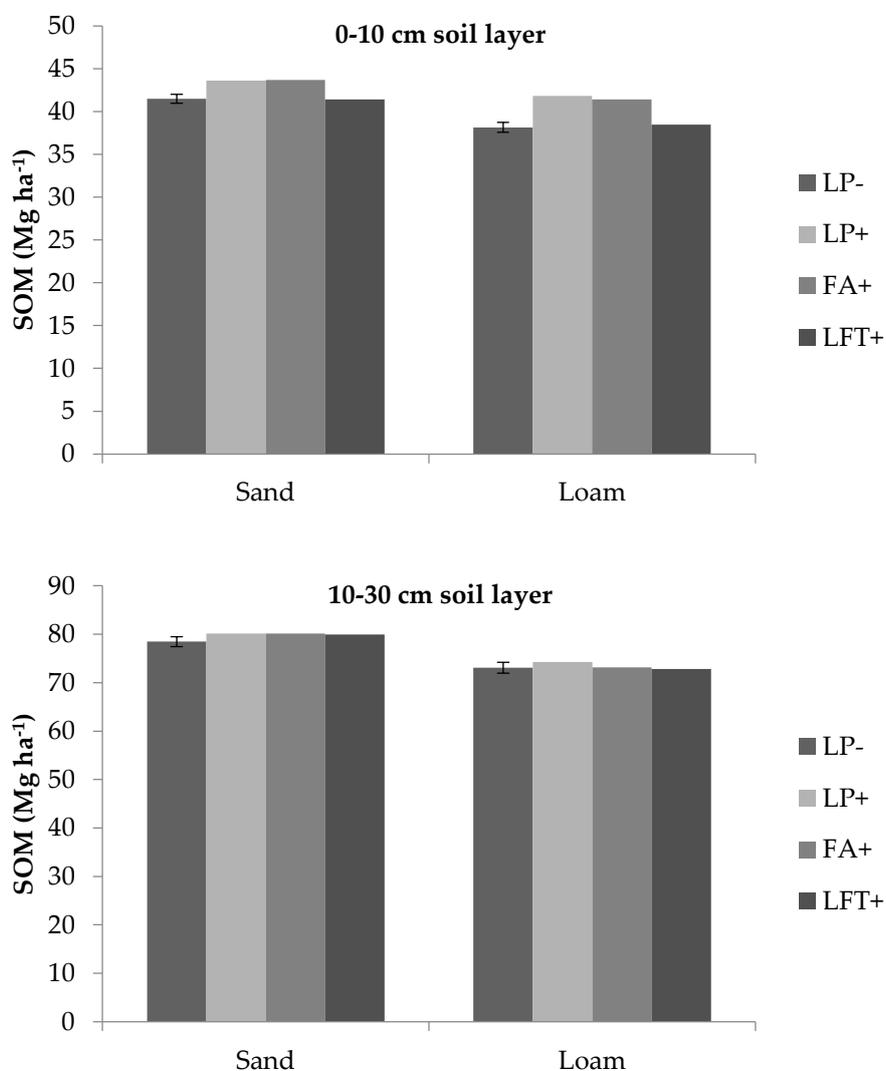
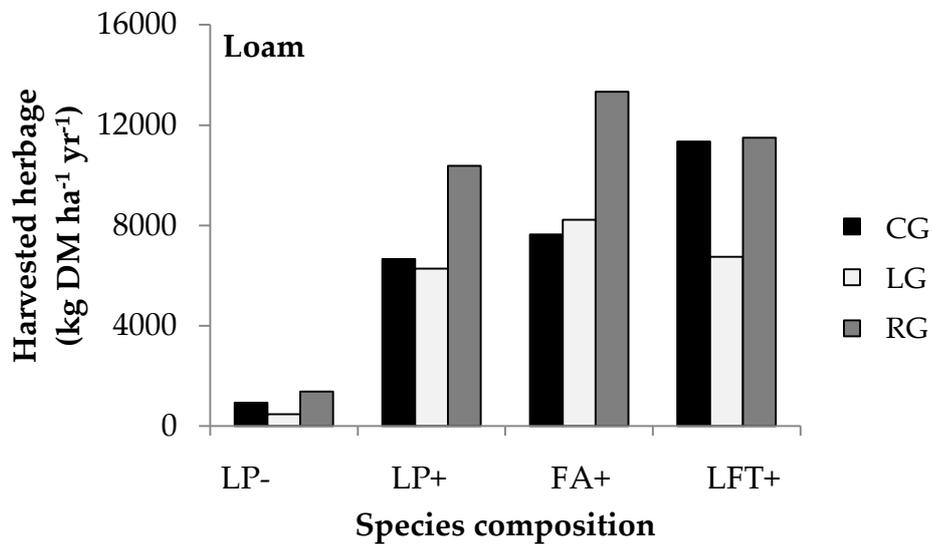
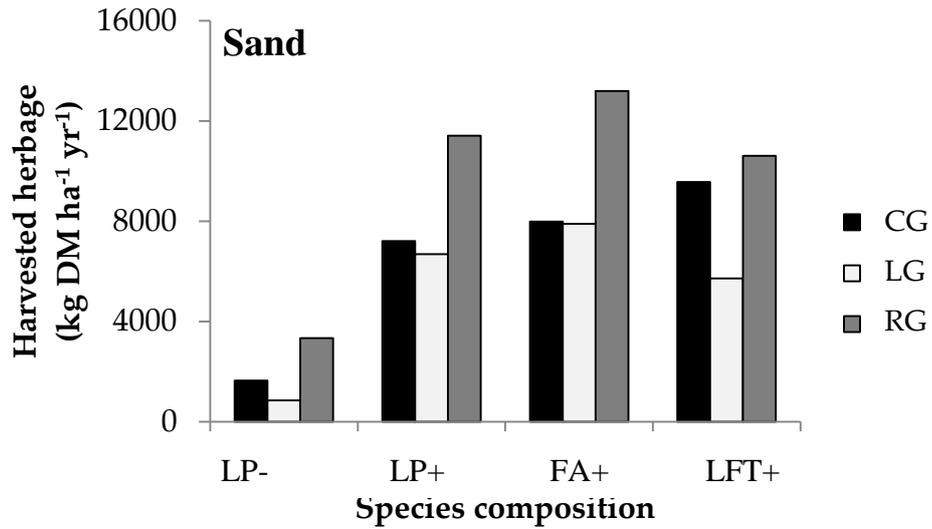


