



RESEARCH ARTICLE

Impact of bare fallow management on soil carbon storage and aggregates across a rock fragment gradient

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Abstract

Background: Our understanding of C storage in soils lacks insights investigating organic matter (OM) depletion, often studied in bare fallow systems. The content of coarse rock fragments is often excluded, whereas it may affect C storage.

Aims: We aim to contribute to a better understanding of the impact of bare fallow on C storage mechanisms in the soil as influenced by its coarse rock fragment contents. We investigated whether bare fallow induced a depletion of C in OM fractions and analyzed to which extent this affected soil aggregate size distribution and the C loading of the clay-sized fraction.

Methods: A comparison of 14 years bare fallow management with adjacent cropped soils located in Selhausen (Germany) provided a gradient of coarse rock fragments of 34%–71%, from which sites with three different fine earth (FE) contents were compared. Across the FE gradient, we isolated particulate OM and mineral-associated OM fractions, obtained microaggregate and macroaggregate size fractions, and quantified the C loading.

Results: Bare fallow management induced an OM depletion at lower contents of FE. There, the management influence was more concentrated onto less FE volume. The contribution of both particulate and mineral-associated OM fractions to the C in the low-FE soils decreased. The C loading increased under bare fallow, compared to cropped soil. In the low-FE soil, we also found less macroaggregates, whereas the C content decreased in some microaggregate size fractions.

Conclusions: A high content of coarse rock fragments can enhance OM depletion decreasing mineral-associated and particulate C under bare fallow.

KEYWORDS

C loading, organic matter depletion, particulate and mineral-associated organic carbon, soil microaggregates

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1 | INTRODUCTION

Soils harbor most terrestrial C, which is about twice the amount of C in the atmosphere and vegetation combined (Scharlemann et al., 2014; Sulman et al., 2018). Soil organic matter (OM) has a critical role in soil ecosystem functions and soil health. As a result, much research has been conducted to investigate the accumulation of C in soils, with many studies focusing on the fate of OM addition (Abiven et al., 2009; Chenu et al., 2019; Paustian et al., 2019). However, there has been much less research investigating C storage mechanisms, related to the dynamic accumulation and decomposition of OM, when OM losses occur (Barré et al., 2018; Doran et al., 1998; Hirsch et al., 2017). A study at the Highfield conversion experiment at Rothamsted (UK) indicated that the decrease of OM after land use change of grassland was much greater than the increase in OM when introducing grassland systems (Jensen et al., 2020). Analyzing the impact of OM depletion can, therefore, provide a novel perspective on mechanisms underlying C storage beyond what is known from studies based on OM addition.

The inclusion of OM in soil structure contributes to C storage as shown by microaggregates $<250\ \mu\text{m}$ being more stable and having longer turnover times in comparison with macroaggregates $>250\ \mu\text{m}$ (Angers et al., 1997; Six et al., 2000; Trivedi et al., 2017). Soil structure, related to the interplay of pores and stable aggregates of different sizes, is an important factor of OM storage by regulating its spatial accessibility and protection within aggregates (Rabot et al., 2018). In turn, when the input of OM is inhibited, the OM in stable aggregates may be depleted likely leading to a breakdown of the soil structure. This was tested in the Rothamsted Highfield conversion experiment, where the conversion of a Chromic Luvisol under bare fallow with regular tillage into grassland without tillage was shown to affect porosity, pore connectivity, and pore surface density based on X-ray microtomography (μCT) assessment (Bacq-Labreuil et al., 2021; George et al., 2021). In the same experiment, the soil aggregate stability measured as dispersibility of clay-sized particles was shown to recover quickly upon grassland introduction. However, the soil aggregate stability degraded slower than the stored OM, which was lost more rapidly than gained (Hirsch et al., 2017; Jensen et al., 2020). This indicates that the interplay of OM depletion and the stability of soil aggregates may be decoupled to some extent after land use change. However, the influence of mechanical disturbance during regular tillage operations of bare fallow management is expected to affect C storage mechanisms, specifically within the particulate OM (POM) fraction (Lee et al., 2009). The consequences of OM depletion on soil aggregates and the interrelated OM storage under bare fallow management warrant further investigations using approaches with minimized influence of mechanical disturbances.

Coarse rock fragments $>2\ \text{mm}$ are routinely excluded from C stock calculations, which are focused on the C storage of the fine earth (FE) fraction in soils (Vos et al., 2019). However, higher proportions of coarse rock fragments may not only dilute the remaining fine particles but management effects may also be concentrated in a much smaller soil volume influencing OM storage in the fine earth (FE) fraction $<2\ \text{mm}$ to a higher extent. The proportion of coarse rock fragments

may thus affect the storage of OM in soils. It is therefore warranted to consider the proportion when conducting bare fallow experiments. The OM storage in the FE fraction is often related to the number of clay-sized particles (Dexter et al., 2008; Prout et al., 2022). The interaction of OM with the high specific surface area (SSA) of clay-sized and fine silt-sized fractions was shown to be an important factor for the OM storage in fine mineral particle fractions (Schweizer et al., 2021; Wagai et al., 2009). The C loading interrelates the amount of C with the mineral surface area of OM and may therefore be affected by OM depletion and different FE contents. Therefore, determining the C loading of fine particle fractions may provide further insights into whether mineral surface area and the association of C with fine mineral particles are involved in mediating the OM storage in soils that are affected by bare fallow and contain higher proportions of coarse rock fragments.

Here, we investigate a long-term experiment with a bare fallow treatment mostly based on herbicide application to induce OM depletion. The herbicide application was used to induce a removal of vegetation cover that allows to assess the effects of OM depletion on the C content stored in OM fractions and aggregate size fractions. Based on previous investigations (Bornemann et al., 2011; Meyer et al., 2017), we conducted a paired comparison of bare fallow and an adjacent cropped site across a rock fragment gradient with 34%–71% coarse fragments. To advance our understanding of C storage mechanisms and how these are influenced by the decreasing FE fraction when comparing topsoil under bare fallow and cropped management, we determined (1) the storage of C in particulate and mineral-associated OM (MOM) fractions, (2) analyzed the impact on C contained in soil aggregates, and (3) investigated the influence on the SSA and C loading of the clay-sized fraction.

