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Cover crops improve soil structure and change organic carbon distribution in macroaggregate fractions

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S1. Long-term history of the Asendorf experimental field site and changes in soil cultivation and OC stocks

The field site was part of a previous livestock farm until 2000. The field site was used for grazing and fodder production, but the crop rotation and site management during that time were not documented. Since 2000, the sites have been rented to an arable farmer who started with low soil fertility and organic matter (OM) content (personal communication). The soils were consequently cultivated with conservation tillage practices (mulch-tillage). The crop rotation was triticale – winter rapeseed – winter wheat. Straw remained on the field and was incorporated into the soil, together with poultry litter (140 kg N), but only before rapeseed planting. The cultivation depth was 10 cm with a chisel plough. The main crops were fertilized with cattle slurry and urea (AHL). The management resulted in enhancement of the soil OM content with approximately 1.8% OC on average in the upper 0-30 cm (Fig. S4).

In 2014, the land was taken over from the DSV (Deutsche Saatveredelung AG). In 2015, the DSV, partner in the project CATCHY (Catch-cropping as agrarian tool for continuing soil health and yield-increase, https://www.bonares.de/catchy), established the field as a long-term experimental site for crop rotations including cover crops (CC) (Fig. S2). At the start of the project, soils were ploughed once by a mould-board plough to 30 cm depth in summer 2014 before the seeding of winter wheat. Thereafter, soils were cultivated only with a chisel plough and a disc harrow to ~15 – 20 cm depth. Fertilization of the main crops winter wheat and maize followed the regular recommendations in the region for mineral fertilizers (wheat in kg ha^{-1}: 140 N, 11 P, 133 K, 81 S, 22 Mg; maize in kg ha^{-1}: 173 N, 39 P, 133 K, 56 S, 9 Mg).

After winter wheat harvest, the straw was incorporated into the soil with a disc harrow and prepared with a seedbed combination. Cover crops were sown until the end of August, and all treatments were fertilized with 40 to 60 kg N ha^{-1}. For consistent N management, this also includes fallow treatments. One year after sampling for evaluation of the aggregate stability (2021), the field sites fell into restricted areas, and legal regulations no longer allow CC fertilization. Maize was harvested as silage maize (Block 1 and 2. Fig. S1). The second crop rotation (Leg+, Fig. S2) replaced silage maize every second crop rotation with fava bean, where the bean straw remained on the field.

For the initial soil characterization in 2015, all plots were sampled in soil increments of 0-10, 10-30, and 30-60 cm. For the sake of common sample acquisition within the CATCHY consortium , we had to change our sampling approach to 10 cm increments thereafter to fit with root and microbiome measurements (Heuermann et al., 2019, 2022). Thus, the 30-60 cm subsoil layer from 2015 cannot be compared with the sampling for aggregate fractionation. Nevertheless, the soil tillage from 2014 resulted in a homogeneous OC distribution in the upper 30 cm with no significant differences between the two upper layers ($F = 0.023$, $p = 0.8802$). Therefore, we assume that the OC concentrations of the 10-30 cm increment from 2015 are comparable to the 20-30 cm increment from the 2020 sampling.

We measured a significant increase in OC concentrations in the 0-10 and 20-30 cm increments (Fig. S3) that resulted in increasing OC stocks from 2015 to 2020. The increase was observed in 80% of the plots and was not connected to the type of CC treatment or fallow (Fig. S4). The increase must consequently be attributable to reasons other than the CC treatments. We consider several possible explanations or their combinations for the increase in OC in the topsoil after five years.

(1) Changes in soil cultivation practices have been shown to change the OC distribution with depth (Haddaway et al., 2017). Long-term organic matter-exhausting management practices before 2000 might have degraded soil OC stocks at a low equilibrium. With the change to conservation tillage and management practices (straw incorporation

with manure and slurry), the soil organic matter content increased and developed to a new level where equilibrium was still not reached.

(2) The remaining wheat straw on the field might contribute to increasing OC stocks in the upper 30 cm. However, the high C:N ratios of wheat straw (C:N between 40 and 80) results in low carbon use efficiency (Sinsabaugh et al., 2016) of the microbial community and, therefore, was not suggested as a measure for OM build-up in soil (Poeplau et al., 2016). In our experiment, the fallow was fertilized in the same way as the CC treatments and achieved 40 kg N ha^{-1} in autumn. This dose of N might stimulate the breakdown of crop residues, increase the carbon use efficiency of the microbiome and finally enhance the capacity to build up stable OM fractions (Li et al., 2022).

(3) Changes in crop rotation and a higher proportion of root-derived OC could also contribute to building up OC stocks. In particular, maize has been shown to contribute strongly to root-derived OC input to the soil (Poeplau et al., 2021).