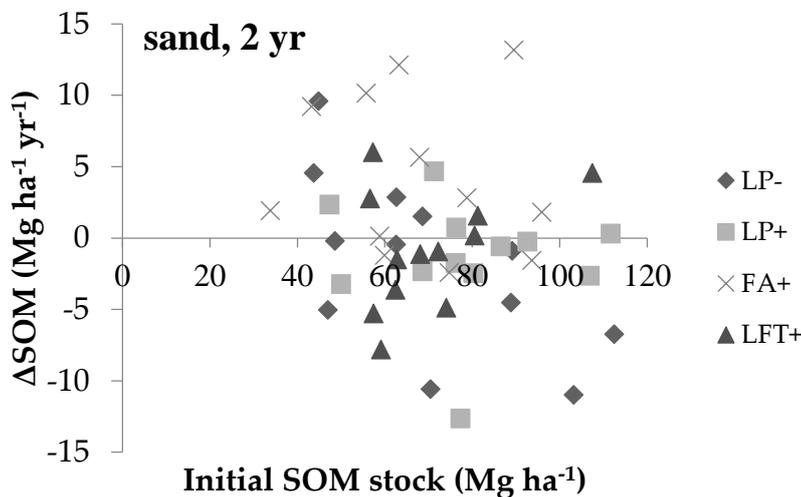
# Do grazing systems and species composition affect root biomass and soil organic matter dynamics in temperate grassland swards?

