

# Addition of sorptive mineral phases to soils decreases short-term organic matter decomposition by reducing microbial access to substrates

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## Abstract

There is increasing evidence that the accessibility of soil organic matter (SOM) to microbial decomposers is more important than chemical recalcitrance for regulating SOM stability. We show that the rapid reduction in SOM decomposition following the addition of sorptive mineral phases to soils in laboratory conditions leads to decreased accessibility of SOM to microbial decomposers due to the formation of organo-mineral complexes. We manipulated SOM accessibility in a short-term microcosm experiment by adding different proportions of a sorptive mineral material derived from an aluminium-rich allophanic soil to a constant mass of soil to determine the effects on SOM decomposition after 1, 4 and 8 days. The decrease in SOM decomposition with increasing proportion of added sorptive mineral phase occurred within 1 day and did not change further at 4 and 8 days. In a second experiment, we added three proportions of the sorptive mineral phases (0%, 15% and 50%) to three soils with different carbon (C) concentrations and measured rates of SOM decomposition, changes in water extractable C, the formation of organo-mineral complexes inferred from pyrophosphate-extractable aluminium, and the natural abundance <sup>13</sup>C isotopic composition of CO<sub>2</sub> derived from SOM decomposition. We confirmed that the proportional decreases in SOM decomposition with increasing organo-mineral complexes and decreasing microbial access to SOM was the same for the three soils, suggesting that the effects are independent of soil C concentration and pH. We also showed that the short-term reductions in SOM accessibility led to microbial decomposition of more <sup>13</sup>C enriched substrates, suggesting preferential stabilisation of plant-derived (<sup>13</sup>C depleted) substrates. Our study demonstrated that SOM accessibility and decomposition could be reduced rapidly and proportionally to the amount of added sorptive mineral phases resulting from increased organo-mineral interactions irrespective of the initial soil organic carbon concentration.

### Highlights

- Addition of sorptive mineral phases reduced short-term soil organic matter (SOM) decomposition by the same proportion for three soils.
- Relatively  $^{13}\text{C}$  depleted SOM was preferentially adsorbed onto the mineral phases.
- The reduction in SOM decomposition was attributed to reduced microbial access due to increased organo-mineral interactions.
- The effects occurred rapidly and proportionally to the amount of added sorptive mineral phases.

### KEYWORDS

$^{13}\text{C}$  natural abundance, microbial access, organo-mineral interactions, soil organic matter decomposition

## 1 | INTRODUCTION

Our ability to predict the effects of climate change on changes in soil organic carbon (C) stocks relies on understanding the mechanisms regulating both the stabilisation and decomposition of soil organic matter (SOM). It is now widely recognised that the interactions of organic molecules with minerals in the soil protect SOM from degradation (Lehmann & Kleber, 2015; Lützow et al., 2006; Schmidt et al., 2011). Protection mechanisms are multiple and include a range of physical, chemical and biological processes, but it is clear that the interactions occurring at the SOM-mineral interface are critical to explain SOM persistence (Kleber et al., 2021). A critical mechanism is the strong chemical adsorption of SOM onto mineral surfaces that results in reduced accessibility of SOM to microbial decomposers and increased protection of SOM (Dungait et al., 2012; Kögel-Knabner et al., 2008; Lützow et al., 2006). Understanding the processes regulating the interactive effects of microbial access to SOM is necessary for improving models that predict the effects of environmental drivers and management practices on SOM formation and decomposition. Ultimately, these effects result in changes in soil carbon stocks that drive carbon-climate feedback responses and the rate of climate change (Bradford et al., 2016).

Direct measurements of SOM decomposition rates from field experiments do not provide sufficient mechanistic insights to reveal the linkages between organo-mineral interactions and microbial access to SOM (Pronk et al., 2017). More analytical approaches to determine the chemical reactions involved in the formation of organo-mineral complexes have involved measurements of the adsorption of solutions of dissolved and extracted SOM onto commercially available clay minerals such as kaolinite and montmorillonite (Kaiser & Guggenberger, 2007;

Wang & Xing, 2005). However, these experiments lack the link to microbial access to SOM. Other experimental approaches to determine the effects of organo-mineral interactions and microbial decomposition of SOM have involved the addition of mineral phases to fresh soils in laboratory conditions. For example, Kalbitz et al. (2005) showed that decomposition was decreased when dissolved organic matter was adsorbed onto mineral soil material taken from a Haplic Podzol. Porras et al. (2018) showed that the temperature sensitivity of glucose decomposition was reduced when glucose was added to soil after adsorption onto goethite and ferrihydrite minerals. Adhikari et al. (2019) showed that the positive priming effect induced by glucose and formic acid addition on SOM decomposition decreased when both compounds were adsorbed onto the mineral ferrihydrite which was then added to the soil. A similar observation reported by Jones and Edwards (1998) showed that microbial decomposition of glucose and citrate was reduced after being adsorbed by minerals and subsequently mixed with soils. Using a slightly different experimental approach, Singh et al. (2019) showed that the decomposition of added organic compounds was reduced when minerals were added to soil samples prior to the addition of the compounds. Moinet, Hunt, et al. (2018) showed that the temperature sensitivity of SOM decomposition was reduced after addition of an allophanic mineral to soil. While these experiments have demonstrated linkages between decomposition and protection by adsorption onto mineral phases of added organic compounds (Adhikari et al., 2019; Jones & Edwards, 1998; Kalbitz et al., 2005; Porras et al., 2018) or of SOM already present in the soil (Moinet, Hunt, et al., 2018; Singh et al., 2019), they have not provided direct evidence that the effects are attributable to a reduction in microbial access to SOM, rather than alternative mechanisms such as enzyme inhibition (Frankenberger & Johanson, 1982).

