

Supplementary Material:

Weed Suppression in Only-Legume Cover Crop Mixtures

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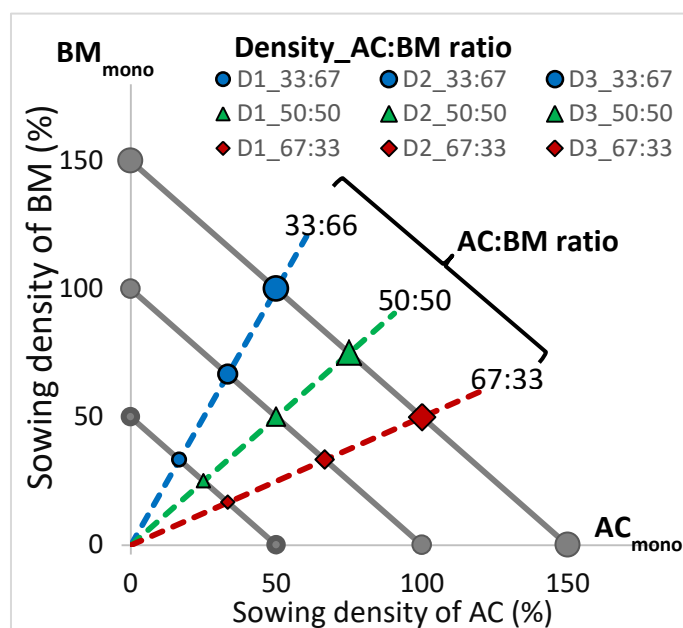


Figure S1. The Mixture design response surface methodology for different combinations of alsike clover (AC) and black medic (BM). The two legume species were sown as sole crops (monocultures; mono) and nine binary mixtures at three relative proportions of AC:BM ratios represented in dashed lines 33:67 (blue), 50:50 (green), and 67:33 (red) sown at three sowing densities (D1 = 50%, D2 = 100%, and D3 = 150% of the recommended seed density). The full grey lines with symbols represent the monocultures of each species at each density.

- **Two legume species:** alsike clover (**AC**) and black medic (**BM**)

Factor (1): 5 mixing ratios (MR) of AC:BM

MR1: mono (100:0)

MR2: Mix (67:33)

MR3: Mix (50:50)

MR4: Mix (33:67)

MR5: mono (0:100)

Factor (2): 3 seed densities (of the recommended seed density; %)

D0: bare soil → only included in the statistical analysis of weed biomass

D1: 50%, **D2:** 100%, **D3:** 150%

- 3 Harvest times per season (**H1**, **H2** and **H3**)

- 2 years (**y1:** 2016, **y2:** 2017)

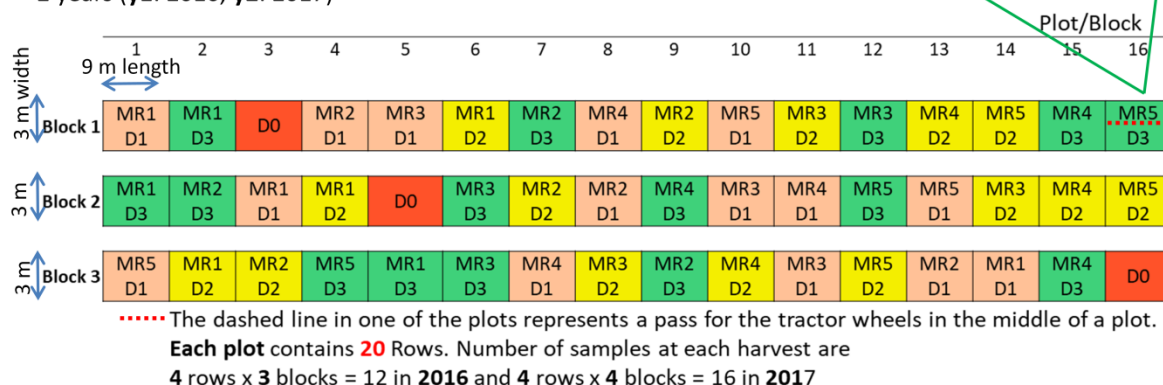


Figure S2. The design of the field trials conducted in 2016 and 2017. Plots with the different treatments the two legume cover crop species: alsike clover (AC) and black medic (BM) was laid out in a randomized complete block design (RCBD) with Factor (1) represents five mixing ratios (MR) of AC:BM (100:0, 67:33, 50:50, 33:67, and 0:100), Factor (2) represents three seed densities (50%, 100%, and 150% of the recommended seed density). Samples from all the 16 treatments were harvested three times across the season (H1 in spring; 40 ± 10 days after sowing (DAS), H2 in summer; 64 ± 10 DAS, and H3 in autumn; 146 ± 26 DAS). Each plot contains 20 rows. Numbers of samples at each harvest are 4 rows \times 3 blocks in 2016 and 4 rows \times 4 blocks in 2017.

