

# Stabilisation of soil organic matter with rock dust partially counteracted by plants

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## Abstract

Soil application of Ca- and Mg-rich silicates can capture and store atmospheric carbon dioxide as inorganic carbon but could also have the potential to stabilise soil organic matter (SOM). Synergies between these two processes have not been investigated. Here, we apply finely ground silicate rock mining residues (basalt and granite blend) to a loamy sand in a pot trial at a rate of 4% (equivalent to  $50\text{ t ha}^{-1}$ ) and investigate the effects of a wheat plant and two watering regimes on soil carbon sequestration over the course of 6 months. Rock dust addition increased soil pH, electric conductivity, inorganic carbon content and soil-exchangeable Ca and Mg contents, as expected for weathering. However, it decreased exchangeable levels of micronutrients Mn and Zn, likely related to the elevated soil pH. Importantly, it increased mineral-associated organic matter by 22% due to the supply of secondary minerals and associated sites for SOM sorption. Additionally, in the nonplanted treatments, rock supply of Ca and Mg increased soil microaggregation that subsequently stabilised labile particulate organic matter as organic matter occluded in aggregates by 46%. Plants, however, reduced soil-exchangeable Mg and Ca contents and hence counteracted the silicate rock effect on microaggregates and carbon within. We suggest this cation loss might be attributed to plant exudates released to solubilise micronutrients and hence neutralise plant deficiencies. The effect of enhanced silicate rock weathering on SOM stabilisation could substantially boost its carbon sequestration potential.

## KEY WORDS

aggregate carbon, basalt, enhanced rock weathering, inorganic carbon, mineral-associated organic matter, particulate organic matter, soil carbon sequestration, soil organic carbon

## 1 | INTRODUCTION

Urgent action is required to avoid the most dangerous impacts of climate change. Such action must include both significant reduction in greenhouse gas emissions and atmospheric greenhouse gas removal largely via land management change (IPCC, 2022). Several promising greenhouse gas removal methods are based on

utilising natural cycles to capture and store atmospheric carbon dioxide (Buss, Yeates, et al., 2021; Fuss et al., 2018). These include plant-derived soil organic carbon formation and enhanced rock weathering.

During natural weathering of Ca- and Mg-rich silicates, bicarbonate ( $\text{HCO}_3^-$ ) is formed, capturing  $\text{CO}_2$  from the atmosphere (largely as  $\text{CO}_2$  respired from plants and soil microorganisms;

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Beerling et al., 2018; Hartmann et al., 2013). Follow-up reactions can produce solid carbonates that sequester carbon for the long term. The mafic rock basalt is one of the most promising rock types for large-scale carbon capture and storage since it is abundant, weathers rapidly and is low in heavy metal contaminants potentially harmful for soil and plant growth. The weathering rates are highly dependent on rock particle size; 1-mm-sized spheres of even the most reactive Ca- and Mg-rich silicates take thousands of years to dissolve (Hartmann et al., 2013). However, grinding rocks to a particle size of  $<100\mu\text{m}$  can result in weathering and carbon drawdown on a societal-relevant scale (Holdren & Speyer, 1985; Renforth, 2012). Unfortunately, across multiple studies into the weathering rates of Ca- and Mg-rich silicates in systems reflecting natural soil–plant conditions,  $\text{CO}_2$  sequestration rates were measured differently and varied by a factor of 1000 (Amann et al., 2020; Haque et al., 2019, 2020; Kelland et al., 2020; ten Berge et al., 2012). This demonstrates the need for further studies that investigate enhanced weathering of globally available materials, such as mining residues, using field soils and plants but grown in controlled conditions and measured consistently across multiple interacting factors.

Soil organic carbon exists in natural systems as soil organic matter (SOM) and mostly enters the soil system as plant-derived particulate organic matter (POM), which is labile and easily decomposed (Lavallee et al., 2020; Poeplau et al., 2018). Soil aggregates can physically protect POM, which is called aggregated organic matter (AggOM), and minerals can sorb partially decomposed POM fragments, so-called dissolved organic matter (DOM), to its surfaces to form mineral-associated organic matter (MAOM; Abramoff et al., 2018; Hemingway et al., 2019; Poeplau et al., 2020). Both processes increase the retention and stability of SOM in soil. Soil aggregates are formed through various soil processes that bind together soil particles, such as activity from fungi hyphae and roots, or soil cementing agents, including Ca, Mg, Al and Fe (Amezketa, 1999). The main components of soil responsible for MAOM formation are clay and short-order Fe and Al minerals formed from weathering of primary minerals (Kleber et al., 2015; Singh et al., 2018). Polyvalent cations, such as Ca, Mg, Al and Fe, also have a key function in facilitating sorption of DOM to mineral surfaces through cation bridging; the connection of (predominantly) negatively charged clay surfaces with negative functional groups of DOM (Kleber et al., 2015; Singh et al., 2018).

Synergies between enhanced rock weathering for both the formation of inorganic carbon for direct carbon drawdown and the formation of secondary minerals and polyvalent cations for stabilisation of SOM could significantly enhance its carbon sequestration potential and have soil health co-benefits. Yet, studies that investigate the factors that could influence the synergistic effects of rock on both inorganic and organic carbon are lacking.

Both rock weathering rates and SOM formation and decomposition reactions are affected by soil water availability and plant activity. Higher precipitation and increased water flow accelerates

rock weathering and hence inorganic carbon formation (Brady et al., 1999; Li et al., 2016; White & Blum, 1995). Water availability also governs microbial processes responsible for SOM decomposition and affects plants that supply carbon into soil. Therefore, precipitation has a strong effect on SOM levels (Luo et al., 2017) and the SOM content is typically higher in areas with more precipitation (Alvarez, 2005; Wiesmeier et al., 2019). Plants can significantly increase rock weathering rates by up 10-fold (Bormann et al., 1998; Cochran & Berner, 1996; Hinsinger et al., 2001). Plants also provide the foundation for SOM formation through rhizodeposits from living plants and litter from dead plants. Yet, they can also accelerate the decomposition of existing SOM through various processes summarised under the term positive priming (Keiluweit et al., 2015; Kuzyakov, 2010). The effects of plants and water could significantly influence the potential synergies of rock weathering on SOM and inorganic carbon sequestration.

In this study, to simulate potential field conditions, bulk mining residues (basalt and granite blend) were applied to a sandy loam and field climatic conditions were replicated in a growth chamber under controlled conditions. The effects of wheat plants and watering on rock weathering, microbial composition and persistence of SOM were investigated. Furthermore, the soil available and plant tissue elemental contents were analysed to understand the mechanisms behind the soil response. The hypotheses were as follows: (i) weathering of Ca- and Mg-rich silicates can increase both inorganic and organic carbon storage, and (ii) high watering and high biological activity (presence of a plant) increase rock weathering rates.

## 2 | MATERIALS AND METHODS

### 2.1 | Soil and rock samples

The soil was agricultural topsoil (0–20 cm) sourced in 2020 from Young in central New South Wales, Australia. The soil was dried and stored for ca. 12 months before it was used in the incubation trial. It had a pH (in water) of 5.68 and was classified as loamy sand (USDA classification). The cation exchange capacity was  $2.3 \text{ cmol}_+ \text{ kg}^{-1}$ , and the total carbon and nitrogen contents were 0.88% and 0.045%, respectively. More details about the soil with full characterisation can be found in Table S1.

The rock mining residues were sourced from Victoria in Australia (Cohuna and Carisbrook) and comprise of both basalt and granite. The rock was ground with a pug mill and sieved to  $<90\mu\text{m}$ . The p80 of the rock was  $50\mu\text{m}$  (80% of particles with a diameter less than specified size). Data on X-ray diffraction and full acid digestion followed by inductively coupled plasma (ICP)—mass spectrometry (method ME-MS61 using perchloric, nitric, hydrofluoric and hydrochloric acids) and particle size using a Mastersizer 2000 (Malvern Panalytical, Malvern, UK) are shown in Table 1 and full particle size distribution of both materials in Figure S1.