In this study, our objective was to demonstrate that the addition of a sorptive mineral phase to soils reduces SOM decomposition rates and that this is attributable to increased formation of organo-mineral complexes leading to decreased accessibility of SOM to microbial decomposers. We undertook the measurements on three soils to determine if the responses to the mineral addition would differ with differences in native C concentration. For each soil we increased the SOM adsorption capacity in the soil matrix directly by mixing a constant mass of soil (constant C concentration for each soil) with increasing proportions of a mineral material derived from an allophanic subsoil with high capacity to adsorb SOM (thereafter referred to as sorptive mineral phase) characterised previously by Parfitt (1990). We determined the effects on microbial decomposition of SOM by measuring rates of soil respiration ( $R_{\text{SOM}}$ ). Concurrently, we measured pH, and hot ( $C_h$ ) and cold ( $C_c$ ) water extractable C as indicators of SOM pools available for microbial decomposition (Ghani et al., 2003; Landgraf et al., 2006). We determined newly formed organo-mineral complexes with the added Al-rich mineral phase by measuring the amounts of pyrophosphate-extractable aluminium, an indicator of organically bound aluminium (Eimil-Fraga et al., 2015; Parfitt & Childs, 1988; Taboada et al., 2019). To provide further evidence of the effects of addition of the sorptive mineral phase on changes in the source of the decomposed substrates, we measured the natural abundance  $\delta^{13}\text{C}$  signature of respired  $\text{CO}_2$  (not requiring the addition of  $^{13}\text{C}$  labelled substrates). Plant material, including roots, root exudates and litter, produce depleted  $\delta^{13}\text{C}$  when decomposed by microbes, and soil respired  $\text{CO}_2$  becomes progressively more  $^{13}\text{C}$  enriched as those plant inputs become part of increasingly stable, protected SOM (Boström et al., 2007; Bowling et al., 2008). This enrichment of stable forms of SOM is associated with a contribution of microbial products to mineral associated organic matter (Lavalée et al., 2020). The natural abundance  $\delta^{13}\text{C}$  signature can therefore be used to determine changes in the dynamics of protected and unprotected SOM pools (Millard et al., 2010; Moinet et al., 2016, 2020; Moinet, Hunt, et al., 2018; Moinet, Midwood, et al., 2018; Zakharova et al., 2014, 2015). We tested the hypotheses that (1) increasing addition of sorptive mineral phases to soils will increase the formation of organo-mineral complexes and reduce SOM accessibility for microbial decomposition, (2)  $R_{\text{SOM}}$  will increase proportionally with increasing amounts of microbially accessible C fractions and decrease proportionally with the formation of organo-mineral complexes, indicating a direct relationship between SOM decomposition and microbial access and (3) the  $\delta^{13}\text{C}$  signature of soil respired  $\text{CO}_2$  will become more depleted with increasing mineral addition, indicating preferential adsorption of microbial

products (relatively  $^{13}\text{C}$  enriched) onto the mineral surfaces.

## 2 | MATERIALS AND METHODS

### 2.1 | Preparation of the sorptive mineral phase for addition to the soils

The source and preparation of the mineral material with high capacity to adsorb SOM (hereafter referred as sorptive mineral phase) was the same as that used previously by Zakharova et al. (2015, 2014) and Moinet, Hunt, et al. (2018). The raw material was collected from an aluminium-rich subsoil from the 2Bw3 horizon of a New Plymouth Brown Loam Allophanic soil (New Zealand classification), Andosol (World Reference Base) (Hewitt et al., 2021), selected for its high capacity to bind and stabilise SOM (Parfitt, 1990). The subsoil was heated to  $500^\circ\text{C}$  for 4 h in a muffle furnace to oxidise all native SOM (Blakemore et al., 1987). The mass of soil for all treatments was constant and silica sand from pure quartz prepared using the same procedure as that for the mineral was used as an inert medium to maintain constant mass for soil–mineral mixtures across all treatments and account for potential dilution effects. Measurements showed that values for  $R_{\text{SOM}}$ , total carbon concentration (C),  $C_c$  and  $C_h$  for the sand and the mineral were below the detection limit and assumed to be zero. Sulphuric acid (10%) was mixed with three subsamples of the preparation and no  $\text{CO}_2$  was emitted, confirming the absence of carbonates and abiotic  $\text{CO}_2$  release during subsequent incubation experiments (Loeppert & Suarez, 1996).

### 2.2 | Sources of soil with different C concentrations

Three soil types were selected for their differences in C identified from data for New Zealand soils given in McNally et al. (2017). The soils were collected from the upper 0.15 m after manual removing of plant material and litter from three locations in eastern regions of the South Island of New Zealand. The soil with the lowest C (Table 1) was collected from a grazed grassland composed of ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) located at Ashley Dene Research & Development Station, Lincoln (latitude  $43^\circ 38.85'\text{S}$ , longitude  $172^\circ 20.76'\text{E}$ ). The soil type is classified as a Pallic Firm Brown (FB) (New Zealand classification), Haplic Cambisol (World Reference Base) (Hewitt et al., 2021). The soil with the intermediate C was collected from an ungrazed grass verge composed of ryegrass and mixed

**TABLE 1** Total soil carbon concentration ( $C$ ), and pH for the three soil types consisting of pallic firm brown (FB), weathered fluvial recent (FR) and typic immature pallic (IP)

Soil	pH	$C$ (mg g soil <sup>-1</sup> )
FB	6.0 ± 0.01 <sup>a</sup>	34.2 ± 0.02 <sup>a</sup>
FR	6.2 ± 0.01 <sup>b</sup>	53.4 ± 0.05 <sup>b</sup>
IP	5.4 ± 0.02 <sup>c</sup>	91.2 ± 0.05 <sup>c</sup>

Note: All values are means ± SE ( $n = 3$ ). Different letters represent significant differences ( $p > 0.05$ ) between soils from the ANOVA.

weed species at Southbridge (latitude 43°50.04'S, longitude 172°19.05'E). This soil type is classified as a Weathered Fluvial Recent (FR) (New Zealand classification), Fluvisol (World Reference Base) (Hewitt et al., 2021). The soil with the high  $C$  was collected from a mixed-species indigenous forest located at the Claremont Reserve, Timaru (latitude 44°41.04'S, longitude 171°18.05'E). This soil type is classified as a typic immature pallic (IP) (New Zealand classification), Cambisol (World Reference Base) (Hewitt et al., 2021). After collection, the soils were stored in sealed plastic bags in the dark at 4°C. Measurements of pH were made in extracts with distilled water (two soil: five water mass/volume).