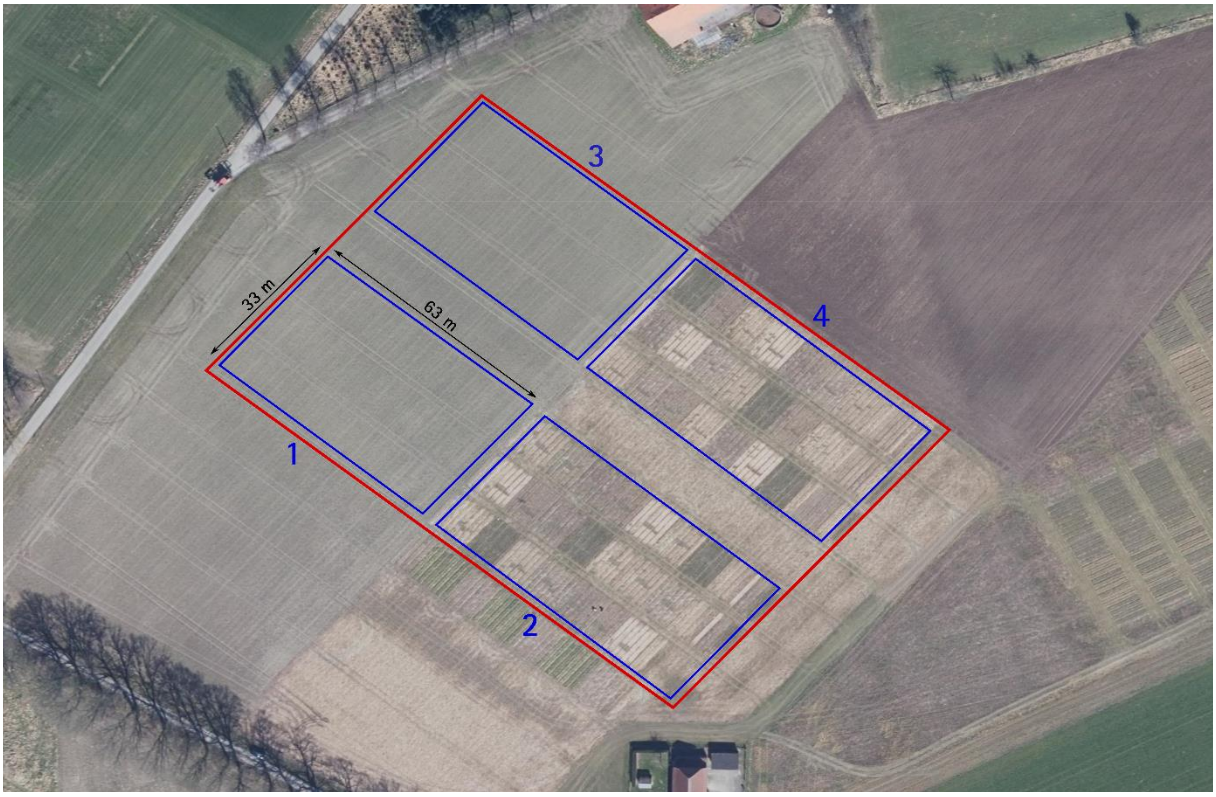

Figure S1. Aerial image of the Asendorf field site (52.76335662176853 N, 9.02475168211285 E). The area in the red rectangle marks the whole experimental area of the randomized split block design. Blocks are marked by blue rectangles and numbers. Blocks 1 and 2 are replicated "Leg-" (Figure S2) crop rotations with a one-year offset. The same is true for Blocks 3 and 4, which represent replications of the "Leg+" rotation (Image from autumn 2021, downloaded from https://opengeodata.lgln.niedersachsen.de/#dop). Samples for aggregate fractionation were taken from Block 2.

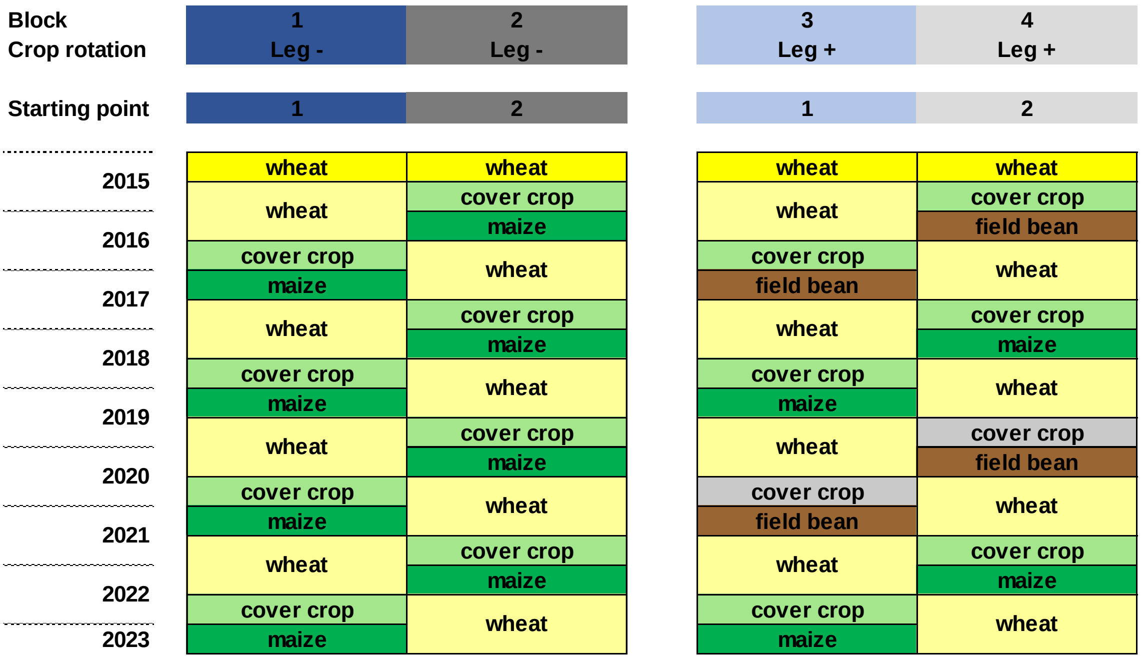

Figure S2. Crop rotation and starting points of the CATCHY long-term field trials. The crop rotation without legume main crop (Leg-) is winter wheat-CC-maize. The second crop rotation with legume as the main crop is winter wheat-CC-maize-winter wheat-CC-fava bean. Crop rotation maize is always harvested as silage maize, while the straw of the fava bean remains on the field.