2 | MATERIALS AND METHODS

2.1 | Field description

The soil sampling sites are located approximately 1 km east of Selhausen (Niederzier) in the Lower Rhine Embayment, Germany, with 104–107 m above sea level at 50.869336° (N) and 6.450797° (E) (WGS84). The climate at the site is part of the temperate maritime climate zone (Cfb Köppen) with a mean annual temperature of 10.2°C and an annual precipitation of 714 mm (Bogena et al., 2018). The site is part of the Terrestrial Environmental Observatories since 2011 (Zacharias et al., 2011) and has been under arable management for at least 100 years before (Bornemann et al., 2011).

There is a gradient of coarse rock fragments in the east–west direction of the site. Across the inclination of approximately 1.7° (at a maximum of 3.5° according to Bornemann et al., 2011; Meyer et al., 2017), fluvial deposits of Pleistocene gravel dominate the soils at the upslope east of the site with lower proportions of FE, whereas Aeolian Pleistocene Loess sediments dominate the downslope soils in the west with higher FE content. The soil type downslope was classified as Stagnic Luvisol, midslope as Orthic Luvisol, and upslope as Dystric Lep-tosols according to the World Reference Base for Soil Resources (WRB

2007) by Bornemann et al. (2010, 2011), where the heterogeneous site properties are described in further details.

We investigated paired comparisons between soils under cropped and bare fallow management. For the cropped management, the main crops in the previous years before sampling were *Triticum aestivum* L., *Zea mays* L., *Brassica napus* L., and *Hordeum vulgare* L., which are common crops in the region (Bogena et al., 2018). For the bare fallow management, glyphosate-based herbicides were applied regularly since 2005 as soon as plant growth was visually detected. In addition, tillage limited to the top 5 cm was applied annually between 2005 and 2010 (Meyer et al., 2017). Since the start of the bare fallow management, the soils were not limed. The adjacent soil under cropping was regularly amended approximately every 3–4 years with Carbokalk at a rate of approximately 4–5 t ha⁻¹. The last liming of the cropped soils was done approximately 3 years before sampling. The soils were sampled in November 2019 after 14 years of bare fallow management by soil pits from the Ap horizon. According to pedogenetic features, the Ap horizon was approximately 0–31 cm, of which the material 5–25 cm was sampled. For sampling, the material >5 cm depth was removed across a surface of 1 m² and consequently approximately 1.3 m³ of material from an equivalent volume was sampled up to a depth of 25 cm. We extracted two field replicates per treatment at three different topographic positions (Figure 1a).

Based on the different topographic positions with approximately 3-m difference across a slope length of 100 m, the erosion of soil upslope and its deposition downslope has shaped the soils, probably throughout its long-standing history under arable management. A previous study measured the ¹³⁷Cs activities after the fallout event of Chernobyl in 1986. Already at the start of the bare fallow management, there were lower ¹³⁷Cs activities upslope of approximately 4 Bq dm⁻³ indicating the loss of soil, compared to the midslope of approximately 5.5 Bq dm⁻³, and downslope indicating a deposition of soil, whereas the endpoint was again lower at approximately 4.5 Bq dm⁻³ (Bornemann et al., 2011). It is likely that the soil erosion under bare fallow might have been higher than the cropped management leading to a higher translocation of soil material from the upslope low FE to medium and high FE further downward. The site was inclined by approximately 1.7° over a length of 100 m, which means that an erosion rate of about 13 t ha⁻¹ would not be exceeded for a bare fallow site in Germany (Auerwald et al., 2009). This would correspond to an approximate loss of 1.3 cm over the 14 years under bare fallow and 0.5 cm at the cropped site. Previous tillage under arable management has overturned the material in the topsoil, based on the detection of the top 0–31 cm as Ap horizons. Since we sampled 5–25 cm, the deposition of approximately 0.8 cm of soil at the bare fallow, compared to cropped soil, probably led to a slightly higher sampling downslope. However, bare fallow tillage did not induce an incorporation into depths >5 cm, and the previous tillage had led to a homogenization of the top 31 cm. Therefore, the recent soil erosion probably has a minor impact on the paired comparison of organic carbon storage of bare fallow and cropped soils at similar topographic positions.

The differences between the bare fallow treatment and cropped soil are not limited to OM depletion but also include potential effects of

tillage, fertilization, pesticide application, and vegetation cover as well as potential changes in soil water retention, soil structure and pore size distribution, nutrient availability, soil temperatures, and soil fauna composition and activity. Since many of these differences are necessarily interrelated with the induced changes of OM input, our study is focused on how this affects C storage in OM fractions and aggregate size fractions. By comparing the bare fallow management with cropped management, our study complements previous studies of the bare fallow site (Bornemann et al., 2011) and the changes of C content over time (Meyer et al., 2017).

2.2 | Analyses of coarse fragments, FE content, and texture

The air-dried soil material was dispersed using pestle and mortar until it passed an 8-mm sieve and then a 2-mm sieve. The mass contribution of coarse fragments >2 mm ranged from 25% to 75% (Table S1). In turn, the FE fraction <2 mm decreased in an east–west direction from 66% downslope (high FE) via 55% midslope (medium FE) to 29% upslope (low FE; Figure 1b).

To determine soil texture, the FE fraction <2 mm was treated with 30% H₂O₂ to remove OM until no further oxidation could be observed. The excess H₂O₂ was removed in an oven at 60°C for 12 h with an additional 500 mL H₂O. The samples were sieved to assess the proportion of sand-sized particles 63–2000 μm. The fraction <63 μm was freeze-dried and 3 g thereof was mixed with 80 mL 0.0125 M Na₄P₂O₇. The content of silt-sized particles 2–63 μm and clay-sized particles <2 μm was determined by X-ray attenuation using a Sedigraph III Plus (Micromeritics). On average, the soil texture consisted of 22.0% sand-sized particles, 53.5% silt-sized particles, and 24.5% that is categorized as silt loam (Figure 1b).