### 2.3 | Manipulation of SOM accessibility and experimental design

In this study, we added different amounts of the sorptive mineral phase to a constant mass of sieved (2 mm) soil with visible roots removed at constant gravimetric water content (25% of water holding capacity). Sand was added to retain all soil mixtures at constant total mass. Each mixture of the soils and sorptive mineral phase addition was considered as a treatment. Before mixing, water was added to the sorptive mineral phase (or sand) to bring the gravimetric water content to the same value as that for the soil samples. This ensured that all the mixtures were maintained at constant gravimetric water content. The soils, mineral and sand were mixed manually for 1 min in a large container, to ensure even distribution. After mixing, each mixture was packed into a glass microcosm (height 96 mm, diameter 15 mm, and mixture depth 40 mm) and pressed gently with a pestle to maintain constant volume and bulk density. To minimise water loss and avoid the need to replace evaporative losses that could result in changes in SOM accessibility (Leitner et al., 2017), the microcosms were placed in a controlled environment cabinet (Fitotron, Weiss Gallenkamp, Loughborough, UK) for 8 days in the dark at an air temperature of 25°C and a relative humidity of

80%. For the incubations, the microcosms were sealed hermetically with a lid fitted with a septum to allow collection of headspace gas for measurement of  $CO_2$  concentration and the natural abundance isotopic  $\delta^{13}C$  signature.

Two successive experiments were undertaken. The objective of the first experiment was to establish the relationship between the proportions of added sorptive mineral phase and  $R_{SOM}$  over time to decide on appropriate proportions of sorptive mineral phase to be used in the second, more detailed experiment. This was conducted using only the FB soil, with three replicates for each of 14 mineral addition treatments ranging from 0% to 200% (the proportional mass of sorptive mineral phase relative to the soil mass in the mixture expressed as a percentage, 100% being equal mass of sorptive mineral phase and soil [Table S1]). Measurements of  $R_{SOM}$  were made at 1, 4 and 8 days after mixing. The pH of the soil mixtures was measured in extracts with distilled water (two soil: five water mass/volume) for the control treatment (no added sorptive mineral phase or sands) and the soil mixtures with 10%, 40% and 200% additions.

The second experiment used a subset of the treatments selected from the first experiment where there were clear differences in the  $R_{SOM}$  (0%, 15% and 50% mineral additions). This aim of this experiment was to determine the relationships between changes in  $R_{SOM}$ , organo-mineral interactions and indicators of accessible  $C$  from measurements of cold ( $C_c$ ) and hot ( $C_h$ ) water extractable  $C$ . Estimates of changes in organo-mineral interactions were made using measurements of aluminium pyrophosphate concentration. Measurements of the natural abundance  $\delta^{13}C$  of respired  $CO_2$  ( $\delta^{13}C_{SOM}$ ) were made on three replicates for each treatment at each of 1, 4 and 8 days after the start of the mixture preparation. Each replicate was used for measurements of  $R_{SOM}$  and  $\delta^{13}C_{SOM}$  and then used for destructive measurements of pyrophosphate-extractable aluminium,  $C_c$  and  $C_h$ .

### 2.4 | SOM decomposition rates

For measurements of the rates of SOM decomposition ( $R_{SOM}$ ), the microcosm lids were sealed and a volume of  $5 \times 10^{-4}$  L of gas from the headspace was sampled immediately with a syringe. One hour after the lid was closed, a second gas sample of the same volume was collected. Both samples were injected into a stream of  $CO_2$  free air leading to an infra-red gas analyser (Model LI-7000, LI-COR Biosciences, Lincoln, NE) to measure  $CO_2$  concentrations and the differences in concentrations over the time period were used to calculate  $R_{SOM}$ .



## 2.5 | Natural abundance $\delta^{13}\text{C}$ derived from $R_{\text{SOM}}$

The gas in the headspace of the microcosms was evacuated and flushed three times with  $\text{CO}_2$  free air. After 1 h (or 3 h for the treatments with low  $R_{\text{SOM}}$ ),  $5 \times 10^{-3}$  L of gas were extracted with a syringe and injected into a stream of  $\text{CO}_2$  free air leading to a tunable diode laser gas analyser (Model TGA1001, Campbell Scientific Inc., Logan, UT) for measurements of the  $\delta^{13}\text{C}$  isotopic signature,  $\delta^{13}\text{C}_{\text{SOM}}$ .

## 2.6 | Cold and hot water extractable C

Fresh soil samples were mixed with distilled water (1 soil:10 water mass/volume) in a  $5 \times 10^{-3}$  L polypropylene tube, shaken in an end-over-end shaker at 250 rpm at  $20^\circ\text{C}$  for 30 min, then centrifuged for 20 min at 3500 rpm ( $1500\times g$ ). The supernatant was collected and filtered through a 0.45 mm cellulose nitrate membrane filter and used for measurements of  $C_c$ . The pellet was then resuspended in the same volume of distilled water (1 soil:10 water mass/volume) and incubated at  $80^\circ\text{C}$  in a water bath for 16 h. The samples were shaken at 250 rpm at  $20^\circ\text{C}$  for 30 min, then centrifuged for 20 min at 3500 rpm ( $1500\times g$ ) and filtered through 0.45 mm cellulose nitrate membrane filters (Ghani et al., 2003). The supernatant was used for measurements of  $C_h$ . Measurements of  $C$  were made using a carbon analyser (TOC, Shimadzu Corporation, Kyoto, Japan).