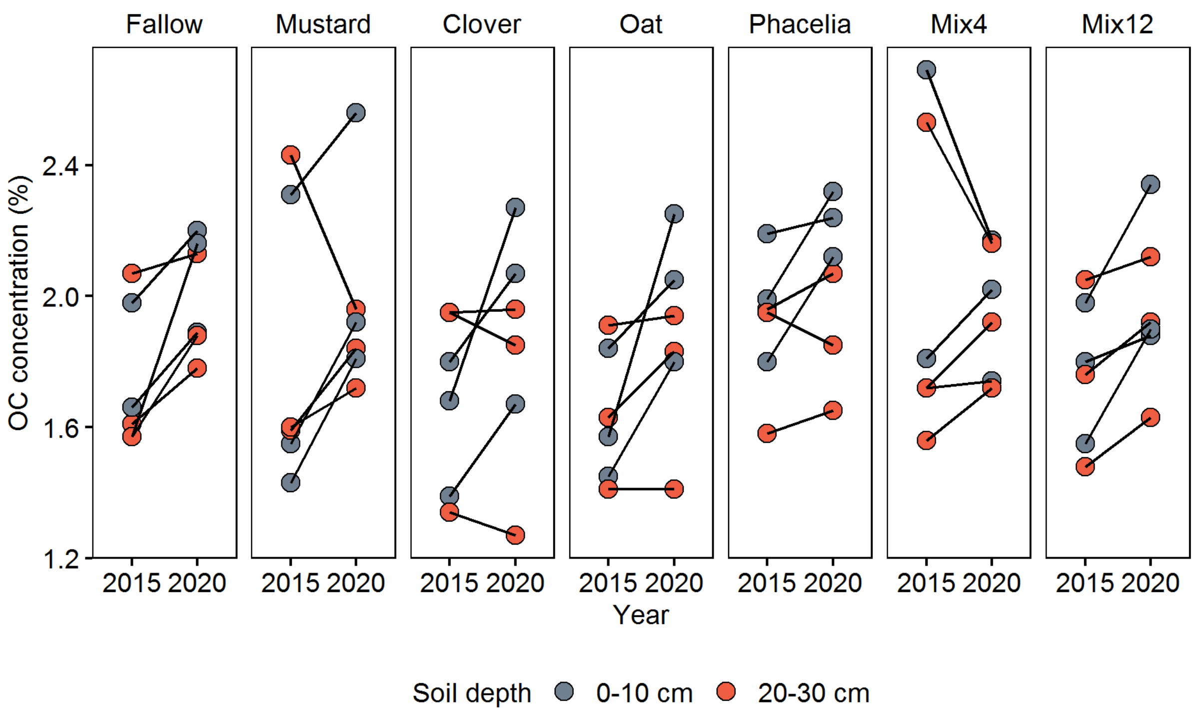

Figure S3. Change in OC concentration from 2015 (start of the field trials) to 2020 (sampling for aggregate fractionation). Line-connected dots indicate the same plots at different time points. Differences between years were evaluated by a LMM with soil depth nested in plot as a random variable (see R scripted for data evaluation). The average OC concentration in the 0-30 cm layer increased significantly ($F = 10.08$, $p < 0.001$) from 1.80 ± 0.04% to 1.95 ± 0.04%.

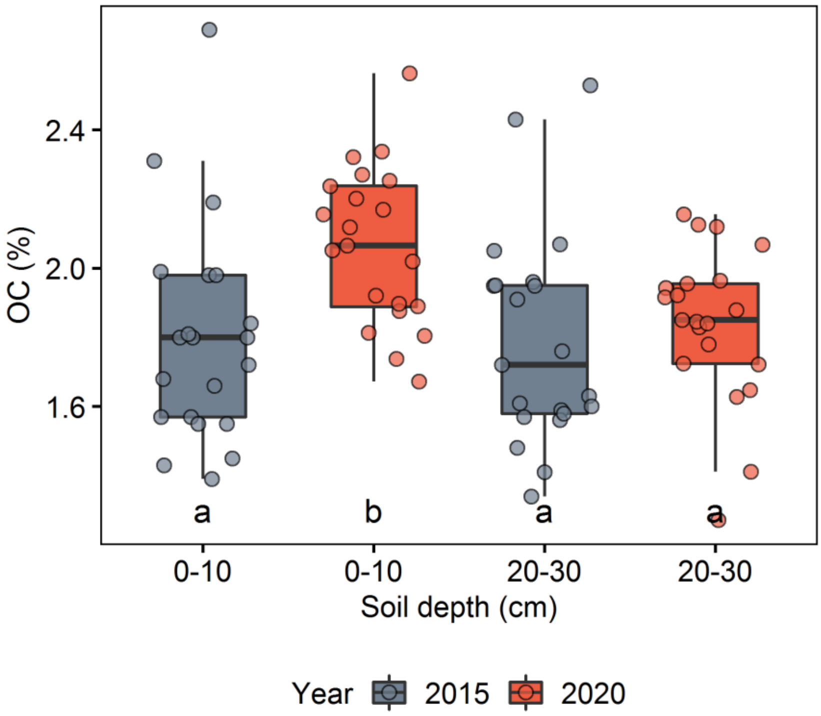

Figure S4: Change in OC concentrations from 2015 to 2020 in the upper 30 cm soil depth. Lowercase letters denote the contribution to significantly different groups based on LMM evaluation.