2.3 | Quantifying carbon storage in OM fractions

To analyze the contribution of C and N stored in particulate and MOM fractions, we applied a combined density and size fractionation slightly modified according to Kölbl and Kögel-Knabner (2004). The term C storage is used in this study synonymously to the C content as well as the proportion and contribution of bulk soil C stored in OM fractions according to Don et al. (2023). After the addition of 3 Na₂WO₄ · 9WO₃ solution of 1.8 g cm⁻³, the floating organic particles were obtained as free POM (fPOM) and the fraction was isolated. The occluded POM (oPOM) was isolated using a step-wise sonication of 100 and another 400 J cm⁻³. We removed all oPOM after the first sonication step to minimize the potential dispersion of POM before sonicating another time to improve the recovery. The sum of both sonication steps amounted to 500 J cm⁻³ of applied ultrasound energy. The MOM fraction >1.8 g cm⁻³ was split further into size fractions of >20 μm by wet sieving and into 6.3–20, 2–6.3, and <2 μm using sedimentation. All POM and MOM fractions were washed with deionized H₂O using a pressure filtration until <1 μS cm⁻¹. The bulk C and N contents and the

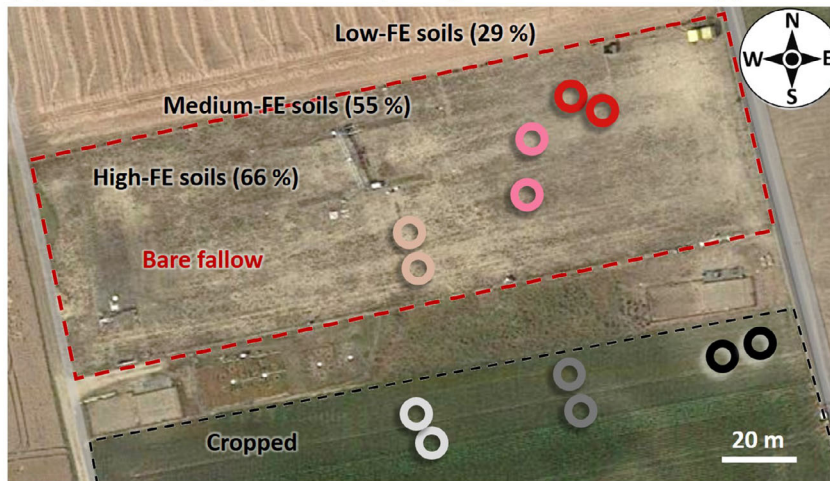
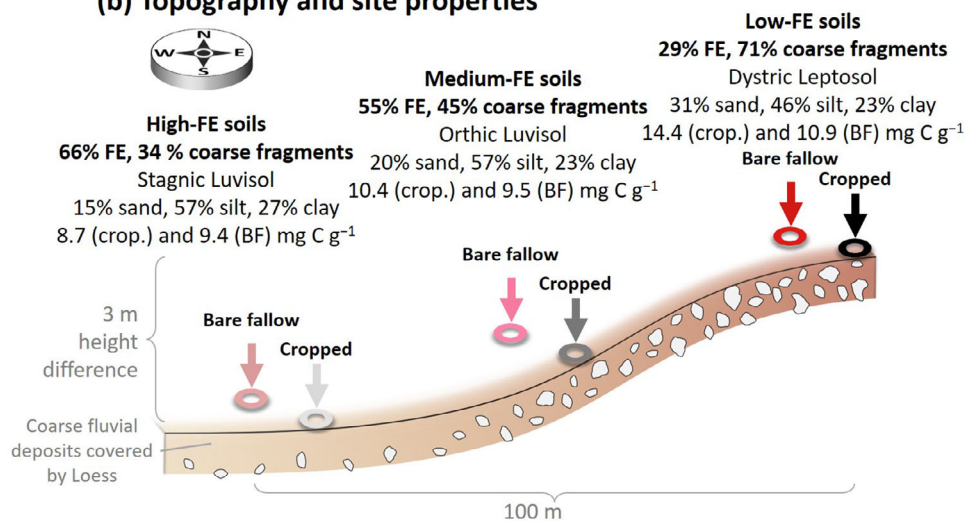
(a) Map of sampling site comparing bare fallow and cropped**(b) Topography and site properties**

FIGURE 1 Overview of the investigated rock fragment gradient containing soils with different fine earth (FE) content. Soils subjected to bare fallow management (BF) for 14 years were compared with adjacent cropped soils (crop.). (a) Map of sampling site (satellite image from Aerodata International Surveys, GeoBasis-DE/BKG, Maxa Technologies, Google Maps, 2023). (b) Schema of the toposequence and site properties. Samples were taken from the Ap horizon at a depth of 5–25 cm. Soil types according to Bornemann et al. (2011).

C and N contents of the POM and MOM fractions were determined by dry combustion at 1000°C using a Vario EL CN analyzer (Elementar). To determine the proportion of inorganic C, the same analysis was conducted after heating the samples in a muffle furnace at 550°C for 4 h to remove organic C using the FE fraction samples. Since the proportion of inorganic C was equally low across the rock fragment gradient and the mass of some POM and MOM was limited, no further differentiation of inorganic C was determined for the OM fractions. Across the rock fragment gradient on average, 95 ± 2.0 SD% mass and 85 ± 10.6 SD% C were recovered in the OM fractions, compared to the bulk soil C. The C stocks of a 30-cm Ap horizon were calculated using the bulk density measured by core sampling and the total C content of the FE <2 mm, whereas we accounted for the coarse rock fragment content by multiplication with the percentage of FE content.

2.4 | Mass contribution and C in soil aggregate fractions

In order to determine the influence of bare fallow management on soil aggregates and the C stored therein, we applied a microaggregate fractionation method according to Krause et al. (2018) and Kösters et al. (2013). Briefly, field-fresh material sieved <8 mm that was stored at 4°C until fractionation was pre-wetted for 5 min. The wet sieving was conducted using an automatic sieve tower submerged in water with mesh sizes 2800, 2000, 250, 53, and 20 µm for 10 min. Accordingly, the free microaggregates 53–250, 20–53, and <20 µm were isolated. The fractions >250 µm was sonicated at 60 J mL⁻¹ to disperse macroaggregates according to Amelung and Zech (1999) and subjected to a similar wet sieving as described. The previously

occluded microaggregates 53–250, 20–53, and <20 μm were isolated as well as the remaining macroaggregates >250 μm . The presence of macroaggregates after sonication indicates an incomplete dispersion of microaggregates, which may be related to a relatively high aggregate stability.

The C and N content of free and occluded microaggregate as well as macroaggregate fractions were determined by dry combustion at 1000°C using a Euro EA CHNS analyzer (Hekatech). Inorganic C was determined using muffled samples that were prepared as described earlier. The proportion of inorganic C of total bulk C was 6% on average across all fractions. On average, 90 ± 6.1 SD% of the total bulk soil C across the rock fragment gradient was recovered in the aggregate fractions.

