Beyond inorganic carbon: Soil organic carbon as key pathway for carbon sequestration in Enhanced Weathering

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1. Supplementary: Evidence of weathering but limited inorganic CO_2 removal

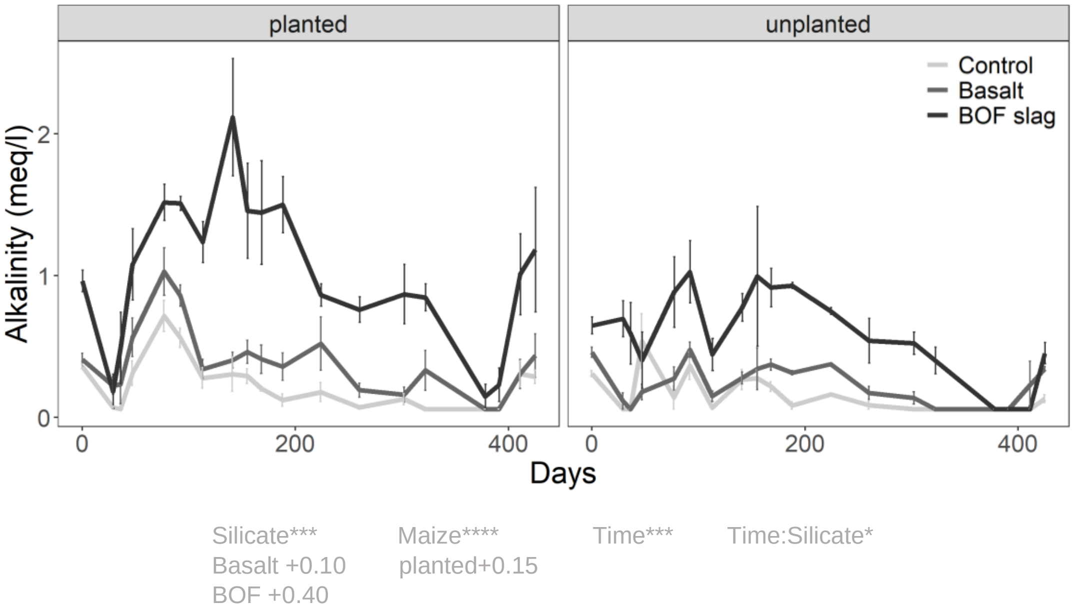

Figure S1: Temporal dynamics of alkalinity concentrations in porewater of topsoil. Error bars display standard errors; p-values <0.05, <0.01 and <0.001 are represented by *, ** and ***; values below p-values represent the change relative to control (for silicate effect) and unplanted (for maize effect) treatments.

Table S1: Results of a mixed model analysis on Ca, Mg, Na and K concentrations in various soil pools. If an effect is significant then the change relative to control (for silicate effect) and unplanted (for maize effect) treatments is shown below p-values.

	Main effects			**Interaction effects**		
	Silicate	**Maize**	**Time**	**Silicate Maize**	**Time Silicate**	**Time Maize**
Exchangeable Ca mol Ca/pot	<0.001 Basalt +0.19 BOF ns	0.559	<0.001		0.002	
Carbonate Ca mol Ca/pot	0.005 Basalt +0.03 BOF ns	0.894	<0.001		<0.001	0.007
(Hydr)oxide Ca mol Ca/pot	0.003 Basalt +0.03 BOF ns	0.851	<0.001		<0.001	
SOM Ca mol Ca/pot	<0.001 Basalt +0.003 BOF -0.003	0.987	<0.001		<0.001	
Exchangeable Mg mol Mg/pot	<0.001 Basalt +0.06 BOF ns	0.135	<0.001		0.004	
Carbonate Mg mol Mg/pot	0.038 Basalt ns BOF -0.007	0.035 -0.003	<0.001		<0.001	0.001
(Hydr)oxide Mg mol Mg/pot	<0.001 Basalt +0.77 BOF ns	0.346	<0.001		<0.001	
SOM Mg mol Mg/pot	<0.001 Basalt +0.05 BOF ns	0.670	<0.001		<0.001	
Exchangeable Na mol Na/pot	<0.001 Basalt +0.02 BOF ns	0.422	<0.001	0.015	<0.001	
Carbonate Na mol Na/pot	<0.001 Basalt +0.04 BOF ns	0.078	<0.001		<0.001	0.008
(Hydr)oxide Na mol Na/pot	<0.001 Basalt +0.02 BOF ns	0.523	<0.001		<0.001	
SOM Na mol Na/pot	<0.001 Basalt +0.002 BOF ns	0.773	<0.001		<0.001	
Exchangeable K mol K/pot	<0.001 Basalt ns BOF -0.004	0.978	<0.001		<0.001	
Carbonate K mol K/pot	<0.001 Basalt +0.02 BOF ns	0.560	<0.001		<0.001	
(Hydr)oxide K mol K/pot	<0.001 Basalt +0.06 BOF +0.06	0.914	0.005		<0.001	
SOM K mol K/pot	0.222	0.626	<0.001		<0.001	

Table S2: Results of mixed model analysis on leachate chemistry. If an effect is significant then the change relative to control (for silicate effect) and unplanted (for maize effect) treatments is shown below p-values.