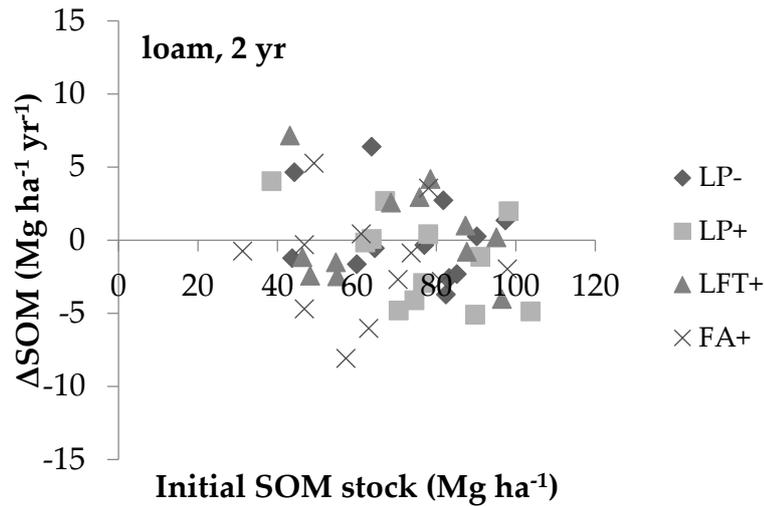


**Figure S1.** Initial soil organic matter (SOM) content ( $\text{Mg ha}^{-1}$ ) in September 2011 per species composition per soil type (sand and loam) and soil layer (0–10 cm and 10–30 cm). LP- = unfertilized perennial ryegrass. LP+ = fertilized perennial ryegrass. FA+ = fertilized tall fescue. LFT+ = fertilized perennial ryegrass, tall fescue and white clover. Analyses were conducted per soil type and per soil layer. The results of the analysis of variance are presented in Table S4. The standard errors depicted in the graph are 0.529 (sand, 0–10 cm), 0.571 (loam, 0–10 cm), 0.998 (sand, 10–30 cm) and 1.107 (loam, 10–30 cm).

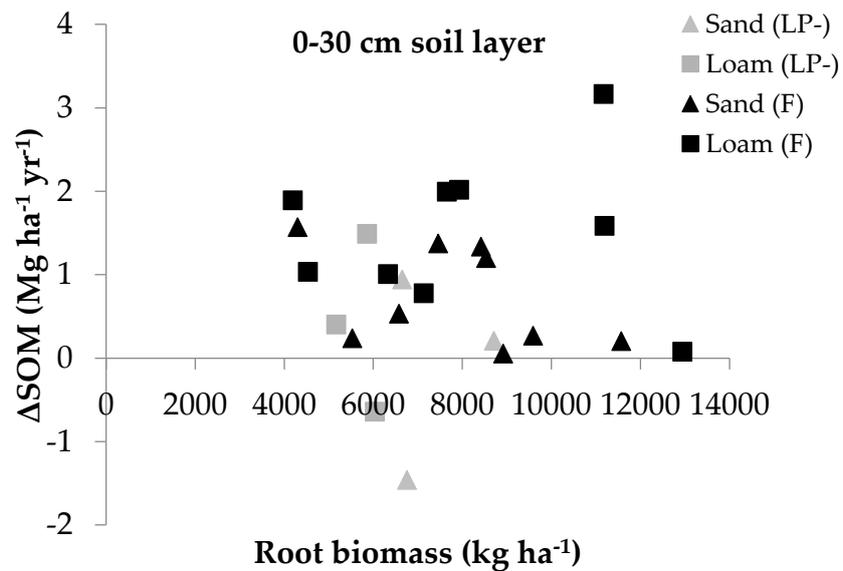


**Figure S2.** Harvested herbage per treatment. Average values of the year 2012 and 2013 are given. LP- = unfertilized perennial ryegrass, LP+ = fertilized perennial ryegrass, FA+ = fertilized tall fescue and ryegrass, and LFT+ = perennial ryegrass, tall fescue and white clover. Three grazing systems were simulated: CG=continuous grazing, LG=lenient grazing and RG=rotational grazing.