TABLE 1 Characterisation of rock sample.

Mineralogy	(%)
Amorphous	19.1
Quartz	16.3
Plagioclase	34.4
K-feldspar	5.7
Clinopyroxene	10.4
Olivine	2
Nepheline	1.2
Analcime	1.3
Apatite	1.3
Kaolinite	0.2
Mica	4.4
2:1 clay (Chlorite, vermiculite or smectite)	3.7
Elemental content	(%)
Al <sub>2</sub> O <sub>3</sub>	13.4
BaO	0.04
CaO	4.67
Cr <sub>2</sub> O <sub>3</sub>	0.05
Fe <sub>2</sub> O <sub>3</sub>	8.12
K <sub>2</sub> O	2.65
MgO	4.13
MnO	0.10
Na <sub>2</sub> O	3.24
P <sub>2</sub> O <sub>5</sub>	0.42
SiO <sub>2</sub>	60.9
SrO	0.05
TiO <sub>2</sub>	1.41
Particle size	(%)
<90	96.5
<70	89.3
<50	80.1
<10	29.6
pH (in water)	9.2
Inorganic carbon (%)	0.05

## 2.2 | X-ray diffraction (XRD) of rock sample

To analyse the mineral composition of the crystallised phases within, the rock was pre-ground and sieved to <90 µm, then spiked with 20wt% Al<sub>2</sub>O<sub>3</sub> (Baikalox polishing corundum) and manually ground finely in an agate mortar in acetone before analysis via XRD. The suspension was pipetted on low-background holders (quartz) and dried. Powder X-ray diffraction analysis was carried out with a Malvern Panalytical Empyrean Series 3 diffractometer that was equipped with Bragg-Brentano<sup>HD</sup> divergent beam optic and a PIXcel<sup>3D</sup> detector (1D scanning mode, 3.347° active length), using CoKa radiation.

## 2.3 | Soil incubation/wheat plant trial

The soil was sieved to <10 mm to remove large root and other plant structures. Soil or crushed rock-soil mix (1.2 kg total) was filled into round pots 11 cm diameter and 11 cm high with drain holes. Crushed rock was premixed with the soil in ziplock bags at a rate of ca. 4% w/w (equivalent to 50 t ha<sup>-1</sup>). After watering, one pregerminated wheat seedling (*Triticum aestivum*, Condo variety) was planted into the centre of each pots (half of the pots were planted).

A full factorial design with treatments of rock/no rock, wheat/no wheat and low/high water was used with eight pot replicates (64 pots in total). No fertiliser was applied, and the pots were kept in a growth chamber in a randomised block design for ca. 6 months simulating the diurnal and seasonal light and temperature regime of central New South Wales, Australia, from which the soil originates (temperature profile of ca. 8–10°C at night and ca. 18–25°C during the day). Pots were watered with predetermined amounts of tap water depending on high- and low-water treatments. The high-water treatment received 100 mL of water three times a week and the low-water treatment 100 and 50 mL water each week. In some weeks, lower/high watering was necessary depending on plant growth stage resulting in 7 and 4 L of water in total for the high- and low-water treatments, which corresponds to ca. 740 and 420 mm precipitation over the 6 months. This is approximately equivalent to the annual lower and higher end of the precipitation in the area the soil and light/temperature regime were adapted from.

At the end of the ca. 6-month period, the plants and their roots were pulled out of the soil and partitioned into roots, shoots and head (seeds/grain), dried at 105°C and weighed. Centrifuge tubes (50 mL) were used to take soil samples from the area under the plant at harvest (3 cm deep core). The soil samples were dried in the oven at 40°C for 3 days and went through the soil analyses pipeline described below. A second soil sample was taken from the bottom of the pot and analysed for total and inorganic carbon to confirm the effects observed at the top of the pot.

## 2.4 | Soil analysis

### 2.4.1 | Carbon analysis

A soil subsample (ca. 400 mg) was ground, and the total contents of carbon and nitrogen were determined with a VarioMax 3000 (Elementar, Germany; peak anticipated N: 210 s, oxygen dosing time: 15 s, oxygen dosing: 70 mL min<sup>-1</sup>, furnace temperatures: 900, 900 and 830°C; helium as carrier gas). The inorganic carbon content was determined through soil acidification using 30% H<sub>3</sub>PO<sub>4</sub>, and the released CO<sub>2</sub> was directly measured with a Skalar Primacs (Skalar Analytical B.V., Breda, Netherlands).

## 2.4.2 | Three-pool soil carbon fractionation

Full details about the soil carbon fractionation that distinguishes free POM, AggOM and MAOM are described previously (Buss, Sharma, et al., 2021). In short: 10 g of soil was shaken with a total volume of 50 mL of deionised water. Wet sieving was performed using 70 µm sieves. From the fraction >70 µm, POM was separated from AggOM through density segregation, using sodium iodide adjusted to a density of 1.8 g cm<sup>-3</sup>. The samples were centrifuged at 2100 × g for 10 min, and POM and AggOM were separated by decanting the content of the tube onto a Whatman No. 2 filter paper. To remove sodium iodide residues, the AggOM fraction was washed with deionised water. The MAOM fraction (derived from the sieving step; <70 µm) was separated from the liquid fraction via centrifugation at 2100 × g for 30 min.

The total carbon content within each fraction was analysed using a combustion method (details above). The aqueous fraction was analysed for electric conductivity (EC) with a ProLab 5000 pH/EC meter (SI Analytics, Germany) and a suite of elements using ICP (details about ICP below). The elemental content determined in this fraction is referred to as 'water-extractable content' in the following.

## 2.4.3 | Two-pool soil carbon fractionation

The AggOM fraction comprises both occluded POM, protected from decomposition, and aggregated clay and silt particles that contain sorbed carbon (MAOM), sourced from either plant exudates or residual POM decomposition (Figure S2). To further separate AggOM into occluded (and free) POM and MAOM, so to investigate whether indeed carbon was occluded in aggregates or whether it was only sorbed to the extra mineral surfaces provided by the weathered rock, a second fractionation based on previous work (Cotrufo et al., 2019) with some modifications was applied on a subset of samples (no rock-no plant; rock-no plant; and rock-plant; all low-water treatments).

A solution with 0.5% hexameta phosphate was prepared, and 30 mL added to 50 mL centrifuge tubes that contained 2.5 g of soil and two glass beads. The tubes were shaken at 150 rpm for 18 h and then sieved through 70 µm sieves (free and occluded POM). The MAOM fraction was subsequently separated from the aqueous fraction through centrifugation at 2100 × g for 30 min. The carbon contents in the free and occluded POM, MAOM and aqueous fraction (DOM) were analysed.

## 2.4.4 | ICP-optical emission spectroscopy (ICP-OES)

The aqueous fractions recovered from the fractionation described under Section 2.4.2 were analysed with an ICP-OES 5110 (Agilent, Santa Clara, CA, USA) for 20 elements. The ICP multielement standard solution Intelliquant Nos. 1 and 2 from Agilent were used for calibration using the following concentrations: calibration blank, 0.01,

0.05, 0.1, 0.01, 1, 5, 10 and 50 mg L<sup>-1</sup>. The 1 pp standard was used as internal quality control.

## 2.4.5 | Soil aggregation test

Soil aggregation was tested on a subset of samples (no rock-no plant; rock-no plant; and rock-plant; all low-water treatments). The goal was to investigate changes in aggregate size classes because of rock and plant addition that explain the elevated AggOM contents. To determine soil aggregates, seven sieves (sizes: 1000, 500, 400, 300, 200 and 70 µm; pluriStrainer, pluriSelect, Leipzig, Germany) were stacked on 50 mL centrifuge tubes. Suction was applied with a syringe to facilitate filtering of 1 g of soil and 500 mL of water through the sieves. Subsequently, the sieves were dried and weighed. The amount of soil in the <70 µm fraction was determined by the difference in soil mass.