2.5 | Surface loading of mineral-associated C

To determine the impact of bare fallow management on the C loading in comparison to the cropped soil, we determined the SSA of the $\text{MOM}_{<2\mu\text{m}}$ and the $\text{MOM}_{2-6.3\mu\text{m}}$ fraction using multi-point Brunauer-Emmett-Teller (BET) method (Brunauer et al., 1938) with N_2 at 77 K at an Autosorb-1 analyzer (Quantachrome). The samples were outgassed for 12 h using He at 40°C, measured two times sequentially, and the mean of these two measurements was reported for each replicate.

The C loading is a measure of the mineral surface area associated with OM. It was quantified as described in Schweizer et al. (2021). Briefly, we measured the SSA of the same sample before and after OM removal using NaOCl, which removed 86 ± 3.4 SD% C. The difference of SSA after NaOCl treatment minus the SSA before treatment was then used as a measure for the SSA loaded with OM. This was interrelated with the difference of C content before minus the C content after NaOCl treatment to determine the C loading as $\text{mg organic C m}^{-2}$ SSA. The measurements of the SSA loaded with OM and the C loading were computed and are reported based on the original values per weight of the soil fraction, with a C content of 29.5 mg C g^{-1} before oxidation and 4.3 mg C g^{-1} after oxidation on average across all samples.

2.6 | Statistical analysis

To evaluate the effects of the bare fallow management in comparison with the cropped sites, we used a model structure that also contained the FE or rock fragment content as a categorical variable. We first considered potential interactions between these two (Duncan & Kefford, 2021) using a full model structure as follows:

$$Y = \text{fine earth content} + \text{management} + \text{interaction term (FE content} \times \text{management)} + \text{error.} \quad (1)$$

In a first step, we computed an analysis of variance (ANOVA) for the full model after testing its assumptions of normality and equal variances. If the interaction term was found significant at $p < 0.05$,

the means of all combinations of FE content and management were compared using Tukey's honest significant difference (Tukey HSD). Accordingly, we evaluated pairwise comparisons of soils under bare fallow versus those under cropping separately at low, medium, and high FE content. If the ANOVA of the full model showed that the interaction term was not significant at $p > 0.05$, we used a reduced model with only FE content and management as predictors. The impact of these predictors was also evaluated using Tukey HSD. If the ANOVA of the full or reduced model provided significant predictor variables or interactions, the p -values of the post hoc comparisons according to Tukey HSD are provided in the graph if $p < 0.05$, provided in brackets if $0.05 < p < 0.1$, or denoted as "ns" if the comparison was not significant in case of $p > 0.1$. All statistical tools were applied using RStudio 1.3.1093 with the R version 4.0.3. Errors given in the text and plotted in the graphs are standard deviations.

3 | RESULTS

3.1 | Soil organic carbon content

The bulk soil C content in the FE <2 mm was 11.7 mg C g^{-1} , and the organic C content was 10.6 mg C g^{-1} on average across all soils. At low FE and high content of coarse rock fragments, the bare fallow soil contained 5.7 mg C g^{-1} less organic C, compared to the cropped soil (9.8 instead of 15.5 mg C g^{-1} ; Figure 2a). At medium FE the bare fallow soil contained only 1.7 mg C g^{-1} less organic C, compared to the cropped soil, whereas at high FE, there was no significant difference in organic C content in the bulk soil (Figure 2a). The response ratios of organic C increased in the soils with lower FE content (Table S2). The proportion of inorganic C from the total C was 9.2% on average across the whole rock fragment gradient, meaning that organic C accounted for 90.8% of the bulk soil C (Figure 2a). For further evaluation of inorganic C please refer to Figure S1a. The bulk N contents and C:N ratio of the FE fraction are found in Figure S1b,c.

Across the whole rock fragment gradient, the pH value was approximately 0.7 lower in the bare fallow soil, compared to the cropped soil, which was, however, only significant for high- and low-FE soils (Figure 2b). When C stocks were computed for the top 30 cm, they did not show significant differences and were 2.8 kg C m^{-2} on average across the rock fragment gradient (Figure S3).

The combined density and size fractionation showed that 28% of the bulk soil C is found in POM, and the remaining 72% of the soil C was mineral-associated on average across the rock fragment gradient (Figure 3a). Most POM was observed in the low-FE soil (Figure 3a), which also showed the highest difference of bulk soil C contents between managed and OM-depleted plots (Figure 2a). The C proportion of POM in the low-FE soil showed a tendency to decrease under bare fallow, compared to the cropped soil, which was however not significant (Figure 3a).

When computing the C contribution of fractions to the absolute C in the bulk soil, the POM fractions contained less C in the medium- and

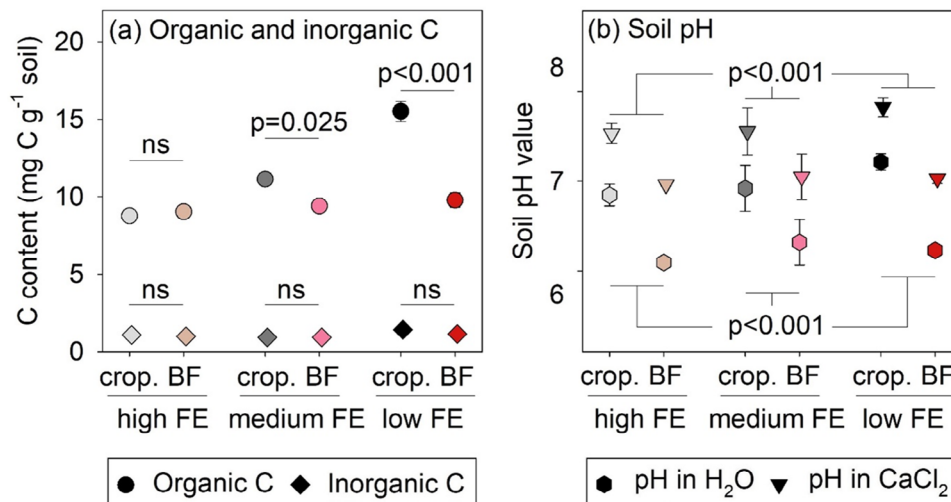


FIGURE 2 (a) Organic and inorganic C contents in the fine earth (FE) <2 mm at different FE contents of 66%, 55%, and 29% across a rock fragment gradient under cropped soil (crop.) and bare fallow (BF). When comparing the effects on organic C, we found a significant interaction between rock fragment content and management ($p < 0.001$). Therefore, the effect of management was compared individually for each FE content. “ns” denotes p -value of $p > 0.1$ by Tukey HSD. (b) Soil pH in H₂O and CaCl₂. The interaction term as well as the FE content were not significant factors of the analysis of variance model. Therefore, the management factor was directly tested, and the p -values are provided in the graph.

