study. One can argue that a first mowing in late June, at the end of the flowering peak, may be a problem for insect conservation. However, a meta-analysis by Humbert et al. (2012b) revealed that there was generally minimal advantage of delaying the first mowing date beyond early summer in grasslands. Also, several species are able to regrow after late June-mowing, and therefore extend flower availability in late summer (Kirmer et al. 2018). However, there is a great amount of evidence that keeping unmown refuges is of primary importance for insect conservation in grasslands (Humbert et al. 2012a; Lebeau et al. 2015) and therefore probably in grassland-like wildflower strips too. Such zones were efficient at preserving insects as well as insect-mediated ecosystem services (Buri et al. 2014). Therefore, maintaining an unmown refuge zone of min. 3-m width all along the wildflower strips was retained as compulsory management

in Wallonia (southern Belgium). In case of a second mowing, the refuge zone of the first mowing has to be maintained or enlarged in order to keep a less disturbed overwintering zone (Schmidt et al. 2008). However, it is recommended that it be moved annually within the wildflower strip in order to avoid species richness decrease due to management abandonment (Schmidt et al. 2008; Uchida and Ushimaru 2014). Mowing regime options other than twice-a-year should not be completely rejected as, with the exception of forage production aspects, they were valuable regarding the tested variables. They can therefore be advantageous in farms without livestock, which is increasingly the case in cropland regions where wildflower strips are often implemented.

None of our hypotheses regarding weed control were verified. Their relative abundance in the first year was due to annual weeds from the soil seed bank. Indeed, soil seed bank is usually dense in arable lands and mainly depends on the former management such as crop rotation or herbicide use (Asteraki et al. 2004; Albrecht 2005). Their emergence in wildflower strips mainly relies on the capacity of sown species to outcompete them (Asteraki et al. 2004; De Cauwer et al. 2008), which was the case in our study in the second and third year. From the fourth year, neither increasing grass seed density nor mowing frequency resulted in a decrease of weed cover. Regardless of the treatment, wildflower tended to shelter noxious weeds after 5 years, notably the thistle *Cirsium arvense*. This supports the need to test the efficacy of specific management practices such as selective clipping or spraying, both being allowed in the Walloon AES.

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Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

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