## 2.4.6 | Soil pH measurement

The soil pH was measured by shaking 1.5 g of soil with 30 mL of either deionised water or 0.01 M CaCl<sub>2</sub> in 50 mL tubes at 150 rpm for 1 h. Tubes were left to settle for 20 min before the pH was measured with a ProLab 5000 pH/EC meter (SI Analytics; Germany).

## 2.5 | Plant elemental content

Representative samples of grain, stem and roots (250 mg) were digested in 9 mL concentrated HNO<sub>3</sub> and 1 mL H<sub>2</sub>O<sub>2</sub> in a microwave digester (Milestone ETHOS UP) at 210°C and 1800 W for 35 min. Samples were subsequently diluted to 2% HNO<sub>3</sub> and analysed via ICP-OES (as described above).

## 2.6 | Microbial composition via shotgun metagenomics

Full details about the method to determine microbial composition were described previously (Buss, Sharma, et al., 2021). In short: a commercial DNA extraction kit (DNeasy PowerSoil Pro Kit, Qiagen, Hilden, Germany) was used for extracting DNA from the dried soil and the samples were barcoded with the 'Native Barcoding kit' (Oxford Nanopore Technology, Oxford, UK) and run on a Flongle flow cell (Oxford Nanopore Technology, Oxford, UK). Data were basecalled and demultiplexed with Guppy (version: 5.0.7; Oxford Nanopore Technology, Oxford, UK, and all short (<200 bp) and low-quality (<q7) sequences were removed with NanoPack (De Coster et al., 2018).

Sequences were blasted against the NCBI nucleotide database version 5 (Sayers et al., 2021). The taxonomic ID for the single best blast hit per sequence was extracted. Sequences without a match



and operational taxonomic units (phylum, class or order) that were only detected once in a sample were excluded. Sequences matching an operational taxonomic unit (phylum, class or order) observed in less than eight samples (each treatment had eight replicates) were also filtered out.

## 2.7 | Data analyses/statistics

The data from this trial are openly available ([dataset] Buss et al., 2023a, 2023b). Statistical analyses were conducted in R (2022.12.0) and most visualisations in SigmaPlot (SYSTAT Software Inc., San Jose CA, USA). Analysis of variance and Tukey post hoc tests were conducted in R using the *aov* and *TukeyHSD* functions.

Microbial data were processed with Analysis of compositions of microbiomes with bias correction (ANCOM-BC) on phyla, class and order level using the ANCOMBC2 package in R (Lin & Peddada, 2020). The method normalises the data and checks for statistical differences based on treatment effects. In addition, the row microbial count data (on phylum, class and order level) were used for clustering using Non-Metric Multi-Dimensional Scaling (NMDS; vegan package; metaMDS; Bray–Curtis dissimilarity matrix), and NMDS 1 and 2 were subsequently plotted. PERMANOVA (vegan package in R) was used to check for significant differences as a result of rock, plant and water treatments.

## 3 | RESULTS

### 3.1 | Rock weathering, soil minerals and plant uptake

The inorganic carbon content was elevated as a response to rock addition. This elevation was statistically significant ( $p=.01$ ) but marginal: a 16% relative change or 0.00005% absolute change per weight of soil at a depth of 3 cm (Figure 1a). However, rock dust addition substantially increased soil pH and EC immediately after application to soil (before water addition to the pots; blue—rock dust baseline in Figure 1b,c) and this effect persisted throughout the incubation (significant rock effect on pH and EC). EC decreased over time from the baseline values in both the soil-only and rock-amended treatments. Both plants and high-water treatment accelerated this EC decrease (Figure 1c). Furthermore, there was a statistically significant increase in soil inorganic carbon at the bottom of the pots by 46% with rock addition ( $p=.00000048$ , Figure 1a) that corresponds to an absolute increase of 0.0002% inorganic carbon. This was further accentuated in the presence of a plant ( $p=.001$ ).

Water-extractable Ca and Mg contents in soil increased significantly after rock addition as expected for weathering, but the presence of wheat eliminated this and decreased water-extractable Ca and Mg down to values comparable to the no-rock control treatments (Figure 1d,e). Rock addition also increased the ammonium acetate-extractable (exchangeable) Ca and Mg contents, and the levels

were significantly higher after the incubation for both planted and unplanted treatments (Figure 1g,h). There was clearly an initial peak of Ca and Mg release of fresh rock-sand samples, but we could not detect associated changes with any bi-(carbonate) levels in water extractions (data not shown), predicted as part of alkalinity release during rock weathering. In contrast to the water-extractable contents of Ca and Mg that decreased over the course of the incubation compared with the baseline value (blue line in Figure 1d,e), the ammonium acetate-exchangeable content increased (Figure 1g,h). The average 40% increase in soil-exchangeable Ca is highly significant across the rock treatments ( $p$ -values  $<.0001$ ). For Mg only, the two unplanted treatments were significantly higher than the rock-sand baseline (low water:  $p=.00003$ ; high water:  $p=.00029$ ). The water-extractable Si content did not change as a result of rock addition (Figure 1f), but the exchangeable content increased by three–four-fold (Figure 1i).

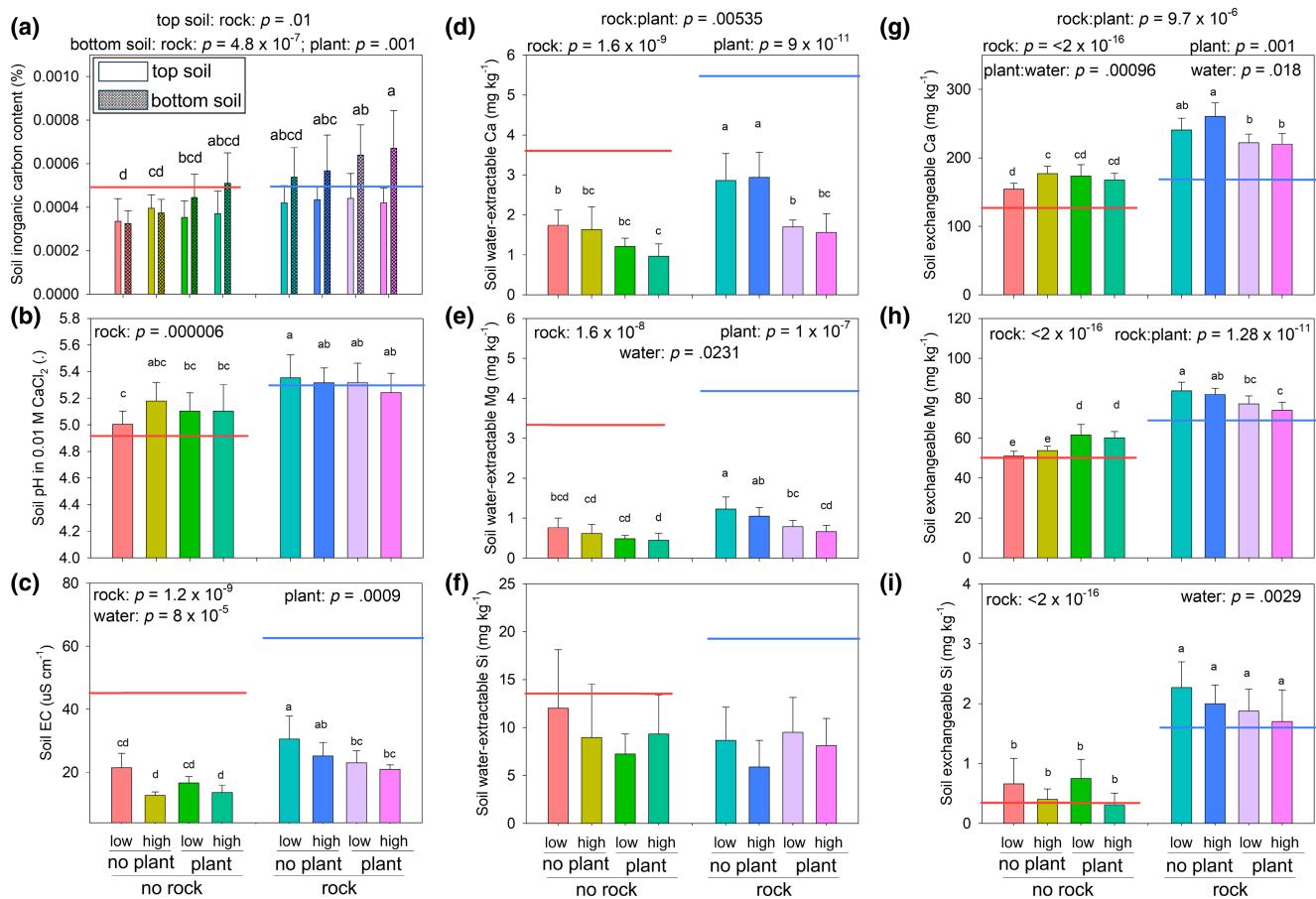
There was no overall effect of rock addition on plant biomass or the individual plant parts (Figure S3) and no effect on uptake (total mass) of Ca and Mg into plant tissue (Figure S4), but the plant grain Ca content (mg Ca per kg plant biomass) and total Ca uptake into grain (mg Ca per plant) significantly increased because of rock addition by 28% and 34%, respectively ( $p=3.9 \cdot 10^{-6}$ ; Figures S4 and S5). Plant tissue contents (stem and root) and total uptake of Si in plant tissue increased significantly by 33% (stem content), 14% (root content) and 27% (total uptake) due to rock application (Figures S4 and S5).