**Figure S3.** Relationship between initial soil organic matter (SOM) stocks and  $\Delta$ SOM ( $\text{Mg ha}^{-1} \text{ yr}^{-1}$ ) per species composition for the 30–60 cm soil layer. Because the relationship was not significant the regression lines were not plotted. LP- = unfertilized perennial ryegrass. LP+ = fertilized perennial ryegrass. FA+ = fertilized tall fescue. LFT+ = fertilized perennial ryegrass, tall fescue and white clover.



**Figure S4.** Relationship between root biomass and rates of change of soil organic matter (SOM) per soil type for the 0–30 cm soil layer. F=all fertilized treatments (monocultures of perennial ryegrass and tall fescue and the mixture of the two grass species with white clover). LP-=unfertilized perennial ryegrass.

**Table S1.** Seeding rates of plant species per treatment. LP- = unfertilized perennial ryegrass, LP+ = fertilized perennial ryegrass, FA+ = fertilized tall fescue and LFT+ = fertilized perennial ryegrass, tall fescue and white clover. T=white clover.

Treatment	Cultivar	Seeding rate (kg ha <sup>-1</sup> )
LP- and LP+	Barflip	8.33
	Barforma	8.33
	Arsenal	8.33
FA+	Barolex	10
	Bariane	40
LFT+	LP: see treatments LP-, LP+	10
	FA: see treatment FA+	20
	T: Alice	1.5
	T: Riesling	1.5

**Table S2.** Dates of fertilizer application during the first two experimental years. Grazing methods were not applicable in 2011. The abbreviations CG, LG and RG refer to continuous, lenient strip and rotational simulated grazing, respectively. LP- = unfertilized perennial ryegrass, LP+ = fertilized perennial ryegrass, FA+ = fertilized tall fescue and LFT+ = fertilized perennial ryegrass, tall fescue and white clover. 'All' refers to all treatments: LP-, LP+, FA+ and LFT+. N-CAN = nitrogen applied as calcium ammonium nitrate.

Dates 2011	Species composition	Fertilizer Type	Amount (kg ha <sup>-1</sup> )
07-06	LP+, FA+	N-Manure granules	120
07-06	LFT+	N-Manure granules	50
15-08	LP+, FA+	N-Manure granules	80
16-08	All	N-CAN	52.5
Dates 2012	Species composition	Fertilizer Type	Amount (kg ha <sup>-1</sup> )
13-04	LP+, FA+, LFT+	N-Manure granules	50
29-03	LP+, FA+	N-CAN	80
25-06	LP+, FA+	N-CAN	30 (CG); 60 (LG, RG)
16-07	LP+, FA+	N-CAN	30

01-08	LP+, FA+	N-CAN	30 (CG); 60 (LG, RG)
13-08	LP+, FA+	N-CAN	30
27-8	All	K <sub>2</sub> O	100
Dates 2013	Species composition	Fertilizer Type	Amount (kg ha <sup>-1</sup> )
02-04	LP+, FA+, LFT+	N-Manure granules	50
05-04	LP+, FA+	N-CAN	80
26-04	All	K <sub>2</sub> O	80
22-05	All	P <sub>2</sub> O <sub>5</sub>	50
22-05	All	Trace elements*	
28-05	LP+, FA+	N-CAN	30 (CG); 60 (LG, RG)
26-06	LP+, FA+	N-CAN	30
30-07	LP+, FA+	N-CAN	30 (CG); 60 (LG, RG)
21-08	LP+, FA+	N-CAN	30

**Table S3.** Annual application rates of micronutrients from 2013 to 2016.

Element	Application rate (kg ha <sup>-1</sup> yr <sup>-1</sup> )
MgO	13.0
B	0.2
Cu	0.9
Fe	13.0
Mn	2.2
Mo	0.03
Zn	0.9
SO <sub>3</sub>	33.0

**Table S4.** Results of the analysis of variance on 288 SOM (soil organic matter) observations. Samples were taken in September 2011. Data were analysed per soil layer and per soil type (sand and loam). Species composition and block were taken as fixed factor and plot within block as random factor. F and *P*- values are presented. df= degrees of freedom.