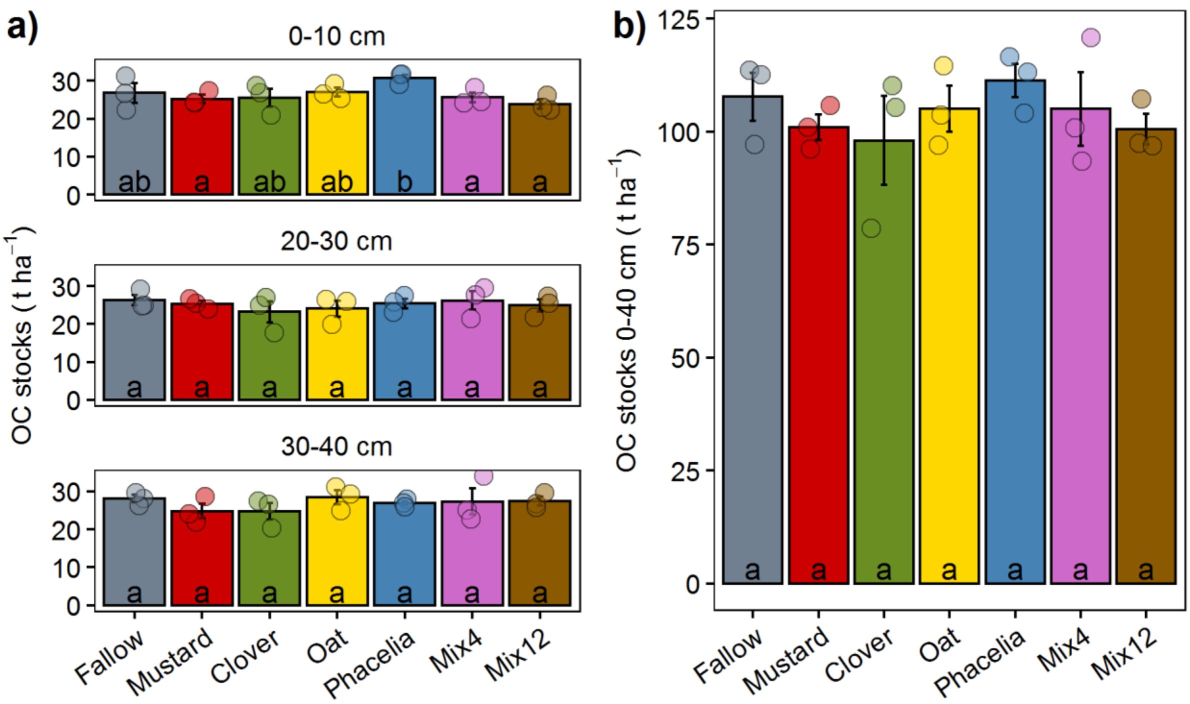

Figure S5. Soil OC stocks in 2020 in individual sampling increments (a) and summed to 40 cm soil depth (b). Note that due to the maximal soil cultivation depth of < 20 cm by a harrow, we assume a homogeneous OC distribution in 0-20 cm and the same OC concentration in 0-10 cm as in 10-20 cm (not sampled during the aggregate sampling campaign). Lowercase letters denote the contribution to significantly different groups based on pairwise t tests.

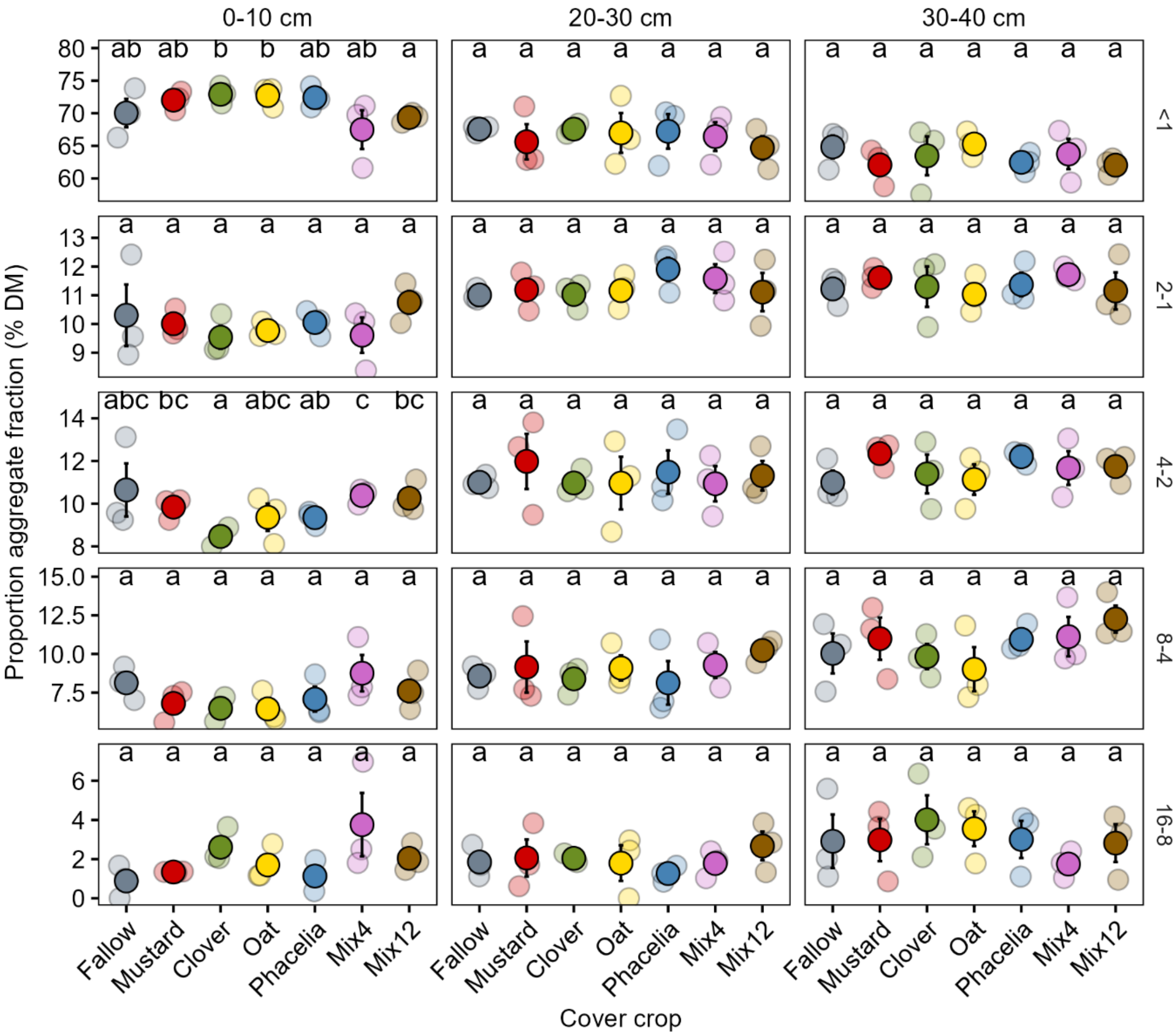

Figure S6. Proportion of individual aggregate fractions to total aggregates in % dry mass (DM). Lowercase letters denote the contribution to significantly different groups based on pairwise t-tests. Pale coloured points represent individual measurements, and full colours summarize mean values and standard errors (error bars).