## 2.7 | Pyrophosphate-extractable aluminium

The degree of organo-mineral interactions was inferred from measurements of the aluminium extracted in sodium pyrophosphate ( $\text{Al}_p$ ) following the extraction procedure described by Blakemore et al. (1987). Briefly, soil was mixed with 0.1 M sodium pyrophosphate buffer (1 soil:100 buffer mass/volume) in  $5 \times 10^{-3}$  L polypropylene ultracentrifuge tube and shaken at 250 rpm at  $20^\circ\text{C}$  for 16 h in an end-over-end shaker.  $4 \times 10^{-5}$  L of 0.4% flocculant solution was added and the samples were shaken vigorously then centrifuged for 30 min at 20,000 rpm ( $40,000\times g$ ). The supernatant was collected and used for measurement of the concentration of Al using inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Model 720-ES, Varian, Victoria, Australia). The concentration of  $\text{Al}_p$  measured for the mineral was (mean  $\pm$  SE)  $3.2 \pm 0.1 \text{ mg g}^{-1}$ ,  $n = 3$  and was undetectable for the sand. The magnitude of new organo-minerals interactions formed from mixing the sorptive mineral phases with the soils were estimated

from differences between the apparent  $\text{Al}_p$  concentrations in the mixtures and the components, calculated as

$$\text{Al}_p = \text{Al}_{p\text{-mixture}} - ((\text{Al}_{p\text{-soil}} \times m_{\text{soil}}) + (\text{Al}_{p\text{-sand}} \times m_{\text{sand}}) + (\text{Al}_{p\text{-mineral}} \times m_{\text{mineral}})), \quad (1)$$

where  $m_i$  is the mass of the relevant component in the mixtures.

## 2.8 | Statistical analyses

Statistical analyses were performed using R v3.5.3 (R Core Development Team, 2017). An exponential decay function was fitted to the data to describe the effect of sorptive mineral phase addition on  $R_{\text{SOM}}$  in the first experiment from

$$R_{\text{SOM}} = R_0 (1 - b)^m, \quad (2)$$

where  $R_0$  is the basal rate of  $R_{\text{SOM}}$  with no sorptive addition of mineral phases,  $b$  is the decay constant reflecting the rate of decrease of  $R_{\text{SOM}}$  per unit addition of sorptive mineral phases and  $m$  is the sorptive mineral phase addition expressed as a percentage. This function was fitted separately to data for days 1, 4 and 8 after addition of the sorptive mineral phases to determine the time responses of  $R_{\text{SOM}}$  to the treatments.

In the second experiment, a multi-way ANOVA was used to test the significance of the effects of addition of sorptive mineral phases, soil type, and their interactions on pH,  $R_{\text{SOM}}$ ,  $C_c$ ,  $C_h$ ,  $\text{Al}_p$  and  $\delta^{13}\text{C}_{\text{SOM}}$  separately for each sampling day. Relationships between  $C_c$ ,  $C_h$ ,  $\text{Al}_p$ ,  $R_{\text{SOM}}$  and  $\delta^{13}\text{C}_{\text{SOM}}$  were tested using linear models. The significance of including soil type as a factor was tested to determine whether the relationships between variables were the same among the soil types.

## 3 | RESULTS

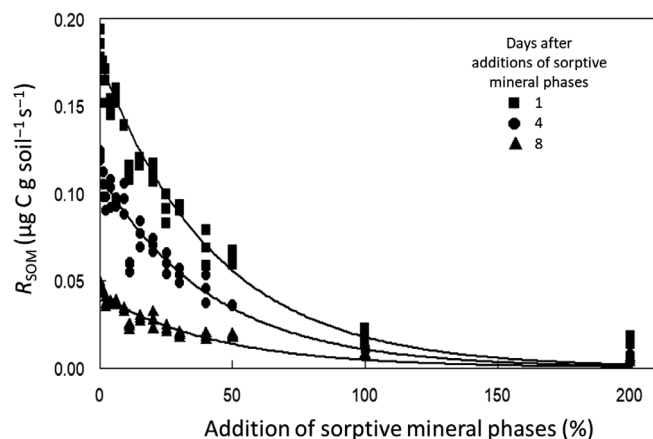
### 3.1 | Soil carbon concentrations

Carbon concentrations in the three soils were significantly different ( $p < 0.05$ ) with the concentration lowest in the grassland FB soil and highest in the native forest IP soil (Table 1).

### 3.2 | Effects of addition of sorptive mineral phases on SOM decomposition with time

In the first experiment,  $R_{\text{SOM}}$  decreased with increasing addition of sorptive mineral phases and the response was

well described by Equation (2) ( $p < 0.001$ ) (Figure 1). Basal respiration rates for soils with no added sorptive mineral phase ( $R_0$ ) decreased strongly with incubation



**FIGURE 1** Soil organic matter (SOM) decomposition rates ( $R_{SOM}$ ) in response to mineral additions. The lines represent the fit from the exponential decay function (Equation 2) with the parameter  $b$  describing the rate of decrease in  $R_{SOM}$  per unit percentage of addition of sorptive mineral phases. The coefficients of determination ( $R^2$ ) (adjusted for the null model) are 0.96, 0.91, 0.89 for day 1, 4 and 8, respectively

time with estimates (mean  $\pm$  SE) of  $0.17 \pm 0.002$ ,  $0.10 \pm 0.002$  and  $0.04 \pm 0.001 \mu\text{g C g soil}^{-1} \text{ s}^{-1}$  at days 1, 4 and 8, respectively. However, the rate of decrease in  $R_{SOM}$  with increasing addition of sorptive mineral phases as shown by the parameter  $b$  was almost identical across the 3 days of measurement (mean  $\pm$  SE)  $0.022 \pm 0.001$ ,  $0.023 \pm 0.001$  and  $0.022 \pm 0.001 \mu\text{g C g soil}^{-1} \text{ s}^{-1} \%^{-1}$  for days 1, 4 and 8, respectively, indicating that the response to the addition of sorptive mineral phases occurred fully and remained the same after day 1 (Figure 1).