Soil layer	Soil type	Species composition		Block		Plot within block	
		F	<i>P</i>	F	<i>P</i>	F	<i>P</i>
0–10 cm	Sand	5.74	0.00	0.42	0.74	5.94	0.00
	Loam	11.374	0.00	4.23	0.05	1.53	0.19
10–30 cm	Sand	0.67	0.58	1.29	0.34	5.51	0.00
	Loam	0.34	0.80	2.87	0.10	3.17	0.01
30–60 cm	Sand	2.08	0.12	1.91	0.21	6.78	0.00
	Loam	1.77	0.17	7.25	0.01	1.26	0.30
		df1	df2	df1	df2	df1	df2
	Sand, Loam	3	33	3	8	8	33

**Table S5.** Average root biomass (kg dry matter/ha) in July 2013 and March 2014 per soil layer per treatment. LP- = unfertilized perennial ryegrass. LP+ = fertilized perennial ryegrass. FA+ = fertilized tall fescue. LFT+ = fertilized perennial ryegrass, tall fescue and white clover. Standard errors of the mean are presented between brackets

Mixture	0-10 cm		10-30 cm		30-60 cm	
	Sand	Loam	Sand	Loam	Sand	Loam
LP-	6451 (655)	4939 (540)	920 (83)	748 (75)	123 (39)	305 (49)
LP+	7183 (655)	6579 (540)	952 (83)	988 (75)	130 (39)	309 (49)
FA+	8596 (655)	10067 (540)	1426 (83)	1698 (75)	616 (39)	648 (49)
LFT+	4565 (655)	4153 (540)	902 (83)	865 (75)	147 (39)	255 (49)

**Table S6.** Estimates and *P*-values for differences between species compositions between SOM rates of change in Mg ha<sup>-1</sup> yr<sup>-1</sup> after a period of 2 years, by soil layer and by soil type.

0-10 cm soil layer				
Species composition	LP-	LP+	FA+	LFT+
LP-	x	<b>-1.71 (0.00)</b>	<b>0.94 (0.00)</b>	<b>-1.40 (0.02)</b>
LP+	<i>-1.32 (0.00)</i>	x	<b>0.78 (0.43)</b>	<b>0.31 (0.05)</b>
FA+	<i>-1.22 (0.01)</i>	<i>0.10 (0.92)</i>	x	<b>0.46 (0.24)</b>
LFT+	<i>-1.27 (0.01)</i>	<i>0.05 (0.84)</i>	<i>0.05 (0.13)</i>	x
10-30 cm soil layer				
Species composition	LP-	LP+	FA+	LFT+
LP-	x	<b>0.28 (0.66)</b>	<b>0.69 (0.37)</b>	<b>0.58 (0.28)</b>
LP+	<i>0.14 (0.81)</i>	x	<b>0.41 (0.65)</b>	<b>0.30 (0.53)</b>
FA+	<i>0.02 (0.53)</i>	<i>-0.12 (0.85)</i>	x	<b>0.12 (0.86)</b>
LFT+	<i>0.37 (0.97)</i>	<i>0.23 (0.70)</i>	<i>-0.35 (0.56)</i>	x
30-60 cm soil layer				
Species composition	LP-	LP+	FA+	LFT+
LP-	x	<b>-0.25 (0.90)</b>	<b>-6.05 (0.00)</b>	<b>-0.90 (0.66)</b>
LP+	<i>1.41 (0.30)</i>	x	<b>-5.80 (0.00)</b>	<b>0.64 (0.75)</b>
FA+	<i>0.22 (0.87)</i>	<i>-1.63 (0.23)</i>	x	<b>5.16 (0.01)</b>
LFT+	<i>-1.83 (0.18)</i>	<i>0.42 (0.76)</i>	<i>-2.05 (0.13)</i>	x