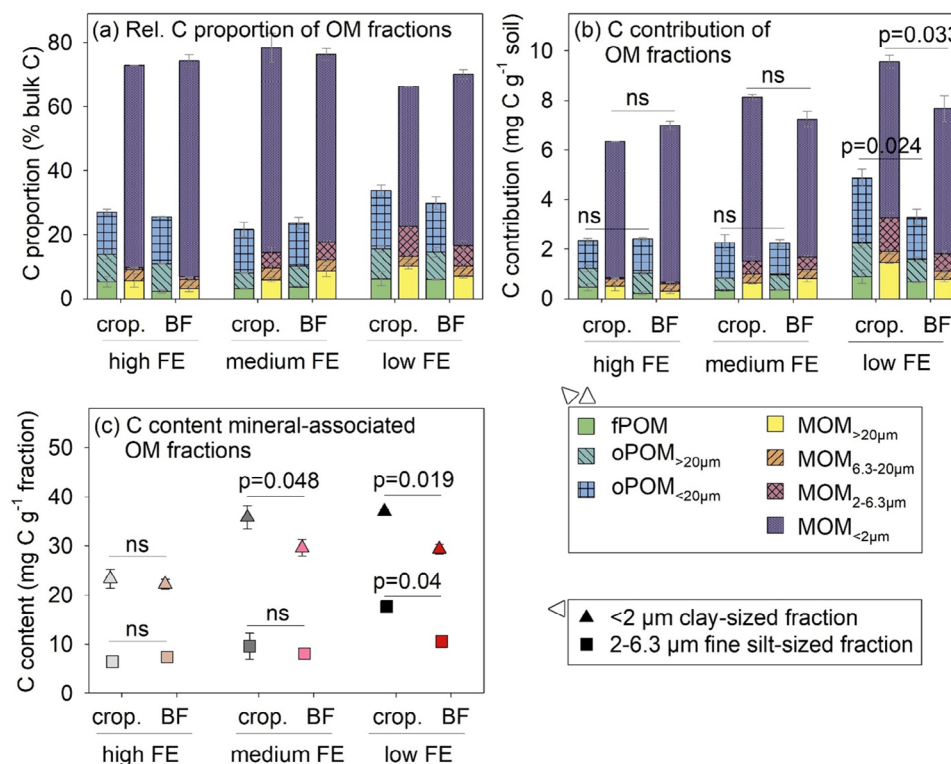


FIGURE 3 Investigation of C contained in particulate OM (POM) and mineral-associated OM (MOM) fractions across a rock fragment gradient with fine earth (FE) contents of 66%, 55%, and 29% testing the effect of 14 years bare fallow (BF) management, compared to soils under cropped (crop.) management at Selhausen (Germany). (a) C proportions of OM fractions (fractions sum up to 100%, mean \pm SD). The total occluded particulate OM (POM) proportion in the low-FE soils was found to be higher than in medium FE ($p = 0.002$) and in high FE ($p = 0.02$), whereas there were no significant interactions or management effects. (b) C contributions (fractions sum up to the absolute organic C content in the bulk soil; mean \pm SD). Due to significant interactions ($p = 0.02$) between FE content and management, we tested the management effect as pairwise combinations at each FE content. The C distribution of particulate and MOM fractions was analyzed by a combined density (1.8 g cm^{-3}) and size fractionation. (c) The C content in MOM fractions (MOM_{2–6.3 μm} and MOM_{<2 μm} ; mean \pm SD). The interaction between FE content and management was found significant for the C content of MOM_{2–6.3 μm} ($p = 0.038$) and showed a tendency for the C content of MOM_{<2 μm} ($p = 0.053$). Therefore, the management effect was tested as pairwise combinations at each FE content using Tukey HSD.

high-FE soils, compared to low FE (Figure 3b). The mineral-associated C contribution increased in soils with lower FE content (6.7, 7.3, and 8.5 mg C g⁻¹ on average), which was lower under bare fallow in the low-FE soil (Figure 3b).

The MOM_{2–6.3 μm} contained 9.5 mg C g⁻¹ and the MOM_{<2 μm} contained 30.0 mg C g⁻¹ on average across all soils (Figure 3c). The effect of bare fallow management mostly decreased the C contents within these fine MOM fractions with increasing differences in the soils with lower FE content. On average the C content in the MOM_{2–6.3 μm} decreased by 2.9 mg C g⁻¹ and in the MOM_{<2 μm} by 4.8 mg C g⁻¹ (Figure 3c).

On average across all sites, the C:N ratios in the POM fractions were 24.3 (fPOM), 21.0 (oPOM_{>20 μm}), and 16.8 (oPOM_{<20 μm}) and in the fine MOM fractions 9.2 (MOM_{6.3–20 μm}), 9.2 (MOM_{2–6.3 μm}), and 7.7 (MOM_{<2 μm}). The C:N ratios of the MOM fractions were lower in the bare fallow soils, compared to the managed ones by 2.5 in MOM_{2–6.3 μm} and 0.3 in MOM_{<2 μm}, which might be a result of an increasingly decomposed state of OM (Figure S1d,e). At the bulk soil scale, the C:N ratios increased in soils with lower FE content (9.7; 9.8; 10.4) in relation to the increasing POM contents (Figure S1b,c).