	Main effects			Interaction effect		
	Silicate	Maize	Time	Silicate Maize	Time Silicate	Time Maize
Leachate DIC mg/L	0.051	0.486	0.091			0.004
Leachate Alkalinity meq/L	0.003 Basalt +0.06 BOF ns	<0.001 +0.06	<0.001			
Leachate pH	0.569	0.002 -0.24	0.006			
Leachate Ca mmol Ca/L	0.355	<0.001 -0.30	<0.001			
Leachate Mg mmol Mg/L	0.198	<0.001 -0. 09	<0.001			
Leachate Na mmol Na/L	0.041 Basalt +0.20 BOF ns	<0.001 -0.20	0.089	<0.001	<0.001	0.068
Leachate K mmol K/L	0.561	<0.001 -0.16	<0.001			
Leachate Si mmol Si/L	0.462	<0.001 -0.10	<0.001			
Leachate Vol mL	0.246	0.003 -118	0.025		0.015	

Table S3: Weathering rates in log(mol total alkalinity/m^2 s) for day 0-9 (before planting), day 9-101 (first growing season) and day 101-435 (winter+second growing season) ± confidence intervals (calculations require the aggregation of replicates, 95% confidence intervals are used to assess the range within which the true parameter is likely to fall, lack of overlap is assumed indicative for significant differences between groups). Basalt/BOF slag weathering rates of day 0-9 are identical since maize has not been planted yet.

	Basalt		
	Day 0-9 t CO_2/ha	**Day 9-101** t CO_2/ha	**Day 101-435** t CO_2/ha
unplanted	-9.2 ± 0.1	-10.3 ± 0.3	0
planted	-9.2 ± 0.1	-10.6 ± 0.6	0

	BOF slag		
	Day 0-9 t CO_2/ha	**Day 9-101** t CO_2/ha	**Day 101-435** t CO_2/ha
unplanted	-10.3 ± 2.7	-9.7 ± 0.2	0
planted	-10.3 ± 2.7	-9.5 ± 0.1	0

Table S4: Change in extractable base cation levels relative to control ± 95% confidence intervals of each soil pool shown in **Figure 3**.

		Basalt		
		Day 9 %	**Day 101** %	**Day 435** %
Leachates	**unplanted**	0	0.05 ± 0.00	0.07 ± 0.03
	planted	0	0.04 ± 0.00	0.20 ± 0.01
Carbonate	**unplanted**	2.82 ± 0.58	6.57 ± 1.74	0.71 ± 0.93
	planted	2.82 ± 0.58	3.37 ± 1.73	0.02 ± 1.67
Exchangeable	**unplanted**	5.78 ± 1.10	8.29 ± 4.20	8.01 ± 5.12
	planted	5.78 ± 1.10	9.08 ± 3.15	3.88 ± 4.35
SOM	**unplanted**	1.85 ± 0.27	1.37 ± 0.53	0.98 ± 0.96
	planted	1.85 ± 0.27	1.09 ± 0.76	0.57 ± 0.90
(Hydr)oxides	**unplanted**	25.29 ± 4.56	48.64 ± 18.34	21.21 ± 15.95
	planted	25.29 ± 4.56	37.18 ± 21.29	13.89 ± 4.96
Plants	**unplanted**	0	0	0
	planted	0	-0.23 ± 0.32	0.12 ± 0.98

		BOF slag		
		Day 9 %	**Day 101** %	**Day 435** %
Leachates	**unplanted**	0	0.44 ± 0.02	0.45 ± 0.17
	planted	0	0.11 ± 0.04	0.51 ± 0.05
Carbonate	**unplanted**	0.73 ± 2.84	9.26 ± 8.65	2.52 ± 4.34
	planted	0.73 ± 2.84	7.44 ± 2.64	0.91 ± 2.92
Exchangeable	**unplanted**	0.93 ± 6.38	36.42 ± 22.42	14.26 ± 21.91
	planted	0.93 ± 6.38	62.25 ± 17.31	24.06 ± 14.95
SOM	**unplanted**	-0.34 ± 0.15	-0.17 ± 0.59	0.05 ± 0.25
	planted	-0.34 ± 0.15	-0.50 ± 0.20	-0.06 ± 0.16
(Hydr)oxides	**unplanted**	0.00 ± 4.20	7.32 ± 7.22	5.49 ± 5.36
	planted	0.00 ± 4.20	5.95 ± 2.45	2.25 ± 3.81
Plants	**unplanted**	0	0	0
	planted	0	0.14 ± 0.98	1.48 ± 2.40

2. Supplementary: Increased SOM decomposition further reduced CO_2 removal efficiency

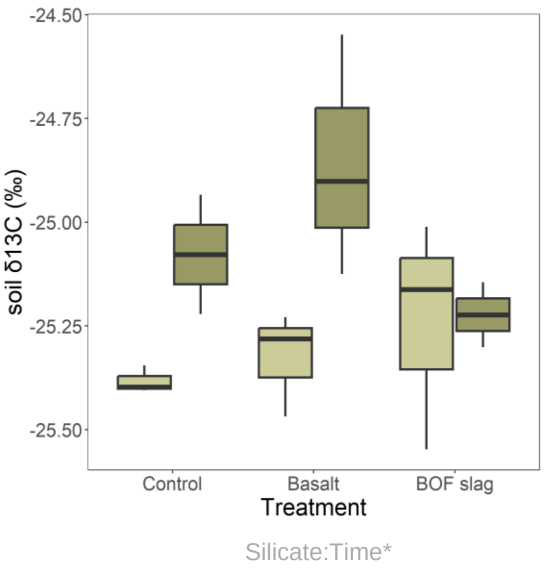

Figure S2: The δ^{13}C signatures of bulk soil. Measurements were taken at two different points in time, day 9 (shortly after start of experiment) and day 435 (end of experiment). P-values <0.05, <0.01 and <0.001 are represented by *, ** and ***.