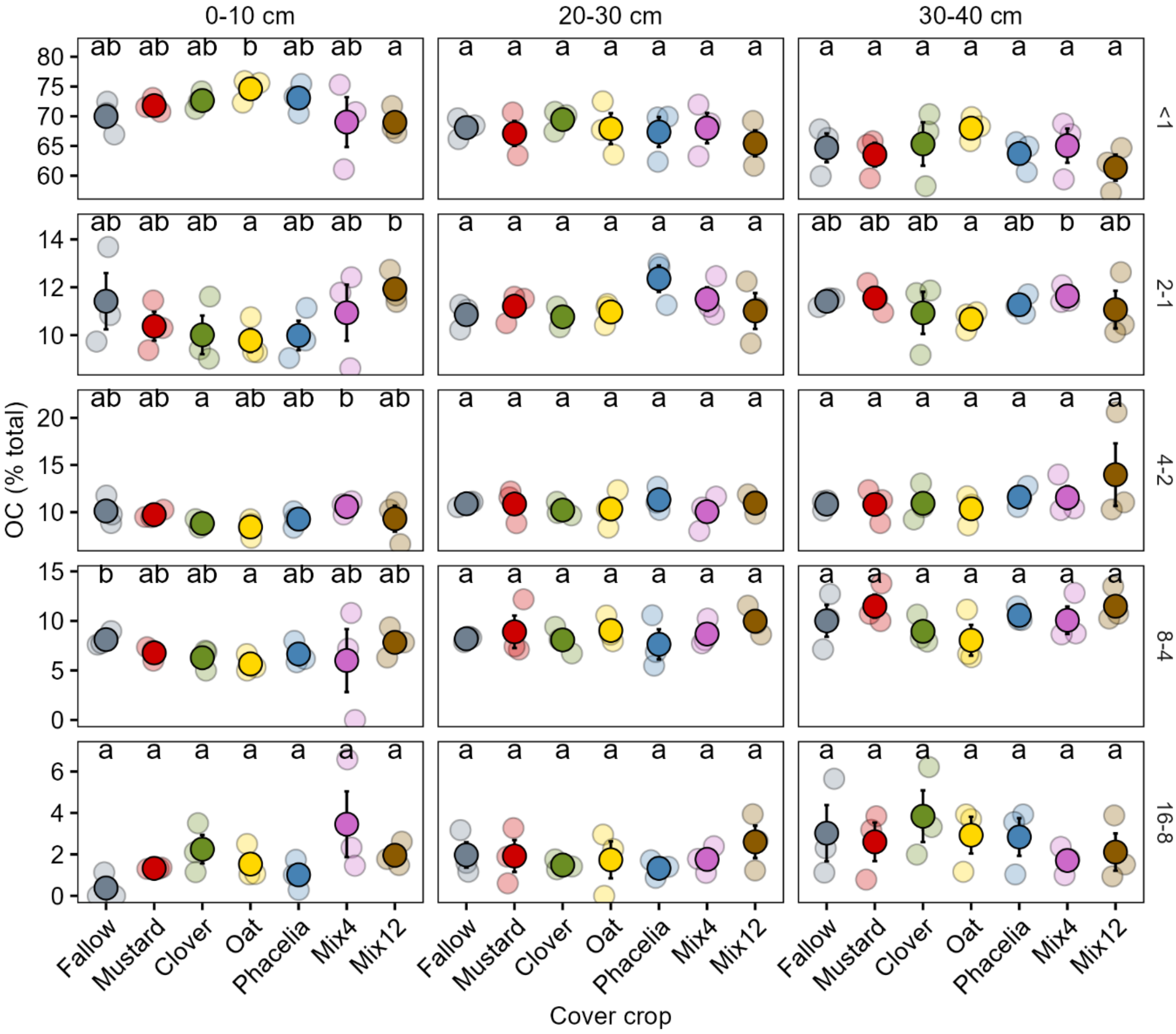

Figure S7. Proportion of OC measured in individual aggregate fractions to OC in total aggregates (in % total OC). Lowercase letters denote the contribution to significantly different groups based on pairwise t-tests. Pale coloured points represent individual measurements, and full colours summarize mean values and standard errors (error bars).