3.2 | Aggregate contributions and C and N contents

The fractionation of free and occluded microaggregate <250 μm as well as macroaggregate >250 μm size fractions showed that the macroaggregate fraction dominated the mass proportions at 26% on average (Figure 4a). Across all sites, the mass proportion of macroaggregates decreased by 4.3% when comparing the cropped with the bare fallow sites ($p = 0.006$; Figure 4a). In turn, the C content of the macroaggregates was one of the lowest at 9.1 mg C g⁻¹, compared to the free microaggregates <20 μm ($p < 0.0001$) and the occluded microaggregates <20 μm ($p < 0.0001$; Figure 4b). The highest effect of bare fallow on organic C content was observed in the low-FE soils as indicated by the free microaggregates 20 – 53 μm and 53 – 250 μm as well as the occluded microaggregates 53 – 250 μm (Figure 4b). In the other aggregate fractions, there were tendencies for C content depletions under bare fallow, compared to the cropped soil, whereas these were not significant (Figure 4b). The inorganic C content was 0.7 mg C g⁻¹ on average (Figure 4b). The inorganic C content was depleted under bare fallow, compared to the cropped soil in the free microaggregates <20 μm and occluded microaggregates 53 – 250 μm, whereas other microaggregate fractions exhibited significant interactions of management (Figure S2a). A detailed evaluation of the total N contents and the C:N ratios of the aggregate size fractions is found in Figure S2.

3.3 | Carbon loading of MOM fraction

The SSA related to OM was approximately 16.7 m² g⁻¹ on average across all samples (Figure 5a). We observed an increasing impact of bare fallow on the SSA the lower the FE content was, as indicated

by the significant interactions between management and FE content (Figure 5a). In the soils with low FE content, the SSA related to OM loading under bare fallow was approximately 8.7 m² g⁻¹ larger than the cropped soil. In the medium-FE soils, the decrease of SSA by bare fallow was 6.2 m² g⁻¹. Independent of FE content, soils under bare fallow indicated a higher C loading in comparison with the cropped sites (Figure 5a). In the samples with less SSA, the C loading was higher as indicated by a significant regression of both properties (Figure 5b).

4 | DISCUSSIONS

4.1 | Bare fallow management affects OM fractions depending on rock content

In this study, we investigated the effect of 14 years of bare fallow management on the storage of C as related to the C content and the C proportion in OM fractions. The soil organic C and other soil properties exhibited a significant interaction factor between management and FE content. This means that the impact of bare fallow, compared to cropped soil, was decisively influenced by the FE content of the soil. We found that the magnitude of OM depletion in soils under bare fallow increased the lower the FE content was in comparison with an adjacent cropped soil (Figure 2a). This is reflected by increasing relative differences between bare fallow and cropped soils the higher the coarse rock fragment content was (Table S2). The higher impact at low FE is, therefore, likely not primarily a result of the higher absolute C contents, compared to the high-FE soils. Instead, the comparison of C storage in POM and MOM fractions may provide further insights to explain how FE content mediates the impact of bare fallow management.

The higher impact of bare fallow management and OM depletion on the bulk soil C is related to shifts in C stored in OM fractions across the rock fragment gradient. The low-FE soil contained relatively more POM (Figure 3a) as also shown earlier across the bare fallow plot using mid-infrared spectroscopy and fractionation (Bornemann et al., 2011). Across both management types, the POM fractions contributed approximately 24% to the bulk soil C at high and medium FE and approximately 32% at low FE (Figure 3b). A recent study quantified that the average contribution of C stored in the POM fraction was 27% in cropped soils in Germany (Vos et al., 2018). A higher content of coarse rock fragments may be related to a higher relative contribution of the POM fractions to the organic C as shown by the low-FE soil in this study. The higher POM content at low FE content may explain the potentially higher vulnerability of C as demonstrated by the chronological observations at the bare fallow site by Meyer et al. (2017). They found that higher losses of organic C occurred over the course of 11 years of bare fallow management in soils with low FE content. In this study, after 14 years of bare fallow management, our comparison with the adjacent cropped soil indicates that the bare fallow management likely decreased the contribution of C in both POM and MOM fractions in the low-FE soil (Figure 3b). The lower MOM contribution under bare fallow, compared to the cropped sites, is related

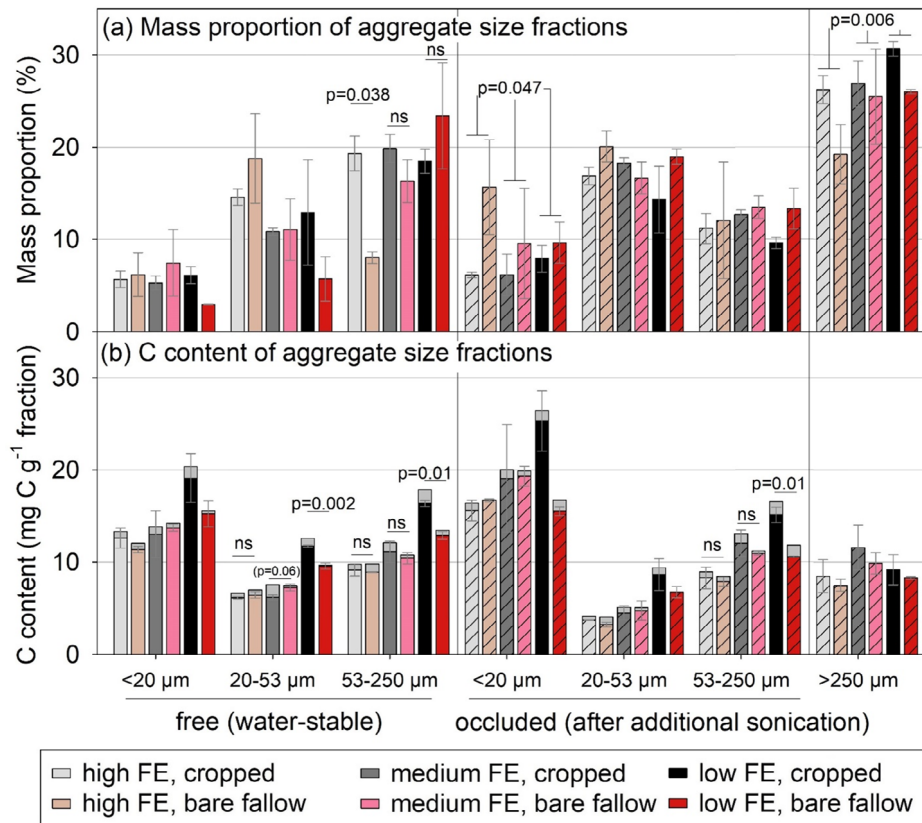


FIGURE 4 (a) Mass proportion of free and occluded microaggregate <250 μm as well as the macroaggregate >250 μm size fractions (mean \pm SD) across the rock fragment gradient comparing cropped and bare fallow. The interaction term was found significant for the free microaggregate fraction 53 – 250 μm ($p = 0.02$). Hence, pairwise comparisons were computed. A significant management effect was found for the occluded microaggregates <20 μm and macroaggregates >250 μm , whereas the interaction term was not significant. (b) Organic (filled bar; mean \pm SD) and inorganic C content (gray bar extension; mean) of aggregate fractions (mean). The interaction term was found significant for the organic C content of free microaggregates 20 – 53 μm ($p = 0.0005$), 53 – 250 μm ($p = 0.02$), and the occluded microaggregates 53 – 250 μm ($p = 0.03$). Pairwise comparisons were therefore computed for these fractions, whereas the other fractions did not show significant interactions or management effects. “ns” denotes p -value of $p > 0.1$ by Tukey HSD, whereas $0.05 < p < 0.1$ is provided in brackets. Open bars indicate free microaggregates after wet sieving and hatched bars indicate occluded microaggregates and macroaggregates after additional sonication.