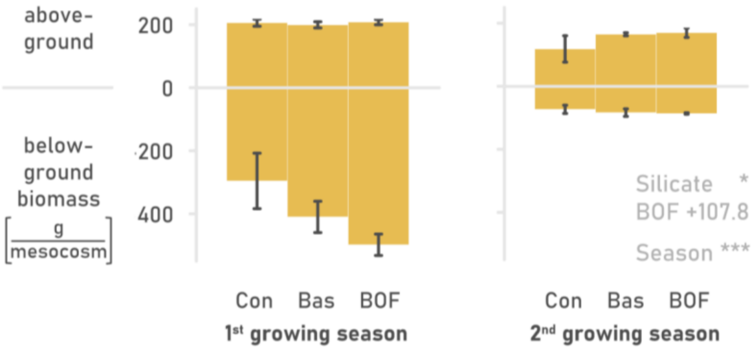

Figure S3: Above- and belowground plant biomass of the first (left) and second (right) growing season. Large differences in root biomass are likely due to different weather conditions (dry first season vs wet second season) and the use of different maize varieties. Error bars display standard errors; Con=control, Bas=basalt, BOF=BOF slag; p-values <0.05, <0.01 and <0.001 are represented by *, ** and ***; values below p-values represent the change relative to the control treatment.

3. Supplementary: Stabilised OC potentially dominating C sink during EW

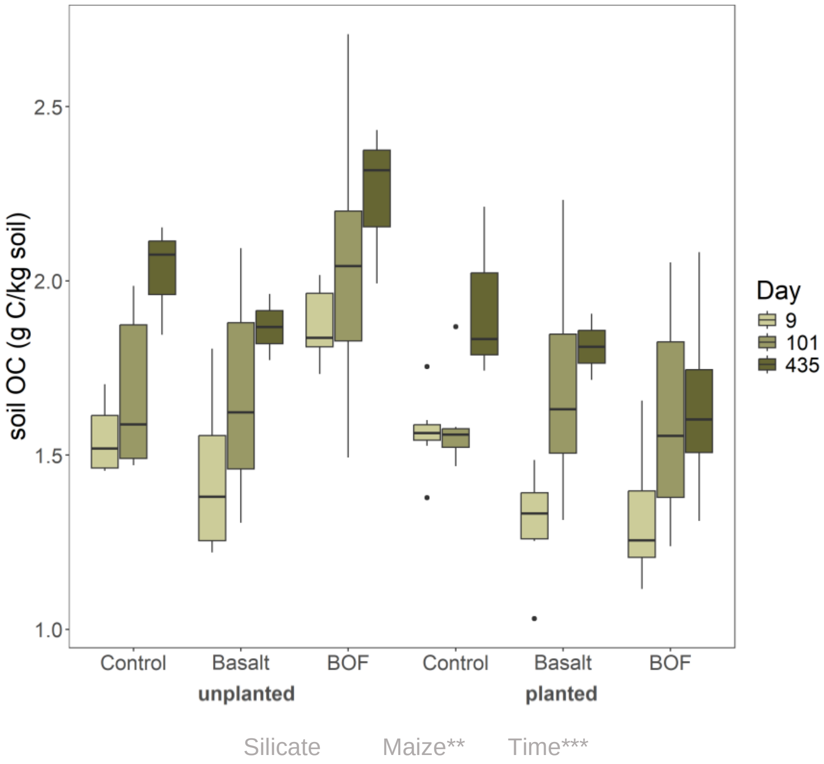

Figure S4: OC associated to amorphous minerals determined based on sequential extractions according to Heckman et al. 2018 (see methods) carried out on soil samples taken on day 9 (=before planting), day 101 (=after 1st growing season) and day 435 (=after 2nd growing season); p-values <0.05, <0.01 and <0.001 are represented by *, ** and ***.