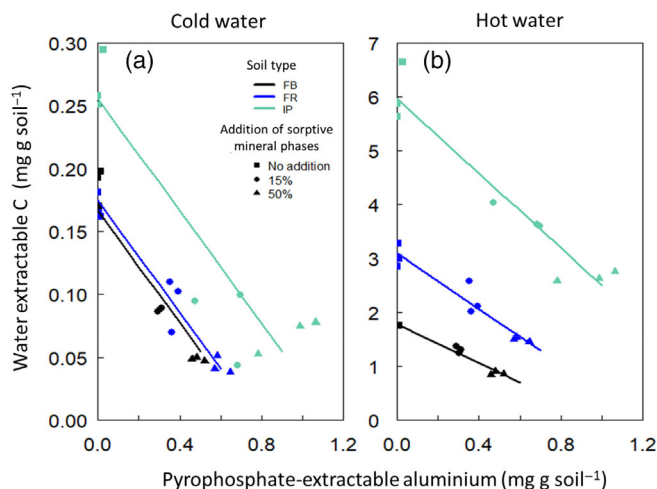
### 3.3 | Addition of sorptive mineral phases and indicators of SOM accessibility

For the second experiment, the effects of addition of sorptive mineral phases on  $R_{SOM}$ ,  $C_c$ ,  $C_h$  and  $Al_p$  for the three soils were analysed separately after days 1, 4 and 8. With increasing addition of sorptive mineral phases,  $R_{SOM}$ ,  $C_c$  and  $C_h$  decreased,  $\delta^{13}C_{SOM}$  became more enriched and  $Al_p$  increased (Tables 2 and S2). The effects were the same for all days of measurement. In all cases, there were significant interactions between soil type and the treatments (Table 2 for day 1 and Table S2 for days 4 and 8). Because of the lack of differences between days, all data

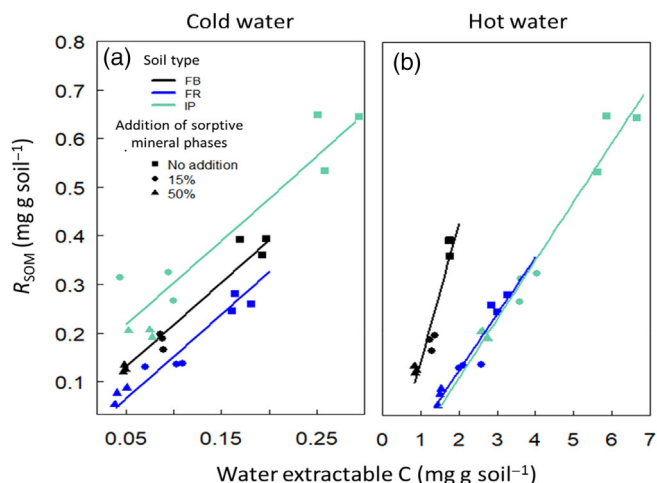
**TABLE 2** Soil organic matter decomposition rates ( $R_{SOM}$ ),  $\delta^{13}C$  isotopic signature of respired  $CO_2$  ( $\delta^{13}C_{SOM}$ ), cold ( $C_c$ ) and hot ( $C_h$ ) water extractable carbon and pyrophosphate-extractable aluminium concentration ( $Al_p$ ) for three soil types incubated with addition of sorptive mineral phases of 0%, 15% and 50%

Soil	Sorptive mineral phases (%)	$R_{SOM}$ ( $\mu\text{g C g soil}^{-1} \text{ s}^{-1}$ )	$\delta^{13}C_{SOM}$ (‰)	$C_c$ ( $\text{mg g soil}^{-1}$ )	$C_h$ ( $\text{mg g soil}^{-1}$ )	$Al_p$ ( $\text{mg g soil}^{-1}$ )
FB	0	$0.38 \pm 0.02^a$	$-28.27 \pm 0.25^a$	$0.19 \pm 0.01^a$	$1.76 \pm 0.00^a$	$0.00 \pm 0.01^a$
	15	$0.18 \pm 0.02^b$	$-26.33 \pm 0.53^b$	$0.09 \pm 0.00^b$	$1.30 \pm 0.07^b$	$0.30 \pm 0.01^b$
	50	$0.13 \pm 0.01^c$	$-23.21 \pm 1.02^c$	$0.05 \pm 0.00^c$	$0.86 \pm 0.03^c$	$0.49 \pm 0.03^c$
FR	0	$0.26 \pm 0.02^a$	$-28.51 \pm 0.61^a$	$0.17 \pm 0.01^a$	$3.05 \pm 0.21^a$	$0.00 \pm 0.01^a$
	15	$0.13 \pm 0.00^b$	$-22.81 \pm 0.68^b$	$0.09 \pm 0.02^b$	$2.24 \pm 0.30^b$	$0.37 \pm 0.02^b$
	50	$0.07 \pm 0.02^c$	$-20.35 \pm 0.88^c$	$0.04 \pm 0.01^c$	$1.50 \pm 0.04^c$	$0.60 \pm 0.04^c$
IP	0	$0.61 \pm 0.07^a$	$-27.72 \pm 0.48^a$	$0.27 \pm 0.02^a$	$6.05 \pm 0.53^a$	$0.01 \pm 0.02^a$
	15	$0.30 \pm 0.03^b$	$-24.19 \pm 0.17^b$	$0.08 \pm 0.03^b$	$3.75 \pm 0.25^b$	$0.62 \pm 0.13^b$
	50	$0.20 \pm 0.01^c$	$-19.08 \pm 0.42^c$	$0.07 \pm 0.01^b$	$2.65 \pm 0.09^c$	$0.94 \pm 0.15^c$
Significance from analysis of variance						
Sorptive mineral phases		***	***	***	***	***
Soil type		***	***	***	***	***
Sorptive mineral phases $\times$ soil type		***	***	***	***	***