Values for the sandy soil are printed in bold; values for the loamy soil are printed in italics. LP-=unfertilized perennial ryegrass, LP+=fertilized perennial ryegrass, FA+=fertilized tall fescue and LFT+=fertilized perennial ryegrass, tall fescue and white clover. A negative difference such as between LP- and LP+ (0–10 cm soil layer; -1.71) means that the annual SOM rate of change of LP- was 1.71 Mg ha<sup>-1</sup> lower than the rate of LP+ (LP-=0.55 Mg SOM ha<sup>-1</sup> yr<sup>-1</sup>; LP+= 2.26 Mg SOM ha<sup>-1</sup> yr<sup>-1</sup>; 0.55-2.26=-1.71). For values of the sandy soil comparisons between treatments are horizontally presented. For the loamy soil comparisons are vertically presented (for instance the SOM rate of change of LP- is 1.32 Mg SOM ha<sup>-1</sup> yr<sup>-1</sup> lower than LP+; 0–10 cm layer).

**Table S7.** Estimates and *P*-values for differences between grazing systems in SOM rates of change in Mg ha<sup>-1</sup> yr<sup>-1</sup> in the loamy (*italics*) and sandy soil (**bold**) for the 0-30 cm soil layer after a period of two and five years, respectively. CG=continuous grazing, LG= lenient strip grazing, RG=rotational grazing. The results of the two year period of the sandy soil are not presented because none of the values were significant.

Grazing system	CG	LG	RG
CG	x	<b>-0.42 (0.17)</b>	<b>0.55 (0.07)</b>
LG	<i>-0.45 (0.54)</i>	x	<b>0.97 (0.00)</b>
RG	<i>-1.16 (0.12)</i>	<i>-1.61 (0.03)</i>	x

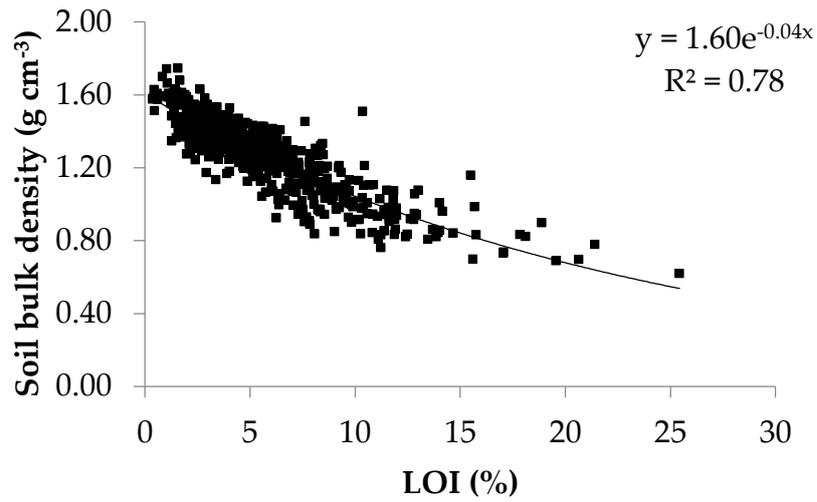
**Table S8.** Estimates for differences between species compositions in SOM rates of change in Mg ha<sup>-1</sup> yr<sup>-1</sup> in the sandy soils after a period of five years. *P*-values are given in parentheses. LP-=unfertilized perennial ryegrass, LP+=fertilized perennial ryegrass, FA+=fertilized tall fescue and LFT+=fertilized perennial ryegrass, tall fescue and white clover. A negative difference such as between LP- and LP+ (0-10 cm soil layer; -0.71) means that the annual SOM rate of change of LP- was 0.71 Mg ha<sup>-1</sup> lower than the rate of LP+ (LP-=1.23 Mg SOM ha<sup>-1</sup> yr<sup>-1</sup>; LP+=1.94 Mg SOM ha<sup>-1</sup> yr<sup>-1</sup>; 1.23-1.94=-0.71).