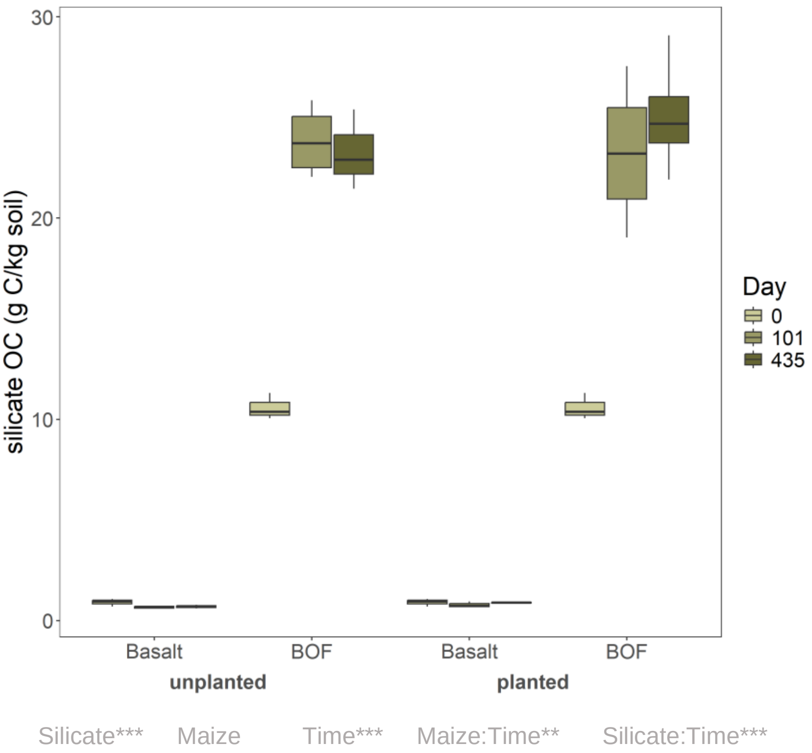

Figure S5: OC associated to amorphous minerals determined based on sequential extractions according to Heckman et al. 2018 (see methods) carried out on silicate samples taken on day 0 (=start of experiment), day 101 (=after 1st growing season) and day 435 (=after 2nd growing season); p-values <0.05, <0.01 and <0.001 are represented by *, ** and ***.

Table S5: Results of a mixed model analysis on SOC found in different aggregate size classes at the end of the experiment. If an effect is significant then the change relative to the control treatment is shown below p-values.

	Main effects		Interaction effect
	Silicate	Maize	Silicate Maize
SOC <63µm %	0.822	0.199	
Weight <63µm g	0.170	0.059	
SOC*weight <63µm g C/kg soil	0.152	0.091	
SOC 63-250µm %	0.033 Basalt ns (p=0.08) BOF +0.012	0.053	
Weight 63-250µm g	0.056	0.547	
SOC*weight 63-250µm g C/kg soil	0.394	0.127	
SOC >250µm %	0.028 Basalt ns BOF +0.019	0.218	0.076
Weight >250µm g	0.011 Basalt +13.10 BOF ns (p=0.09)	0.846	
SOC*weight >250µm g C/kg soil	0.010 Basalt +1.55 BOF ns (p=0.09)	0.955	

4. Supplementary: Methods

4.1 Experimental set-up

Elemental analysis was performed on a WD-XRF Bruker S8 Tiger 4K spectrometer, using milled samples (**Table S6**). To determine mineralogy of applied silicates, XRD measurements (Bruker D8 Avance, Cu Kα radiation at 30 mA and 40 kV, with 2θ ranging from 5 to 70°) were carried out. During sample preparation samples were milled in ethanol for 10 minutes. Rietveld refinement was carried out to quantify both the different crystalline phases present and the amorphous phase. Note that both silicates contain a large amount of amorphous minerals (whose mineralogy could not be characterised in more detail) (**Table S7**).

Table S6: Composition of BOF slag (left) and basalt (right) measured by XRF. Concentrations are given in % and in g/m^2 soil (considering an application rate of 5 t/ha BOF slag and 50 t/ha basalt).

BOF slag

	Concentration %	Concentration g/m^2 soil (5 t/ha)
Ca	42.01	210.02
Fe	18.30	91.51
Si	4.93	24.65
Mn	2.20	11.00
Mg	0.97	4.85
Al	0.90	4.50
P	0.77	3.85
Ti	0.47	2.35
V	0.32	1.60
Cl	0.28	1.40
S	0.13	0.65
Cr	0.12	0.60
Nb	0.03	0.15
Sr	0.03	0.14
Zr	0.02	0.08
K	0.01	0.05
Zn	0.01	0.03

Basalt

	Concentration %	Concentration g/m^2 soil (50 t/ha)
Si	14.70	735.10
Fe	8.58	429.38
Ca	6.42	321.21
Mg	5.88	294.49
Al	5.16	258.17
Na	1.87	93.50
Ti	1.08	54.21
K	0.77	38.54
P	0.26	13.00
Mn	0.15	7.50
Cl	0.08	3.83
Sr	0.07	3.70
Ba	0.06	2.80
Ni	0.04	2.04
Cr	0.04	1.84
V	0.02	1.21
Ce	0.02	0.99
Zr	0.02	0.75
Zn	0.01	0.52
S	0.01	0.47
Cu	0.01	0.38
Nb	0.01	0.31
Rb	0.00	0.12
Y	0.00	0.10
Co	0.00	0.02

Table S7: Mineralogy of crystalline phase of BOF slag (left) and basalt (right), determined via XRD measurements.