Note: The three soil types refer to pallic firm brown (FB), weathered fluvial recent (FR) and typic immature pallic (IP). All values are means  $\pm$  SEs ( $n = 3$ ). Different letters indicate homogeneous groups from post-hoc Tukey analysis between the additions of sorptive mineral phases within each soil type. For the analyses of variance, the  $p$ -values significance levels are shown as  $* \leq 0.05$ ;  $** \leq 0.01$ ; and  $*** \leq 0.001$ .

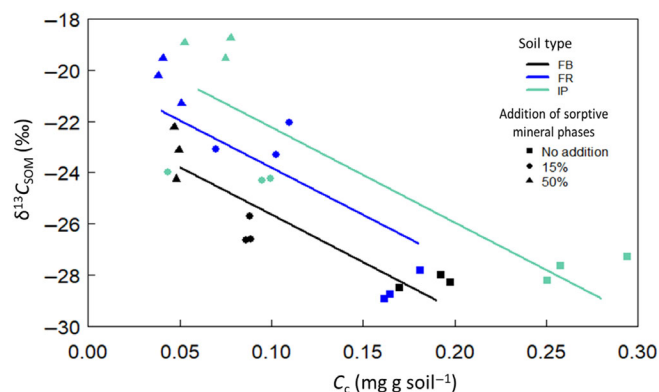


**FIGURE 2** Cold (a) and hot (b) water extractable carbon concentrations in relation to the amounts of organo-mineral complexes measured as pyrophosphate-extractable aluminium concentration. The lines are linear fits ( $p < 0.001$ ) for the three soil types described in Table 1. The coefficients of determination for cold water extractable C are 0.95, 0.96 and 0.86, and for hot water extractable C are 0.98, 0.98 and 0.95, for the firm brown, fluvial recent and immature pallic soils, respectively [Color figure can be viewed at [wileyonlinelibrary.com](#)]



**FIGURE 3** Soil organic matter (SOM) decomposition rates ( $R_{SOM}$ ) in relation to (a) cold and (b) hot water extractable carbon concentrations for three soils mixed with addition of three proportions of sorptive mineral phases. The lines are linear fits ( $p < 0.001$ ) for the three soil types described in Table 1. The coefficients of determination are 0.97, 0.95 and 0.91 for cold water extractable C, and 0.98, 0.98 and 0.98 for hot water extractable C, for the firm brown, fluvial recent and immature pallic soils, respectively [Color figure can be viewed at [wileyonlinelibrary.com](#)]

shown below are those at 1 day. Mean values and significance of analyses of variances for data from days 4 and 8 are shown in Table S2 and the relationships



**FIGURE 4** Relationship between  $\delta^{13}C$  isotopic signature of respired  $CO_2$  derived from soil organic matter decomposition ( $\delta^{13}C_{SOM}$ ) and cold water extractable carbon concentration ( $C_c$ ). The lines represent the fit ( $p < 0.001$ ) of the relationship for three soil types described in Table 1. The coefficients of determination are 0.95, 0.90 and 0.86 for the firm brown, fluvial recent and immature pallic soils, respectively [Color figure can be viewed at [wileyonlinelibrary.com](#)]

between variables for data for days 4 and 8 are shown in Figures S1–S3.

### 3.4 | SOM accessibility and SOM decomposition

Both indicators of accessible SOM ( $C_c$  and  $C_h$ ) were strongly negatively correlated with  $Al_p$  ( $p < 0.001$ ) (Figure 2). There were significant differences in the intercepts ( $p < 0.001$ ) for the relationship between  $C_c$  and  $Al_p$  among the soil types (mean  $\pm$  SE  $0.18 \pm 0.01$ ,  $0.17 \pm 0.02$  and  $0.24 \pm 0.02$   $mg\ g\ soil^{-1}$  for the FB, FR and IP soils, respectively). There were no significant differences ( $p = 0.33$ ) in the slopes of the relationships between the addition of sorptive mineral phases and  $Al_p$  for the three soils (mean  $\pm$  SE  $-0.2 \pm 0.007$   $mg\ C\ g\ soil^{-1}\ mg\ Al_p^{-1}$ ). For the relationship between  $C_h$  and  $Al_p$ , there were significant differences among the three soils in both the intercepts ( $p < 0.001$ ) (mean  $\pm$  SE  $1.78 \pm 0.16$ ,  $3.09 \pm 0.23$  and  $5.09 \pm 0.23$   $mg\ g\ soil^{-1}$ ) and slopes ( $p = 0.01$ ) ( $-1.80 \pm 0.4$ ,  $-2.56 \pm 0.6$  and  $-3.4 \pm 0.5$   $mg\ C\ g\ soil^{-1}\ mg\ Al_p^{-1}$  for the FB, FR and IP soils, respectively).