Table S1. Model output of the linear mixed model describing mixing effects of alsike clover and black medic on cover crop and weed aboveground biomass (CCB and WB, respectively). The two legume species were sown in five mixing ratio (MR) of AC:BM (100:0, 67:33, 50:50, 33:67, and 0:100) and three seed densities (Den) representing 50%, 100%, and 150% of the recommended seed density at three harvest times (H1: 40 ± 10 days after sowing (DAS), H2: 64 ± 10 DAS, H3: 146 ± 26 DAS) during the summer season of 2016 and 2017. Fixed effects included MR, Den, and MR \times Den. F and P values refer to ANOVA tests of each factor separately in each year and in each harvest and the interactions between them (N=1680).

Year	Source of variance	DF	CCB (g DM m ⁻²)					
			H1		H2		H3	
			F-value	P-value	F-value	P-value	F-value	P-value
2016	MR	4,160	24.33	<.0001	0.71	0.59	85.98	<.0001
	Den	2,160	22.46	<.0001	9.62	0.0001	0.92	0.40
	MR \times Den	8,160	0.56	0.81	0.65	0.74	4.12	0.0002
2017	MR	4,220	19.86	<.0001	7.37	<.0001	3.15	0.02
	Den	2,220	44.92	<.0001	14.46	<.0001	1.18	0.31
	MR \times Den	8,220	1.66	0.11	1.38	0.21	1.40	0.20
			WB (g DM m ⁻²)					
			F-value	P-value	F-value	P-value	F-value	P-value
2016	MR	4,215	1.35	0.25	0.22	0.9264	2.70	0.03
	Den	3,215	4.92	0.003	9.10	<.0001	422.68	<.0001
	MR \times Den	12,215	1.35	0.19	0.56	0.87	1.64	0.08
2017	MR	4,295	4.75	0.001	4.37	0.00	7.50	<.0001
	Den	2,295	21.80	<.0001	60.52	<.0001	253.04	<.0001
	MR \times Den	12,295	2.30	0.01	1.06	0.40	2.14	0.01

Table S2. List of the most common weeds in 2016 recorded in the fallow plots and at high density (150% of the recommended seed density) of alsike clover (AC) and black medic (BM) in monocultures. Values in the table represent an average percent ground cover by weeds of n = 12 (4 rows × 3 blocks) and was assessed visually on a scale of 0% (no weeds present) to 100% (weeds covers a row of 0.5 cm).

Weeds name	Fallow plots	AC (150%)	BM (150%)
<i>Chenopodium album</i>	52.1	48.5	23.8
<i>Echinochloa crus-galli</i>	12.7	9	5.3
<i>Galinsoga parviflora</i>	4.4	3.8	2.8
<i>Stellaria media</i>	4.2	0.3	0.5
<i>Fallopia convolvulus</i>	1.9	< 0.1	< 0.1
<i>Polygonum persicaria</i>	1.2	0	< 0.1
<i>Portulaca oleracea</i>	0.8	0	< 0.1
<i>Equisetum arvense</i>	0.6	0	0
<i>Polygonum aviculare</i>	0.5	0	0.5
<i>Capsella bursa-pastoris</i>	0.4	0.2	0.5
<i>Sonchus oleraceus</i>	0.4	0.2	< 0.1
<i>Lamium amplexicaule</i>	0.3	0	0.2
<i>Poa annua</i>	0.3	0	0.2
<i>Brassica napus</i>	0.2	0.4	0.3