BOF slag		
Calcite	$CaCO_3$	8.66%
Srebrodolskite	Ca_2FeO_5	8.36%
Beta-C2S	Ca_2SiO_4	7.06%
Bredigite	$Ca_{14}Mg_2(SiO_4)_8$	4.41%
Wuestite	FeO	3.93%
Portlandite	$Ca(OH)_2$	2.38%
Periclase	MgO	1.92%
Magnetite	Fe_3O_4	1.81%
Quarz	SiO_2	1.11%
Iron	Fe	0.62%
Hematite	Fe_2O_3	0.27%
Crystalline phase		40.53%
Amorphous phase		59.47%

Basalt		
Clinopyroxene	$CaMg_{0.641}Fe_{0.759}Si_{1.6}O_6$	21.22%
Forsterite	$MgSiO_4$	14.45%
Hedenbergite	$CaFeSi_2O_6$	6.98%
Labradorite	$Ca_{1.34}Na_{0.66}Si_{4.6599}Al_{3.24}O_{16}$	5.70%
Magnetite	Fe_3O_4	2.73%
Pigeonite	$Mg_{1.36}Fe_{0.52}Ca_{0.12}Si_2O_6$	2.36%
Grossmanite	$CaMg_{0.39}Al_{0.86}Ti_{0.48}Si_{1.27}O_6$	1.29%
Albite	$NaAlSi_3O_8$	1.29%
Analcime	$Na_{1.241}Al_{1.33}Si_{2.667}O_{9.333}$	0.93%
Enstatite	$MgSiO_3$	0.66%
Ankerite	$CaMg_{0.32}Fe_{0.68}(CO_3)_2$	0.18%
Quartz	SiO_2	0.07%
Crystalline phase		57.86%
Amorphous phase		42.14%

4.2 Soil sequential extractions

Two different types of soil sequential extractions were carried out. Sequential extractions adapted from Tessier et al. (1979) and Uhlig and Von Blanckenburg (2019) aim at quantifying the cation content of four different soil pools (**Table 2**). During the first extraction step, 10 mL of 1 M ammonium acetate (NH_4OAc) was added to 0.5 g of ground, air-dried soil. After 1 hour of shaking samples were centrifuged for 10 minutes at 4400 rpm and supernatant was collected after filtration through a 0.2 µm poresize polyethersulfone syringe filter. During this step, cations sorbed to the soil exchangeable complex are exchanged for NH_4^+ and can be quantified in the supernatant. In the second step, carbonates are dissolved by adding 5 mL of 1 M acetic acid (CH_3COOH) to the same soil samples. After 2 hours of shaking 1 mL of 3 M ammonium acetate (NH_4OAc) was added to prevent resorption. Samples were brought up to 10 ml with ultrapure milliQ water (18.2 Ω cm-1), centrifuged again and supernatants collected as above. This step is thought to target carbonate minerals. In the next extraction step a solution of 5 mL of 0.05 M hydroxylamine (NH_2OH) in 1 M hydrochloric acid (HCl) was added to the samples and they were placed in an 80 °C water bath for 5 hours. During this time samples were shaken manually every 30 minutes. As above, 3 M ammonium acetate was added to each sample again and they were brought up to 10 mL with milliQ water before supernatants were collected after centrifugation. This extraction step targets cations found in oxides and hydroxides. During the last extraction step 4 mL of 9.8 M hydrogen peroxide (H_2O_2) was added to the samples. Again, they were put in a water bath (70 °C) and shaken manually every 30 minutes. After three hours 3 M ammonium acetate (NH_4OAc) was added and samples were brought to 10 mL with milliQ water after which samples were centrifuged and collected. This extraction step targets cations bound to SOM. Between all extraction steps soil samples were washed twice with milliQ water. Mg, Ca, Na, K, Fe, Al and Si concentrations were determined for each extraction step with either a Varian 720-ES or SpectroGreen ICP-OES, both hosted at the Helmholtz Laboratory for the Geochemistry of the Earth Surface (HELGES) in GFZ-Potsdam. Samples were diluted by a factor of four to a 0.3 M HNO_3 matrix. Standards were gravimetrically prepared from single element ICP standards, also in 0.3 M HNO_3. Blanks and in house quality control standards – including those designed to match the residual sample NH_4OAc, acetic acid, hydroxylamine, or peroxide matrix – were measured at least every 10 samples to track instrumental drift.

Sequential extractions following the protocol of Heckman et al. (2018) aim at quantifying SOC bound to amorphous minerals. As none of the extractants contains carbon, DOC can be measured to quantify SOC released during each of the two extraction steps. The sum of both extraction steps is assumed to quantify SOC bound to amorphous minerals (**Table 3**). During the first extraction step, 35 mL of 0.1 M sodium pyrophosphate ($Na_4P_2O_7$) was added to 1 g of air-dried and ground soil. After shaking 16 h, samples were centrifuged for 4 minutes with 3000 rpm and the supernatant was collected. All samples were washed with deionized water, before the next extraction step was carried out on the same soil samples. During the second extraction step 35 mL of 0.1 M hydroxylamine (NH_2OH) in 0.25 M HCl was added to each sample. Shaking and sample collection was carried out the same way as during the previous step. The extractant solutions had a pH (25°C) of 9.9 (sodium pyrophosphate), 0.4 (hydroxylamine). Collected supernatants from both extraction steps were analysed for DOC using a continuous flow analyser (Skalar, SAN++). To give additional insights into mineralogy and interactions with SOC we also determined Fe, Al, Ca, Mg and Si concentrations using an ICP-OES (Thermo Scientific, iCAP 6300 duo).