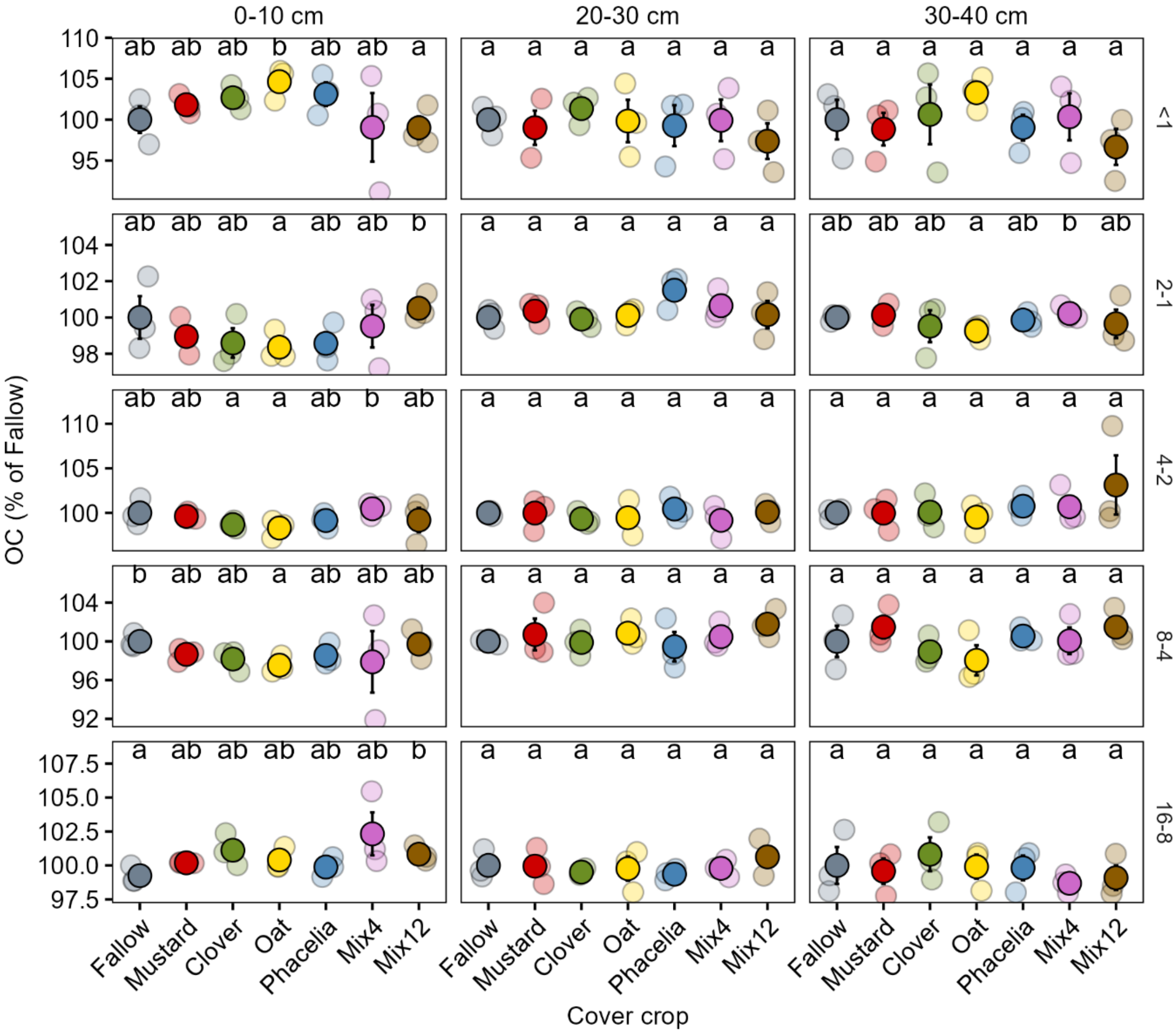

Figure S8. Statistical evaluation from Fig. 1. The mean OC proportion of the fraction made up 100% of the fallow. Therefore, the fallow was set to 100%, and the mean value of fallow was subtracted from the proportion of OC from individual aggregate fractions. Pale coloured points represent individual measurements, and full colours summarize mean values and standard errors (error bars).

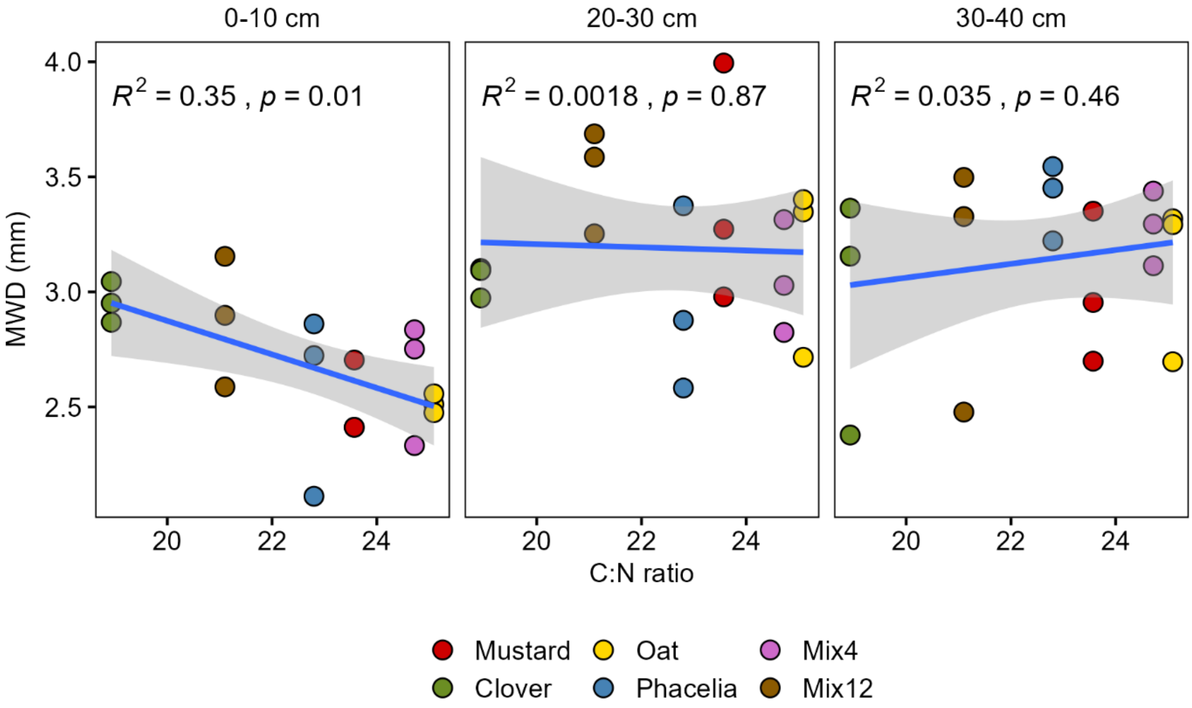

Figure S9. Correlation between MWD and the C:N ratio of litter material from different CCs. Data on litter composition were published in Gentsch et al. (2022) and in Table S3 below..