Species compositions	0-10 cm	10-30 cm	30-60 cm	0-30 cm	0-60 cm
LP- vs. LP+	-0.71 (0.00)	0.07 (0.81)	-1.33 (0.13)	-0.65 (0.07)	-1.96 (0.05)
LP- vs. FA+	-0.15 (0.38)	-0.01 (0.97)	-2.63 (0.00)	-0.17 (0.64)	-2.77 (0.01)
LP- vs. LFT+	-0.34 (0.05)	-0.10 (0.73)	-0.91 (0.31)	-0.44 (0.22)	-1.32 (0.18)
LP+ vs. FA+	0.56 (0.00)	-0.08 (0.78)	-1.30 (0.14)	0.48 (0.18)	-0.82 (0.40)
LP+ vs. LFT+	0.37 (0.03)	-0.16 (0.56)	0.43 (0.63)	0.21 (0.56)	0.64 (0.51)
FA+ vs. LFT+	-0.18 (0.28)	-0.08 (0.76)	1.72 (0.05)	-0.27 (0.45)	1.45 (0.13)

**Table S9.** Average rates of change of soil organic matter (SOM) in Mg ha<sup>-1</sup> yr<sup>-1</sup> per soil layer per simulated grazing system for the period September 2011 –January 2014 (sand and loam, 2 years) and the period September 2011 – May 2016 (sand, 5 years). Simulated grazing systems: CG=continuous grazing, LG=lenient strip grazing and RG=rotational grazing.

Soil type	Period (yr)	Layer (cm)	$\Delta$ SOM (Mg ha <sup>-1</sup> yr <sup>-1</sup> )		
			CG	LG	RG
Loam	2	0–10	1.8	2.2	1.6
		10–30	-0.3	-0.3	-1.3
		30–60	0.4	-0.5	-1.5
		0–30	1.5	1.9	0.3
		0–60	1.9	1.5	-1.2
Sand	2	0–10	1.8	1.5	1.4
		10–30	-1.2	-0.9	-1.1
		30–60	-0.8	-0.3	1.3
		0–30	0.6	0.6	0.3
		0–60	-0.2	0.3	1.6
Sand	5	0–10	1.6	1.7	1.3
		10–30	-0.2	0.1	-0.5
		30–60	1.0	1.8	1.4
		0–30	1.4	1.8	1.0
		0–60	2.4	3.7	2.6

Box S1 Soil bulk density measurements and the conversion from SOM concentrations to stocks  
 Soil bulk density (BD) measurements were needed to convert SOM concentrations to SOM stocks. BD measurements were taken in the experimental fields using the core method at the end of the ‘two year period’ (see Materials & Methods). All collected samples for SOM could not be accompanied by BD measurements as no surface would remain at the end of the experiment. For this reason, BD values had to be estimated for all individual SOM measurements that were used to derive SOM trends. A strong relationship exists between the SOM content and soil bulk density value of sample. SOM increases with decreasing BD because the true density of organic matter is much lower as compared to mineral particles [51]. A dataset that contained both LOI and BD values was used to derive a relationship between LOI and BD (Figure S5). The equation was used to estimate BD values of the experimental fields based on SOM contents. Next, SOM contents were converted to SOM stocks with the estimated BD values.



**Figure S5.** Relationship between soil bulk density and Loss-on-ignition values (n=684). Data was obtained from the 0–30 cm soil layer of 19 grasslands in the neighborhood of Wageningen, The Netherlands.

#### Reference

Adams, W.A. The effect of organic matter on the bulk and true densities of some uncultivated podzolic soils. *Eur. J. Soil Sci.* **1973**, *24*, 10–17.