$R_{SOM}$  increased linearly with increasing  $C_c$  and  $C_h$  for the three soils ( $p < 0.001$ ) (Figure 3). For the relationship between  $R_{SOM}$  and  $C_c$ , there were significant differences ( $p < 0.001$ ) in the intercepts (mean  $\pm$  SE  $0.03 \pm 0.02$ ,  $0.006 \pm 0.04$  and  $0.12 \pm 0.03$   $mg\ g\ soil^{-1}$  for the FB, FR and IP soils, respectively) but there were no significant differences ( $p = 0.52$ ) among the slopes (mean  $\pm$  SE  $1.84 \pm 0.2$   $\mu g\ C\ g\ soil^{-1}\ s^{-1}\ mg\ C_c^{-1}$ ). There were significant differences in the relationships between  $R_{SOM}$  and  $C_h$  for the

three soils in both the intercepts ( $p < 0.001$ , mean  $\pm$  SE  $-0.14 \pm 0.04$ ,  $-1.0 \pm 0.23$  and  $-0.13 \pm 0.05$  mg g soil $^{-1}$ ) and slopes ( $p < 0.001$ ,  $0.28 \pm 0.03$ ,  $0.11 \pm 0.03$  and  $0.12 \pm 0.05$   $\mu$ g C g soil $^{-1}$  s $^{-1}$  mg C $_h$  $^{-1}$  for IP, FB and FR soils, respectively).

There was a linear depletion (decrease) in  $\delta^{13}\text{C}_{\text{SOM}}$  with increasing  $C_c$  ( $p < 0.001$ ) (Figure 4) with significant differences among the three soils for the intercepts ( $p < 0.001$ ) (mean  $\pm$  SE  $-22.4 \pm 1.17$ ,  $-17.57 \pm 1.69$  and  $-19.37 \pm 1.54$  mg g soil $^{-1}$  for the FB, FR and IP soils, respectively), but not the slopes ( $p = 0.54$ ) (mean  $\pm$  SE  $-30 \pm 2.1\%$  mg C $_c$  $^{-1}$ ).

## 4 | DISCUSSION

Our findings show that the addition of increasing proportions of sorptive mineral phases derived from an allophanic subsoil resulted in decreased rates of SOM decomposition. The effect appeared rapidly within 1 day after mineral addition and remained proportionally the same through 8 days (Figure 1). These findings are consistent with previous studies showing that the addition of various mineral phases resulted in decreasing decomposition rates of SOM (Feng et al., 2014; Sanderman et al., 2014). We showed that the decrease in SOM decomposition rates with added sorptive mineral phases were attributable to reductions in the amounts of carbon substrates (decreasing cold and hot water extractable C) accessible for microbial decomposition (Figure 3). Further, we showed that the treatments resulted in increased pyrophosphate-extractable aluminium which we interpret to indicate increased formation of organo-mineral complexes (Figure 2). The proportional changes in these variables were the same for three soils with contrasting properties, providing evidence that the mechanisms are independent of soil C concentration, pH and microbial community composition and diversity.

### 4.1 | Pyrophosphate-extractable aluminium and organo-mineral interactions

We hypothesised that the addition of the mineral phases derived from an allophanic clay soil (Parfitt, 1990) would increase the adsorption of SOM onto the surfaces of the mineral clay microstructure to form complexes with aluminium (Kaiser & Guggenberger, 2007; Lützow et al., 2006; Schneider et al., 2010). Although our experimental approach did not allow direct determination of the mechanisms leading to the increase in SOM stabilisation, we interpreted the fraction of aluminium dissolved in

sodium pyrophosphate as a quantitative indicator of organo-mineral complexes that leads to SOM stabilisation (Eimil-Fraga et al., 2015; Masiello et al., 2004; Parfitt & Childs, 1988; Taboada et al., 2019). However, the use of pyrophosphate-extractable aluminium as an indicator of the amounts of organo-mineral complexes has been questioned because small amounts of aluminium can be extracted directly from allophane or aluminium hydroxides (Kaiser & Zech, 1996). This suggests that not all the dissolved aluminium in sodium pyrophosphate extracts is derived from organo-mineral complexes and explains the background Al $_p$  we observed in the mineral with no soil present and where organic C was not detectable. We accounted for this effect by subtracting the value of aluminium pyrophosphate for the mineral alone from the treatments with mixtures of soil and the mineral, and we interpreted this as the value of newly formed organo-mineral complexes. Furthermore, although we mixed the same mass of sorptive mineral phases to the three soils, the absolute amount of Al $_p$  was higher in the soil with the highest amount of water extractable C (Figure 2 and Table 2). This provides evidence that Al $_p$  formation was related specifically to the amount of water extractable C in each soil prior to mixing. Further, the increasing amount of Al $_p$  with decreasing amounts of water extractable C for each soil (Figure 2) supports our interpretation that the amount of Al $_p$  is representative of the newly formed organo-mineral complexes.

### 4.2 | SOM accessibility and decomposition

For the three soil types, we showed similar proportional decreases in SOM accessibility ( $C_c$  and  $C_h$ ) within 1 day after the addition of increasing amounts of the mineral phases to the soils (Figures 2 and 3). Previous studies by Zakharova et al. (2014, 2015) reported decreases in SOM accessibility of 59%–62% from measurements of  $C_h$  after 14 days for soils incubated with 50% mineral addition. The strong negative correlations between both  $C_c$  and  $C_h$  and Al $_p$  (Figure 2) highlight the rapid formation of organo-mineral complexes and the rapid stabilisation of SOM onto organo-mineral interactions. This suggests that the proximity of C substrates and mineral surfaces is a key driver for C stabilisation by reducing accessibility to microbial decomposers. Other processes that affect the proximity in field conditions are likely to be the rates of SOC translocation following leaching, the fragmentation of soil aggregates by soil fauna (Mayes et al., 2012), root distribution and the release of root exudates (Sokol & Bradford, 2019), bioturbation (John et al., 2015), and structural and variable (pH- and adsorbed-ion-dependent)



properties of the minerals (surface charge, surface topography, size, and shape) (Kleber et al., 2021).