4.3 Measurement and analysis of soil CO_2 efflux

Soil CO_2 efflux and its $\delta^{13}C$ signature was measured throughout the experiment. To partition fluxes in rhizosphere respiration and SOM decomposition $\delta^{13}C$ signature of root and SOM end members are needed (**Equation 2**).

The measured $\delta^{13}C$ of maize roots amounted to -13.127 ± 0.13 ‰ and was adjusted with a 4.4 ‰ correction to account for fractionation during CO_2 diffusion. Therefore, -8.727 ‰ was assumed to be the signature of CO_2 originating from roots. While this diffusion correction is commonly done (e.g. Moyes et al., 2010; Schindlbacher et al., 2019), we could also confirm it with experimental measurements. At the beginning of the experiment soil CO_2 efflux had a $\delta^{13}C$ signature of -20.729 ± 0.57 ‰, while soil samples taken at the same time had a $\delta^{13}C$ signature of -25.096 ± 0.36 ‰, suggesting that there was a diffusion induced shift in the delta value of 4.367 ‰.

The $\delta^{13}C$ of SOM was determined from unplanted treatments. As the CO_2 efflux of all three unplanted treatments changed over time ($p<0.001$, **Figure S6**) the signature of the respective day was used as end member, instead of using the signature of the first CO_2 efflux measurement or the signature measured in soil samples. This approach allows to take seasonal effects into account, therefore facilitating more precise partitioning of soil CO_2 effluxes.

Further, during the first growing season the three different unplanted treatments (control, basalt, BOF slag, **Figure S6**) displayed different $\delta^{13}C$ signatures. Silicate application led to an increase in the $\delta^{13}C$ signature ($p=0.004$, control = -20.182 ± 0.36 ‰, basalt = -19.082 ± 0.30 ‰, BOF = -18.387 ± 0.45 ‰) possibly due to increased SOM decomposition or dissolution of carbonates contained in the silicates. The maximum amount of CO_2 that could be released due to carbonate dissolution amounts to 0.20 and 0.27 t of CO_2/ha (assuming complete dissolution, this contributes only ~1.5% of the total CO_2 efflux). Considering overall flux sizes and isotopic signatures of silicates such large shifts in CO_2 $\delta^{13}C$ signatures cannot be explained by carbonate dissolution alone. It is thus likely a combination of both processes, carbonate dissolution and SOM decomposition. To take these shifts in silicate amended treatments into account and avoid an overestimation of SOM decomposition during the first season, the signature of the respective treatment of the respective day was used as input for the SOM signature, instead of only using the signature of the control treatment of the respective day.

Partitioning was not carried out outside of the growing seasons since the fractionation due to diffusion is known to change over time (Zhou et al., 2018) and since CO_2 effluxes during cold months were very low, hampering precise determination of the $\delta^{13}C$ of the soil CO_2 efflux. Especially since rhizosphere respiration dropped to zero mid-October (a little over a month after the harvest), CO_2 was considered to only originate from SOM decomposition from this point onwards. Also during the already warmer month of May (shortly before replanting) rhizosphere respiration remained zero. In other words, for the period from one month after the harvest until replanting, SOM decomposition was therefore assumed to equal soil CO_2 efflux.

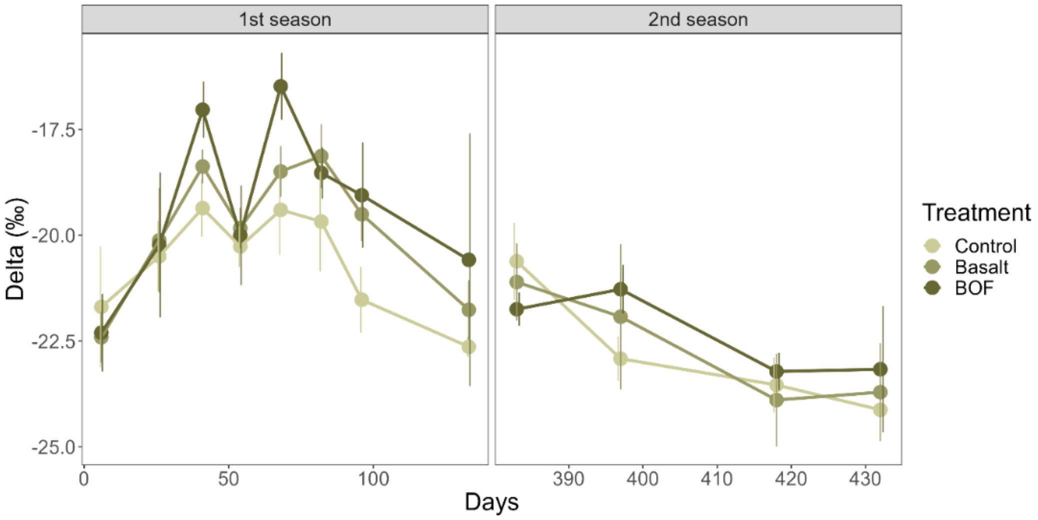

Figure S6: Temporal dynamics of the $\delta^{13}C$ signatures of soil CO_2 effluxes of unplanted treatments measured during the first and second growing season. Error bars display standard errors.