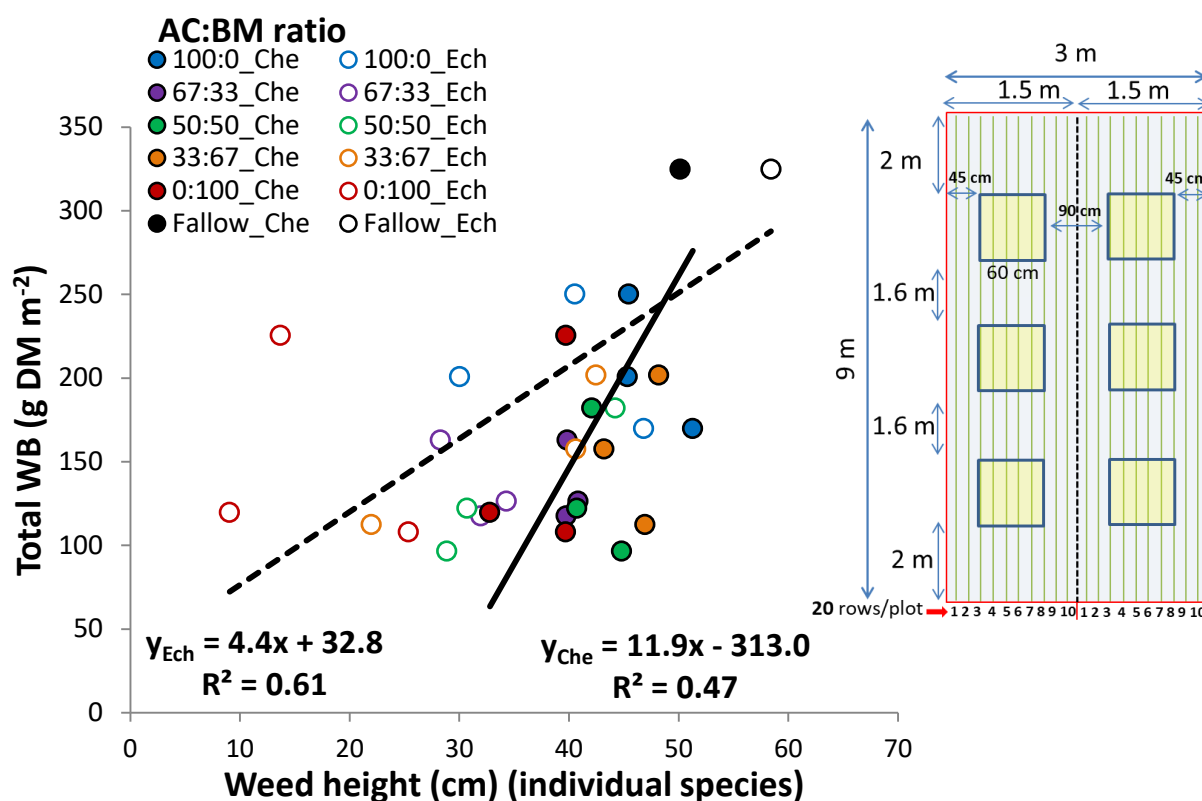


Figure S3. Relationship between weeds' heights of Fat-hen (*Chenopodium album* L.; Che) and Barnyard grass (*Echinochloa crus-galli* L.; Ech) and total weed biomass (WB). Weed biomass has been harvested at H2 (on 04–07 July 2017; 74 days after sowing) in the field trial 2017 and weeds' height was measured a week later (on 13–16 July 2017; 83 days after sowing). Both weeds and the cover crops were at the flowering stage. The selected two weeds were measured in the fallow plot (bare soil) and 15 treatments of binary mixture of cover crops of alsike clover (AC) and black medic (BM). The cover crop treatments included five mixing ratios of AC:BM (100:0, 67:33, 50:50, 33:67, and 0:100) sown at three seed densities (50%, 100%, and 150% of the recommended seed density). The empty circles are for Ech and the filled circles are for Che. The different colors are for different AC:BM ratio and the fallow as in the legend of the graph. Each dot represents the mean of $n = 18$ (six spots per plot × three blocks). **Methodology:** as graphically described in the upper right part of the figure, weeds sampling has been conducted in six spots (60 × 60 cm) per plot (9 m length × 3 m width). An area has been left from all sides of each plot to avoid edge effect (2 m from top and bottom longitudinal sides and 45 cm from the left and right horizontal sides). Inside the plot, a distance of 70 cm has been left between two horizontally adjacent spots and of a 1.6 m between two longitudinally adjacent spots.