Rock amendment significantly decreased soil-exchangeable contents, plant tissue levels and plant uptake of micronutrients Mn and Zn (Figure 2). The rock treatment did not significantly reduce exchangeable Fe levels (Figure 2a3), yet within the rock treatments, plant addition decreased exchangeable Fe (Figure 2a3) and Zn (Figure 2a2). Rock significantly decreased the content and total uptake of Fe into grains (Figure 2c3).

### 3.2 | Soil carbon content and three-pool soil carbon fractionation

The total soil carbon content decreased over the course of the incubation in all treatments (red/blue lines in Figure 3a). The rock-amended soils started with a slightly lower soil carbon content (blue line) than the control (red line) because the mass of rock addition diluted the soil carbon content (rock 0.075% C; soil 0.88% C).

By the end of the experiment, rock addition led to a 16% higher total carbon content across all treatments (rock effect:  $p=.00007$ ; Figure 3a) and an even 32% higher content when only the non-planted treatments are considered. Plants partially counteracted the rock effect by decreasing soil carbon content (rock: plant effect:  $p=.00006$ ). Overall, the three treatments and their interactions explained 56% of the variance in soil carbon content of which 35% was explained by rock, 5% by water, 9% by plants and 19% by rock: plant interactions. The significant effects of rock and rock: plant interactions were confirmed in soil samples taken from the bottom of the



**FIGURE 1** Soil indicators for weathering of Ca- and Mg-rich silicate rocks at the end of 6-month incubation. (a) Inorganic carbon content, (b) pH, (c) electric conductivity (EC) and water-extractable (d) Ca, (e) Mg and (f) Si contents and ammonium acetate-extractable (exchangeable) (g) Ca, (h) Mg and (i) Si contents. Soil was analysed at the end of a 6-month incubation study using a full factorial design with high/low water, unplanted/planted soil and soil only/rock addition. Red and blue lines indicate baseline values from the soil at the start of the trial without and with rock addition (ca. 4%). Different letters indicate significant differences among the treatments determined via Tukey post hoc test.

pot (Figure S6). These soil samples also demonstrated a lower soil carbon content at the bottom of the pots (0.59% C) compared with the top (0.69% C) across all treatments.

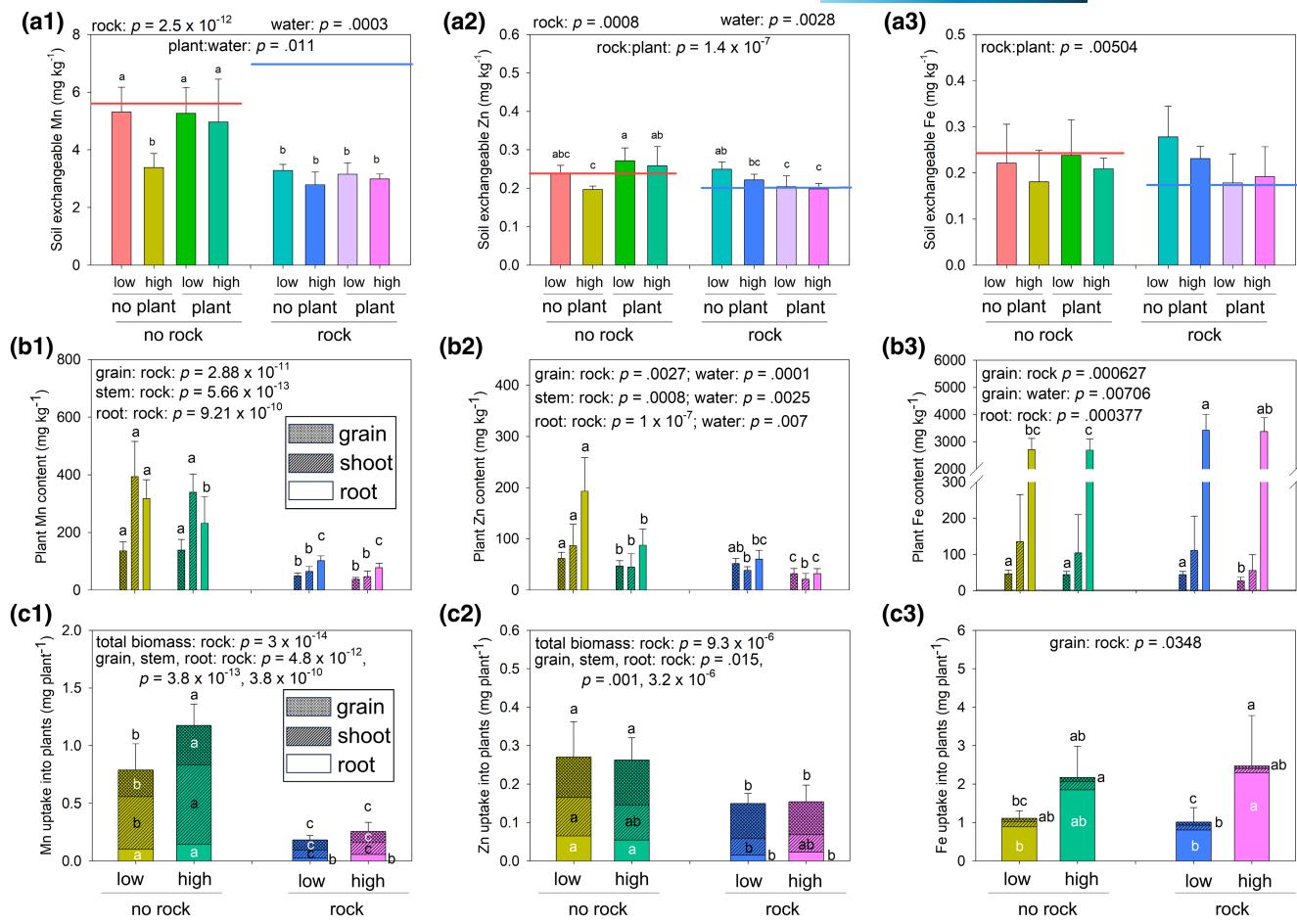
We next conducted a three-pool soil fractions to separate labile (free) POM, mineral and aggregate components (Figure S2). The carbon content associated with the free POM fraction decreased drastically over the course of the trial in all treatments from ca. 0.3% carbon per amount of soil in the baseline (red/blue line) to ca. 0.1% (Figure 3b). None of the treatments affected the free POM loss. Rock addition significantly increased C associated with AggOM by 25% over the nonamended control and by a massive 46% when only the nonplanted treatments are considered (Figure 3c). Plants counteracted the effect of rock amendment on AggOM (rock: plant effect;  $p = .0002$ ). The carbon content associated with the MAOM fraction increased by 22% because of rock addition and by 32% when only the nonplanted treatments are considered (Figure 3d). Rock amendment significantly increased the amount of soil recovered in the  $<70\text{ }\mu\text{m}$  fraction (Figure S7a) but

decreased the concentration of carbon within the MAOM fraction by 14% ( $p = .0002$ ; Figure S7b).