After partitioning, the weekly/bi-weekly measurements of soil CO_2 efflux were used to reconstruct daily CO_2 effluxes based on daily soil temperature and soil water content measurements (**Figure S7**). Reconstruction was done for SOM decomposition (**Figure S8**) and the total CO_2 efflux (**Figure S9**) using the model of **Equation 5**. Due to the relatively short growing seasons, rhizosphere respiration itself was not reconstructed as model fits were not satisfactory.

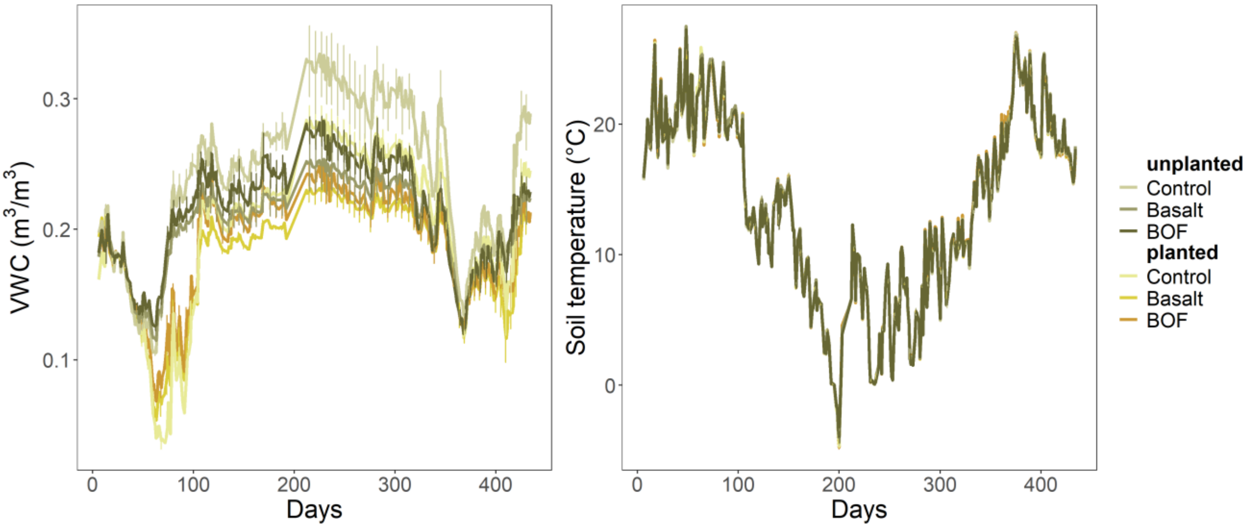

Figure S7: Temporal evolution of volumetric water content (left) and temperature (right) in the soil. Error bars display standard errors.

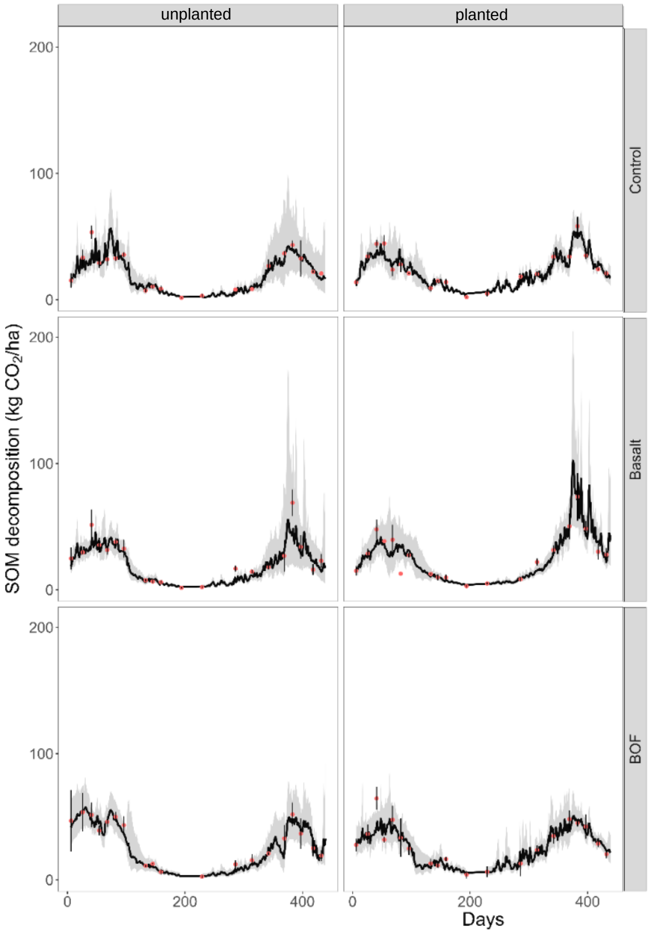

Figure S8: Daily reconstructed SOM decomposition (black line) including 95% confidence intervals (shaded in grey). Red dots show mean of actual measurements for each treatment with standard errors. Note that soil temperature and soil water content during the CO_2 measurement slightly differed from the daily average soil temperature and soil water content. Actual measurements can therefore deviate slightly from reconstructed effluxes.