to a lower C content of the fine silt-sized and the clay-sized MOM fraction of the low-FE soil (Figure 3c). Across the rock fragment gradient, the C content in fine MOM fractions increased with decreasing FE content, which indicates a more concentrated C storage in the soils with higher rock content (Figure 3c). A study at the medium-FE soil, showed that the post-modern ¹⁴C content decreased under bare fallow, which indicates older OM on average, compared to the cropped sites (Siebers et al., 2024).

Bare fallow management induced a loss of both POM and MOM fractions at the low-FE soil. A higher proportion of coarse rock fragments dilutes the FE content in a given soil volume. Thus, the effect of OM depletion is concentrated on a smaller amount of the fine particle fractions of the soil that are mostly related with OM storage. Previous studies found a higher macroporosity and air capacity in soils with lower FE content (Gargiulo et al., 2016; Li et al., 2021). However, a potentially higher macroporosity in the low-FE site of this study does not seem to be related with an increased decomposition of OM since the organic C content was higher than in the medium- and high-FE sites. Hence, the higher concentration of OM in a smaller amount of FE may

explain higher POM proportions in soils with more coarse rock fragments. The loss of C from both POM and MOM in the low-FE sites indicates that the depletion under bare fallow management may be accelerated and thus rendered more vulnerable to OM depletion in soils with a higher content of coarse rock fragments.

In comparison with bare fallow studies in the literature (Bacq-Labreuil et al., 2021; Doran et al., 1998; Paradelo et al., 2016), in our experiment, the direct effect of tillage was excluded since biomass was mainly removed using herbicides. Our soil sampling at 5–25 cm was below the shallow tillage operations limited to the top 5 cm in 2005–2010. The impact of bare fallow on POM fractions was likely lower in our study than in other studies where bare fallow was achieved by tilling the soils. Tillage is known to induce a mechanical breakdown of macroaggregates and therefore most of the OM loss was attributed to lower POM contributions (Six et al., 1999). We observed that the soils under bare fallow management contained less C stored in the POM and MOM fractions at low FE content (Figure 3b). This shows that both the POM and MOM fractions may be vulnerable to decrease when the input of OM is eliminated. Further research is warranted

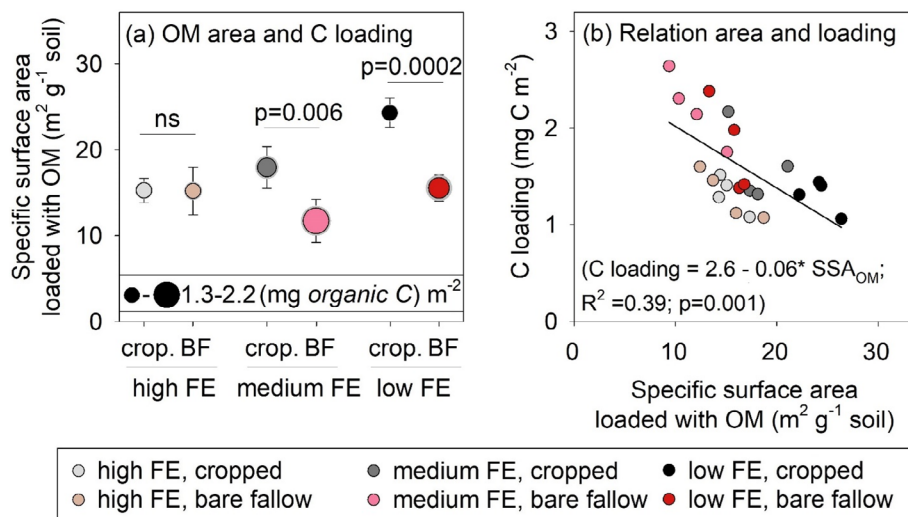


FIGURE 5 Analyses of surface properties of bare fallow (BF) management, compared to soils under cropped (crop.) management. (a) Specific surface area (SSA) loaded with OM and amount of C loading (symbol size) of the clay-sized $<2 \mu\text{m}$ MOM fraction. The SSA indicated significant interactions between fine earth (FE) content and management ($p = 0.002$). Therefore, pairwise evaluations of the management effect on the SSA are shown for the three different FE contents. The amount of C loading indicated an increase under bare fallow management, compared to the cropped sites ($p = 0.008$) without significant interactions with FE content. (b) Scatter plot and regression of the SSA and C loading across all measurements.

to distinguish the effect of OM depletion from mechanical influences since our experiment did not consider different tillage practices.

The pH was lower under bare fallow, compared to the cropped soil across the whole rock fragment gradient (Figure 2b), which is likely attributed to different times since the last lime application. The soils under bare fallow were not limed for 14 years, whereas the cropped soils are commonly amended with lime as part of the cropping activities. However, the inorganic C contents (on average 1 mg g^{-1}) did not differ between different management types (Figure 2a). Therefore, independent of inorganic C, the depletion of fresh OM input may have led to a lower number of base-acting cations from fresh OM input. This was also found in the Rothamsted Bare Fallow experiment where the soil pH measured in H_2O declined from 6.3 to 5.2 over the course of 55 years (Barré et al., 2010).

The impact of soil erosion likely affected the comparison of bare fallow and cropped soil to a minor extent since we sampled the soil 5–25 cm. Yet the removal of C upslope and its deposition downslope may have enhanced the differences between bare fallow and cropped soil at low-FE sites and counteract differentiation at high FE content. This may help to explain why we observed significant differences in C in both POM and MOM at low FE, whereas we could not find differences at medium and high FE.