High-water treatment resulted in significantly lower soil carbon levels than low-water treatment ( $p = .00148$ ; Figure 3a). Water treatment only affected the carbon content associated with MAOM fraction (Figure 3d;  $p = .002$ ). This loss of carbon was not associated with a lower carbon content within the MAOM fraction (Figure S7b), but instead with less soil recovered in this fraction (Figure S7a; water effect:  $p = .0006$ ).

### 3.3 | Two-pool soil carbon fractionation and soil aggregates on a subset of samples

Using a two-pool soil carbon fractionation technique that ensures full disaggregation (and hence separation of the AggOM pool into MAOM and free plus occluded POM) on a subset of samples (Figure 4a; schematic Figure S2), both planted and unplanted rock



**FIGURE 2** Micronutrients Mn (1), Zn (2) and (3) Fe in plant and soil at the end of 6-month incubation. (a) Ammonium acetate-exchangeable contents for soils in all eight treatments, and (b) plant tissue contents of grain, shoots and roots and (c) total plant uptake in grain, shoot and roots for the planted treatments. Red and blue lines indicate baseline values from the soil at the start of the trial without and with rock addition (ca. 4%), respectively. Main effects determined using one-way ANOVAs (results shown at the top of each figure). Different letters indicate significant differences among the treatments determined via Tukey post hoc test.

treatments significantly increased the carbon content associated with the MAOM fraction by 18 and 24%, respectively, compared with the no-rock treatment. Rock addition also increased the POM fraction that includes both occluded and free POM (Figure 4a1;  $p=.006$ ), but only the unplanted, rock-amended treatment was different to the control ( $p=.005$ ; Figure 4a1). The aqueous fraction that is used to extract the soil, contained DOM in the range of 0.12–0.15% carbon per unit of soil (Figure S8). There was a marginally statistically significant increase in the DOM pool in the rock, non-planted treatment compared with the control treatment (ANOVA:  $p=.049$ ; Tukey:  $p=.046$ ).

In the same subset of samples, we analysed soil aggregation within aggregate size classes of  $<70$  to  $>1000\text{ }\mu\text{m}$  (Figure 4b). There was a statistically significant increase in microaggregates of size  $70\text{--}200\text{ }\mu\text{m}$  from 14.1% in the control to 22.2% in the unplanted, rock-amended (Tukey post hoc test:  $p=.004$ ). Plants reduced the percentage of microaggregates to 14.2%, fully counteracting the increase in microaggregation induced by rock addition (Figure 4b).

### 3.4 | Soil DNA and microbial composition

The extractable soil DNA content was significantly higher in rock-amended treatments compared with unamended treatments (Figure S8a). The DNA content decreased over the course of the trial compared with the baseline indicating that soil DNA was lost. There was a significant correlation between soil DNA and soil carbon content (Figure S8b).

The total number of continuous DNA fragments (reads) extracted and sequenced per sample was between 4000 and 50,000 with an average read length of 600–3000 base pairs (Table S3), generating  $>1\text{Gb}$  of sequence. The abundant microbial high-level taxonomic groups detected via shotgun metagenomics and long-read sequencing did not change significantly as a result of rock addition as shown via clustering (Figure S10b) and percentage composition (Figure S11). We also did not detect specific changes due to basalt or plant effects on individual microbial taxa (Table S4). Water treatment, however, significantly affected clustering of the samples based on phylum, class and order level (phylum level clustering in

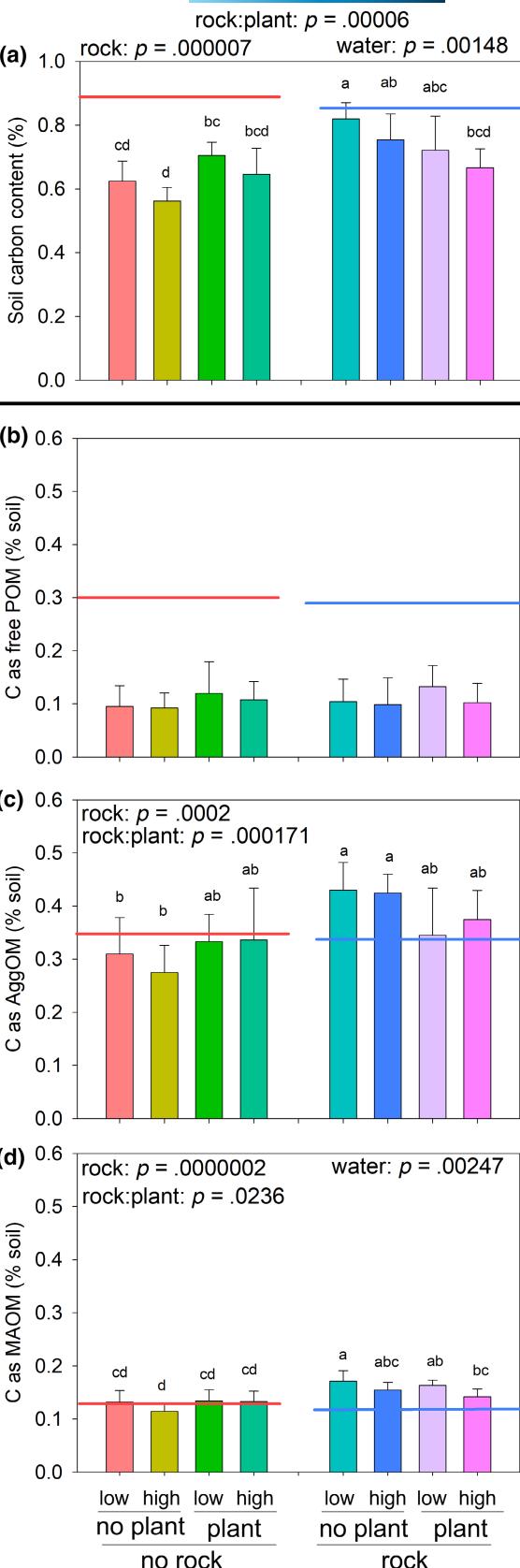


Figure S9a (PERMANOVA results in Table S5). There were also significant effects within the microbial taxa due to water treatment at the class and order level (Table S4).

**FIGURE 3** (a) Total soil carbon content and (b-d) three-pool soil carbon fractionation data at the end of 6-month incubation. (b-d) Show carbon associated with (b) particulate organic matter (POM), (c) aggregate organic matter (AggOM) and (d) mineral-associated organic matter (MAOM). A full factorial design of high/low water, unplanted/planted soil and soil only/rock addition was used. Red and blue lines indicate baseline values from the soil at the start of the trial without and with rock addition (ca. 4%), respectively. Main effects determined using one-way ANOVAs (results shown at the top of each figure). Different letters indicate significant differences among the treatments determined via Tukey post hoc test.

### 3.5 | Associations of soil properties with SOM fractions and SOM transformations

There was a highly significant correlation between soil-exchangeable Ca and Mg contents and soil carbon content ( $p=2.4 \times 10^{-7}$  and  $p=2.4 \times 10^{-10}$ ; Figure 5a,d) and a significant correlation with Fe content ( $p=.029$ ; Figure 5g). Exchangeable Ca and Mg contents also correlated highly significantly with both carbon in AggOM (Figure 5b,e) and MAOM (Figure 5c,f). Exchangeable Fe only correlated highly significantly with carbon as AggOM ( $p=.005$ ; Figure 5h).