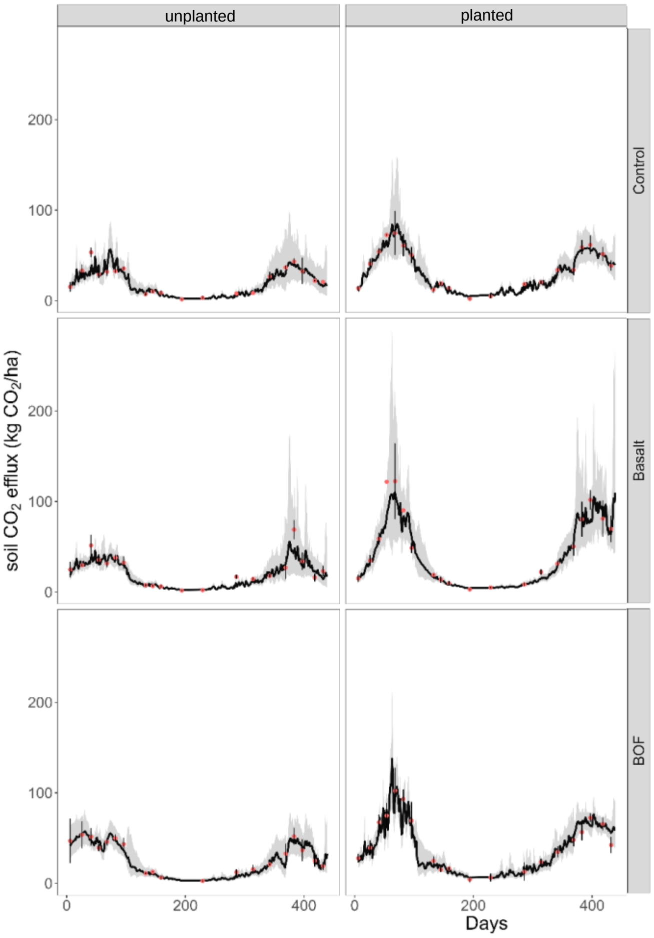

Figure S9: Daily reconstructed CO_2 efflux (black line) including 95% confidence intervals (shaded in grey). Red dots show mean of actual measurements for each treatment with standard errors. Note that soil temperature and soil water content during the CO_2 measurement is likely not the same as the daily average soil temperature and soil water content. Actual measurements can therefore deviate slightly from reconstructed effluxes

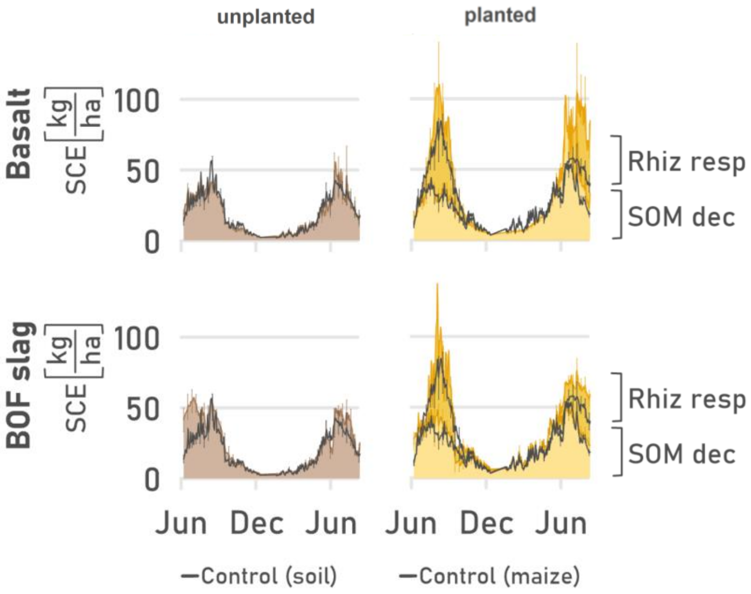

Figure S10: Temporal dynamics of reconstructed soil CO_2 effluxes (SCE) partitioned into rhizosphere respiration (darker colour) and SOM decomposition (lighter colour). Error bars show standard errors, measured SCE is shown in **Figure 5**.

4.4 Calculation of weathering rates

Weathering rates were calculated based on cation release of applied silicates. To this end, changes in concentrations of control treatments were subtracted from silicate amended treatments. The amount of cations, is thus expressed relative to non-amended treatments. All pools were considered, thus cations in plant, exchangeable, carbonate, hydr(oxide), SOM and leachates. Weathering rates (Wr, expressed as $\log_{10}$(mol total alkalinity/m^2 s)) are then calculated using **Equation S1** (Niron et al., 2024; Vienne et al., 2023). The SSA of the applied basalt and BOF slag corresponds to 6.374 and 6.492 m^2/g respectively. The application rate was 980 and 98 g/mesocosm (corresponding to 50 and 5 t/ha), respectively.

$$\mathrm{Wr} \;= log_{10}\left(\frac{\sum 2 * (Ca + Mg) + (Na + K)}{\text{time} * \text{silicate application rate} * \text{SSA}}\right) \quad (\boldsymbol{S1})$$