4.2 | Interplay of soil aggregates and C storage

We used a stepwise approach to isolate free (water-stable) microaggregates. Water-stable macroaggregates were sonicated for further dispersion into occluded microaggregates and sonication-resistant macroaggregates as also applied in Krause et al. (2018). In both this

study and Krause et al. (2018), the aggregate isolation approach highlighted recurring size distribution patterns in both free and occluded microaggregates. Across all treatments, we observed increasing average mass proportions in the order <20 , $20\text{--}53$, $53\text{--}250$, and $>250 \mu\text{m}$ (Figure 4a). In contrast to the mass proportions, the C contents were highest in the $<20 \mu\text{m}$ fraction (Figure 4b). This may be related to lower contents of large primary particles diluting more C-rich fractions as observed by Felde et al. (2021). The inorganic C content in the macroaggregate fraction was lower under bare fallow, which may explain the lower mass contribution of macroaggregates in comparison to the cropped soil (Figure 4; Figure S2a). However, the bare fallow management did not affect the inorganic C content in the bulk soil (Figure S1a). The stepwise aggregate fractionation approach provides insights into the interactions of the free and occluded aggregate size fractions with stabilizing agents by interrelating the fraction mass proportions with their organic and inorganic C contents. In the soils with low FE content, most aggregate breakdown is likely attributed to the OM depletion under bare fallow, compared to the cropped soil.

Under bare fallow management, the mass of macroaggregates in the low-FE soil decreased, which may be interrelated with the highest loss of organic C, compared to the cropped soil. In turn, the total mass proportion of microaggregates under bare fallow increased by 4.8% in the low-FE soil, whereas the C content decreased in the free and occluded $53\text{--}250 \mu\text{m}$ microaggregate fractions, compared to the cropped soil (Figure 4). The depletion of OM and the decrease of OM fractions may be directly interrelated with the impact of bare fallow on aggregate size fractions since OM acts as gluing agents of the aggregate fractions (Amelung et al., 2023; Tisdall & Oades, 1982). The soil with low FE content lost C stored in both POM and MOM fractions, which may be directly related with the proportions of free and occluded aggregates.

Under bare fallow management, we observed a breakdown of macroaggregates into microaggregates at all FE contents when comparing bare fallow to the cropped soil. However, at high FE, the organic C contents did not exhibit differences between bare fallow and cropped soil (Figures 2a and 3c). This indicates that in the high-FE soils, bare fallow management may have led to a loss of the gluing effect of OM on aggregation, whereas the remaining OM may have remained protected. This may be related with a change in the OM composition such as the observation of a higher relative contribution of microbial sugars in unfertilized OM-depleted soils in comparison with fertilized soils from various long-term agricultural experiments in Europe (Kiem & Kögel-Knabner, 2003). Further studies are warranted to analyze the interactions of OM composition and its effect on soil aggregate stability.

4.3 | Impact of bare fallow on carbon loading of mineral-associated OM

The effects of OM depletion through bare fallow exhibited an interaction with coarse rock fragments, which also affected the SSA associated with OM (Figure 5a). In the high-FE soil, we did not observe a significant difference in the SSA associated with OM between bare fallow and cropped soil. However, in the low-FE soil with 71% coarse rock fragments, the SSA associated with C decreased by $9 \text{ m}^2 \text{ g}^{-1}$ under bare fallow, compared to cropped management (Figure 5a). When less SSA was associated with OM, the C loading increased independent of the FE content (Figure 5b), when comparing bare fallow and cropped soil. The increase of C loading up to 2.1 mg C m^{-2} is higher than the monolayer-equivalent C loading, which was estimated to be $0.5\text{--}1.0 \text{ mg C m}^{-2}$ (Keil et al., 1994; Mayer, 1994). This means that to some extent, the storage of mineral-associated C in the clay-sized fraction seems to shift toward a thicker and more piled-up arrangement. A similar mechanism of increased mineral-associated C loading was found in the clay-sized fraction when the clay content decreased from 18% to 5% in a previous study (Schweizer et al., 2021). The lower SSA of mineral surfaces associated with OM indicates that mainly the extension of OM associated with mineral surfaces has shrunk in the low-FE soil under bare fallow in comparison to the cropped management. A previous study of a bare fallow experiment with tillage in France used X-ray absorption spectroscopy to show that the coverage of OM across illite particles decreased over time, whereas mixed-layer illite/smectite and smectite surfaces were still associated with OM (Lutfalla et al., 2019). Our finding of the lower mineral surface associated with OM storage under bare fallow at the low-FE soil highlights the important role of the actual arrangement of OM across mineral surfaces for C storage in the MOM fraction.

5 | CONCLUSION

Our results demonstrate how bare fallow management induces OM depletion and affects OM storage after 14 years in comparison with

an adjacent cropped soil. Across the rock fragment gradient with 34%–71% coarse fragments, we observed that a higher coarse rock fragment content was related to an enhanced impact of bare fallow, compared to the cropped soil. Significant interactions between the FE content and the management were found. This led to the observation of a higher impact of bare fallow in the low-FE soils at the upper end of the rock fragment gradient, whereas no differences were found in the high-FE soil at the lower end of the gradient. The dilution of the soil matrix with coarse rock fragments led to a concentration of the impact of bare fallow on the C contained in the FE fraction $<2 \text{ mm}$. The C of both the particulate and the MOM fractions was depleted under bare fallow management, compared to the cropped soil at low FE. This shows that both OM fractions seem to be similarly vulnerable in the absence of mechanical disturbance by tillage in contrast to other bare fallow studies. Free and occluded microaggregates reflected the C loss at the low-FE soil, indicating a breakdown of macroaggregates into smaller units. In addition, at the high-FE soil, we found less stable aggregates under bare fallow, compared to cropped soil, despite the similar bulk soil C contents. This indicates a subsided effect of the existing OM gluing agents. Accordingly, by using N_2 -sorption, we observed that the mineral surface area loaded with OM decreased under bare fallow in the medium and low FE. The C loading consequently increased under bare fallow in comparison with cropped soil. Soils with higher coarse rock fragment contents may interact with OM depletion leading to a higher vulnerability of OM affecting C storage, aggregate size distribution, and the mineral association of OM.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in “figshare” at <https://doi.org/10.6084/m9.figshare.22221235>.

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