The schematic in Figure 6a shows the effects of rock and rock-plant interactions on soil carbon of different stability compared with the soil-only baseline and is based on Figure 3b-d. Low- and high-water treatments were pooled to focus on plant and rock effects. Carbon associated with POM was reduced in all treatments compared with the baseline. However, depending on the treatment, the carbon was either lost or converted into different soil carbon fractions (Figure 6a). In the no-rock treatments, free POM was lost over time without any conversion into AggOM or MAOM (C content as AggOM and MAOM same as in the baseline). In the rock-amended treatment, some free POM was instead transformed to MAOM and AggOM. In the nonplanted, rock-amended treatment 12% of POM was lost and 19% in the treatment with plant. Hence, the rock-amended, nonplanted control retained 23% of SOM lost in the control sample in the form of more stable soil carbon fractions.

## 4 | DISCUSSION

### 4.1 | Inorganic carbon sequestration

Previous rock weathering studies have found it is challenging to directly detect changes to soil inorganic carbon content, and therefore, typically proxies are used to assess rock weathering and associated drawdown of atmospheric CO<sub>2</sub>. Such proxies include Ca and Mg mass balance approaches based on both pore water or ammonium acetate-extractable cations and changes in pH and EC compared with a nonamended control (Amann et al., 2020; Amann & Hartmann, 2022; Kelland et al., 2020; ten Berge et al., 2012). In this study, we found elevated levels of water-extractable and exchangeable Ca and Mg contents and pH and EC levels in soil 6 months after rock addition. However, water-extractable Ca and Mg did not

**FIGURE 4** (a) Two-pool soil fractionation data and (b) soil aggregates in a subset of samples at the end of 6-month incubation. Treatments: no rock without plant, rock with and without plants (all low-water treatments). (a) Soil carbon fractionation using hexameta-phosphate extraction for full soil disaggregation at the end of a 6-months incubation. Carbon associated with the (a1) free and occluded POM fraction and (a2) MAOM fraction. Main effects determined using one-way ANOVAs (results shown at the top of each figure). Different letters indicate significant differences among the treatments determined via Tukey post hoc test. (b) Soil aggregate classes. For statistical analysis (ANOVA, followed by Tukey's post hoc test), data were centred log-ratio-transformed.

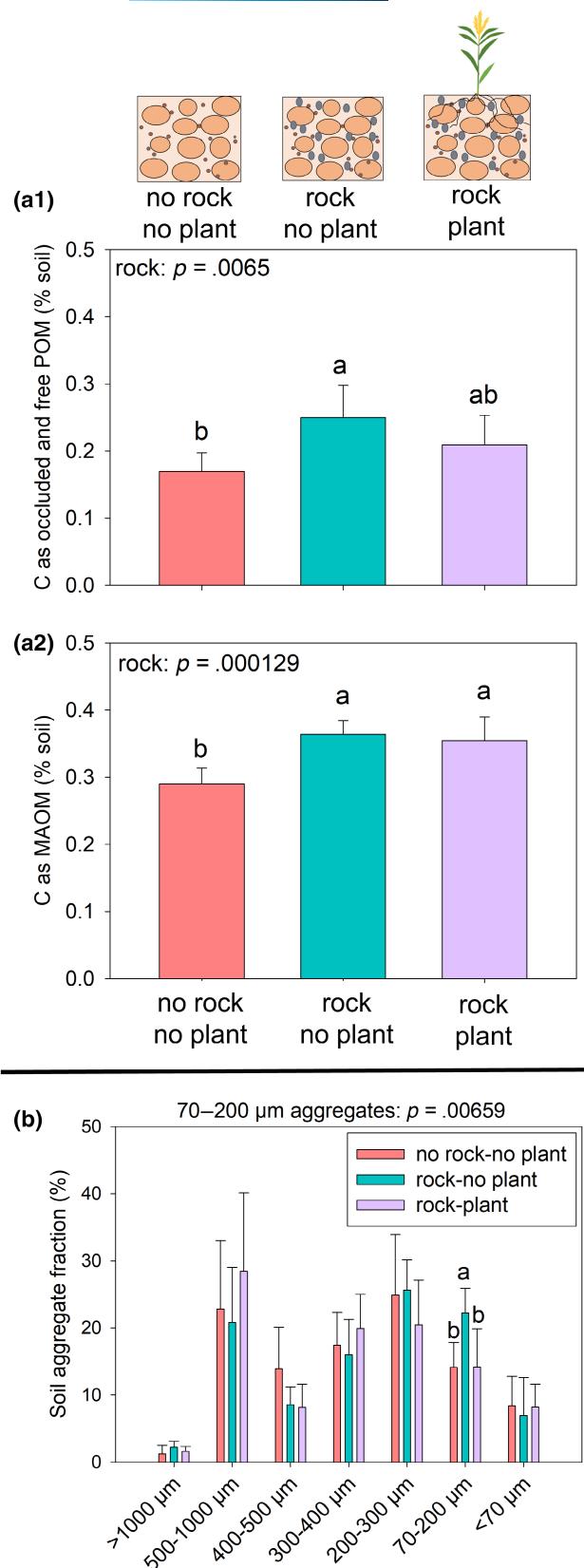
indicate additional weathering of our mining residues since the levels peaked at the start of the incubation (fresh rock-soil mix) and this peak was not associated with any (bi)carbonate formation (product of Ca- and Mg-rich silicate reaction with carbonic acid). Our mining residues had preweathered and contained 19.1% amorphous material and 3.9% secondary minerals, which likely contained cations that were only sorbed to mineral surfaces and were released without reacting with carbonic acid. These readily available cations would be responsible for this initial Ca and Mg release. Preweathering eliminates the Ca and Mg mass balance approach as proxy for new carbon drawdown and may be unsuitable for mining residues, at least if water extraction or pore water values are used.

There is some evidence for rock weathering based on exchangeable Ca and Mg contents, which were higher at the end of the trial compared with the rock-soil baseline. Importantly, there is also extra inorganic carbon detected in the soil layer at the bottom of the pot at the end of the incubation. This carbon likely accumulated as a result of bicarbonate formation and subsequent carbonate precipitation due to altered chemical conditions at the bottom of the pot. The presence of a plant increased inorganic carbon formation confirming our hypothesis that plants increase rock weathering. Across the four treatments (low/high water and no plant/plant), the average extra carbon after rock addition corresponds to  $0.005\text{t CO}_2 \text{ha}^{-1}$  at a soil bulk density of  $1.2\text{ g cm}^{-3}$  and assumed accumulation of extra inorganic carbon in a layer of 0.05 m (ca. half the pot). Within the various studies measuring and calculating carbon drawdown from Ca- and Mg-rich silicate application, this value is at the low end of the range (Kelland et al., 2020).