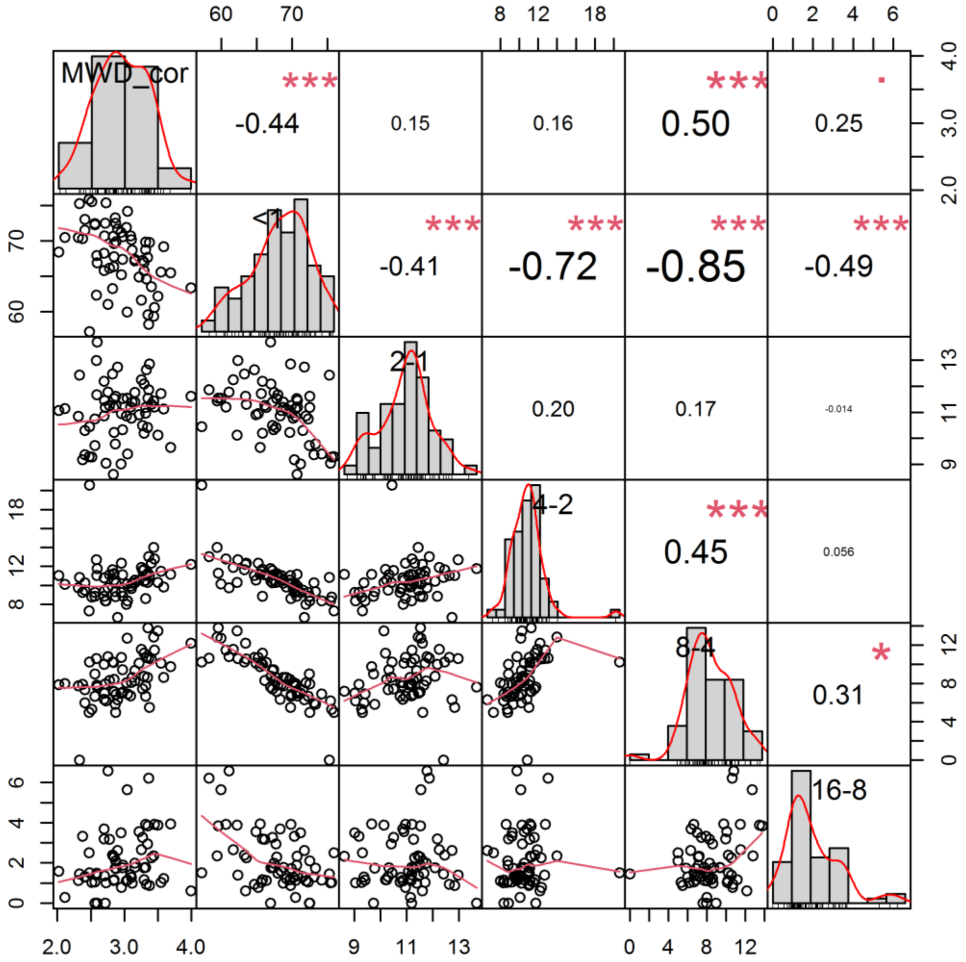

Figure S10. Correlation matrix of the MWD and the percentage of OC in each fraction. Numbers present Pearson's correlation coefficient with asterisks showing different p values: * p < 0.05, ** p < 0.01, *** p < 0.001.

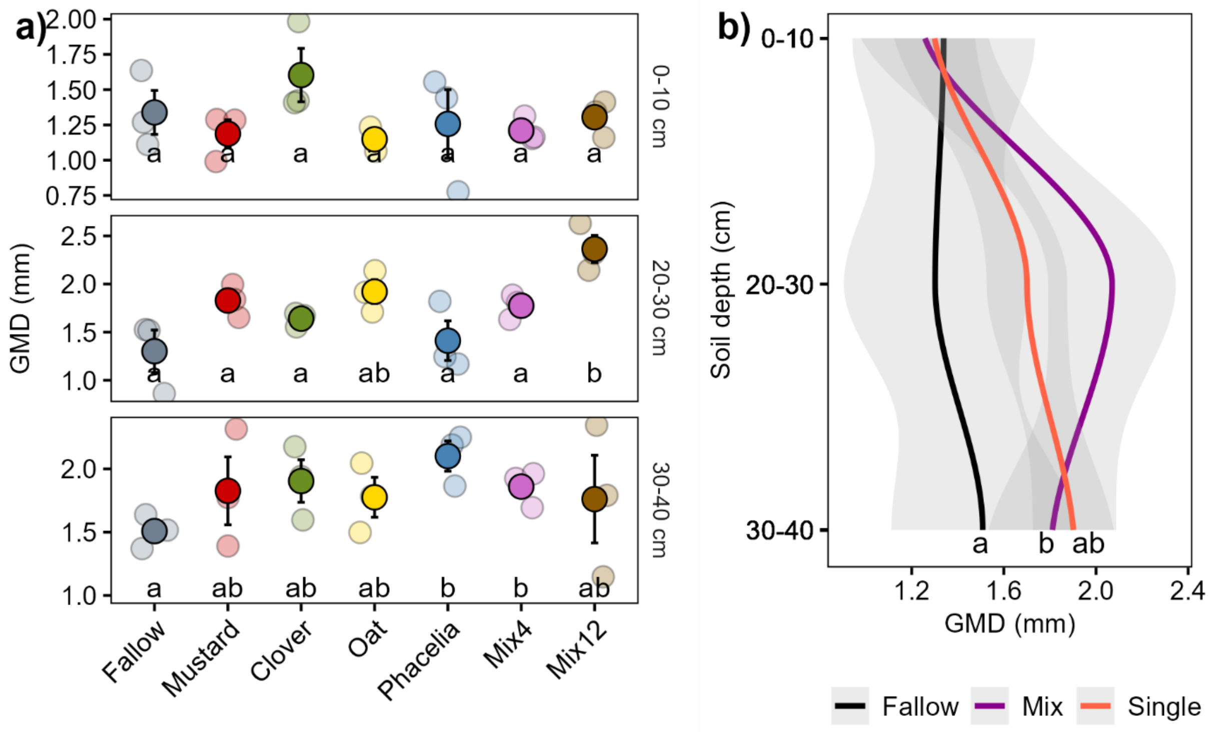

Figure S11. Geometric mean diameter (GMD) of soil aggregates after wet sieving from different soil depths. Lowercase letters denote significant differences between CC (cover crop) treatments by pairwise comparison (a) and overall effects of CC from an LMM (b). Translucent points represent the individual measurements, and opaque colours are mean values (±SE).