The identical slopes in the decrease in  $C_c$  with increasing  $Al_p$  for all three soils suggest a constant proportional decrease in  $C_c$ , independent of the initial available C concentrations, as indicated by differences in the intercept on the  $C_c$  axis (Figure 2). In contrast, the slope of the relationship between  $C_h$  and  $Al_p$  decreased as the initial value of  $C_h$  (intercept) decreased (Figure 2). This suggests that  $C_c$  represents a C pool that is more directly accessible to adsorption onto minerals surfaces than the pool estimated from  $C_h$  and this is consistent with studies showing that hot water extracts contain substrates that are not immediately available for decomposition (Ghani et al., 2003).

SOM decomposition rates were strongly and positively correlated with the size of accessible C pools ( $C_c$  and  $C_h$ ). Similar to the relationships with  $Al_p$ , the slopes of the relationships between  $R_{SOM}$  and  $C_c$  remained constant across soil types but differed between soil types for the relationships between  $R_{SOM}$  and  $C_h$  (Figure 3). Further, the intercepts for the relationship between  $R_{SOM}$  and  $C_h$  were negative, meaning that the slope and fit were overestimated. This also confirms previous suggestion that microbial activity is related more directly to  $C_c$  than to  $C_h$  (Landgraf et al., 2006).

### 4.3 | $\delta^{13}C$ natural abundance of respired $CO_2$

With increasing proportions of added sorptive mineral phases and decreasing amounts of  $C_c$  and  $C_h$ , the  $\delta^{13}C$  isotopic signature of respired  $CO_2$  became more enriched (less negative) (Figure 4). This suggests preferential adsorption of C substrates with more depleted (more negative) signatures onto mineral surfaces, leaving more enriched substrates accessible for microbial decomposition, consistent with previous findings (Kaiser et al., 2001). This negates our third hypothesis. Two concepts have been proposed to explain the stabilisation of organic substrates in interactions with minerals in the soil (Liang et al., 2017; Sokol et al., 2019). First, the “direct sorption pathway” or “ex vivo pathway” suggests that organo-mineral interactions occur by direct sorption of plant compounds onto mineral surfaces. Second, the “in vivo microbial turnover pathway” proposes that SOM associated with minerals is constituted mainly of microbial residues that are relatively  $\delta^{13}C$  enriched compared to the values of compounds derived from plant residues. The higher proportion of a more enriched  $\delta^{13}C$  signature in organo-mineral complexes compared with that in other SOM pools provides evidence to support the microbial

formation pathway (Miltner et al., 2012). In contrast, the presence of plant-derived materials on minerals bypassing microbial assimilation would lead to more depleted  $\delta^{13}C$  values for organo-mineral complexes and support the direct adsorption pathway (Kramer et al., 2012). It is tempting to interpret our relative  $\delta^{13}C$  enrichment of respired  $CO_2$  with decreasing C accessibility as being attributable to preferential adsorption of plant derived compounds onto the increasing amounts of organo-mineral interactions, thus providing support for the direct adsorption pathway. However, it is problematic to extend findings from the experimental conditions in the laboratory with no plants present to interpret mechanisms operating directly in field conditions. Curtin et al. (2014) and Zakharova et al. (2015, 2014) showed that sieving soil during preparation of soil samples, as was done in our study, releases previously protected substrates that lead to a rapid and strong depletion of soil respired  $\delta^{13}CO_2$ . The authors attributed this to the disruption of soil aggregates containing plant derived material that became immediately accessible as substrates for microbial decomposition. Protection of these previously aggregate-protected substrates by subsequent addition of sorptive mineral phases would result in preferential adsorption of plant-derived compounds released artificially by disruption onto the mineral surfaces. However, this would not occur in field conditions where the substrates are likely to be unavailable for adsorption. Sokol et al. (2019) suggested that both direct adsorption and microbially mediated pathways coexist, and the dominance of either pathway depends on the location within the soil matrix that defines the relative availability of plant and microbial products for organo-mineral interactions. Our experimental conditions may be representative of soil hotspots with high availability of plant derived material deposited prior to sampling and mineral surfaces that promote direct adsorption of substrates. Nonetheless, the  $\delta^{13}C$  enrichment of respired  $CO_2$  with decreasing C accessibility and increasing formation of organo-mineral complexes can be interpreted as a change in the composition of accessible SOM substrates, leading to the decomposition of more microbially derived and enriched substrates in our experimental conditions (Boström et al., 2007; Sanderman et al., 2008).

## 5 | CONCLUSIONS

We have provided evidence in laboratory conditions that the addition of sorptive mineral phases to soils decreases the rate of microbial decomposition of SOM and that this is attributable to increased organo-mineral interactions leading to a reduction in SOM accessibility for decomposition.

We demonstrated strong linear relationships between the decrease in C accessibility and SOM decomposition, with cold water extractable C more directly related to the rate of decomposition than hot water extractable C. SOM accessibility and decomposition were reduced rapidly and proportionally to the amount of added sorptive mineral phases resulting from increased organo-mineral interactions irrespective of the organic carbon concentration of the soils tested.

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT



The data that support the findings of this study are available from the corresponding author upon reasonable request.

## AUTHOR CONTRIBUTIONS

**Jonathan Nuñez:** Formal analysis (lead); investigation (lead); methodology (lead); writing – original draft (lead). **Gabriel Moinet:** Conceptualization (lead); formal analysis (supporting); investigation (supporting); methodology (supporting); supervision (equal); writing – review and editing (equal). **Scott Graham:** Formal analysis (supporting); methodology (supporting); supervision (supporting); writing – review and editing (equal). **Matthew Turnbull:** Funding acquisition (equal); supervision (equal); writing – review and editing (equal). **Gwen-Aëlle Grelet:** Formal analysis (supporting); supervision (supporting); writing – review and editing (equal). **David Whitehead:** Conceptualization (supporting); funding acquisition (lead); supervision (equal); writing – review and editing (equal).

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