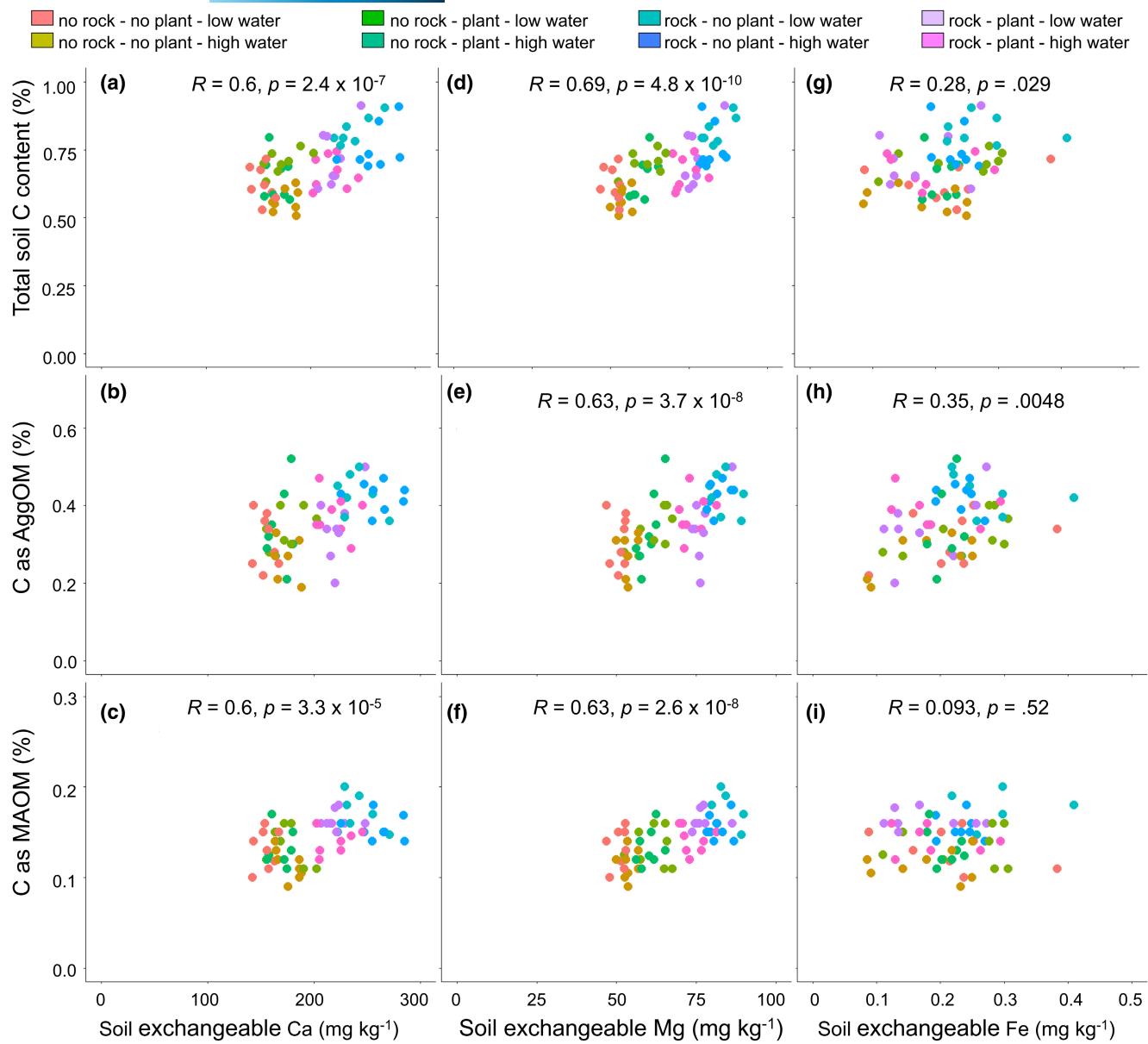
Only around half of the rock was composed of basalt and the percentage of the fast-weathering mineral olivine was only 2%, which expectantly did not result in a strong weathering signal at an application rate of  $50\text{ t ha}^{-1}$ . Given the large range of weathering rates and associated carbon drawdown rates in the literature using different measurement techniques, it is clear that the method for determining weathering rates needs refinement. Other indirect weathering effects, as presented here for SOM, do show potential.

## 4.2 | Rock addition increases organic carbon protection

POM in sandy soil has little protection from decomposition, and subsequently, 2/3 of the POM in the baseline soil was lost after 6 months



of incubation under growing conditions (Figure 6a). If leaching was a major factor of this loss, we would expect soil carbon at the bottom of the pot to be either equal to, or greater than, soil carbon in the top 3 cm. Given that the soil carbon content at the bottom of the pot was



**FIGURE 5** Relationship between soil-exchangeable contents and soil carbon (fractions) at the end of 6-month incubation. Exchangeable (a–c) Ca, (d–f) Mg and (g, h) Fe with (a, d, g) total soil C content, (b, e, h) C associated with AggOM and (c, f, i) C associated with MAOM. Treatment labels are no rock/rock – no plant/plant – low/high water. Pearson correlation coefficient and *p*-value shown, respectively.

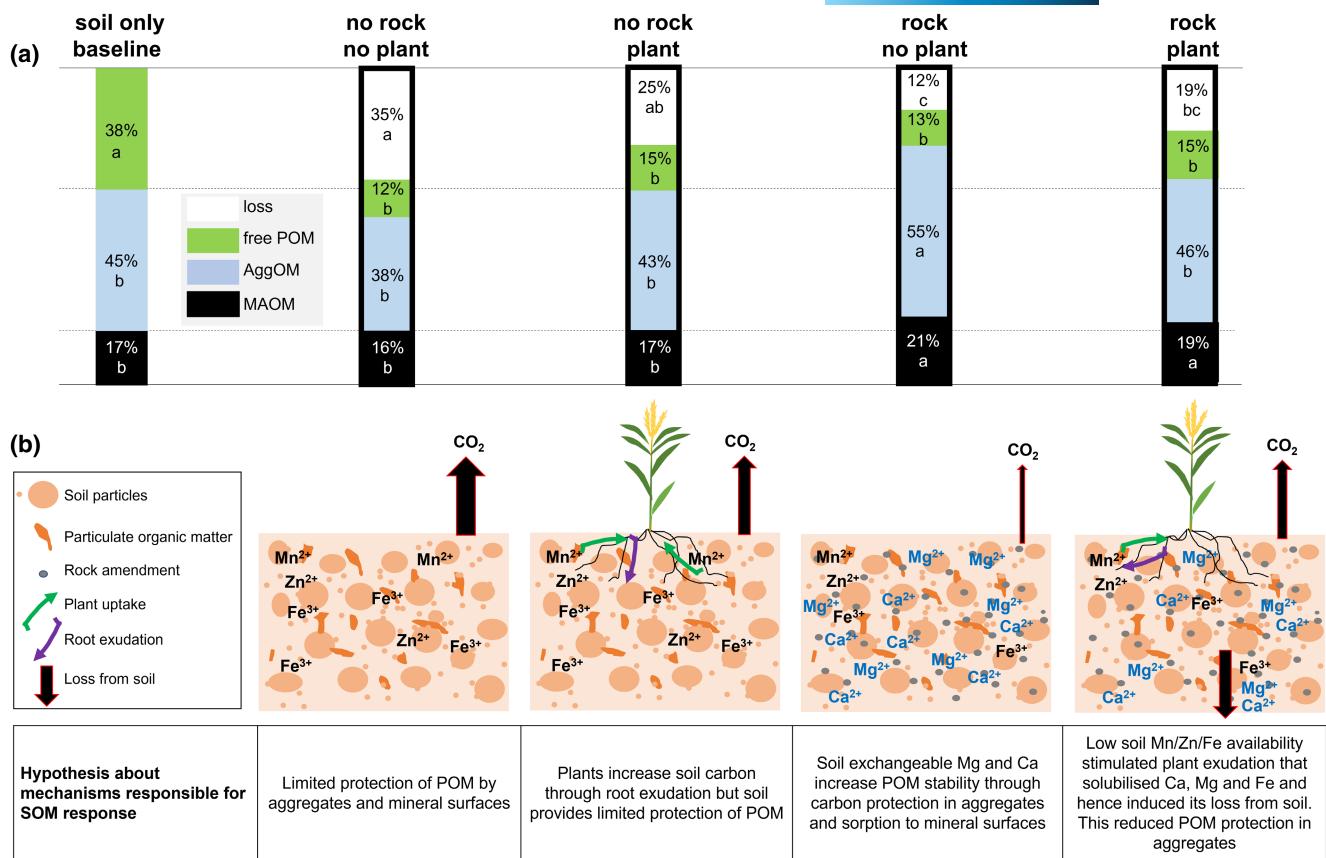
lower than at the top of the pot and that watering treatment only explained a small proportion of the variance in soil carbon (5%) at the top of the pot, it is unlikely that the carbon was lost through leaching and was instead converted into  $\text{CO}_2$ .