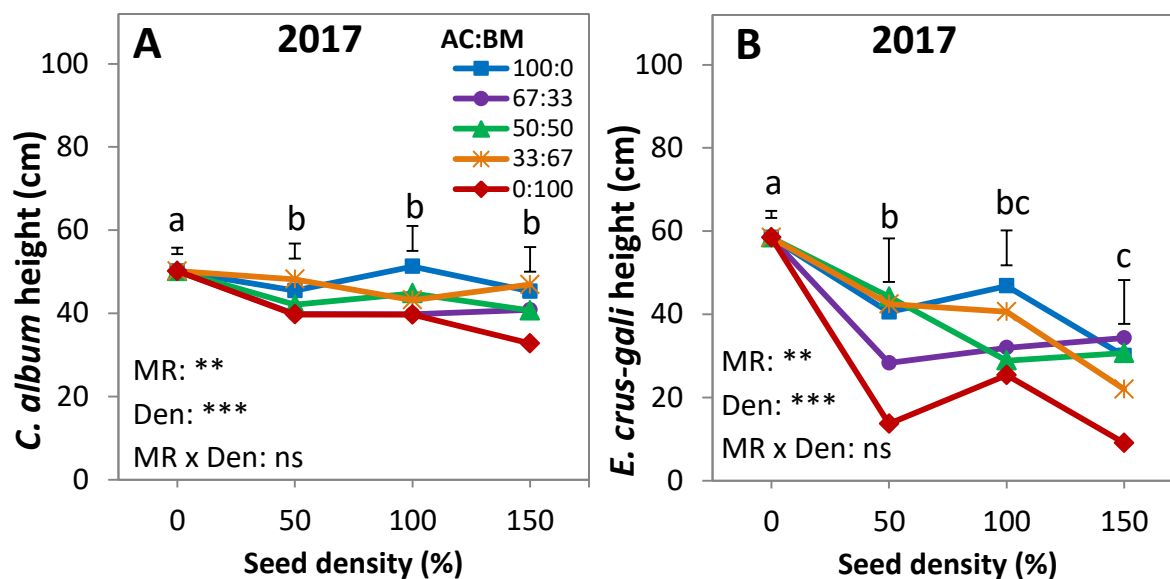


Figure S4. Weed height of Fat-hen (*Chenopodium album* L.; panel A) and Barnyard grass (*Echinochloa crus-galli* L.; panel B) in response to cover crops of alsike clover (AC) and black medic (BM) in monoculture and three binary mixtures. Measurements of weed height were done one week after H2 (on 13-16 July 2017; 83 days of sowing). Both weeds and the cover crops were at the flowering stage (~50%). The selected two weeds were measured in the fallow plot (bare soil) and 15 treatments of binary mixture of cover crops included five mixing ratios (MR) of AC:BM (100:0, 67:33, 50:50, 33:67, and 0:100) sown at three seed densities (Den) of 50%, 100%, and 150% of the recommended seed density. The data represents the mean of $n = 18$ (six spots per plot x three blocks). Vertical bars represent Tukey's HSD tests ($p < 0.05$) at a given seed density. Different letters above the vertical bars indicate significant differences among the seed densities, based on ANOVA followed by Tukey HSD test ($p < 0.05$). Asterisks indicate significant effects of MR, Den, and the interaction between them according to ANOVA; *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, and ns = not significant. Methodology is as described in Figure S3.

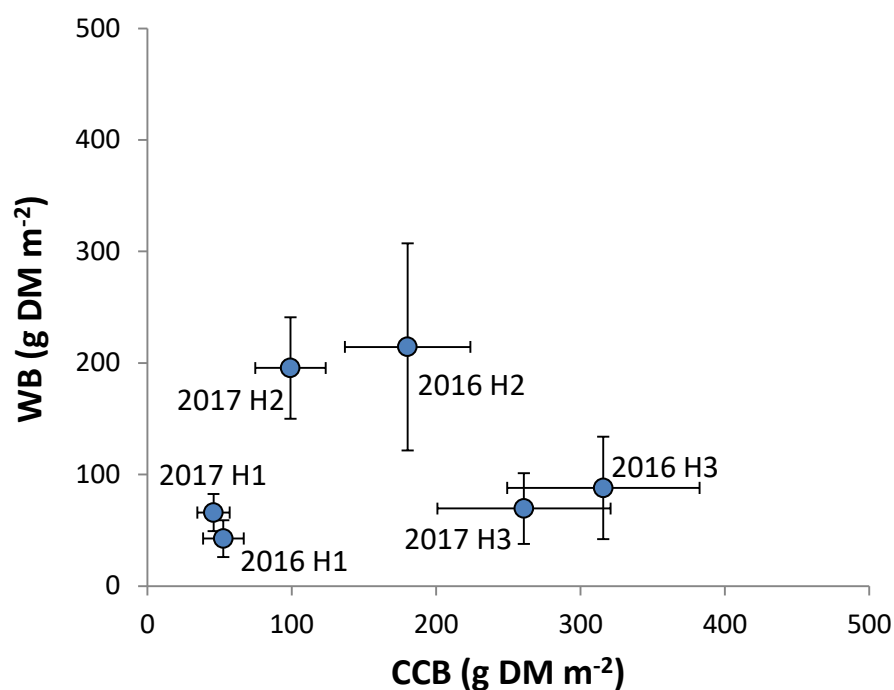


Figure S5. Weed biomass (WB) and cover crop aboveground biomass (CCB) averaged across all treatments at three harvest times in 2016 and 2017. The treatments are one fallow and 15 treatments of cover crop legume species of alsike clover (AC) and black medic (BM) contained five mixing ratios of AC:BM (100:0, 67:33, 50:50, 33:67, and 0:100) sown at three seed densities (50%, 100%, and 150% of the recommended seed densities). The three harvest times across the growing season are H1: in spring (40 ± 10 days after sowing; DAS), H2: in summer (64 ± 10 DAS), and H3: in autumn (146 ± 26 DAS) in two years 2016 and 2016. The Vertical and horizontal error bars represent standard error ($SD/3$). The data represented in each point are means of $n=240$ in 2016 and 320 in 2017 at each harvest.