Table S1 Basic soil properties from the initial soil survey in 2015, the start of the Experiment. se = standard error.

Soil depth (cm)	pH		Conductivity (μS)		Sand (%)		Silt (%)		Clay (%)		CEC_{eff} ($cmol_c$ kg^{-1})		OC (%)		TN (%)		N
	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se	
0-10	6.061	0.030	114.342	2.667	19.366	0.192	72.883	0.221	7.750	0.117	21.513	0.215	1.729	0.033	0.154	0.003	84
10-30	6.167	0.032	119.956	2.907	19.333	0.225	73.067	0.252	7.600	0.116	21.675	0.202	1.710	0.033	0.151	0.002	84
30-60	6.411	0.022	72.500	1.179	20.149	0.454	73.771	0.499	6.080	0.148	20.140	0.194	0.970	0.030	0.086	0.002	84

Table S2. Factor loadings on components of a PCA with Eigenvalues >0.9. The exploratory PCA helped to select for latent variable construction of the SEM.

	Comp.1	Comp.2	Comp.3
BD	0.36	0.4	0.17
Clay	-0.17	-0.43	-0.74
OC	-0.38	-0.24	0.2
OC1	-0.5	0.2	0.08
OC2_1	0.19	-0.57	0.49
OC4_2	0.39	-0.39	-0.06
OC8_4	0.43	-0.07	-0.12
OC16_8	0.27	0.29	-0.34
Variance explained	0.45	0.16	0.12
Cumulative variance explained	0.45	0.61	0.73
Eigenvalue	3.58	1.29	0.97

Table S3. Sawing and harvest dates at the experimental site Asendorf. Note: harvest date of cover crops only refer to the date of biomass determination. Cover crops were left in the field for frost termination and incorporated to the ground before seeding.

Year	**Crop**	**Sawing date**	**Harvest date**
2015	wheat	spring wheat	GPS
2015	cover crop	2015-09-03	2015-10-23
2016	wheat	2015-10-21	2016-07-27
2016	cover crop	2016-08-22	2016-10-28
2016	maize	2016-05-04	2016-09-27
2017	wheat	2016-10-17	2017-08-02
2017	maize	2017-04-28	2017-10-09
2017	cover cropp	2017-08-15	2017-10-24
2018	wheat	2017-11-03	2018-07-24
2018	cover crop	2018-08-16	2018-10-28
2018	maize	2018-04-26	2018-08-23
2019	wheat	2018-10-19	2019-07-23
2019	cover crop	2019-08-14	2019-10-28
2019	maize	2019-04-25	2019-09-24
2020	wheat	2019-10-15	2020-07-29
2020	cover crop	2020-08-24	2020-11-04
2020	maize	2020-04-22	2020-10-06
2021	wheat	2020-10-16	2021-07-26
2021	cover crop	2021-08-14	2021-11-03
2021	maize	2021-04-27	2021-10-20
2022	wheat	2021-10-27	2022-07-29
2022	cover crop	2022-08-23	2022-11-10
2022	maize	2022-04-25	2022-09-15
2023	wheat	2023-10-04	2023-08-09
2023	cover crop	2023-08-11	2023-11-02
2023	maize	2023-05-02	2023-10-04

Table S4: Plant biomass data of shoots, roots and total biomass from different catch crop treatments (dry weight). Mean values of six plots per treatment and standard error (SE) are shown. Methods are described in detail in Heuermann et al. (2019).

Cover cop	**OC (mg g^{-1})**		**TN (mg g^{-1})**		**C:N ratio**		**Biomass (t ha^{-1})**		**Root:shoot ratio**	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Root										
Mustard	387.5	20.4	8.9	0.9	45.7	6.7	1.5	0.4	-	-
Clover	353.4	25.2	29.0	3.1	12.4	0.6	0.8	0.4	-	-
Phacelia	399.9	9.5	8.3	1.1	51.1	8.0	1.1	0.4	-	-
Oat	238.9	8.6	6.8	1.5	41.1	9.4	1.5	0.3	-	-
Mix4	362.5	13.4	9.2	1.1	40.9	5.3	5.2	1.5	-	-
Mix12	360.3	8.5	16.4	2.0	23.0	2.9	19.5	3.2	-	-
Shoot										
Mustard	406.8	5.1	21.5	2.5	20.4	2.6	2.9	0.4	-	-
Clover	399.3	9.5	37.0	1.6	10.9	0.4	0.8	0.3	-	-
Phacelia	379.7	3.5	21.1	2.4	19.3	2.4	2.3	0.3	-	-
Oat	402.3	4.3	22.5	2.9	19.3	2.2	1.7	0.5	-	-
Mix4	401.1	4.4	22.7	1.8	18.3	1.5	8.2	1.3	-	-
Mix12	387.3	3.7	29.9	2.9	13.6	1.3	25.6	4.7	-	-
Total										
Mustard	665.1	85.4	27.4	1.6	25.2	4.3	3.9	0.7	0.54	0.4
Clover	634.9	72.9	56.3	5.8	11.2	0.4	1.3	0.6	0.98	0.3
Phacelia	646.3	85.2	26.6	1.6	25.3	4.4	3.0	0.6	0.51	0.4
Oat	561.6	46.9	27.0	1.9	21.8	3.1	2.7	0.8	0.88	0.4
Mix4	642.7	77.9	28.9	1.4	22.5	2.9	11.7	2.6	0.64	1.4
Mix12	627.5	77.4	40.9	2.6	15.4	1.9	38.6	8.5	0.76	4.0

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