Rock dust addition decreased SOM losses by transformation of POM into more durable soil carbon fractions, and the effects were linked to changes in soil chemistry. We failed to detect substantial abundance or composition shifts in the microbiome as a result of rock addition (Figure S9). Our microbiome assay using shotgun, long-read sequences via Oxford Nanopore Technology is robust and can detect differences among treatments in environmental samples (Hamner et al., 2019; Loit et al., 2019; Petersen et al., 2020). Our study shows that water treatment did have an effect on microbial composition as expected. The soil response to rock addition can,

however, be explained by three main chemical changes that fostered SOM protection.

First, our rock mining residues were clearly preweathered (Table 1); therefore, the rocks provided secondary silicate minerals immediately after application that were able to sorb DOM directly. The concentration of carbon within MAOM of these rock-amended soils was lower than their no-rock counterparts, which suggests potential for further carbon sorption and hence MAOM formation with continuing rock weathering.

Second, rock provided Ca and Mg that are key cations involved in polyvalent cation bridging and associated MAOM formation, along with Fe and Al. Ca and Mg mostly operate in soil at neutral pH and Fe and Al in acidic conditions (Rowley et al., 2018; Singh et al., 2018). Our baseline soil pH was slightly acidic with a pH of 5.7 in water and



**FIGURE 6** Summary of soil (carbon) responses to rock and plant additions relative to the soil baseline. (a) Conversion of SOM fractions in the baseline scenario (soil before trial) into other soil carbon fractions based on data in Figure 3 (low- and high-water treatments pooled together,  $n=16$ ). Letters show significant differences among the treatments. (b) Schematic summary of the key results.

4.9 in 0.01 M CaCl<sub>2</sub>, which increased to 6.2 in water (data not shown) and 5.2 in 0.01 M CaCl<sub>2</sub> at the end of the incubation across all treatments. At this pH range, both groups of cations are similarly important in cation bridging, yet given our correlation analysis (Figure 5), exchangeable Ca and Mg seemed to play a more important role in MAOM formation in our case.

Third, the results from both fractionation assays showed that rock clearly increased SOM occluded within microaggregates. This increase in microaggregates and associated AggOM can be explained by the supply of available Ca and Mg by the rock (Figure 6b), which facilitated the formation of soil aggregates and carbon protection (Baldock, 1989; Clough & Skjemstad, 2000; Rowley et al., 2018; Totsche et al., 2018). Our strong correlation between soil-exchangeable Ca and SOM content (Figure 5a) has also been seen in previous studies (Rasmussen et al., 2018; Rowley et al., 2018). In our trial, rock addition protected an extra 17% (Figure 6; or ca. 0.15% C in absolute values (Figure 3)) of soil carbon within AggOM that was lost without rock addition. This effect could play a significant role in protection of POM from decomposition, in particular in soil with low degree of aggregation and soil-exchangeable Ca and Mg levels.

Overall, the soil carbon content after 6 months of incubation was 32% higher after rock amendment, equivalent to an absolute increase of 0.2% per weight of soil, compared to the no-rock control. At a soil bulk density of 1.2 g cm<sup>-3</sup> and a soil depth of 0.1 m (depth of

rock dust incorporation), this corresponds to 2.4 t of extra carbon or 8.8 t of CO<sub>2</sub> stored/stabilised per hectare. While these results so far are only valid for sandy soils of similar chemistry and cannot be extrapolated to larger areas globally, it does clearly demonstrate the potential of ground rock application for sequestering additional organic carbon. Plants, however, partially counteracted this effect.

### 4.3 | Plant counteraction of protection of carbon in aggregates due to micronutrient deficiency

In the absence of rock, plants increased the soil carbon content (Figure 3). However, under the altered chemical conditions after rock amendment, plants reduced the protection of SOM in aggregates (Figure 6b). We suggest this is linked to changes in pH and exchangeable cation levels initially induced by rock addition and a plant response to counteract this effect.

Rock addition increased soil pH by ca. 0.2 units in a range (5–6.5) where any pH rise exponentially decreases the availability of the plant micronutrients Mn and Zn through an increase of adsorbed Mn and Zn to soil surfaces (Basta et al., 2005; Blume et al., 2016). This can substantially affect plant micronutrient supply to plants (Blume et al., 2016). The dramatic drop in soil-exchangeable micronutrient levels in our study after rock addition, as also observed for Mn in a

previous study after basalt application (Anda et al., 2015), indeed resulted in lower plant uptake (Figure 6b). We hypothesise that because of this reduced soil micronutrient availability, root exudation of organic acids was altered.

Plants, including wheat, can increase organic acid exudation as a response to nutrient deficiency to solubilise micronutrients, such as Mn, Zn or Fe, to increase their availability and uptake (Awad et al., 1994; Cakmak & Marschner, 1988; Gherardi & Rengel, 2004; Li et al., 2018). Zn deficiency in various plant species, for example, increased root exudation by a factor of 2 on average (Cakmak & Marschner, 1988). Such exudates include oxalate, tartrate, L-malate, lactate, citrate and succinate (Gherardi & Rengel, 2004). This, however, can also accelerate the turnover of aggregates, that is, induce not only aggregate formation but also destruction (He et al., 2020; Ma et al., 2022; Wang et al., 2020) and break up MAOM.

Organic acids can strip polyvalent cations from their metalorganic ligand complexes that result in both loss of cations and carbon (Keiluweit et al., 2015; Li et al., 2018, 2021). Fe does play a particularly important role in the formation of microaggregates (52–250 µm; Lin et al., 2022; Xue et al., 2019), and hence, loss of Fe in addition to Ca and Mg can explain the reduced microaggregation and carbon in AggOM in our study. With a loss of Ca, Mg and Fe as mediators between clay surfaces and soil organic carbon, soil aggregation decreased and hence less soil organic carbon was stabilised.

This shows the complexity of the system and how soil chemistry changes can alter the effect of plants on SOM content, which in this case resulted in loss of soil carbon in rock amended soil. Addressing micronutrient deficiencies should avoid the effect plants had on soil aggregation and associated carbon contained within aggregates. Future studies should be designed to specifically investigate this hypothesis. The results presented here so far were only confirmed in pots that maximise root density and nutrient deficiency, and therefore, future studies should also investigate other conditions and cover field trials.

## 5 | CONCLUSION

We found some evidence that a blend of granite and basalt applied to a sandy soil weathered during a 6-month incubation, as demonstrated by soil-exchangeable Ca and Mg and inorganic carbon contents that were elevated compared with the baseline values. Rock addition further increased SOM stabilisation through the release of Ca and Mg and provision of secondary minerals. A growing wheat plant partially counteracted this effect, which we attribute to the potential release of plant root exudates induced by reductions in micronutrient levels, Mn and Zn, after rock addition and its associated pH increase. The loss of Ca, Mg and Fe that are typically involved in aggregate stabilisation, freed up carbon previously protected in aggregates. Still, the application of Ca- and Mg-rich silicates could be a valuable tool to stabilise SOM, particularly in sandy soil and when micronutrient deficiencies are addressed. This could substantially improve the carbon sequestration potential of ground rock application on agricultural land. Higher soil organic carbon levels can

have further soil and plant benefits, such as increasing nutrient and water retention. These findings could boost the economic and environmental attractiveness of enhanced rock weathering as a global method for carbon dioxide removal.

## AUTHOR CONTRIBUTIONS

**Wolfram Buss:** Conceptualization; data curation; formal analysis; investigation; methodology; project administration; visualization; writing – original draft; writing – review and editing. **Heath Hasemer:** Investigation; visualization; writing – review and editing. **Scott Ferguson:** Formal analysis; methodology; writing – review and editing. **Justin Borevitz:** Formal analysis; funding acquisition; supervision; writing – review and editing.

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## CONFLICT OF INTEREST STATEMENT

The authors declare to have no competing interests.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Dryad at <https://doi.org/10.5061/dryad.djh9w0w67> and NCBI at <https://www.ncbi.nlm.nih.gov/sra/PRJNA1036354>.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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